ABSTRACT BOOK

ICAR2020+2

Bologna, Italy

19th International Congress
on Animal Reproduction

BOLOGNA (ITALY), 26th-30th JUNE 2022

CONTENTS

INVITATION

Dear Colleagues, dear Friends,
on behalf of the Local Organising Committee, it is my pleasure to welcome you to the 19th International Congress on Animal Reproduction at the Palazzo della Cultura e dei Congressi in Bologna (Italy) from 26th to 30th June 2022.

As you surely know, the Congress was to be held in Bologna from 28th June to 2nd July 2020; however, due to the Coronavirus outbreak that has paralyzed the scientific community (and not only) worldwide, it has been at first moved to 2021; after that, being the situation still uncertain, we decided to postpone again the Congress to the above mentioned dates and to refer to the Congress as ICAR2020+2.

The International Congress on Animal Reproduction (ICAR) is a nonprofit international organization that conducts conferences on animal reproduction.
Founded in 1948, ICAR has no members but is governed by a Standing Committee made of scientists representative of over 40 countries and has held a major conference every four years in many countries throughout the world.
When ICAR Board decided to move ICAR2020 to 2022, contextually decided to move the subsequent Congress from 2024 to 2026.

The focus of the Congress is animal reproduction, including animal physiology, animal pathology and reproductive technologies.

The conference is organized in individual plenary sessions followed by concurrent symposia and workshops as well as poster sessions.
A trade exhibition will remain open throughout the conference.

At ICAR 2020+2 we are more than 700 delegates (the number could have been much higher but unfortunately limitations to travel still exist in many Countries) who will attend a 4-day Scientific Programme organized in 5 Plenary Sessions, 12 Symposia 23 Workshops and 3 Poster sessions.

Welcome to ICAR 2020+2!

Prof. Carlo Tamanini
Chair of the Organising Committee

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SYMPOSIUM 1 The nonconformist: conceptus-maternal communication in the dog

O01
A HIGH CONCENTRATION OF PROGESTERONE ENHANCES THE MEIOTIC MATURATION OF CANINE OOCYTES WITHOUT INFLUENCING THE GENE EXPRESSION OF CX37 AND CX43

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BACKGROUND-AIM
Progesterone concentrations are especially high in the preovulatory period in canines as the follicles are luteinized before ovulation. The mechanisms by which progesterone modulates the final oocyte development remain to be characterized in dogs. This study aimed to evaluate the effect of a high concentration of progesterone supplementation on meiotic development in relationship to connexin 37 (Cx37) and 43 (Cx43) gene expression in canine cumulus oocytes complexes (COCs) during in vitro maturation (IVM).
METHODS
A total of 180 ovaries from adult bitches were collected following routine ovariohysterectomy. In each experiment, a group of COCs retrieved from antral follicles was subjected to IVM under different progesterone supplementation: 1) Control, without progesterone supplementation; 2) 50 µg progesterone, 3) 100 µg progesterone. A half of COCs recovered at 0 h and 72 h of IVM from each group were subjected to evaluation of the meiotic development, and the other half was submitted to q-PCR to analyze connexins gene expression. Data were analyzed with ANOVA.
RESULTS
The addition of exogenous progesterone to the culture medium increased (P<0.05) the meiotic resumption (GVBD) and the percentage of oocytes at the MII stage compared to the control group. Higher (P<0.05) percentage of oocytes in the control group remained arrested at the GV stage compared to those cultured with progesterone. The highest rate of MII stage was observed with the highest doses of progesterone. Although a significant decrease in the mRNA levels of both connexins was noticed after culture, there was no effect in Cx37 and Cx43 gene expression when adding exogenous progesterone.
CONCLUSIONS
High doses of progesterone to the culture media enhance meiotic development during IVM, but it seems to have no direct effect on Cx37 and Cx43 gene expression
Supported by Grant FONDECYT 1211285

O02
GENOME-WIDE TRANSCRIPTIONAL EFFECTS IN DOG UTERINE STROMAL CELLS DURING DECIDUALIZATION AND FOLLOWING ANTIGESTAGEN TREATMENT

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BACKGROUND-AIM
By expressing the nuclear progesterone (P4) receptor (PGR), maternal stroma-derived decidual cells play key roles in the maintenance of canine pregnancy as the decreased circulating P4 levels at term, or the preterm blocking of PGR with antigestagens, lead to termination of pregnancy. In vitro decidualization of immortalized dog uterine stromal (DUS) cells showed upregulation of several decidualization markers, e.g., IGF1, PTGES and PRLR, and are a valuable model for investigating underlying mechanisms. Type II antigestagens, aglepristone and mifepristone, have detrimental effects on the expression of these markers and on the proliferative capacities of DUS cells. However, despite being strongly related chemically, the two antigestagens appear to differ to some extent in the effects they exert upon target gene expression.
METHODS
Here, we aimed to deepen our understanding of the mechanisms involved in decidualization and the initiation of parturition (associated with the withdrawal of PGR function). Genome-wide changes (deep RNA sequencing; RNA-seq) associated with decidualization, and antigestagen treatment in decidualized cells, were assessed in DUS cells. Following decidualization (0.5mM cAMP, 72h), cells were treated for 6h with antigestagens. Differentially expressed genes (DEGs), functional terms, networks and pathways were analysed following RNA-seq.
RESULTS
Decidualization increased the presence of 1500 DEGs (P<0.01, FDR<0.01), mainly associated with extracellular matrix organization and cell proliferation; 1320 DEGs, including those involved in apoptosis, were downregulated. A total of 1118 and 1341 DEGs were found after treatment with aglepristone and mifepristone, respectively, predominantly associated with reversing the cAMP-mediated effects (up to 74.5% of DEGs). Only 54% of the antigestagen-affected DEGs were common to both antigestagens, implying differences in their functionality in decidualized cells.
CONCLUSIONS
Cumulatively, antigestagens appear to greatly reverse the transcriptional changes resulting from decidualization, underlying the importance of cAMP signalling. Despite mifepristone and aglepristone causing the same outcome in vivo, i.e. the termination of pregnancy, these results provide further support for them having functional differences. SNFS:31003A_182481.

O03

A MODIFIED AGLEPRISTONE PROTOCOL FOR MID-PREGNANCY TERMINATION IN LARGE DOGS

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BACKGROUND-AIM

Aglepristone, a competitive progesterone antagonist, is one of the available drugs to effectively terminate mid-pregnancy in dogs. The recommended protocol consists of two subcutaneous injections of Aglepristone (SC) at 10 mg/kg of body weight administered 24 hours apart (Thomas & Fontbonne, 2008). A week later, animals are submitted to an ultrasound examination to confirm abortion. If not achieved, the protocol should be repeated. The reported effectiveness of Aglepristone-induced abortion at mid-pregnancy is close to 95% (Thomas & Fontbonne, 2008). In the practice, however, failure to abort after one cycle of Aglepristone at mid-pregnancy seems to be higher. In our experience, we perceived it as higher in larger-sized female dogs bearing large litters, driving the need for an additional cycle. That uncertainty on the success of the treatment in particular situations leads us to test an alternative, Aglepristone-modified protocol.

METHODS

The Aglepristone-modified protocol was tested in 10 healthy female dogs presented for pregnancy termination at a private clinic. The day of breeding and the embryonic morphology were used to estimate the age of the pregnancy. Aglepristone was administered at 10 mg/kg on days 1 (at presentation), 3, and 5. Ultrasound was performed on day 12 to evaluate the response to the treatment. Data from these females were compared with retrospective data from the clinic, using a group of 10 mid-pregnancy female dogs treated for mismating with similar ages and size.

RESULTS

The age of the females submitted to the new protocol ranged between 10 months and 4 years old, and body weight between 28.2 to 37.6 kg. Unwanted pregnancies were estimated between 28 and 30 days, and all presented a large number of fetuses (between 8 and 11). The use of the modified Aglepristone protocol successfully interrupted pregnancy in all the cases with one cycle treatment, compared with a 70% success rate obtained with the standard protocol.

CONCLUSIONS

Preliminary results show that in large-sized bitches bearing larger litters, the aglepristone protocol described herein may be more efficient to induce abortion than the traditional one.

REFERENCE LIST:

Thomas, P. G., & Fontbonne, A. (2008). Drugs and reproduction. Small Animal Clinical Pharmacology, 528–545. doi:10.1016/b978-070202858-8.5

O04

RECENT ADVANCES IN UNDERSTANDING UTERINE INERTIA IN THE DOG: FOCUSING ON SELECTED CONTRACTILE AND CONTRACTILITY-ASSOCIATED PROTEINS IN THE UTERUS

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BACKGROUND-AIM

The etiology of canine primary uterine inertia (PUI) is still unclear. This compilation summarizes our findings on blood glucose (BG) and ionized calcium (iCa) levels as well as on uterine expression of selected contractility-associated proteins in PUI.

METHODS

BG and iCa were compared between dogs diagnosed with PUI (n = 14; no/very weak contractions on tocodynamometry and/or vaginal feathering, no pups born, no obstruction), secondary uterine inertia (SUI, n=6; pups born but contractions ceased, no obstruction) and obstructive dystocia (OD, n=6; still strong contractions). Expression of smooth muscle (SM) α- and γ-actin, SM-myosin, leptin (Lep), Lep receptor (LepR), RhoA and its kinases (ROCK1, ROCK2; involved in calcium sensitization) and members of the prostaglandin (PG) pathway (PTGS2, PTGFS, PTGFR, PTGES, PTGER2 and 4, HPGD, PGT) was compared between PUI (n=10-12) and OD (n=5-8) in full-thickness interplacental uterine biopsies collected at C-sections; qPCR (relative gene expression, RGE/ratio) and, depending on availability of antibodies, immunohistochemistry were applied. One-way ANOVA, t-test, Mann-Whitney U test and linear regression with significance $P<0.05$ were used. The influence of litter size was also studied.

RESULTS

BG and iCa were similar between groups and not affected by litter size. The dam's body weight was related to iCa ($R2=0.241$, $P=0.013$). Whereas ratios of none of the members of the PG pathway differed between PUI and OD, uterine RGE of SM-γ-actin, SM-myosin, ROCK1 and ROCK2 was significantly higher in PUI than OD. RGE of LepR was undetectable in all OD and in 5 PUI dogs. Litter size affected RGE of SM-γ-actin and PGES. All proteins investigated were expressed in the myometrium and immunoreactivity did not differ between groups.

CONCLUSIONS

Low iCa may have contributed to PUI in some small size

bitches. Alterations of the PG pathway seem not to be involved in PUI. Differences in uterine RGE of some contractility-associated proteins between PUI and OD may reflect abnormal labor progression in PUI with only absent or weak contractions. It is unclear if these alterations are due to an intrinsic error of the uterus not becoming committed to labor or the consequence of inadequate endocrine or mechanical stimuli. Litter size may affect expression of some contractile elements. Low animal numbers are a limitation of this study.
*,#equal contribution; Grant: Agria and SKK Res. Found. N2014-0002

SYMPOSIUM 2 Devising new gonadotropins for the future...which strategies, which uses?

O05
EFFECT OF 2000 IU OF SYNTHETIC ECG-LIKE GLYCOPROTEIN ON THE FOLLICULAR DEVELOPMENT AND OVULATION IN BEEF COWS (BOS TAURUS)

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BACKGROUND-AIM
The objective of this study was to determine the biological activity of a synthetic eCG-like glycoprotein as an alternative to native eCG in cattle.
METHODS
Fourteen cyclic Angus/Hereford cows, 3 to 5 years of age were used. The trial was conducted in two periods in a crossover design (Periods 1 and 2). All cows were treated on Day 0 of treatment with an intravaginal device (DIB, 1 gram progesterone, Syntex, Argentina) plus 2 mg im of estradiol benzoate (Gonadiol, Syntex). On Day 4, cows were divided into two groups and received 2000 IU im of eCG (Novormon, Syntex; eCG group; n = 13) or 2000 IU im of synthetic eCG-like glycoprotein (Syntex; eCG-like group; n = 14). On Days 6.5 and 7, 500 µg im of Cloprostenol sodium (Cyclase, Syntex) was administered. On Day 7, DIB devices were removed and on Day 8, 100 µg im of Gonadorelin acetate (Gonasyn, Syntex) was administered. Twenty-seven days after the end of Period 1, cows were treated with the same protocol in Period 2, except that cows in the eCG group were treated with eCG-like and cows in the eCG-like group were treated with eCG. The animals were scanned by transrectal ultrasonography (Chison 500; 7.5 MHz, Doppler) on Day 8 of treatment and all follicles > 8 mm diameter (pre-ovulatory) were recorded. Daily ultrasound examinations were continued until Day 11 to determine the number of ovulations, defined as the disappearance of follicles larger than 8 mm present in the previous examination. The average number of follicles > 8 mm on Day 8 and the number of ovulations between Days 8 and 11 were compared by ANOVA.
RESULTS
There was no effect of Period (P = 0.17) or Period*Group interaction (P = 0.69) for follicle numbers, but there tended to be an effect of Period (P = 0.06) on the number of ovulations, but again, no Period*Group interaction (P = 0.85). No differences () were found in number of follicles > 8 mm on Day 8 (eCG group - 14.5 ± 2.33 vs eCG-like group - 15.0 ± 1.94; P = 0.9) or in number of ovulations (eCG group - 13.7 ± 2.54 vs eCG-like group - 13.3 ± 1.79; P = 0.83).
CONCLUSIONS
We conclude that the synthetic eCG-like glycoprotein has the same biological activity as native eCG.

O06

EFFICIENCY OF REPEATED SUPERSTIMULATIONS ON OVUM PICK-UP AND IN VITRO PRODUCTION (OPU-IVP) OF CATTLE EMBRYOS AND DONOR HEALTH

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BACKGROUND-AIM
In preliminary studies, we used abattoir-derived ovaries to optimize media for oocyte recovery and maturation, fertilization and embryo culture. Our custom SOF medium yielded higher cleavage rates than TCM (91 vs 42%; p<0.05) and higher blastocyst rates than TCM or KSOM (49, 12 and 35% respectively, p<0.1). SOF-derived embryos (n=70) were frozen (programmable freezer), thawed and cultured in vitro for 48 h; 76% re-expanded and 50% hatched. Objectives of this study were to evaluate impacts of repeated superstimulation on OPU-IVP and donor reproductive health.
METHODS
Cross-bred beef heifers (n=6) and cows (n=3) were subjected to superstimulation and OPU-IVP (Day 0: CIDR insertion; Day 2: follicle ablation; Days 4 to 6: FSH 12 h apart; Day 8: OPU) twice monthly for 1 y. The oocytes recovered were in vitro fertilized and in vitro cultured in our custom-made SOF medium for 7 days. The resulting embryos were transferred fresh or frozen for subsequent transfer to estrous synchronized heifers. Pregnancy diagnosis was done using ultrasonography on Day 22 to 30 following embryo transfer.
RESULTS
Mean (±SEM) number of follicles, oocytes recovered, cleavage rate (%) and blastocyst rate (%) had substantial ranges among heifers (5±0.8 to 18±1.8; 2±0.6 to 9±1.6; 40±13.6 to 58±11.3; and 10±5.5 to 24±11.4, respectively) and cows (9±1.2 to 56±1; 4±0.8 to 17.5±3.5; 54.5±16.5 to 89±6.2; and 20±1 to 78±7.7). However, repeated trials did not significantly affect OPU-IVP efficiency. Embryo viability was based on pregnancy rates (PR) on Days 22 to 30 after transfer of fresh or frozen-thawed embryos (n=20 each) and live births. The PR for OPU-IVP derived fresh embryos (51%) were similar to that of fresh in vivo embryos (63%, 607/964), whereas transfer of OPU-IVP derived frozen embryos reduced PR (33%) compared to frozen-thawed in vivo embryos (54%, 606/1124; P<0.05). All recipients diagnosed pregnant delivered a viable calf, suggesting developmental competence of the embryos. Repeated OPU-IVP
had no apparent deleterious effects, as all donors had viable calves after ET.
CONCLUSIONS
We concluded that multiple OPU-IVP cycles can be effectively performed for a prolonged interval to maximize embryo production from elite donors.

SYMPOSIUM 3 Cloning and genome editing

O07

EFFECT OF OOCYTE SOURCE ON THE DEVELOPMENT OF EQUINE EMBRYOS PRODUCED BY SOMATIC CELL NUCLEAR TRANSFER

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BACKGROUND-AIM
The production of equine embryos by somatic cell nuclear transfer (SCNT) is limited by the availability of immature oocytes. Considerable resources are required to retrieve immature oocytes from live mares programmed for ovum pick up (OPU). Alternatively, immature oocytes can be recovered from abattoir-sourced ovaries; however, the stage of follicle development at the time of slaughter is usually unknown, such that inferior quality oocytes may be collected. The aim of this study was to compare the development of equine SCNT embryos produced using oocytes either retrieved by OPU or recovered from abattoir-sourced ovaries.
METHODS
For the study, a total of 1,128 oocytes were used, of which 663 were from abattoir-sourced ovaries and 495 were from live mares programmed for OPU. The harvested ovaries were transported to the lab within 1 h for processing. The methods used for in vitro maturation (IVM), SCNT and embryo culture in vitro were identical for both sources of oocytes. The rates of maturation, cell fusion, cleavage and blastocyst formation at Day 7 were evaluated. Transferrable grade blastocysts were transferred to recipient mares and pregnancy was diagnosed at Days 14 and 42 by ultrasound.
RESULTS
The maturation rate of OPU-derived oocytes was lower than that of abattoir-derived oocytes (50.3 ± 2.7% vs 61.9 ± 3.4%; P < 0.05). However, the rates of cell fusion (90.7 ± 2.6% vs 81.9 ± 5.2%), cleavage (68.8 ± 3.9% vs 61.9 ± 5.0%) and blastocyst formation (34.3 ± 2.8% vs 25.4 ± 2.1%) were greater (P < 0.05) for OPU-derived embryos compared with abattoir-derived embryos. While similar proportions of OPU- and abattoir-derived blastocysts initiated pregnancy at Day 14, a greater proportion of OPU-derived blastocysts developed to Day 42 of gestation (13 of 50 embryos) compared with abattoir-derived blastocysts (3 of 27 embryos; P < 0.05).
CONCLUSIONS
The results show that abattoir-sourced oocytes subjected to IVM have the potential to support the development of SCNT embryos that can initiate pregnancy. However, given the observed difference in subsequent in vivo development, the use of OPU-derived oocytes for equine SCNT embryo production is recommended. Further research is needed to understand the cause of the deficiency and improve the developmental potential of abattoir-sourced oocytes.

O08

THE USE OF APHIDICOLIN, A REVERSIBLE DNA REPLICATION INHIBITOR, REDUCE MOSAICISM RATE AND EMBRYO DEVELOPMENT OF KNOCK-OUT TPC1 PORCINE EMBRYOS EDITED BY CRISPR/CAS9

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BACKGROUND-AIM

The most important limitation of the use of CRISPR/Cas9 in embryos is mosaicism. To solve this problem, we carried out strategies such as delivering CRISPR/Cas9 before fertilization and obtained homozygous knock-out pigs (Navarro-Serna et al. 2021, CRISPR Journal). However, we still find embryos with mosaicism. For the current study, we decided to test the use of aphidicolin (Ap) to increase the time in which CRISPR/Cas9 can generate cuts before the start of DNA replication and reduce mosaicism. For this study, a sgRNA against two-pore channel 1 (TPC1) was used. TPC1 is a cation-permeable channel present on endosomes and is involved in different physiological processes; thus the pig is a good animal model to study the mechanism of action and pathophysiological function of this gene in a whole animal.

METHODS

Porcine oocytes collected from the slaughterhouse and matured in NCSU37 for 40-44h were electroporated in the absence (E) or presence (EAp) of CRISPR/Cas9 anti-TPC1, and in vitro fertilized, as was an unelectroporated control (C). The EAp group was incubated with 0.5µM of Ap for 6 to 20 hours post insemination and in vitro cultured up to 6 days. Cleavage rate and blastocyst yield (blastocyst/oocyte) were evaluated, and mutation rates were analysed by fluorescent PCR-capillary gel electrophoresis. 7 replicates were performed.

RESULTS

As expected, cleavage rate was higher in groups electroporated (E and EAp, 73.3% and 66.8%) respect to C group (59.4%, $p<0.01$). Nevertheless, blastocyst yield was similar between C (23.1%) and E groups (20.7%), and lower in EAp group (13.4%, $p<0.01$). Mutation rate ($p=0.02$) and mosaicism rate ($p=0.01$) were significantly higher in E (72.3% and 29.8%) than in EAp group (55.0% and 14.1%). Although mosaicism was significantly lower with Ap treatment, due to toxic effect in embryo development, just 11.5% of initial oocytes reached the condition of non-mosaic mutant blastocyst respect 14.5% of non-treated with Ap ($p>0.05$).

CONCLUSIONS

Despite the use of Ap reducing mosaicism, it does not offer an improvement in the overall generation of non-mosaic modified embryos. This is the first report about the use of aphidicolin in porcine embryos for gene editing and the first generation of porcine embryos with a KO in the TPC1 gene.

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SYMPOSIUM 4 New aspects of corpus luteum regulation toward successful pregnancy

O09

ARE L-LACTATE EFFECTS DURING THE FOLLICULO-LUTEAL TRANSITION MEDIATED VIA NMDA RECEPTORS IN BOVINE GRANULOSA CELLS?

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BACKGROUND-AIM

L-lactate was shown to act as a signaling molecule in cultured bovine granulosa cells (GCs) inducing specific alterations of the gene expression and hormone profile thus mimicking the folliculo-luteal transition (Baufeld and Vanselow 2018 RB&E 16:15). In neurons different signaling pathways have been shown to mediate L-lactate effects: (I) a G-protein coupled receptor (HCAR1) and (II) L-lactate transporters with N-methyl-D-aspartate receptors (NMDARs). From a microarray study we obtained several hints that NMDAR signaling might be present in GCs (Baufeld et al. 2019 BMC Genomics 20:273). In this study we aim to clarify the mechanisms behind the signaling action of L-lactate in bovine GCs.

METHODS

The presence of G-protein coupled receptor HCAR1 transcripts in granulosa and theca cells isolated from small (4-5 mm), medium (9-10 mm) and large (> 14 mm) follicles was analyzed by RT-PCR besides liver as a positive control. In another approach focusing on NMDARs, bovine GCs were cultured in a serum-free cell culture model supplemented with FSH, IGF-1 and androstenedione for 8 days (37°C, 5% CO2) either with 30 mM sodium L-lactate or 30 mM NaCl as vehicle control. The GCs were pre-treated for 48 h with NMDAR antagonists D-AP5 and MK801 (both 10, 25 and 50 µM). Subsequently, GCs were characterized by analyzing the transcript abundance of selected marker genes (CYP19A1, FSHR, LHCGR, RGS2 and VNN2).

RESULTS

Firstly, HCAR1 transcripts could not be detected in bovine GCs, but were present in theca, highly in GCs of medium follicles, and liver. Secondly, inhibitor studies in vitro (n = 3) indicate that L-lactate induced effects on the expression of CYP19A1, FSHR, LHCGR, RGS2 and VNN2 can be partially abolished by pre-treatment with the antagonists D-AP5 and MK801.

CONCLUSIONS

From the obtained results it can be concluded so far that L-lactate action in bovine GCs is not mediated via the G-protein coupled receptor HCAR1. This is also in line with our previous observations that the effects of L-lactate can be almost completely abolished by inhibition of L-lactate transporters. Instead, the action of L-lactate might be mediated via NMDARs. These novel insights in molecular mechanisms of L-lactate action in GCs are not only interesting for bovine reproductive physiology, but also for other monovular species such as humans.

O10

SYSTEMIC ADMINISTRATION OF β-NGF FROM LLAMA SEMINAL PLASMA AT LH PEAK ENHANCES CORPUS LUTEUM FUNCTION IN DAIRY HEIFERS

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BACKGROUND-AIM

Progesterone plasma profile after ovulation is associated with fertility in dairy cattle. We have purified a protein molecule (nerve growth factor-β; β-NGF) from llama seminal plasma with ovulatory and luteotrophic effects after intramuscular administration. The main goal of this study was to determine if systemic administration of purified llama β-NGF at a LH peak induced by estradiol affect corpus luteum (CL) function in dairy heifers.

METHODS

Semen was collected from adult llamas using ovine artificial vagina. Purification of seminal plasma β-NGF was performed using a combination of hydroxyl apatite and gel filtration chromatography. Holstein-Friesian heifers (n=15) weighing between 320 and 330 Kg received 2 mg of estradiol benzoate (EB) plus an intravaginal progesterone device (DIB®, Pfizer, Chile) at day 0. At day 8, the device was removed along with an i.m dose of 500 ug of cloprostenol (Boviprost®, Anasac, Chile) and 1 mg of EB was given at day 9. At day 10, heifers were randomly assigned to receive: β-NGF (1 mg i.m., n=8) or saline (Control, n=7). Ovaries were examined daily by B-Mode ultrasonography from DIB removal to ovulation (disappearance of dominant follicles) and then at days 6, 9, 12, 15 after ovulation to determine CI diameter. Blood samples were collected at the same days after ovulation to determine plasma progesterone concentration. Proportional and Non-serial data were analy zed using Fisher's exact test and student t Test respectively whereas serial data were analyzed using PROC MIXED procedure in SAS.

RESULTS

Results showed that neither follicle size (13.0±0.5 vs.14.0±0.6 mm respectively; P=0.20); the proportion of ovulated heifers (7/8 (87.5%) vs. 6/7 (85.7%), P= 0.9); nor the CL profile diameter (P=0.8) differed between NGF and Control groups. However, plasma progesterone concentration was higher in the NGF compared to Control heifers respectively (day 6: 9.7±0.9 vs. 6.5±1.0 ng/ml; day 12: 8.7 ± 0.7 vs 6.5 ± 0.8 ng/ml; day 15: 8.8 ± 0.5 vs 6.2 ± 0.4 ng/ml, P=0.01) and still tended to be higher at day 9 (9.6 ± 0.8 vs. 7.5 ± 0.8 ng/ml; P=0.07).

CONCLUSIONS

We conclude that systemic administration of β-NGF at LH peak induces an earlier increase of luteal progesterone compared to untreated dairy heifers.

This study was supported by Grant Fondecyt Regular 1190980.

WORKSHOP 5 Improving livestock production: beyond genetic again

O11

EFFECT OF THE SPERM SELECTION METHOD ON BASIC SPERM PARAMETERS AND IN VITRO FERTILIZATION OUTCOMES

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BACKGROUND-AIM

Porcine in vitro embryo production (IVEP) protocols have traditionally relied on density gradient centrifugation (DGC) as a sperm selection method to achieve a successful fertilization of the oocytes in vitro (IVF). However, the toxicity of the solutions used and the centrifugation needed for DGC selection can negatively affect the quality of the sperm. Microfluidic chip-based sperm (MCS) sorting is proposed as an alternative technique for the selection of high-quality sperm with the aim to improve reproductive outcomes in IVF. This device does not require of centrifugation or any toxic solution to prepare the sample for fertilization so the sample is not subjected unnecessary stress.

METHODS

For the analysis of the basic parameters, boar semen samples were divided into three groups: unselected semen in extender (EX), sperm selection by MCS, and sperm selection by DGC. Data collection included concentration, morphology, total motility and progressive motility. For IVF outcomes samples were divided in 2 groups and data was collected for cleavage and blastulation rates.

RESULTS

Expected reduction in the sperm concentration after sperm sorting (31.9 mil in EX) but there are not significant differences between the concentration obtained in MCS and DGC (4.5mil and 9.31 mil respectively). There was a similar total motility between the 3 groups (65.8%/74.0% /77.2% for EX/DGC/MCS). Significant differences were found in progressive motility when comparing EX (19.5%) with DGC and MCS, but not between MCS and DGC (53.37%* and 58.67%*, respectively). MCS enriched samples for sperm with significantly lower proportion of abnormalities (22.50%/15.50%** for DGC/MCS). After fertilization, no statistical differences were found between the 2 for cleavage (p=0.827) and blastulation rates (p=0.223).

* Significant improvement for MCS or DGC compared to EX, ** significant improvement for MCS compared to DGC, $p < 0.05$ (GLM Test).

CONCLUSIONS

Here we demonstrated that sperm selection using MCS is as least as good as the standard DGC for in terms of capacitating the sperm and increasing progressive motility and fertilising oocytes. Furthermore, we showed that MCS as the strongest sperm selection method reducing significantly the concentrations of abnormal sperm.

O12

INTERACTION OF POLYUNSATURATED FATTY ACIDS AND MELATONIN DURING BOVINE IN VITRO MATURATION ON CUMULUS-OOCYTE METABOLISM AND EMBRYO DEVELOPMENT

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BACKGROUND-AIM

β-oxidation of fatty acids (FAs) is an important energy source during oocyte maturation. Additionally, polyunsaturated fatty acids (PUFAs) have functional roles related to cell signalling and biosynthesis. During in vitro maturation (IVM) the uptake and metabolism of FAs, together with mitochondrial function, are modulated by the inclusion of melatonin. Here we report the effects of specific PUFAs (linoleic (LA) and a-linolenic (ALN) acids) and melatonin (M) in defined IVM media on (i) mitochondrial oxygen consumption by cumulus-oocyte complexes (COCs) and (ii) subsequent embryo development.

METHODS

Base IVM media was TCM199 as described previously (Tutt et al. Theriogenology, 2021;161:108-19) with 4 mg/ml rHSA replacing serum and BSA. This was supplemented with LA (25 μM) + ALN (25 μM), and/or 10-7 mol/L M in a 2 x 2 factorial arrangement; thus FA+ vs FA-; M+ vs M-. Oocytes were collected from sexually mature heifers by transvaginal follicular aspiration; COCs from 8 heifers over 4 cycles were matured, fertilised and embryo development assessed. COCs (trimmed pools of 6) from two cycles were subjected to oxygen consumption and extracellular flux analysis (Seahorse XF-Mitro-Stress test). Proportions were analysed by logistic regression and respiration by repeated measures ANOVA.

RESULTS

An interaction between FAs and M meant that basal (P=0.02) (FA-/M- 13.9, FA-/M+ 6.9, FA+/M- 2.1, FA+/M+ 21.3 pmol/min/COC; SED=9.76) and maximum (P=0.058) (FA-/M- 24.0, FA-/M+ 15.8; FA+/M- 12.3, FA+/M+ 30.6 pmol/min/COC; SED=10.89) respiration during maturation was supressed when FAs or M were incorporated independently, but increased when incorporated together. Similarly, the proportion of embryos that developed beyond Day 6 (P=0.028) (FA-/M- 0.760 ± 0.0522, FA-/M+ 0.535 ± 0.0570, FA+/M- 0.460 ± 0.0531, FA+/M+ 0.680 ± 0.0531) and hatched (P=0.021) (FA-/M- 0.229 ± 0.0543, FA-/M+ 0.077 ± 0.0299, FA+/M- 0.075 ± 0.0289, FA+/M+ 0.143 ± 0.0392) was suppressed when FAs and M were incorporated independently but not when added together.

CONCLUSIONS

Adding M or FAs individually to defined IVM media reduces embryo development, possibly due to altered mitochondrial performance. This effect is mitigated when M and FAs are added together. Further molecular and metabolic analyses are underway.

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O13 - 1

MORPHOLOGICAL PARAMETERS OF EXTRACELLULAR VESICLES SECRETED IN VITRO BY BOVINE EMBRYOS CAN BE ALTERED BY PORES OF ZONA PELLUCIDA

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BACKGROUND-AIM

Pre-implantation bovine embryos secrete extracellular vesicles (EVs) must likely to interact with the environment throw their cargo (microRNAs, mRNAs, proteins, DNA). It seems that morphological characteristics of EVs population as particles mean size and concentration might vary during preimplantation development due to the itself changes of development and the pores of zona pellucida (ZP). During the early development, ZP has pores ranging 155-438 nm what could restrict the pass of bigger vesicles, but quantity and diameter of pores depends on embryo stage. However, after hatching EVs secretion is not limited by the ZP. The aim of this study was to evaluate how the presence of the ZP may modulate secretion of EVs to culture medium by in vitro produced embryos.

METHODS

Bovine embryos were produced by in vitro fertilization and cultured individually in EVs-depleted SOF according experimental group; W1: day 1 to 3, W2: day 3 to 5, W3: day 5 to 7 and W4: day 7 to 9. Embryo morphology was evaluated at day 7 or 9 depending on the group and culture media from embryos that reached blastocyst stage at day 7 or 9 were analyzed. Particles were isolated and then evaluated by nanoparticle tracking analysis (NTA) to determine their characteristics. Presence of EVs was confirmed by presence of classical markers and morphology by transmission electron microscopy. Data were analyzed using Wilcoxon test. Data were considered statistically different with $p<0.05$.

RESULTS

Particle concentration was 5.9x107/mL, 9.39x108/mL, 2.43x109/mL and 5.26x109/mL, meanwhile mean size was 197.8, 109.4, 106.6 and 114.9 in W1, W2, W3 and W4 respectively. Particles concentration was statistically different between groups; as the embryo develops it began to secrete higher concentration of extracellular vesicles, probably because number of pores from ZP increased in more developed stages. Size of EVs from embryos without ZP was bigger, but without statistical significance. Unexpectedly, mean size of EVs secreted during day 1 to 3 (W1) was higher to the other groups ($p<0.05$).

CONCLUSIONS

This is a preliminary study but indicates that the ZP modulate the secretion of EVs of pre-implantion embryos. Supported by Fondecyt 1170310 and 1210334, and National Doctoral Scholarship 21191050 from Chile.

O13 - 2
REMOVAL OF ZONA PELLUCIDA DOES NOT AFFECT EXTRACELLULAR VESICLES SECRETION FROM BOVINE EMBRYOS

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BACKGROUND-AIM
Zona pellucida (ZP) is an extracellular matrix that surrounds oocytes and early embryos. ZP has several functions, during fertilization allows spermatozoa binding and prevents polyspermy while during early development facilitates the passage of the embryo through the oviduct preventing the blastomeres dispersion and protecting the embryo from viral infection. ZPs also modulate the embryonic-maternal interaction, acting as a barrier to the exchange of molecules.The ultrastructure of the zona pellucida changes across the development and it is hypothesized that this influences the secretion of embryo derived extracellular vesicles (e-EVs). This study was aimed to evaluated concentration and mean size of particles secreted by embryos cultured with (EVs-ZI) or without (EVs-ZF) zona pellucida.
METHODS
Bovine embryos were produced by in vitro fertilization (IVF) and cultured individually in EVs-depleted SOF up to day 8 of development. Removal of ZP was carried out 20 h after IVF using pronase treatment (2mg/ml during 8 min) and zona free embryos were cultured in well of the well system on individual drops using SOF media depleted of EVs. Blastocyst rate and embryo morphology was evaluated at day 7 and 8. Only culture media from embryos that reached blastocyst stage were collected and analyzed by nanoparticles tracking analysis (NTA) to determine concentration and mean size of e-EVs.
RESULTS
Blastocyst rate at day 8 in individual culture of ZF embryos was 5.94% whereas in individual culture of ZI embryos was 8.33%. Embryo stage and quality as well blastocyst rate was similar between groups ($p>0.05$). The EVs nature of isolated nanoparticles was confirmed by the positivity to classical molecular markers (CD9, CD63, CD81) and by their morphology evaluated by transmission electron microscopy. Concentration and mean size of isolated nanoparticles were 1.92x108 and 256 nm respectively in EVs-ZF, whereas in EVs-ZI were 2.35x108 and 250 nm. No statistical differences were observed for concentration neither for particles size between groups ($p>0.05$).
CONCLUSIONS
Although quantity and diameter of ZP pores changes during the early developmental period, its seems that its presence does not inhibits secretion of e-EVs. Supported by Fondecyt 1210334 from Chile.

O14
ANALYSIS OF MOSAICISM IN BOVINE EMBRYOS USING PREIMPLANTATION GENETIC TESTING (PGT-A) ALGORITHMS

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BACKGROUND-AIM
In advanced cattle breeding programmes, SNP (Single Nucleotide Polymorphism) data obtained at the embryonic stage is used to calculate the genetic value of an animal and improve selective breeding. SNP genotyping also provides a cost-effective aneuploidy screening tool and previous studies using SNP based PGT-A reported an aneuploidy incidence of 14-24% in cattle blastocysts. However, embryonic karyotypes display considerable variability as, during development, mosaicism can arise following a chromosome segregation error or partial aneuploidy correction, leading to the formation of two or more karyotypically distinct cell lines in the embryo. Mosaicism is estimated to affect between 2-50% of human embryo biopsies at the blastocyst stage, with recent approaches showing an average incidence of about 30%. However, similar estimates have seldom been reported in other species.
METHODS
Here, we applied a novel PGT-A algorithm based on signal intensity metrics to review SNP data from n=320 bovine blastocysts, detect whether mosaicism was present, and quantify its level. Trophectoderm biopsies were obtained from embryos of stages 7 to 8 and quality grades 1 or 2 (IETS grading). An aneuploidy percentage was calculated per chromosome; chromosomal errors displaying a percentage between 20-80% were considered mosaic, while a complete aneuploidy was defined by a percentage >80% (euploid chromosomes had a percentage <20%).
RESULTS
The overall aneuploidy incidence was 20.31% (65/320), and 33.8% (22/65) of the aneuploid embryo biopsies were mosaics. We detected n=74 individual chromosomal errors, the most common of which was trisomy (39.2%, 29/74). The incidence of mosaicism differed according to the type of error (generalised linear model, $p<0.01$); the error with the highest incidence of mosaicism was hypotriploidy (59.1%, 13/22). Chromosomal errors occurring during oogenesis (78.4%, 58/74 and 68.2%, 15/22 for non-mosaic and mosaic errors, respectively) were more frequent than during spermatogenesis (14.9% 14/74 and 18.2%, 4/22, respectively).
CONCLUSIONS
These results indicate that mosaicism is present at similar levels in bovine and human embryos and that mosaicism screening in livestock embryos can be performed using the same genomic data collected by breeding companies while selecting their animals.

SYMPOSIUM 5 Reproduction in non-domestic and endangered species

O15
ENIGMATIC STORY OF EMBRYONIC DIAPAUSE BEHIND THE SCALY ARMOR: NON-INVASIVE ASSESSMENT OF REPRODUCTIVE CYCLE IN WILD TAIWANESE PANGOLIN (MANIS.P. PENTADACTYLA) USING FECAL STEROID METABOLITES

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BACKGROUND-AIM
Pangolins are extensively studied species to combat illegal trade, whereas its basic endocrinological and reproductive biology is relatively unexplored. Currently, several discrepancies in the major reproductive events (mating, gestation period, and parturition) from captive records of the Chinese pangolin (Manis pentadactyla) have been witnessed. This study attempted to generate the annual endocrinological profile of wild Taiwanese pangolin (M. p. pentadactyla) in both males and females using fecal samples.
METHODS
A total of 38 female and 33 male fecal samples were obtained by radio-tracking from the Coastal Mountain Range of Taitung, southeastern Taiwan, from 2010 to 2016. The sample size for each month ranged from 1 to 6. We used EIA to analyze estradiol-17β, Pregnanediol-3-Glucuronide (PDG), and epiandrosterone (EpiA).
RESULTS
The results indicated that there is a significant decrease in the levels of PDG with significant elevation in the levels of estradiol-17β around November and December in females, which corresponds to its birth season advocated by field observations. At the same time, increases in levels of estradiol-17β (November- December) indicated that spontaneous ovulation followed by mating may occur shortly after giving birth. Furthermore, the levels of both estradiol-17β and PDG were found lower during the months of February to April, suggesting that the Formosan Pangolin might exhibit phenomena of embryonic diapause (delayed implantation). Whilst, subsequent elevation in the levels of estradiol-17β and PDG was witnessed from the month of May suggested blastocyst implantation might commence during this time of the year. At the same time, males represent sync in spermatogenesis regarding the female reproductive cycle to exhibit copulation during the brief reproductive window at the end of the year.
CONCLUSIONS
The obtained fecal hormonal profile aligns with serum endocrinological analysis for both male and female pangolin species. This study has a potential application in the reproductive management of pangolin.

O16
ANTI-MÜLLERIAN HORMONE (AMH) LEVELS IN AMAZONIAN MANATEES (TRICHECHUS INUNGUIS): SEX AND AGE CLASS VARIATIONS

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BACKGROUND-AIM
The Amazonian manatee (Trichechus inunguis) is a threatened aquatic mammal endemic of the Amazon basin. Although the increase of knowledge about the reproductive physiology of this species in the last decade, more studies are necessary to support future assisted reproductive programs. The Anti-Müllerian hormone (AMH) has been reported as a promising marker of potential fertility in humans and animals. In aquatic mammals, variations on AMH levels were already reported for some cetacean species and for Florida manatees, demonstrating species-specific differences. Therefore, the aim of this study was to determine AMH levels in Amazonian manatees evaluating sex and age class differences.
METHODS
Serum samples were collected from 39 female and 39 male Amazonian manatees (from 1yr to over 40 yrs old) housed at the National Institute of Amazonian Research – INPA. Levels of AMH were determined using a commercial ELISA kit (AMH Gen II; Beckman Coulter) following the manufacturer's instructions. The results were statistically compared between males and females and between two age categories (prepubertal: <6 yrs old; reproductive age: >5 yrs old) for each sex.
RESULTS
The range of AMH levels for males and females were 14.45 – 1247.57 ng/ml and <0.03 – 1.82 ng/ml, respectively, with male levels markedly higher than females (279.17±273.66 vs 0.34±0.40 ng/ml; P<0.0001, t-Test). For both sexes, prepubertal animals showed lower AMH levels than reproductive age animals (males: 132.85±124.12 vs 392.25±305.05; P< 0.001; females: 0.12±0.19 vs 0.45±0.44; P< 0.001, Mann-Whitney test).
CONCLUSIONS
The difference in AMH levels between sexes observed in this study corroborates with those described for other mammalian species. Additionally, the higher levels of AMH in reproductive females than prepubertal ones suggest a relationship with ovarian activity. However, the high levels in reproductive age males corroborate with the previous report for Florida manatees, different from other mammals, suggesting the action of AMH as a marker of Sertoli cells activity in these species.
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SYMPOSIUM 6 Immune regulation of oviduct/ uterine function

O17
THE ROLE OF INTERLEUKIN-1 AS OVULATION INDUCING FACTOR IN RABBITS (ORYCTOLAGUS CUNICULUS)

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BACKGROUND-AIM
Various studies report that rabbit seminal plasma components are involved in male and female reproductive processes (Maranesi et al. 2018). Nerve growth factor (NGF) is a well-known ovulation-inducing factor, but other seminal plasma cytokines may affect the female reproductive tract, so inducing ovulation and enhancing the reproductive success.
METHODS
Five male rabbits and 5 females were used. This study investigated the expression of interleukin 1 (IL1)/IL1 receptor (IL1R) system in the testis, prostate, seminal vesicles, deferens ampullae and uterus of rabbits by IHC and RT-PCR. Seminal plasma IL1 presence was evaluated by WB. The crosstalk between IL1 and NGF was observed in vitro by incubating uterine tissue with NGF and IL1 alone or with their cognate receptor antagonist and assaying their production and that of prostaglandin F2alpha (PGF2alpha) and PGE2 by ELISA. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls t-test.
RESULTS
IL1 and IL1R immunoreactivity was detected in all male and female reproductive organs examined. In particular, IL1R positive immunostaining was observed in the Leydig cells cytoplasm and in that of annexed glands secretory epithelium, whereas IL1 positive immunoreaction was localized in the secreting gonadal tissues. IL1 gene transcript was greater ($p<0.05$) in the deferens ampullae than in other tissues, whereas that for IL1R was higher ($p<0.05$) in the prostate ($p<0.05$). By WB, IL1 seminal plasma presence was confirmed by a 30-35 kDa strong band. The in vitro system showed that IL1 increased basal uterine NGF production, whereas NGF did not affect that of IL1; in addition, IL1 enhanced also PGF2alpha and PGE2 uterine secretion.
CONCLUSIONS
This study evidence that the IL1/IL1R system is expressed in the reproductive tissues of both rabbit sexes and that the seminal plasma IL1 presence modulates the uterine endocrine activity. In particular, these data suggest a cooperative ovulatory role for IL1 and NGF. In conclusion, as postulated by Duffy et al. (2019), our findings support the idea that ovulation is an inflammatory-like response. A deeper knowledge of these complex cellular mechanisms will be useful to improve reproductive performance of farm animals and humans too.
Duffy et al. Endocr Rev 2019 40:369
Maranesi et al. Biol Reprod 2018 98:634

O18
MATING INDUCES DOWN-REGULATION OF MIR-671, A KEY REPRESSOR OF BINDING FUNCTION BY FIBRONECTIN TYPE 1 (FN1) IN THE SOW INTERNAL GENITAL TRACT.

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BACKGROUND-AIM
Endogenously expressed miRNAs are small non-coding RNA molecules whose main function is the post-transcriptional regulation of gene expression. Here we studied gene expression changes of miRNAs in the sow genital tract during estrus. miR-671 is a repressor of fibronectin type 1 (FN1), a plasma protein that is involved in binding (GO:0005488) to both cell surfaces and compounds as fibrin, actin, collagen and heparin, an action linked to the epidermal growth factor (EGF) (molecular and transducer activity (GO:0060089); binding (GO:0005488)).
METHODS
Tissues from cervix (Cvx), uterus (DistUt and ProxUt), utero-tubal junction (UTJ), isthmus (Isth), ampulla (Amp) and infundibulum (Inf), were surgically removed from oestrous sows, 24 h after natural mating (n=4) with fertile boars, artificial insemination with the first 10 mL of the sperm rich fraction (SRF; P1-AI, n=4), infusion with sperm-free seminal plasma from the SRF (SP-P1, n=4) or of the entire seminal plasma (SP-Ejac, n=4). Infusion of Beltsville Thawing Solution (BTS) was used as negative control (Control, n=4). Trizol isolated RNA was explored for global transcript analysis using microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix). The data was normalized (Robust Multiarray Average) and analysed with the Transcriptome Analysis Console (RMA-method, -1> fold changes >1, $p<0.05$), with molecular processes identified by PHANTER.
RESULTS
Mating significantly down-regulated miR-671 expression in all tissues. In contrast, miR-34 was up-regulated (DistUt (Mating, P1-AI), UTJ (Mating) and Inf (Mating; SP-P1)). miR-let-7a was down-regulated by Mating and SP-Ejac in DistUt and ProxUt.
CONCLUSIONS
miR-671 down-regulation could induce FN1 expression, increasing the binding-molecular function, perhaps in relation to male-female interaction. miR-34 up-regulation could be related to apoptosis and cell cycle, for its relation to p53 and the repression of the transcription factor FOXP1. miR-let-7a down-regulation might be involved in the suppression of IL-17-associated autoimmune inflammation, thus reducing the IL-6, pro-inflammatory cytokine, avoiding rejection of foreign spermatozoa by the female.
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O19

MATERNAL AGE RESULTS IN ALTERED ENDOMETRIAL MIRNA PROFILE IN A NOVEL BOVINE MODEL.

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BACKGROUND-AIM
Maternal age is associated with reduced fertility rate and can have negative impacts on both pregnancy and offspring outcomes. The endometrium is susceptible to the ageing process, and changes at the molecular level may result in diminished fertility. The endometrial-embryo dialogue is integral to embryo implantation, and disturbances to this may explain failed implantation after embryo transfer. Endometrial microRNAs (miRNAs) play an important role in establishing an optimal uterine environment, and thus the successful establishment of a pregnancy. This study aimed to determine the variability of endometrial miRNA with maternal age, using a novel bovine model that removes potential confounders of nuclear genetics, environment and diet.
METHODS
Endometrium samples of five young (4 years old) and five old (11 years old) cloned Holstein Friesian cows with an identical genetic background, and managed as one herd, were collected on day 7 of a natural cycle, and total RNA was extracted.
RESULTS
Through Next-Generation Sequencing, 786 differentially expressed transcripts were identified between the young and old group. Of those, 46 were significantly different after FDR correction ($P < 0.05$). qRTPCR confirmed the increased expression of miR-125a, miR-140, and miR-145 in the old group ($P < 0.01$). KEGG pathway analysis reveals that these miRNAs are involved in fatty acid, extracellular matrix and transforming growth factor β pathways. Furthermore, the target mRNAs of these miRNAs have previously been identified in endometrial dysfunction.
CONCLUSIONS
Improved understanding of the molecular regulation of the endometrium will further our understanding of implantation and pregnancy failure and may allow for interventions to improve fertility rate.

O20

EFFECT OF GROWTH FACTORS DERIVED FROM COMMERCIAL OVINE PLACENTAL EXTRACT ON EARLY PREGNANCY LOSS IN MARES

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BACKGROUND-AIM
Early pregnancy loss(EPL)is the most common form of mare's infertility in horse breeding industry, which occurs at various stages of pregnancy. Several hormones play important roles in fertilization, implantation and maintenance of pregnancy. The role of insulin-like growth factor I (IGF-I) as a paracrine-autocrine modulator of steroidogenesis in the conceptus was investigated. IGF-I and IGF-II displayed different spatial and temporal patterns of expression during early pregnancy and had a role in the process of attachment and implantation. Uterine expression of IGF-I is widely documented. Growth factors play critical roles in normal physiology; IGF-I increased embryo cleavage rates in several species (Guler et al.,2000). Hormonal and cytogenetic analysis is essential tools in the evaluation of early pregnancy loss. The aim of this study was to evaluate the extent and effect of growth factors in management of EPL.
METHODS
Twenty-four non pregnant Holstein mares aged from 10 to 15 years were used for this study. All mares had a history of early pregnancy loss at between 15 to 35 days of pregnancy without any clinical signs of uterine problems in previous two cycles. Mares were divided in two groups: treatment and control. Mares in the treatment group were treated weekly with ovine fractionated placental extract (SGF1000, VECTA animal health international, Australia) intravenous injection for six weeks. Mares in both group were inseminated with fresh semen 12 hours before ovulation. Mares were periodically examined with ultrasound at days 14,20,35,45 and 60 after ovulation for investigation of follicular development, ovulation and pregnancy. Blood samples were collected from mares for detection of hormones and cytogenetic analysis. Incidence of EPL was recorded in all mares with ultrasound.
RESULTS
The incidence of EPL in the treatment group was significantly lower (14%) than in control group (70%) ($p<0001$). Progesterone levels in mares were significantly higher at 14, 20, and 60 days after ovulation in mares which did not present EPL ($p<0001$).
CONCLUSIONS
Further studies are needed to determine dysfunction in hormone production in relation to early pregnancy loss and their relationship with infection causing pregnancy loss.

SYMPOSIUM 7 Effects of heat stress on reproduction: from conception to lactation

O21
EFFECTS OF TEMPERATURE RISE DURING IN VITRO MATURATION ON EMBRYO PRODUCTION IN DAIRY AND BEEF CATTLE.

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BACKGROUND-AIM
It is well documented that heat stress compromises dairy cattle fertility. Among a series of adverse effects, it has been shown that exposure of dairy cattle oocytes to high temperatures reduces their in vitro developmental competence. However, it is unclear if the latter apply for beef cattle. Here, we studied the effects of short lasting, moderate temperature rise during IVM of Holstein (H) and Limousine (L) oocytes on embryo yield, and gene expression at various stages of IVP.
METHODS
H and L abattoir derived oocytes were matured for 24 hours in TCM199 plus FCS and EGF at 39oC (C-controls, T treated); HC (n=450) and LC (n=333) or at 41oC from hour 2 to hour 8 of IVM (HT, n =651, and LT n=443). Matured oocytes were fertilized by the same frozen/thawed swim-up separated sperm. Denuded zygotes were cultured in groups of 25 at 39oC in SOF with FCS for 9 days. Cleavage and blastocyst yield were evaluated at 48 hours PI and on days 7,8,9 respectively (7 replicates). Relatlive mRNA abundance of genes related to thermal and oxidative stress, metabolism, apoptosis, and placentation was evaluated in oocytes, cumulus cells (cc) and blastocysts by qRT-PCR. Reference genes (YWHAZ, EEF1A1, UBA52) were used for normalization and their suitability was checked with the geNorm program. Cleavage, blastocyst formation rates and gene expression between groups were tested by t-test.
RESULTS
No difference was detected in cleavage rate or in blastocyst formation rate (%) among HC and LC (cleavage 86.2 vs 87.92; d7 blastocysts 31.0 vs 25.1). In both breeds, exposure of oocytes to high temperatures decreased ($p<0.05$) blastocyst yield (d7 24.5 vs 13.9, d8 27.8 vs 17.0, d8 29.9 vs16.8 for H and L respectively, $p<0.05$), but at all days the suppression in embryo yield was higher in L ($p<0.01$). In group H altered gene expression was detected in cc (G6PD, Glut1) and blastocysts (PLAC8) while in L group differences were found in oocytes (G6PD, HSP90AA1), in cc (CPT1B, HSP90AA1, SOD2, and blastocysts (DNMT, HSP90AA1, SOD2).
CONCLUSIONS
In both breeds oocytes are sensitive to HS. However, it appears that Holsteins are more tolerant than Limousines, possibly due to compulsory –production driven- selection.
Acknowledgments: Work co-financed by the EU and Greek national funds (RESEARCH – CREATE – INNOVATE, code:T1EDK-1078).

O22
ELEVATED ENVIRONMENTAL TEMPERATURE INCREASES THE INCIDENCE OF UTERINE DISEASE IN DAIRY COWS INDEPENDENT OF ELEVATED BACTERIAL LOAD

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BACKGROUND-AIM
Global temperatures are increasing, and heat-stress negatively affects milk production of the dairy cow. In parallel, 30% of postpartum cows suffer from uterine infection, resulting in metritis or endometritis. Heat-stress impairs immunity in cows, and so we aimed to determine if uterine disease incidence was increased in summer months. Evaluation of 3,509 calving events, over 6 years in Florida, demonstrate an increased incidence of metritis by 4.4% in summer months when environmental temperatures are increased. In addition, we sought to determine if changes to infection prevalence was due to increased abundance of bacteria as a result of increased environmental temperature.
METHODS
Disease incidence, production data and vaginal mucus were collected from study cows in cool months (n=51, avg max temp, 22.5°C) or warmer months (n=51, 31°C). All cows were housed on pasture during the dry period prior to calving, with no heat abatement. After calving all cows were housed free-stall barns equipped with soakers and fans. Production data were recorded for 60 days in milk, and vaginal mucus was collected on day 7 and day 21 postpartum to quantify bacterial DNA by qPCR.
RESULTS
Cows had reduced milk production in warmer months (5.2 kg/day) or if they had a uterine infection (6.7 kg/day), however there was no interaction between warmer months and infection. During warmer months a higher proportion of study cows had persistent uterine disease (58.8% vs. 29.4%), and an increased incidence of clinical endometritis 21 days postpartum (64.7% vs. 43.1%). The concentration of bacterial 16S rRNA in vaginal mucus was higher in cows with endometritis compared with healthy cows at day 21 (2.35 vs. 0.04 ng/mg mucus) but did not differ between the warmer and cooler months (2.12 vs. 2.68 ng/mg mucus). The abundance of 16S rRNA, Truperella pyogenes, and Fusobacterium necrophorum was increased in cows with disease but did not differ between the warmer or cooler months.
CONCLUSIONS
These data suggest that uterine infection is more prevalent in warmer months, but this is not due to increased bacterial abundance. We propose that increased environmental temperature and subsequent heat-stress reduces the capacity of cows to tolerate the presence of pathogenic bacteria, resulting in the development of uterine disease.

O23

LACTATION STATUS DURING GESTATION IS NEGATIVELY ASSOCIATED WITH THE SIZE OF THE OVARIAN RESERVE OF FEMALE OFFSPRING IN DAIRY CATTLE

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BACKGROUND-AIM
Evidence indicates that the total number of healthy follicles and oocytes in ovaries of mammals (ovarian reserve) is positively associated with several measures of fertility and that the ovarian reserve can be programmed by the conditions encountered in early fetal life. The hypothesis that maternal lactation status (lactating vs non-lactating) and lactation number are associated with the size of the ovarian reserve in their daughters in dairy cattle was addressed.
METHODS
A single blood sample was collected to measure serum anti-Müllerian hormone (AMH; n = 310) and the number of follicles > 3 mm (Antral follicle count, AFC) was assessed by transrectal ovarian ultrasonography (n = 258) on a random day of the estrous cycle in Holstein-Friesian heifers (16.1 mo of age ± 1.32; mean ± SD). Relations among variables were analyzed with Pearson Correlation with SAS. Serum concentrations of AMH and AFC, were analysed with a mixed model (Proc MIXED procedure of SAS) considering the main effects of maternal lactation status (non-lactating heifers vs lactating cows), lactation number of the dam (0, 1 or ≥ 2) and their interaction. Tukey test was used for comparisons.
RESULTS
Maternal lactation status was associated with AMH concentrations (R = 0.14; P = 0.0147) and AFC (R = 0.19; P = 0.002) of their daughters. Both AMH serum levels (P < 0.01) and AFC (P < 0.05) were lower in young adult heifers born to dams that were not lactating (n = 147; 441.53 ± 28.63 pg/mL; 9.64 ± 0.43 follicles) compared to cows that were lactating during gestation (n = 163; 576.47 ± 35.97 pg/mL, 11.49 ± 0.47 follicles). Lactation number of the dam was also associated with AMH circulating concentrations (R = 0.11; P = 0.05) and AFC (R = 0.16; P = 0.008) of their female offspring. Heifers born to dams that were not being milked during gestation had lower AMH levels and AFC compared to offspring of cows on their first lactation (P < 0.05), whereas no difference was detected with daughters of cows on second or greater lactation. AMH and AFC were similar between offspring of cows on their first and second or greater lactation.
CONCLUSIONS
In conclusion, the ovarian reserve may be smaller in heifers born to non-lactating heifers compared to lactating cows.
Funded by RAS, LR7-2015.

SYMPOSIUM 8 Cryopreservation: freezing, vitrification, freezing drying

O24

EFFICIENT AND SAFE SEPARATION OF STALLION AND BULL SPERM WITHOUT CENTRIFUGATION

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BACKGROUND-AIM
Conventional separation of sperm includes various technologies such as centrifugation and swim up or dilution into freezing extender. These techniques expose the sperm to several noxious conditions as reactive oxygen species (ROS), media or seminal fluid contaminants, light, radiation and temperature. Here, we used the Easy Separation device (E. Sep), a new technology which based on special micropores and thermo taxis and operates in a closed system.
METHODS
Semen samples (3 replicates) from 5 different bulls were pooled and subjected to 'swim-up' procedure using regular centrifugation and swim-up (CENT) or E. Sep separation device. Sperm membrane potential (ΔΨm), ROS level and acrosome integrity were evaluated using specific kits (IMV Technologies, France). In addition, 20ml of stallion semen was separated into two groups. In the first, sperm (10ml) was allowed to swim directly through the E. Sep into the 5ml freezing extender (Minitub, Germany) during 20 min at 37°C. In the second group, sperm was centrifuged and diluted with freezing extender to reach concentration of 200m/ml.
RESULTS
Bull sperm showed significantly higher ΔΨm, in E. Sep group compared to CENT, (0.7 ±0.3vs.0.3±0.01;P<0.02). Oxidation level, i.e., the percentage of viable spermatozoa exhibiting ROS, was significantly lower in E. Sep (23.5±5.8%) compared to CENT (53.0±2.0;P<0.05). Acrosome integrity, i.e. was significantly higher in E. Sep (13.1±3.0%) compared to CENT (3.2±1.6%; P<0.05). In the stallion study, the concentration was similar between the E. Sep group and raw semen (200m/ml), while that of CENT was only 60% motility with a lot of debris and leukocytes. Further findings (i.e., membrane potential, ROS and acrosome integrity) will be presented in the ICAR meeting.
CONCLUSIONS
Using this device, the separated sperm obtained a higher performance of motility compared to swim up and more importantly less mitochondrial damage, higher acrosome integrity and lower ROS, indicating less DNA damage. These outcomes indicate that the sperm directly swims into the syringe or into the freezing extender in the tube (E. Sep) which can then be used for insemination, IVF or freezing. The device is safe and operates in a closed system without the need for centrifugation.

O25

ANTIFREEZE PROTEIN TYPE I IN EXTENDER FOR POTENTIAL CRYOSURVIVAL ENHANCEMENT OF FROZEN/THAWED SHEEP SEMEN

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BACKGROUND-AIM

Antifreeze proteins (AFP) are efficient cryoprotectant agents to avoid the growth of ice crystals during cryopreservation. Thus, this study assessed if the addition of AFP I enhances ram sperm cryopreservation.

METHODS

This study was approved by Ethics Committee for Use of Animals (#3696250121). Semen collection was performed by electroejaculation daily for 6 d on 10 Santa Inês rams. All ejaculates presenting motility ≥70% were cryopreserved (n=43). Each ejaculate was diluted to a final concentration of 100 × 106 sperm per straw (0.25 mL) and allocated into: CONT (diluted in TRIS-egg yolk-glycerol extender), or AFP (the same extender added with 0.1 µg/mL of AFP type I). Cryopreservation was carried in a freezing machine and straws were stored in cryogenic cylinder. After thawing, evaluations of sperm kinetics, plasma membrane integrity and functionality, capacitation, and sperm perivitelline binding test were performed. Data were submitted to Shapiro-Wilk test; non-normal distribution was corrected using log transformation. Data were analyzed using generalized linear mixed models. Results are presented as mean ± SEM.

RESULTS

The AFP and CONT samples had similar percentages of fast (P=0.14) and medium (P=0.11) sperm velocities. However, the percentage of slow sperm velocity was higher in AFP (27.2 ± 2.2 vs 23.6 ± 2.0%, P=0.048), resulting in a greater percentage of total motile sperm (31.1 ± 2.9 vs 26.2 ± 2.4%, P=0.03). Moreover, the AFP also promoted significant benefits on the percentage of sperm with integral plasma membrane (33.2 ± 2.0 vs 26.6 ± 2.0%, P=0.001), number of sperm binding to egg perivitelline membrane (888.3 ± 175.1 vs 642.8 ± 111.9 sperm/mm², P=0.02) and motility cryoresistance (32.3 ± 2.9 vs 27.1 ± 2.3%, P=0.02). Wobble coefficient tended to be greater in AFP (65.4 ± 1.4 vs 63.4 ± 1.6%, P=0.07). No differences were observed in sperm membrane functionality and capacitated sperm for AFP and CONT, respectively (16.3 ± 1.8 vs 16.5 ± 1.7%, P=0.89; and 18.5 ± 2.4 vs 18.7 ± 2.8%, P=0.90).

CONCLUSIONS

The use of AFP type I increased the viability and sperm cell protection during cryopreservation, with no adverse effect on membrane functionality and capacitation, indicating that it can be included in ram sperm cryopreservation programs.

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O26

EFFECT OF DHA AND DHA-LOADED CYCLODEXTRIN ON THE EQUINE FROZEN SEMEN QUALITY

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BACKGROUND-AIM

The addition of docosahexaenoic acid (DHA) to the frozen semen extender improve total motility of sperm. Thus, the cyclodextrin is a protein that promotes a complex of inclusion with lipids, facilitating the penetration to the plasma membrane of sperm. Therefore, this report investigated if the addition of DHA and DHA-loaded cyclodextrin (DCL) to the frozen semen extender of stallions increase the motility and viability sperm parameters.

METHODS

The semen of 22 stallions was divided in 5 groups. Before the frozen, the semen were treated with Botucrio® (CT), 400 µg/ml (DCL and DHA 400) and 200 µg/ml (DCL and DHA 200) concentrations. Sperm motility was evaluated by computer assisted semen analyses (HTMA-IVOS-12), for the following parameters: percentage of total motility (TM), progressive motility (PM), and rapid sperm (RAP). The analysis of plasma membrane integrity was performed by flow cytometer (LSR Fortessa, BD Biosciences) using the combination of fluorescent probes Hoechst 33342 and Sitox Green. To oxidative status, the identification of generation of peroxide (MitoSox Red, Life Technologies), and high mitochondrial potential (MitoStatus Red, BD Pharmigen) were measured. The GraphPad Prism 6 checked data as the normality through the Kolmogorov-Smirnov test. For the average obtained with normal distribution, the paired t test was used and for results without normal distribution the Wilcoxon test was applied and differences were considered significance when P<0,05.

RESULTS

There was improvement of TM (CT 51,9±3,1 DCL 200 55,8±3,1; DHA 200 58,2±2,8), PM (CT 21,4±1,6; DCL 200 24,5±1,9; DHA 200 24,4±1,7) and RAP (CT 36,1±3,6; DCL 200 39,4±3,2; DHA 200 41,2±3,1; P<0.05) in the groups treated with DCL and DHA 200, compared to the CT. No differences were found in sperm membranes between all groups. Similarly, no difference mitochondrial generation of peroxide was founded. However had more high mitochondrial potential in the groups treated with DCL and DHA 200, compared to the CT group.

CONCLUSIONS

In conclusion, the addition of 200 µg/ml of DCL and DHA improve sperm motility and high mitochondrial potential. However, it was not possible to prove the antioxidant status of DHA, by the decreases of mitochondrial generation of peroxide.

O27

THE OXIDATIVE STRESS INDEX (OSI) IN SEMINAL PLASMA COULD BE A FREEZABILITY MARKER IN DONKEY SEMEN

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BACKGROUND-AIM

Donkey sperm are particularly sensitive to oxidative stress due to the large amount of polyunsaturated fatty acids present in the plasma membrane. Oxidative stress results from the imbalance between the production of reactive oxygen species (ROS), which are a by-product of sperm metabolism, and the enzymatic and non-enzymatic antioxidant capacity of seminal plasma (SP). However, SP is removed before cryopreservation. The seminal oxidative stress index (OSI) is a parameter that accurately measures the oxidant/antioxidant ratio. Therefore, the objective of this study was to measure seminal OSI in donkey SP and relate it to sperm freezability.

METHODS

The ejaculates of 15 Catalonian jackasses were separated into two aliquots of equal volume: one was used to isolate the SP by centrifugation at 1,500 × g and 4 °C for 10 min (Medifriger BL-S; JP Selecta SA, Spain) at least five times, and the other was cryopreserved with BotuCRIO® freezing medium (Botupharma, Brazil) at a concentration of 200 × 106 viable sperm/mL. After thawing, ejaculates were classified hierarchically as of good (GFE) or poor freezability (PFE) based on the percentages of total motility (TM) and of sperm with an intact plasma membrane (SYBR14+/PI-). The seminal OSI (arbitrary units) was calculated from the measurement of total oxidative status (TOS, µmol H2O2 equiv./L) divided by the Trolox equivalent antioxidant capacity (TEAC, mmol Trolox equiv./L). A t-test for independent samples was run to compare the activity levels of seminal OSI between GFE (n = 8) and PFE (n = 7) using the R software (V4.0.3, R Core Team, Austria).

RESULTS

Ejaculates classified as GFE presented significantly lower activity levels of seminal OSI ($P < 0.01$) compared to those classified as PFE. The values ranged between 0.88 to 3.73, and between 3.15 to 9.38 in GFE and PFE, respectively.

CONCLUSIONS

Seminal OSI is related to sperm cryotolerance in the donkey and could be used as a freezability marker.

SYMPOSIUM 9 New approaches in buffalo reproductive management

O28
MELATONIN IMPLANTS OR PROGESTERONE DEVICE ADDED TO THE OVSYNCH PROTOCOL IN DAIRY BUFFALOES

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BACKGROUND-AIM
Melatonin was reported to increase ovarian activity in anestrous buffaloes experiencing summer heat stress in tropical South Asia. The present study examined whether pretreatment with melatonin would stimulate ovarian follicular activity and increase the response to estrus synchronization and fixed-time AI (TAI) in seasonally anestrous lactating dairy buffaloes in a subtropical environment.
METHODS
In Experiment 1, buffaloes were assigned to three groups: control (n=12), melatonin (n=13) and progesterone (P4) (n=15). The melatonin group were implanted with melatonin (216 mg) on Day -20 (D-20). From D0 to D9, buffaloes underwent estrus synchronization using a standard Ovsynch protocol (control, melatonin) or a P4-based Ovsynch protocol (P4).
In Experiment 2, buffaloes at four commercial farms were subjected to the same three treatments as in Experiment 1, and additionally underwent TAI after estrus synchronization. A bull was placed with the buffaloes at each farm at D20.
RESULTS
There were no differences (P>0.05) among groups for the presence of a corpus luteum (CL) at D0, size of the largest follicle at D0, ovulation to GnRH injection at D0 and D9, or the time to ovulation after injection of GnRH at D9.
The P4 group had a higher (P = 0.001) pregnancy/AI (50/83, 60%) than the control (11/65, 17%) and melatonin (18/77, 23%) groups which did not differ. The P4 group also had a larger (P = 0.005) CL at D20 (19.2 ± 03 mm) compared with the control (17.4 ± 0.6 mm) and melatonin (17.6 ± 0.5 mm) groups. There were no differences among groups in pregnancies to natural mating.
CONCLUSIONS
The findings showed that pretreatment with melatonin did not increase ovarian activity or the response to estrus synchronization and TAI in anestrous lactating buffaloes in a subtropical environment.

O29

METABOLIC STATUS AND PREGNANCY RATE IN ITALIAN MEDITERRANEAN BUFFALOES

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BACKGROUND-AIM
Aim of the present study was to evaluate the influence of metabolic status on Italian Mediterranean Buffaloes pregnancy rate.
METHODS
The animals involved in the trial (n= 1561) were reared in eight commercial buffalo dairy farms located in the mozzarella cheese PDO area. All animals were fed diets with similar nutritional caractheristic (crude protein 15%, NDF 40%, NSC 31%, UFL/kg 0,90) and have an average of days in milk (DIM) of 109.9±0.1. Milk yield was recorded monthly and two milk samples (during morning and afternoon milking) were collected to evaluate milk quality and urea, acetone and β-Hydroxybutyrate (BHBA). Buffaloes underwent synchronization of ovulation with Ovsynch-TAI program and artificially inseminated. Pregnancy diagnosys were carried out 27 and 45 days post AI to assess embrionic and foetal mortality.
RESULTS
Data analysis carried out between pregnant (n = 825; average DIM = 109.0±2.7) vs. non-pregnant (n = 736; average DIM = 110,9±3,2) subjects, showed there were no significant differences in days in milk, parity, urea and acetone dosed in milk. In contrast, milk BHBA was higher in non-pregnant compared to pregnant buffaloes (0.17±0.01 vs 0.15±0.01; P <0.05). The logistic regression analysis shows that as the BHBA increases, the likelihood of pregnancy decreases (odds ratio = 0.406; P <0.05). In particular, the estimated probability of being pregnant, for subjects with values greater than 0.25 mmol/L of BHBA, is less than 50%. On the basis of this data we considered it appropriate to divide the buffaloes into two groups according to the levels of BHBA in milk, at high risk (≥0.25 mmol/L) and at low risk (≤0.25 mmol/L) of ketosis. The incidence of subjects with high values (≥0.25 mmol/L) of BHBA on the total was 22%. In particular, pregnancy probability was lower than 50% in buffaloes with BHBA values higher than 0.25 mmol/L. It is interesting to note that, the pregnancy rate after instrumental insemination increased significantly in buffaloes with low levels of BHBA compared to those that showed higher values (55.8 vs 45.3%; P <0.01).
CONCLUSIONS
In conclusion, the possibility of using milk BHBA levels as a indicator for an increased likelihood of pregnancy in the buffalo may improve buffalo reproduction.

SYMPOSIUM 11 New imaging systems for assessing gamete and embryo quality

O30

IDENTIFICATION OF SECRETORY PROTEINS FROM BOVINE CUMULUS OOCYTE COMPLEXES DURING IN VITRO MATURATION

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BACKGROUND-AIM
Analysis of proteins secreted by cumulus oocyte complexes (COCs) into the maturation medium gives a valuable insight into metabolism of COCs. Identification of secreted proteins can contribute to the discovery of markers for developmental competence of the oocytes and identify potential media requirements. The aim of the present study was the characterization of COC secretory proteins through a qualitative proteomics approach.
METHODS
Maturation medium for this study was harvested from 250 µl drops of medium, which hosted 0, 15 or 30 COCs for 23 hours (n=6 for each group). Collected samples were measured by a shotgun proteomics approach (LC-MS/MS). A qualitative data analysis was performed to identify secreted candidates. Replicates were analysed in two clusters (1-3; 4-6). Identified candidates had to be present in at least two of three replicates in both clusters, as well as absent in at least two of the three controls in both clusters. Candidates identified in the 15 and 30 COCs group were identified as highly secreted, whereas proteins present only in the 30 COCs group were described as low secreted candidates.
RESULTS
A total of 543 proteins were confidently identified in the samples with at least two peptides. Out of these, 7 low secreted candidates were only identified for the medium holding 30 COCs, whereas 7 highly secreted candidates were also identified for the medium holding 15 COCs. One highly interesting candidate amongst the low secreted proteins was Inhibin A (alpha chain and beta A chain), already known as secretory protein of the COC. Nevertheless, it's role in successful maturation was not finally elucidated. A novel protein, not detected in maturation medium up to now, was Uterine Milk Protein (SERPINA14), which was identified as highly secreted protein in the 15 and 30 COC group. Gene expression of this protein was already documented for cumulus cells, and seems to be under the regulation of LH and ovarian steroids.
CONCLUSIONS
The study identified 14 secreted proteins, deserving further attention regarding their role in COC maturation. Especially for the two candidates Inhibin A and Uterine Milk Protein, studies relating their secretion directly to the developmental competence of the oocyte are desirable.

O31

THE CRYOSURVIVAL OF BLASTOCYSTS SIGNIFICANTLY IMPROVES AFTER EXPOSURE TO OLEIC ACID DURING THE FIRST FIVE DAYS OF IVP

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BACKGROUND-AIM

Blastocysts originating from in vitro produced (IVP) embryos show a reduced quality in comparison to in vivo derived blastocysts. Developmental competence has primarily been related to oocyte quality, but embryo quality was demonstrated to be affected by embryo culture conditions. In the current study it is investigated whether quality of IVP embryos and thus cryosurvival is influenced by supplementation of free fatty acids (FFA) during the first 5 days of embryonic development, i.e. the physiological period that the embryo would reside in the oviduct

METHODS

Cumulus-oocyte-complexes (COCs) were collected from bovine slaughterhouse ovaries and 23h matured and fertilized (IVF=day 0; according to standard protocol). Embryos were cultured from day 1-5 in fatty acid free (FAF) SOF without (control) or with 25µM stearic acid (C18:0) or oleic acid (C18:1; n≥805 COCs per group), and from day 5-8 embryos in fresh FAF SOF. At day 8, grade 1 and 2 blastocysts (IETS) were slow frozen. Cryosurvival, expansion blastocoel 24h post-thawing, was compared with in vivo blastocysts (donated by CRV). By confocal microscopy the number of lipid droplets (LD540), total (Hoechst 33342) and damaged cells (EthD-1 and TUNEL) of blastocysts was determined. Statistical analysis (R version 4.0.5) was performed with a binomial logistic regression model for blastocyst rate, cryosurvival and proportion of damaged cells. A mixed effect regression model was used for number of lipid droplets and cells.

RESULTS

Blastocyst rates were lower after embryo culture in FAF SOF (23.7 ± 7.2%) compared to C18:1 (30.8 ± 8.4%). Interestingly, cryosurvival blastocysts of the C18:1 group with a high lipid content and the FAF SOF (respectively 70.1% and 67.4%), likewise those of in vivo blastocysts (68%), was significantly higher than the cryosurvival of C18:0 exposed embryos (17.6%). The number of damaged cells after thawing was in all blastocysts higher in comparison to fresh, but significantly higher in the group exposed to C18:0 (43.2%) in comparison to C18:1 (26.0%) and FAF SOF (26.5%).

CONCLUSIONS

The current study demonstrates higher cryosurvival for embryos exposed to C18:1, despite their high lipid content, in comparison to C18:0 and stresses the importance to identify specific fatty acids in order to improve embryo quality.

SYMPOSIUM 12 Novel insights on uterine immunology during pregnancy and disease

O32

ASSESSMENT OF THE ANTI-INFLAMMATORY POTENTIAL OF HORSE ENDOMETRIAL AND ADIPOSE MESENCHYMAL STEM CELLS IN AN VIVO MODEL OF MARE INDUCED ENDOMETRITIS

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BACKGROUND-AIM

Horse mesenchymal stem cells (hMSCs) are potential candidates for anti-inflammatory treatment of uterine pathologies like post-mating induced endometritis (PMIE) of mares. In this research hMSCs isolated from the endometrium or subcutaneous fat of the same donor were infused into mares with experimentally induced PMIE. The aim of this research was to assess in vivo the anti-inflammatory action of hMSCs in an experimental model of PMIE

METHODS

Cells: endometrial (e) and adipose (a) hMSC derived from biopsies of mares in ovulatory season were used in passage 4. Animals: Nine gynecologically healthy mares during ovulatory season were selected, biopsied and graded as I in Kenney-Doig´s category. Procedures: In all mares PMIE was induced by uterine infusion of 500×106mL−1 dead sperms in 20 mL saline. Inflammatory markers PMNs, IL-1a, 6, 8, 10, TNFa and COX2 were analyzed in uterine lavages and/or biopsies immediately before and 3h after infusion of sperm. After 24h, 3 mares were instilled intrauterine with 20 mL of 0.9% sterile saline, the rest received the same volume of saline plus 2×107 heMSCs (n=3) or haMSCs (n=3). After 48h another biopsy and lavage were done and the same parameters analyzed. Measurements: PMN (cytology), proteins IL-6 and TNFa (ELISA in the lavages) and immunostaining (biopsies). Transcripts of IL-1a, 6, 8, 10, TNFa and COX2 (qPCR of pelleted lavages).

RESULTS

Dead sperms markedly increased PMNs counts, IL-6 and TNFa expression in the immunostaining and ELISA ($p<0.05$). After treatment with haMSCs, a significant decline of IL-6 and TNFa was detected in the ELISAs, while in mares treated with heMSCs expression of IL-6 dropped ($p<0.05$ in all cases). Transcript analysis in the pelleted uterine lavages showed a significant increase ($p<0.05$) of all proinflammatory cytokines but IL-1a, in all mares receiving dead sperm and a marked reduction of these markers after infusion of haAMSC along with a significative increase in IL-10 expression. In heMSC treated mares the same pattern was observed for IL-6 and 8, but not for IL-1a. COX-2 was unchanged at all times. In the saline group, IL-10 increased after 48h of infusion

CONCLUSIONS

We conclude that inoculation of hMSCs significantly reduced inflammation independently of the origin of the cells. ACKNOWLEDGEMENTS. Supported by Fondecyt 1150757 grant AND FONDEF IT19I1007

O33
UTERO-PLACENTAL IMMUNE EVENTS IN THE DOG DURING PREGNANCY

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BACKGROUND-AIM

Maternal tolerance towards the embryo is crucial for the maintenance of pregnancy. However, events like implantation and parturition are associated with increased inflammatory activity in several species. Nevertheless, in contrast with other species, information regarding the uterine immune milieu during canine pregnancy is still scarce.

METHODS

Thus, in the present work, the gene availability of several immune factors was assessed in canine utero-placental compartments collected from the pre-implantation uterus (days 10-12) and corresponding non-pregnant controls, during implantation (day 17), post-implantation (days 18-25), mid-gestation (days 35-40), and prepartum luteolysis (term). Additionally, differences between natural and preterm induced parturition/abortion were assessed in samples collected 24h and 72h after administration of aglepristone to terminate gestation in mid-pregnant bitches.

RESULTS

Among the main findings, embryo presence prior to implantation was associated with an apparent increase in immune activity, suggested by higher transcriptional levels of MHCII, CD4, CD25, NCR1, IL6, -8 and -10, CCR7, IDO1 and AIF1 ($P<0.05$). An apparent shift towards anti-inflammatory events during implantation was suggested by upregulation of FoxP3 and IL12a ($P<0.05$), concomitant with the downregulation of CD4, IL8, -10 and CCR7 ($P<0.05$). Maintenance of pregnancy was associated with decreased immune activity, suggested from the decreased availability of MHCII, CD206, FoxP3 and NCR1, IL12a, TNFR1 and TLR4 during post-implantation ($P<0.05$), and further decreased IL1β in mid-gestation ($P<0.05$). Both natural and induced luteolysis were associated with increased availability of CD163, CD206, CD4, IL8, CCL3 and TLR4, while IL6 was downregulated ($P<0.05$). Prepartum luteolysis was further marked by the upregulation of TNFR1 and CCL13 ($P<0.05$). In contrast, MHCII, CD25, IL10, TNFa, AIF1 and IDO1 were upregulated after aglepristone treatment ($P<0.05$), but not at term. Despite some differences between natural and induced luteolysis, both appear to represent pro-inflammatory events.

CONCLUSIONS

Altogether, the present work provides new insights into uterine and placental pro- and/or anti-inflammatory signals during the establishment, maintenance and termination of canine pregnancy.

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TOPIC 1 Avian species reproduction

M01
EFFECT OF A SYNTHETIC ECG-LIKE GLYCOPROTEIN ON FOLLICULAR DEVELOPMENT AND OVULATION IN ANESTROUS EWES

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BACKGROUND-AIM
The objective was evaluate the effect of a synthetic eCG-like glycoprotein as an alternative to native eCG in ewes during seasonal anestrous.
METHODS
Anestrous multiparous ewes were used during nonbreeding season. Absence of corpus luteum (CL) was confirmed by transrectal ultrasonography (Mindray M7; 7,5 MHz) and all ewes were treated with an intravaginal progesterone device (0.3 g, DICO, Syntex, Argentina) treatment for 7 days. On day five of treatment the ewes were assigned to three experimental groups: Control group (n= 10) receiving placebo (5 ml im), eCG group (n=10) receiving 1000 IU of eCG (5 ml im, Novormon, Syntex), and eCG-like group (n=11) receiving 1000 IU of a synthetic eCG-like glycoprotein (5 ml im, Syntex). On day 7 the intravaginal device was removed and on day 8 all ewes received a dose of GnRH analogue (Gonadorelin acetate 100 µg, Gonasyn, Syntex) im. The antral follicular population (> 2 mm) was evaluated by transrectal ultrasonography on day 0, 5, 7 and 8 of treatment. Six days after GnRH administration the ovulatory response was evaluated by laparoscopy. Statistical analysis was performed by using ANOVA.
RESULTS
The number of total follicles (>2 mm) and large follicles (follicles ≥ 5 mm) was not different in the three experimental group on day 0 and 5. The number of large follicles was 3.3±0.4; 4.0±0.6 and 0,9±0.3 on day 7 (P< 0.05) and 3.8±0.3; 4.6±0.6 and 1.2±0.3 on day 8 (P< 0.05), for eCG group, eCG-like group and Control group respectively. Two and three days after of given of eCG (i.e. days seven and eight) the number of large follicles were higher in the eCG group and eCG like group than Control group (P< 0.05). But no difference was found in large follicles between eCG and eCG-like group (P> 0.05).
Ovulation occurred in all treated ewes for eCG (10/10) and eCG-like group (11/11), and in five treated ewes (5/10) for the control group (P<0.05). The number of CL in ovulated ewes was higher in the eCG group and eCG-like group than Control group (5.2±0.7; 6.6±0.7 and 1±0.0 respectively; P< 0.05), and no difference was found between eCG group and eCG-like group (P> 0.05).
CONCLUSIONS
In conclusion, the synthetic eCG-like glycoprotein has similar biological activity than native eCG in terms of follicular stimulation and ovulation rate in anestrous ewes.

M02
SPERM MORPHOLOGY AND FLAGELLUM MORPHOMETRY ON EJACULATES OF TWO SUBSPECIES OF PEREGRINE FALCON

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BACKGROUND-AIM
In the recent years, the use of artificial insemination (AI) has experienced a strong increase in raptors species, especially in Peregrine falcon. Knowledge of the sperm characteristics is crucial for understanding their reproductive biology and for the further development of AI. The aim of this study was analyzed the morphology and flagellum morphometry of sperm cells in two subspecies of falcon (F. peregrinus brookei and F. peregrinus peregrinus) during breeding season.
METHODS
Semen samples (n=16) were collected by cloacal massage from four mature and healthy falcons and stained with Diff-Quick stain. The percentages of normal, abnormal and immature sperm cells were identified by bright field optical microscopy. The morphometric analysis of the flagellum and midpiece were performed with ImageJ free software. A minimum of 200 spermatozoa were analyzed per semen sample.
RESULTS
The length of the flagellum was significantly higher in sperm from F. brookei than F. peregrinus (37,82µm±3,64 vs 29,85µm±4,32), as well as the length of the midpiece (1,54µm±0,21 vs 0,93µm±0,17) (p<0.05). The percentages of the three categories of sperm cells were similar in the ejaculates of F. brookei and F. peregrinus. High values of normal sperm cells (46.7% vs 53.3%) were found in comparison to immature sperm cells (30% vs 26.7%), respectively. The mean of abnormal sperm cells was similar in two subspecies (23.3% vs 20%).
CONCLUSIONS
Falcon ejaculates showed high values of sperm pleomorphism, although no differ between subspecies. On the contrary, we found significant differences in flagellum morphometric analysis. This finding might be important in the future development of conservation protocols for falcon sperm.

M04
TESTICULAR HISTOMORPHOMETRY OF COCKS WITH DIFFERENT WATTLE PRESENTATION

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BACKGROUND-AIM
This is a case report of the incidence of different wattle appreance in a flock of Black and Brown Harco cocks in Nigeria, raised for the purpose of free range breeding under small holder production system. A tiny number of the chicken population showed either a single or pair of undeveloped wattles, while the majority of the flock showed full wattle development. We were therefore interested to know if the unilateral and bilateral unwattled cocks would have the same testicular histomorphology compared to the normally wattled cocks.
METHODS
Three each of matured Black and Brown Harco cocks in the following categories (bilaterally wattled; unilaterally wattled and; bilaterally unwattled) were weighed and then sacrificed to harvest their two testes for morphometric measurement and histological preparation. The right and left testes were measured separately and then sectioned with microtome for histological staining by H&E method. The sectioned tissues were fixed on glass slides, stained with H&E and then sealed with mountantant for viewing under the under a light microscope at 100 magnification.
RESULTS
Wattle length in the bilateral wattled (BW), unilateral wattled (SW) and bilaterally unwattled (UW) equals 3.9 ±0.05cm, 3.5 ± 0.03cm and 0.5 ± 0.01cm, respectively. whereas live body weights were 2.68 ± 0.69kg , 2.6 ± 0.58kg and 2.58 ± 0.64kg, respectively. There was no significant difference ($P > 0.05$). in the weights of right testes between BW (12.5g), SW (12.2g) and UW (11.7g) as well as left testes (BW, 11.8g; UW, 11.5g and UW, 11.9g). Histological sections revealed that there were no developmental lapses in the testes of the three groups of cocks. In all the sections, the seminiferous tubules were well formed and sperm cells at different stages of spermatogenesis were evident. These results indicated that the appearance of the wattles had no relation to the testes in the chickens. The undescended wattle could just be a simple mutation among the population with no particular effect on the maleness of the cock.
CONCLUSIONS
These results showed that wattle presence or absence have no effects on the functionality of the testes in Harco breed of cocks.

M05
PACAP INCREASES SURVIVAL RATE AND HBEGF EXPRESSION OF BLASTOCYSTS WHEN USED AS AN ADDITIVE DURING VITRIFICATION

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BACKGROUND-AIM
Since the discovery of pituitary adenylate cyclase-activating polypeptide (PACAP) in 1989 in sheep hypothalamic extracts, a variety of functions and possible effects have been discovered. Due to its antiapoptotic effect and its widespread presence in the organ system, PACAP is considered as a general cytoprotective peptide. The fact that peptide was found in high levels in the gonads suggests the peptide might play a central role in reproduction. The aim of our study is to assess the application possibilities of PACAP treatment during embryo vitrification in the view of developmental rate and the HB-EGF gene expression.
METHODS
12 weeks old BDF1 female mice were superovulated (7.5 IU eCG i.p., followed by 7.5 IU hCG i.p. 48 hours later) and paired with males for a night. The zygotes were collected on the subsequent morning, and cultured in G1 medium for 72 hours. Afterwards, developmental stage was examined and blastocyst stage embryos were vitrified. Embryos were treated with 1 and 2 µM PACAP1-38 (groups VF1 and VF2, respectively) during the vitrification and after thawing, in the culture medium (groups IVC1 and IVC2, respectively). After 24 hours of culture, survival rate was investigated in each group and HB-EGF gene expression level was analysed with qPCR.
RESULTS
Survival rate after thawing was 69.87% in the vitrification control group and 70.87% in fresh control group. Significant differences from the control groups in the case of PACAP treatment during vitrification were observed. In VF 1, 65.42% of embryos reexpanded or developed. In contrast, in VF 2, when embryos were treated with higher dose of PACAP (2 µM), significant increase was found, a survival rate of 90.23%. No significant difference was found between the control groups and the groups of PACAP treated after vitrification.
CONCLUSIONS
Our results showed a higher rate of survival and higher level of HB-EGF gene expression in the group of higher concentration of PACAP-treatment during vitrification compared to both the vitrified and fresh control groups, indicating that PACAP treatment during vitrification has a beneficial effect on embryo survival and HB-EGF gene expression, thus on probability of implantation in dose-dependent manner.

TOPIC Bovine reproduction

M06
EVALUATION OF HEMODYNAMIC CHANGES OF UTERINE ARTERY USING DOPPLER ULTRASONOGRAPHY DURING DIFFERENT STAGES OF PREGNANCY IN BOS INDICUS COWS

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BACKGROUND-AIM
Doppler ultrasonography has provided an insight with diagnostic imaging from an anatomical to the physiological basis in veterinary reproduction. The objectives of the study were to evaluate hemodynamic changes and their relationships among ipsilateral (IPS) and contralateral (CONT) uterine arteries (UA) during different stages of pregnancy in Bos indicus cows.
METHODS
.Multiparous pregnant cows (n = 40) having gestation length 30.47 ± 0.54 (mean ± SD) were randomly enrolled, and subjected to Doppler ultrasonography sequentially at 1st, 2nd, 4th, 6th, and 8th months of gestation. Blood flow indices including diameter of UA (cm), blood flow volume (BFVo, mL/min), blood flow velocity (BFVe, cm/sec), time-averaged maximum velocity (TAMV, cm/sec), pulsatility index (PI), and resistance index (RI) were recorded. Data were analyzed with mixed models using the PROC MIXED procedures, and Pearson correlation coefficients were calculated using the PROC CORR statement in SAS. The final statistical models included the fixed effects of side of UA, gestation month, and the interaction between side of UA and gestation month.
RESULTS
Results revealed that the mean diameter of the UA (12.13 ± 0.22 vs. 10.09 ± 0.22), BFVo (1236.33 ± 0.55 vs. 770.41 ± 0.55), BFVe (17.18 ± 0.42 vs. 15.58 ± 0.42), and TAMV (17.11 ± 0.44 vs. 15.77 ± 0.44) were higher ($P < 0.05$) in IPS as compared to CONT side of the UA in cows. However, PI and RI did not differ between IPS and CON arteries of uterus in cows. A very high and positive correlation ($r = 0.89$; $P < 0.05$) existed between diameter of UA and BFVo starting from 1st to 8th months of gestation in IPS as well as CONT sides of UA. Moreover, TAMV was highly and positively correlated ($r = 0.91$; $P < 0.05$) with BFVe throughout the gestation.
CONCLUSIONS
Blood flow velocity (BFVe) and Timed average maximum velocity (TAMV) were higher in IPS as compared to CONT side of the UA in cows. However, PI and RI did not differ between IPS and CON arteries of uterus in cows. These hemodynamic changes in the UA could be used as a valuable validity tool to differentiate the compromised pregnancy in Bos indicus cows.

M07
PREGNANCY AND HORMONAL RESPONSE IN SUCKLED BEEF COWS ADMINISTERED HIGH-CONCENTRATION PROSTAGLANDIN F2ALPHA IN A 5-DAY CO-SYNCH + CIDR SYNCHRONIZATION PROGRAM

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BACKGROUND-AIM
There is evidence that two injections of prostaglandin $F2_{\alpha}$ (PGF) in a 5-d CO-Synch+CIDR timed-AI protocol (5-d CIDR) is necessary to induce complete luteolysis and reduce serum progesterone (P4) concentrations by the time of AI in beef cattle. The objectives were to examine the effects of a single high-concentration dose of PGF in a 5-d CIDR protocol on P4 concentration and pregnancy per AI (P/AI) in suckled beef cows.
METHODS
Angus-Hereford cows (n=404) were synchronized (d0) with a 5-d CIDR protocol and randomly assigned to receive either one injection of high-concentration PGF (HC; 12.5 mg/mL; total dose: 25 mg i.m; n=203) or two injections of conventional PGF (2PG; 5 mg/mL; Each dose: 25 mg i.m.; n=201), at CIDR removal (d5) and 8h later. Estrous behavior was monitored using estrus detection aids and visual observation from d5 until AI (d8). All cows were inseminated at a fixed time 72±2 h after PGF treatment. Pregnancy was determined by ultrasound 48-63 days after AI. To quantify P4, blood samples were collected seven days before (d-7, on day of protocol initiation (d0), and at AI. Samples on d-7 and d0 were used to determine cyclicity (serum P4 ≤1 ng/mL).
RESULTS
Cyclicity status and proportion of cows with a CL at protocol initiation did not differ between treatments ($P > 0.53$). Proportion of cows detected in estrus was greater ($P = 0.01$) for 2PG (63.1%) than HC (49.3%). Treatment did not affect P/AI ($P = 0.87$), as mean P/AI was 51% vs. 52% for HC and 2PG, respectively. Cows detected in estrus had increased P/AI (62.4 vs. 40.5%, $P < 0.01$). Cyclic cows tended ($P = 0.09$) to have improved P/AI (57.0 vs. 46%). Serum P4 at AI also affected ($P < 0.01$) P/AI; as P4 concentration increased, P/AI decreased. A ROC curve analysis showed a greater P/ AI was achieved when serum P4 was ≤ 0.43 ng/mL, with a 96.8% sensitivity and 23.0% specificity. Fewer ($P < 0.01$) HC cows (84%) had serum P4 at AI ≤ 0.43 ng/mL than 2PG cows (97.0%). Nonetheless, mean serum P4 concentrations at AI were 0.36 ± 0.03 and 0.13 ± 0.03 ng/mL for HC and 2PG, respectively.
CONCLUSIONS
Although two doses of conventional PGF (2PG, total dose: 50 mg) more effectively reduced P4 to ≤ 0.43 ng/mL by the time of AI than one dose of high-concentration PGF (HC, total dose: 25 mg), subsequent P/AI was not different between treatments.

M08
EFFECTS OF ONE OR TWO PROSTAGLANDIN F2α INJECTIONS ON LUTEAL FUNCTION AND PREGNANCY OUTCOME IN DAIRY COWS RESYNCHRONIZED WITH A 5-D + CIDR SYNCHRONIZATION PROTOCOL

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BACKGROUND-AIM
Evidence shows that two injections of prostaglandin F2a (PG) in a 5-d CIDR timed-AI protocol is necessary to induce complete luteolysis by the time of AI and improve pregnancy rate in cattle. The objectives of this study were to compare the effects of a single dose of high-concentration PG (HC) or two doses of conventional PG (2PG) on luteolysis, serum progesterone (P4) concentrations, and pregnancy per AI (P/AI) in Holstein cows re-synchronized with a 5-d CIDR program.
METHODS
Upon non-pregnancy diagnosis, cows were re-synchronized receiving 50 ug of GnRH (d 0). On d 5, cows were stratified by parity and number of inseminations and assigned to receive either one injection of HC (12.5 mg/mL; total dose: 25 mg, s. c.; n = 247) or two injections of conventional PG 24 h apart (2PG; 5 mg/mL; Each dose: 25 mg i.m.; n = 242), at CIDR removal. Estrual behavior was monitored from d 5 to d 8, and if cows were detected in estrus, they were bred on that day. On d 8, cows not detected in estrus were administered a second dose of GnRH and inseminated at a fixed time AI (TAI). Blood samples were collected, on d 0, 5 and 8 to measure serum P4 concentrations. Pregnancy was confirmed via ultrasonography 40 d after TAI.
RESULTS
On d 0 there was no difference in mean P4 (P=0.54) between treatments. The mean % of cows with an active CL (P4 ≥0.7 ng/mL) on d 0 were not different between HC and 2PG (74.3% vs. 71.2%, respectively). On the day of CIDR removal (d 5), there was no difference in mean P4 concentrations (P=0.53) between treatments. Using 489 cows, no difference in P/AI was detected between treatments (P = 0.12; 20.0% vs. 25.7% for HC and 2PG, respectively). There was a treatment by parity effect on P4 concentrations on d 8 (P<0.05). Serum P4 concentration at TAI was greater (P<0.05) in HC compared with 2PG, only in multiparous cows. Regardless of parity, P4 at TAI was <0.5 ng/mL for both treatments. Based on P4 on d 5 and 8, incidence of luteolysis was greater (P<0.05) in 2PG (97%) than HC (87%) cows.
CONCLUSIONS
Overall, 2PG was more effective at inducing luteolysis before TAI than HC. Given the number of animals used, no difference in P/AI was detected between treatments. Thus, one dose of HC may potentially be used as an option in the 5-d CIDR re-synchronization program with fewer animal handlings, injections, and labor compared with two doses of PG.

M10
INCREASED CONCENTRATION OF PROGESTERONE PRIOR TO LUTEOLYSIS INCREASES CELLULAR IMMUNE RESPONSE AND BRANCHED-CHAIN AMINO ACIDS DEGRADATION IN THE UTERUS DURING THE SUBSEQUENT LUTEAL PHASE IN BEEF COWS

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BACKGROUND-AIM
In cattle, fluctuations in the concentrations of sex-steroids throughout the estrous cycle modulate endometrial function, receptivity to the embryo, and fertility. However, the influence of sex-steroids on the subsequent estrous cycle is understood poorly. Objective was to compare the luminal epithelial transcriptome 4 days after estrus (d 4) between cows that were exposed to contrasting concentrations of progesterone (P4) before estrus.
METHODS
Sixty-four Bos indicus-influenced cows received either 1) a new CIDR for 7 days and an injection of GnRH at CIDR insertion (d -9; high P4 treatment; HP4) or 2) a used CIDR for 7 days and injections of PGF2a and GnRH at CIDR insertion (low P4 treatment; LP4). All cows received PGF2a at CIDR removal. Ovarian ultrasonography and blood collections were performed on d -9, d -2, d -0.5, d 0 (estrus), d 4, d 7, and d 14 for measurement of ovarian structures and concentrations of P4 and estradiol (E2). Uterine luminal cells were collected using a cytology brush on d 4, d 7, and d 14 for RNAseq. Functional enrichment analyses were performed using Ingenuity Pathway Analysis. The effect of treatment on dominant follicle diameter, CL area, and concentrations of P4 and E2 were analyzed by split-plot ANOVA.
RESULTS
As expected by design, on d -2, CL area and the concentrations of P4 were greater in HP4. The HP4 cows presented greater concentration of estradiol on d -0.5. No differences were observed in the size of ovarian structures or in P4 and E2 concentrations on d 4, d 7, or d 14 (P > 0.05). On d 4, d 7, and d 14, 424, 705, and 320 genes were differentially expressed between groups, respectively. Canonical pathways related to cellular immune response were upregulated for HP4 on d 4, branched-chain amino acids degradation and cholesterol biosynthesis were upregulated whereas cell signaling and movement were downregulated for HP4 on d 7, and cell signaling and immune cell trafficking were upregulated for HP4 on d 14.
CONCLUSIONS
Increased progesterone concentrations during the luteal phase of the previous estrus cycle modify the transcriptome of the luminal epithelium reflected in up-regulation of the immune response, cell signaling, cholesterol biosynthesis, and degradation of amino acids in a day-dependent manner. Implications for embryo receptivity and reproductive outcomes deserve further investigation.

M11
EFFICIENCY OF REDUCED SIZE P4 INTRAVAGINAL DEVICE IN ANIMAL WELFARE AND PREGNANCY RATE IN NELORE HEIFERS (BOS INDICUS)

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BACKGROUND-AIM
The aim of this study was to evaluate the impact of P4 intravaginal device (DIP4) size on animal welfare and on TAI pregnancy rate (PR) of Nelore heifers (Bos indicus).
METHODS
Thus, 3 experiments (Exp.) were carried out. In Exp.1, heifers [14 months (n=296) and 24 months (n=309)] were submitted to a synchronization protocol. On day 0 (D0), heifers were randomly assigned to receive a DIP4, DIB0.3 [reduced size (12 cm/width), 0.3g P4, Syntex®] or DIB0.5 [conventional size (18 cm/width), 0.5g of P4, Syntex®]. After insertion of DIP4, discomfort behaviors (raised tail, dorsal arching) were evaluated. In Exp.2, heifers [16 months (n=802)] were submitted to a TAI protocol to assess the PR. On D0, animals were randomly distributed in a 2x2 design: device (DIB0.3 and DIB0.5) and moment of TAI (36 and 48 hours). Also, all heifers received an application of 2mg of EB (Gonadiol®) and 12.5mg of dinoprost (Lutalyse®) i.m. On day 8, DIP4 was removed, and animals received 12.5mg of dinoprost, 0.5mg of EC (ECP®) and 200UI of eCG (Novormon®), i.m. In Exp.3, heifers [16 months (n=1,821)] were submitted to a TAI protocol to evaluate the PR considering results obtained in Exp.2. On D0, animals were distributed according to cyclicity to receive DIB0.3 or DIB0.5. Heifers treated with DIB0.3 were inseminated after 36 hours and with DIB0.5 after 48 hours following withdrawal of DIP4. Data analysis was performed using SAS®.
RESULTS
In Exp.1 the behavioral results were (DIB0.3/14m, DIB0.5/14m, DIB0.3/24m, DIB0.5/24m, respectively): Raised tail: 52% (78/149), 73% (107/147), 51% (78/153), 62% (96/156), PDev<0.0001, PAge=0.09, Dev*Age=0.17; Dorsal arching: 22% (33/149), 54% (79/147), 30% (46/153), 38% (60/156), PDev<0.0001; PAge=0.55, Dev*Age=0.003. In Exp.2 there was an interaction between treatments [DIB0.3/36h= 53.3% (112/210); DIB0.5/48h= 43.2% (82/190); DIB0.3/36h= 46.6% (89/191), DIB0.5/48h= 49.8% (105/211); PDev*Proc=0.04]. In Exp.3 the PR was higher in heifers that received DIB0.3 with insemination after 36 hours [35.5% (320/901)] when compared to DIB0.5 group [29% (267/920; PDev=0.001].
CONCLUSIONS
It can be concluded that DIB0.3 caused less discomfort than DIB0.5, especially in 14-month-old heifers. Also, animals treated with DIB0.3 and inseminated 36 hours after withdrawal of DIP4 demonstrated higher PR.

M12
INFLUENCE OF PARITY (CATEGORY) AND AGE OF DAIRY GIR COWS ON COLOSTRUM AND MILK COMPOSITION

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BACKGROUND-AIM
Colostrum and milk composition of Gir dairy cows are not fully stablished, with special regards to the protein and immunological features. Zebu breed cows are referred to present low milk yield and late sexual maturity, which may influence the immunological content of the colostrum. Therefore, the aim of this study was to evaluate and compare the protein and immunological composition of colostrum and milk of dairy Gir cows in regards to the animal category (parity) and age group.
METHODS
Thus, Gir cows and their calves were assigned to two experimental groups according to the number of calvings (parity): Primiparous (n=12 cows at first calving; n=7 calves) and Pluriparous (n=16 cows with more than 1 calving; n=10 calves). According to the maternal age, cows and calves were also divided into: Young (n=6, mean age of 24-36months; n=5 calves), Adult (n=15, mean of age 37-91months; n= 8 calves) and Senior (n=7 females, mean age of 96-137months; n=4 calves). After delivery (0 hours), colostrum samples were obtained. Transitional milk was collected after 24 hours and after 5 days, the milk. At 3 days of life, blood samples were obtained from the calves. Milk were analyzed for density, total solids concentration, pH, total protein concentration, protein fractions and immunoglobulin G (IgG) concentration. Blood from calves were evaluated for total solids concentration, total protein concentration, liver function, and IgG concentration. LSD test was used considering P<0.05.
RESULTS
Primiparous and pluriparous had progressive decrease in total solids concentration among colostrum, transitional milk and milk. However, heifers had lower albumin concentration compared to cows. Young cows had higher colostrum and milk total solids concentration compared to adult and senior. Young females had lower a-lactalbumin concentration compared to Senior Group. Considering lactoferrin and albumin concentrations, colostrum and transitional milk was higher than milk, while colostrum a-lactalbumin concentration was lower in transitional and milk. Calves of senior cows had higher serum albumin concentration.
CONCLUSIONS
In conclusion, parity of dairy Gir cows does not modify colostrum and milk immunological content. However, cow's age interfere protein composition of the colostrum, transitional milk and milk.

M13
REPRODUCTIVE PERFORMANCE OF NORTH AMERICAN AND NEW ZEALAND BIOTYPE HOLSTEIN DAIRY COWS IN PASTURE-BASED SYSTEMS OF URUGUAY THROUGHOUT THREE YEARS

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BACKGROUND-AIM
In the last decades, New Zealand Holstein genetics have been used in Uruguayan dairy farms. The farmers are seeking cows better adapted to the pasture-based systems and more efficient to convert pasture to valuable milk traits. However, Uruguay lacks information about the reproductive performance of the New Zealand Holstein (NZH), comparing year-over-year and under the same management conditions of North America Holstein (NAH) biotype cows. The objective of this study was to compare the reproductive performance of NAH and NZH cows throughout three years.
METHODS
The experiment was conducted at the research facilities of INIA-LE, Uruguay, between 2017 to 2019. All animals were submitted to the same nutritional, health, and reproductive management system and annual production targets. The NAH (n=60) represents the national herd, with 80% of NA genetics, and the NZH (n=60) with at least 75% of NZ genetics. Cows were selected and paired according to lactation number, calving date, and health status. The voluntary waiting period was 45 days and animals were inseminated after estrus detection or timed-AI. Individual data were daily recorded using DairyPlanC21. The parameters, calving to first service interval (CFS, days), calving to conception interval (CCI, days), number of services per conception (SPC), first service conception rate (FSC), and overall pregnancy rate (PR) were used for data analysis. Statistix v.9 was used for statistical analyses using the ANOVA and Tukey's test for comparing the means and the Chi-square test for the proportions.
RESULTS
Similar (P>0.05) results between NAH and NZH were recorded to CFS (70.6±1.6 and 72.5±1.7 days), CCI (100.6±3.3 and 98.2±2.9 days), FSC (37.8 and 45.0%), and PR (78.9 and 82.9%). Moreover, the year and the interaction between biotypes and years did not affect (P>0.05) the abovementioned parameters. Nonetheless, the NZH presented lower (P<0.05) SPC than NAH (1.6±0.08 and 1.9±0.08, respectively). Although a lower (P<0.05) overall SPC in 2019 (1.6±0.09) when compared to 2017 (1.9±0.1), no interaction of biotypes and years was detected.
CONCLUSIONS
Therefore, we demonstrated that the NZH biotype had lower SPC than NAH and may maintain a better reproductive performance throughout the animal's lifetime in the pasture-based systems of Uruguay.

M14
EFFECTS OF PURIFIED ECG CONCENTRATION IN ECG/PGF2α-BASED SOV PROTOCOLS ON OVARIAN STRUCTURES AND IN VIVO EMBRYO PRODUCTION IN NELORE CATTLE

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BACKGROUND-AIM
Superovulation (SOV) is a frequent approach to maximize the number of embryos available per individual for increasing profitability and genetic gains in bovine species. The main aim was to study the effect of different purified eCG (eCG-p) concentrations in eCG/PGF2a-based SOV protocols in order to test the differential effects obtained regarding ovarian structures and in vivo embryo production in Nelore (Bos indicus) cattle.
METHODS
A total of 24 Nelore cows (BC: 3-3.5) were divided randomly into 2 groups: NH (n=12; high eCG-p dose= 2,500 IU) and NL (n=12; low eCG-p dose= 2,000 IU). The SOV protocol was applied as follows: Day 0: intravaginal P4 device (CIDR: 1.38 gr) + 2 mg intramuscular (i.m.) Estradiol Benzoate E2B + 50 mg P4 (i.m.); Day 4: eCG-p (2,500 or 2,000 IU for NH and NL group, respectively); Day 6: PGF2a i.m. (300 µg D-cloprostenol); Day 7: CIDR removal (36h post- 1st PGF2a application) + PGF2a i.m. (150 µg D-cloprostenol); Day 8: 500 µg GnRH (48h post-1st PGF2a application) + FTAI. Ovarian structures (follicles (FL) and corpora lutea (CL)) were recorded by ultrasonography on Day 8 and Day 15 (embryo collection day). The traits assessed were: total follicles (TFL > 8mm), total corpora lutea (TCL), and no-ovulated follicles (NOFL). Embryo-derived parameters were: ovulation rate (OR; %), recovery rate (RR; %), total structures (TS), transferable embryos (TE), freezable embryos (FE), unfertilized oocytes (UFOs) and degenerated structures (DS).
RESULTS
No differences were observed in TFL, TCL or NOFL ovarian-derived traits between groups (p>0.05). Significant differences were observed in OR (40.8±6.0% vs. 58.4±5.3%), TE (4.4±0.6 vs. 6.9±1.0), and FE (3.8±0.7 vs. 6.7±0.9) embryo-derived traits for NH and NL, respectively (p<0.05). No differences between groups for any other embryo-derived parameters were observed (p>0.05).
CONCLUSIONS
In conclusion, although no differences were observed regarding ovarian-derived parameters between protocols, the most efficient eCG/PGF2a-based SOV protocol regarding embryo-derived traits was the low-eCG-p-dose protocol (NL group) providing a higher number of transferable and freezable embryos per individual in Nelore breed cows.

M15
TIMING EFFECTS OF CIDR REMOVAL IN ECG/PGF2α- SOV PROTOCOLS ON IN VIVO EMBRYO PRODUCTION IN BOS INDICUS AND BOS TAURUS X BOS INDICUS CATTLE BREEDS

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BACKGROUND-AIM
Equine chorionic gonadotropin-serum (eCG) has been used systematically in Bos taurus SOV but not as frequent in Bos indicus and its crossbreeds. The objective was to study the timing effects of CIDR removal in order to elucidate the differential effects on in vivo embryo production in Bos indicus and Bos taurus x Bos indicus cattle breeds.
METHODS
A total of 32 individuals [breeds: Brangus (BGUS; n=16); Brahman (BMAN; n=16); BC: 3-3.5] were divided randomly into 4 groups [BGUS-A (n=8); BMAN-A (n=8); BGUS-B (n=8), and BMAN-B (n=8)]. Two SOV protocols (A and B) were applied: Day 0: intravaginal P4 device (CIDR: 1.38 gr) + 2 mg Estradiol Benzoate E2B (im) + 50 mg P4 (im); Day 4: eCG-p (2,000 IU); Day 6: PGF2a im (2 doses; 150 µg D-cloprostenol each, am/pm); Day 7: CIDR removal (24 or 36h post-1st PGF2a application for A and B protocol, respectively); Day 8: 500 µg GnRH (48h post-1st PGF2a application) + FTAI. Embryo-derived traits scored: total structures (TS), transferable embryos (TE), degenerated structures (DS) and unfertilized oocytes (UFOs).
RESULTS
Significant differences were observed in TS, being higher in BMAN-A (12.0±1.8) compared to BGUS-A (10.0±1.9), BGUS-B (10.2±1.8), and BMAN-B (9.5±1.4) groups ($p<0.05$). Significant differences in TE were observed in BMAN-A group (9.2±1.1), being the highest when was compared to BGUS-A (7.0±1.9), BGUS-B (5.5±2.0), and BMAN-B (4.2±0.6) ($p<0.05$). Moreover, differences were detected regarding DS being higher in BMAN-B (4.5±0.4) compared to BMAN-A (1.7±0.4), BGUS-A (1.3±0.6), and BGUS-B (0.5±0.1)($p<0.05$). Regarding UFOs, differences were observed among groups, being higher in BGUS-B (4.2±1.1) compared to BGUS-A (2.1±0.7), BMAN-A (1.0±1.0), and BMAN-B (0.5±0.3) ($p<0.05$). When just breed was compared differences were detected in DS and UFOs being higher in BMAN (3.1±0.4 vs. 0.9±0.3) and BGUS (2.9±0.7 vs. 0.7±0.5) for the first and the second trait, respectively ($p<0.05$). When just SOV protocols were considered, differences were observed in TE between A and B protocols (8.1±1.1 vs. 4.8±1.0; $p=0.04$).
CONCLUSIONS
Important differences were observed in in vivo embryo production traits among breeds and SOV protocols. The A protocol was the most efficient for both breeds, being Brahman breed response the best. These differences may be related to the interaction between the timing of CIDR removal together with genetic intrinsic factors such as cattle breed.

M16
PARA- POLAR BODY SITE SPERM INJECTION IN PIEZO-ICSI CAN IMPROVE SUBSEQUENT EARLY DEVELOPMENT OF BOVINE EMBRYOS

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BACKGROUND-AIM
The orientation of the injection site in conventional and piezo-intracytoplasmic sperm injection (ICSI) are generally performed with the first polar body (PB) of a metaphase II (MII) oocyte in either the 6 or 12 o'clock position. However, it is possible that the ooplasmic cell membrane is damaged during the drilling of the zona pellucida by piezo pulses in piezo-ICSI, thus resulting in a reduction in blastocyst rate. Here, we describe a new piezo-ICSI method in which the PB is set at either the 2 or 4 o'clock position. In this new method, zona drilling and sperm injection is performed through the para-PB site, which is the widest position in the perivitelline space.
METHODS
In experiment 1, we evaluated the effect of injection site in bovine ICSI on the survival rate of the oocyte; we also investigated developmental competence and chromosomal integrity at the blastocyst stage. In experiment 2, we examined the effect of injection site on integrity of the meiotic spindle in MII oocytes. In experiment 3, we examined the effect of piezo pulse upon the ooplasmic cell membrane with regards to subsequent development.
RESULTS
In experiment 1, .the survival rate in the para-PB piezo group (90.1%) was significantly higher than that in the conventional piezo group (75.0%, $P<0.05$). There were no significant differences in the blastocyst rate of the surviving oocytes or the chromosomal integrity. In experiment 2, there were no significant differences in meiotic spindle integrity. In experiment 3, the survival rate of the oocytes decreased as the distance between the micropipette and ooplasmic cell membrane became smaller.
CONCLUSIONS
In conclusion, the present study revealed that our new piezo-ICSI method (para-PB site piezo-ICSI) can improve current ICSI technology.

M17
ACCURACY OF THE USE OF DOPPLER ULTRASOUND AND THE EXPRESSION OF INTERFERON-TAU-STIMULATED GENES FOR EARLY PREGNANCY DIAGNOSIS IN HEIFERS AND DAIRY COWS

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BACKGROUND-AIM
The bovine conceptus secretes interferon-tau (IFN-tau), which stimulates the transcription of several genes and participates in the process of maternal recognition during pregnancy. The aim of this study was to compare the accuracy of using IFN-tau stimulated gene abundance (ISGs) in peripheral blood mononuclear cells (PBMC) with Doppler ultrasound (Doppler US) in dairy cows and heifers at 21 days (D21) after fixed-time artificial insemination (FTAI) for early pregnancy diagnosis
METHODS
144 cows and 32 heifers of the Holstein breed, were subjected to a hormonal ovulation synchronization protocol and FTAI. At D21, PBMCs were isolated via blood samples and CL blood perfusion was assessed by color Doppler US. The abundance of the target genes (ISG15 and RSAD2) was determined by RT-qPCR, which were normalized with the endogenous genes GAPDH and PPIA. Confirmatory pregnancy diagnosis was performed at 32 days (D32) post-FTAI by visualization of the embryo by B-mode US. The abundance of transcripts was evaluated by analysis of variance (ANOVA considering fixed effects of group (pregnant or non-pregnant), category (cow or heifer) and group and category interaction using SAS PROC MIXED. For ISG15 and RSAD2 the points cut-off values were established by analyzing the ROC curve using MedCalc® Software (version 19.1; Medcalc Software).
RESULTS
The abundance of ISG15 was greater in the pregnant female group (0.120 ± 0.013) compared to the non-pregnant group (0.084 ± 0.010; P=0.0003). There was also a significant difference in the abundance of RSAD2 for pregnant females compared to non-pregnant females (P=0.0008). Furthermore, a difference was observed for the abundance of genes in relation to the animal category (P=0.001). A positive and significant linear correlation was found between the expression of ISG15 and RSAD2 genes (r=0.53; P<0.05). The Roc Curve analysis indicated that the abundance of genes ISG15 (AUC= 0.70; P<0.0001) and RSAD2 (AUC=0.71; P<0.001), and of US Doppler (AUC= 0.69; P<0.0001) were good predictors of early pregnancy in PBMCs in dairy cows and heifers
CONCLUSIONS
. We conclude that Doppler US and gene expression of the evaluated ISGs are significant methods for diagnosing pregnancy at D21 post-FTAI in dairy females, and ISGs can be used as a potential early marker of pregnancy.

M18
INTRINSIC OVARIAN FACTORS ASSOCIATED WITH EMBRYO DEVELOPMENT AND QUALITY IN AN INDIVIDUAL CULTURE SYSTEM

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BACKGROUND-AIM
The oocyte developmental capacity may be affected by intrinsic ovarian factors such as corpus luteum (CL), dominant follicle (DF), and the respective progesterone and estradiol concentrations in neighbouring follicles. We evaluated the effect of CL and DF (>12 mm) on cumulus expansion, embryo development, and quality in a serum-free individual culture system. Furthermore, we evaluated the progesterone and estradiol concentrations in follicles (4 to 8 mm) from ovaries with and without CL or DF.
METHODS
Bovine genital tracts were collected from slaughtered cows, and healthy non-pregnant uteri were selected. Follicular fluid (FF) from follicles between 4 to 8 mm was collected (and pooled), and COCs with at least three layers of cumulus cells and homogeneous cytoplasm (n=1,772 COCs from 108 ovaries) were selected for in vitro maturation, fertilization, and culture in individual 20 µL droplets under paraffin oil.
RESULTS
Oocytes from ovaries without a CL (CL-) showed greater cumulus expansion (232 ± 3.99 µm) compared to those oocytes derived from CL bearing ovaries (CL+) (216 ± 4.33 µm; P = 0.0001). CL- ovaries resulted in greater cleavage and day 8 blastocyst rates (61.4 ± 2.6 and 23.1 ± 1.5%, respectively) compared with CL+ ovaries (53.7 ± 2.59 and 12.2 ± 1.1%, respectively; P > 0.003). Oocytes from CL- ovaries resulted in blastocysts with higher total cell number (TCN; 100.7 ± 0.6%), trophectoderm (60.4 ± 0.4%), and inner cell mass cells (37.3 ± 0.6%) compared to CL+ ovaries (92.5 ± 0.8, 57.7 ± 0.5, and 34.8 ± 0.7%, respectively; P > 0.0001). In CL- ovaries, the apoptotic cell (AC) number (2.61 ± 0.05%) and AC/TCN ratio (2.6 ± 0.1%) were lower than CL+ (3.28 ± 0.07, 3.8 ± 0.1%, respectively; P > 0.0001). The presence of a DF did not affect any parameter (P < 0.3). The progesterone concentration of FF from CL+ ovaries was higher (108.3 ± 9 ng/mL) than in CL- ovaries (52.6 ± 9 ng/mL; P = 0.0002) and the estradiol concentration of FF from ovaries with a DF was higher (3.78 ± 0.9 ng/mL) than ovaries without a DF (2.24 ± 0.68 ng/mL; P = 0.04).
CONCLUSIONS
Since intrinsic ovarian factors can affect embryo development, we recommend using COCs derived from ovaries with similar intrinsic characteristics when assessing biomarkers associated with development.

M21
ASSOCIATION BETWEEN EARLY LACTATION CLINICAL MASTITIS AND PREGNANCY RATE AND ITS INTERACTION WITH PREPARTUM NEFA CONCENTRATION.

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BACKGROUND-AIM
Clinical mastitis affects fertility. We aimed to study the association between early lactation clinical mastitis and pregnancy rate and its interaction with prepartum NEFA concentration (pre-NEFA).
METHODS
Holstein primiparous (n = 375) and multiparous (n = 663) cows from four grazing dairy farms were followed from 7 days before the expected calving, when blood samples were drawn for NEFA determinations, until 305 days in milk (DIM). Clinical mastitis during the first 30 DIM (CM), uterine disease (UD: retained placenta, metritis or both) and endometritis were diagnosed according to standard definitions. Time to pregnancy was defined as the interval in days from calving to the last insemination before the pregnancy diagnosis. Pregnancy diagnoses were performed by transrectal palpation or ultrasonography by the farm veterinarian.
Cox's proportional hazards regression models were used to analyze pregnancy rate within 305 DIM: parity (1, 2, and 2+), pre-NEFA (Low ≤ 0.3, High > 0.3 mM), calving month (February/March, April, May, June, July and August/ September), CM (yes/no), UD (yes/no) and endometritis (yes/no) were included as class variables. Farm, also as a class variable, was included as a random effect. Two-way interactions between pre-NEFA and clinical disease were checked for significance.
RESULTS
Prepartum NEFA concentration was not associated with pregnancy rate (HR = 0.87, P = 0.17), but interacted with CM: in High NEFA cows, CM was associated with a reduced pregnancy rate (HR = 0.62, P = 0.04), while in low pre-NEFA cows CM was not associated with pregnancy rate (HR = 0.99, P = 0.94). Compared to parity 2, parity 1 tended to be associated with a higher pregnancy rate (HR = 1.19, P = 0.07), while parity 2+ was associated with a lower pregnancy rate (HR = 0.77, P = 0.008). Uterine disease was associated with a lower pregnancy rate (HR = 0.76, P = 0.008), and endometritis tended to be associated with a lower pregnancy rate (HR = 0.72, 0.10).
CONCLUSIONS
Recently, it was proposed that increased NEFA concentrations are a mere sign of immune activation; whether High pre-NEFA cows are less able to deal with CM or whether the high pre-NEFA concentrations are a sign of immune activation due to dry period intramammary infection warrants further research.

M22
EFFECT OF THE ADDITION OF GNRH AND A SECOND PROSTAGLANDIN TREATMENT ON PREGNANCY RATES IN LACTATING DAIRY COWS SYNCHRONIZED WITH AN ESTRADIOL/PROGESTERONE-BASED PROTOCOL

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BACKGROUND-AIM
Two experiments were designed to evaluate whether the addition of GnRH at the beginning of the synchronization protocol and a second dose of prostaglandin F2a (PGF) the day before the removal of a progesterone (P4) releasing device improves pregnancy rate to timed-AI (P/TAI) in lactating dairy cows synchronized with an estradiol/P4-based protocol.
METHODS
Holstein cows (43.8±9.3 Kg of milk, 68.3±8.1 DIM, BCS of 3.1±0.3), were allocated into 1 of 4 treatment groups. On Day 0, cows were scanned (67.5% CL) and treated with a CIDR-B device (Zoetis, Argentina) and 2 mg of estradiol benzoate (Gonadiol, Zoetis). Half of the cows also received 200 µg gonadorelin (GnRH, Gonasyn, Zoetis) at the same time. On Day 7, cows were further subdivided to receive 500 µg cloprostenol (PGF, Ciclase DL, Zoetis) or no PGF treatment. On Day 8, CIDR-B devices were removed, and all cows received PGF,1 mg estradiol cipionate (Cipiosyn; Zoetis), 400 IU eCG (Novormon; Zoetis) and an estrus detection patch (Fasco AP, Argentina). In Experiment 1, cows (n=76) were examined by ultrasonography every 12 h from the time of CIDR-B removal until ovulation and were bled for plasma P4. In Experiment 2 (n=1036), cows with >50% of the patch rubbed-off by 48 h were TAI at that time, whereas those not in estrus received 100 µg of GnRH and were TAI 12 h later.
RESULTS
In Experiment 1, the interval to ovulation was 71.7±1.5 h and did not differ among groups. However, cows that received 2 injections of PGF had greater (P<0.01) estrus rate and lower (P<0.01) P4 concentrations at TAI than those that received 1 PGF (estrus rate: 86.8%, 33/38 vs 68.4, 26/38 and P4: 0.12±0.01 vs 0.36±0.07 ng/mL, for those with 2 or 1 PGF, respectively). In Experiment 2, cows treated with 2 PGF had a greater estrus rate (84.7%, 438/517) than those with 1 PGF (65.7%, 341/519; P<0.01). Furthermore, there was a GnRH by PGF treatment interaction (P<0.05) on P/TAI, that was attributed to a greater P/TAI in cows that received GnRH on Day 0 and 2 PGF treatments than in those in the other three treatment groups (EB+1 PGF: 45.2%, 119/263; EB+2 PGF: 45.8%, 119/260; EB+GnRH+1 PGF: 45.7%, 117/256 and EB+GnRH+2 PGF: 57.2%, 147/257).
CONCLUSIONS
The addition of GnRH on Day 0 and a second dose of PGF improves P/TAI in dairy cows synchronized with an estradiol/P4-based protocol.

M23
PREGNANCY RATES IN SUCKLED BEEF COWS SYNCHRONIZED WITH TWO DIFFERENT PROGESTERONE/ESTRADIOL-BASED PROTOCOLS AND INSEMINATED WITH CONVENTIONAL OR SEXED-SORTED SEMEN

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BACKGROUND-AIM
An experiment was designed to determine pregnancy rates (P/AI) in suckled beef cows synchronized with two different progesterone (P4)/estradiol-based protocols and time-inseminated (TAI) with conventional (non-sexed) or sexed-sorted semen.
METHODS
Angus and Angus-cross cows (n=777), 60-90 days postpartum, with a body condition score of 2.5 to 3.5 (scale of 1 to 5) and a CL (44%) or a follicle >8 mm in diameter (56%) detected by ultrasonography received a P4 device (DIB 0.5 g P4, Zoetis, Argentina) and 2 mg estradiol benzoate (Gonadiol, Zoetis) on Day 0 and were allocated randomly into two treatment groups. Cows in Group 1 (J Synch group) had the DIB removed, received 400 IU eCG (Novormon, Zoetis), 500 µg cloprostenol (PGF, Ciclase, Zoetis) and were tail painted on Day 6. Cows with >50% of the tail-paint rubbed off (defined as in estrus) by 72 h after DIB removal were AI with either conventional (25 million sperm) or sexed-sorted (SexedUltra™ 4 M, 4 million sperm) semen from one of two Angus bulls. Those with the tail-paint intact (defined as not in estrus) received 100 µg of gonadorelin (GnRH, Gonasyn, Zoetis) at that time and were AI with either conventional or sexed-sortedsemen 12 h later. Cows in Group 2 (the ECP group) had the DIB removed and received eCG, PGF, 0.5 mg estradiol cypionate (Cypiosyn, Zoetis) and had tail paint applied on Day 7. Cows with the tail paint rubbed off by 60 h were AI with either conventional or sexed-sorted semen and those not in estrus received GnRH and were AI with conventional or sexed semen 12 h later. Pregnancy was diagnosed by ultrasonography 30 days after AI. Data were analyzed by GLMM for binary data with a logit link.
RESULTS
There was no significant effect of the protocol on P/AI (Group 1 - J-Synch 53.8 %, 205/381 vs Group 2 - ECP 51.8%, 205/396; P>0.1). However, cows that showed estrus had a higher P/AI (295/517, 57.1%) than those that did not (115/260; 44.2% P <0.01). In addition, P/AI was greater in cows inseminated with conventional (196/398, 49.2%) than in those inseminated with sex-sorted semen (171/379, 45.1%).
CONCLUSIONS
Both treatment protocols result in comparable pregnancy rates in beef cows inseminated with conventional or sex-sorted semen. Furthermore, expression of estrus affects P/AI significantly, regardless of protocol or type of semen used.

M24
A COMPARISON OF PREGNANCY RATES IN LACTATING HOLSTEIN COWS TREATED WITH ONE OF TWO TIMED-AI PROTOCOLS WITH PROLONGED PROESTRUS

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BACKGROUND-AIM
An experiment was designed to compare pregnancy rates to timed-AI (P/TAI) in lactating Holstein cows synchronized with two protocols with prolonged proestrus: an estradiol-based (J-Synch) or a GnRH-based (Web-Synch).
METHODS
Lactating Holstein cows (n=179), producing 41.3±0.9 kg of milk per day, with 139.0±8.3 days in milk, 1.9±0.1 lactations, body condition score 3.0±0.1 and managed in a confinement system, were randomly allocated into one of two treatment groups. On Day 0, cows in the J-Synch group received 2 mg estradiol benzoate (Estradiol, Over, Argentina) and a vaginal device containing 1 g progesterone (P4, Sincrover, Over). On Day 6, P4 devices were removed, and cows received 150 µg D (+) cloprostenol (PGF, Prostal, Over) and 400 IU eCG (Novormon, Zoetis). On Day 7, a second dose of PGF was administered. Cows in the Web-Synch group were treated with PGF and a P4 device on Day -5 and 20 µg buserelin (GnRH, Gestar, Over) on Day 0; device removal, PGF and eCG was done on Day 6, and a second dose of PGF was administered on Day 7. Cows in both groups were tail painted for estrus detection and were TAI 80 h after P4 device removal on Day 6. Cows without the tail-paint rubbed-off received 10 µg GnRH at TAI. Ovarian ultrasonography was performed on Days -5, 0 and 6, and all cows were also scanned 30 d after TAI for pregnancy diagnosis. Data were analyzed using the GLM mixed model procedure for binary data and a logit link.
RESULTS
Overall, expression of estrus was 49.7% (89/179) and P/TAI did not differ (P>0.6) whether cows showed (42.6%, 38/89) or did not show (36.6%, 33/90) estrus at TAI. However, both the expression of estrus and P/TAI were greater (P <0.05) in cows in the Web-Synch group (59.3%, 54/91 and 49.4%, 45/91, respectively) than in the J Synch group (39.7%, 35/88 and 29.5%, 26/88, respectively). The proportion of cows with a CL on Day-5 did not differ between groups (J-Synch: 60.2%, 53/88 vs Web-Synch: 63.7%, 58/91; P>0,6). However, the proportion of cows with a CL in the Web-Synch group was 12.1% on Day 0 and 76.9% on Day 6, indicating that at least 64.8% of the cows ovulated in response to the first GnRH.
CONCLUSIONS
Between the two protocols with prolonged proestrus tested in lactating dairy cows in the present study, the Web-Synch protocol resulted in greater P/TAI than the J-Synch.

M25
BLOOD ACETYLCHOLINE AND BETA-ENDORPHIN CONCENTRATION IN DAIRY COWS AFFECTED BY OVARIAN CYSTIC DISEASE

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BACKGROUND-AIM
The etiopathogenesis of cystic ovarian disease (COD) is multi-factorial and represents the most frequent technical pathology (5-30%) in post-partum cows with high dairy production. Endorphin-mediated pathologies can also appear because of the altered hormone-receptor binding system with an increase of endogenous opioids and changes in Ca2+ turnover. Reproduction is also regulated by GnRH, gonadotropins and by the cholinergic system; alterations of this synergism can lead to the development and persistence of ovarian cysts. Aim of the work is to evaluate the involvement of acetylcholine (Ach) and beta endorphin (β-End) in COD and the efficacy of an epidural GnRH/PGF2a treatment in its resolution.
METHODS
After 60 days from partum, cows were clinically examined to select 150 Holstein subjects with COD. After 10 days, 30 cows were randomly selected and treated by an epidural administration of 2ml, 0.9% NaCl (control, group C). The remaining animals were treated by an epidural GnRH administration (2ml, 50µg lecirelin). At the 21st d, plasma P4 concentration was used to evaluate cyst luteinization to repeat the same pharmacological treatment (group A) or in the case of high P4 (>2ng/ml) to administer PGF2a (2ml, 150 µg d-cloprostenol, group B). Blood samples were obtained from all animal at day: 0, 10, 11, 12, 17, 21 to determine P4, Ach, β-End, and LH (on 10th day).
RESULTS
In group A: 32/120 (27%) cows returned in oestrus; 22/120 (18%) received a 2nd GnRH treatment, while 9/120 (8%) were not responding cows. In group B: 52/120 (43%) cows received the PGF2a treatment while 5/120 (4%) were not responding. In group C: 2/30 (6%) showed a spontaneous cyst regression and in the remaining subjects, cysts persisted. β-End concentration in blood increased at day 10 and slowly returned at basal level at day 17. LH concentration increased (P<0.001) 120 min after GnRH treatment. Blood Ach decreased (P<0.01) starting from day 12. Fertility index was measured evaluating the pregnancy rate in group A 37/54 (68%) in group B 38/52 (73%) in group C 0/30 (0%).
CONCLUSIONS
We have shown the involvement of Ach and β-End that inhibit the follicular wall contractility in COD. Futhermore we demonstrated the efficacy of the epidural GnRH and PGF2a administration in COD.

M26
PRESYNCHRONIZATION USING AN INTRAVAGINAL PROGESTERONE DEVICE IMPROVES FERTILITY IN SUCKLED BEEF COWS SUBMITTED TO FIXED TIME ARTIFICIAL INSEMINATION

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BACKGROUND-AIM
Presynchronization treatments administered before imposing a CO-Synch type regimen are designed to improve ovulatory response and synchronization of follicular development. The objective of this study was to determine effects of various presynchronization treatments before initiation of a 6-day CO-Synch on estrous expression and fertility to timed artificial insemination (TAI).
METHODS
Suckled beef cows (n=645) with moderate body condition scores (6; 1 to 9 scale) were blocked by age and postpartum interval and randomly assigned to treatments: presynchronization with prostaglandin F2a (PGF Presynch); presynchronization with progesterone (P4) and PGF (P4+PGF Presynch); or no presynchronization (Control). Cows in the P4+PGF Presynch group were administered a once-used P4 intravaginal device (CIDR, Zoetis) on D-17 and the CIDR was removed on D-11 concurrently with administration of 500 µg of cloprostenol (PGF, Parnell). Cows in the PGF Presynch group were administered PGF on D-11. On D-9 all cows were administered a new CIDR and 100 µg of gonadorelin (GnRH, Parnell). Six days later (D-3) CIDRs were removed, and cows were administered 1000 µg of PGF and an estrous detection patch (Estrotect, Rockway). At 72 h after CIDR removal, 100 µg of GnRH were administered, and cows were inseminated. Pregnancy was determined using ultrasonography on D35 and D90. Data were analyzed using generalized linear mixed models.
RESULTS
Percentage of cows expressing estrus was greater (P=0.04) in the PGF Presynch group (83.9%: 182/217) than P4+PGF Presynch (75.7%; 159/210) and Control (74.8%,163/218) group. There was, however, no difference on interval between CIDR removal and estrus (60.1 ± 0.8 h; P=0.41). Pregnancies per AI (P/AI) on D35 was greater (P<0.01) for cows in the P4+PGF Presynch (66.7%; 140/210) than PGF Presynch (55.3%; 120/217) and Control (50.9%; 111/218) group. Similarly, the P4+PGF Presynch resulted in greater (P=0.01) P/AI at D90 (61.4%; 129/210) compared to that for the PGF Presynch (51.9%; 112/216) and Control (49.1%;107/218) group. There were no differences (P=0.41) in pregnancy loss among groups (5.9%; 22/370).
CONCLUSIONS
In conclusion, presynchronization with P4 and PGF prior to initiation of a 6-day CO-Synch treatment regimen improves fertility as a result of TAI in suckled beef cows.

M27
FOETAL SEX RATIO IN LACTATING DAIRY COWS FOLLOWING TIMED ARTIFICIAL INSEMINATION (TAI) OR TIMED ET (TET) WITH FRESH OR FROZEN IN VITRO PRODUCED (IVP) EMBRYOS

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BACKGROUND-AIM
Published data indicate IVP bovine embryos have a sex ratio biased towards males. The aims of this study were to determine the sex ratio of (1) Day 7 and 8 IVP blastocysts, and (2) 62-day-old foetuses in lactating dairy cows bred using either TAI or TET.
METHODS
IVP blastocysts were generated from oocytes collected by ovum pick-up from genetically elite Holstein-Friesian (n=29), Jersey (n=11) and Angus (n=21) donors and oocytes from slaughtered commercial crossbred beef heifers (n=119). Following in vitro maturation, fertilisation (conventional unsorted semen) and culture, single Grade 1 blastocysts were transferred either fresh or following freezing and on-farm thawing. A total of 1192 recipients (lactating dairy cows, predominantly Holstein-Friesian) were synchronised with a 10-d Progesterone-Ovsynch protocol, of which 240 (20%) were assigned to receive AI (16 h after second GnRH, conventional semen) and 952 (80%) were assigned to receive ET on Day 7. Between Day 62-65 after synchronised ovulation, foetal sex was determined by trans-rectal ultrasonography in 436 of the cows that had previously been diagnosed pregnant on Day 32-35. Data were analysed using generalised linear mixed models including service treatment (TAI vs. TET) as a fixed effect.
RESULTS
The sex ratio (M:F) of foetuses derived from TET (n=328 pregnancies) was 60.8:39.2. In contrast, that of foetuses derived from TAI was 42.7:57.3 (n=108) (P=0.002). There was no difference in sex ratio across the different IVP-ET treatments (i.e., beef vs. dairy, fresh vs. frozen). Day 7 (n=63) and Day 8 (n=40) IVP blastocysts produced over 3 repetitions of IVP using abattoir-derived ovaries as the source of oocytes were snap-frozen, and their sex was determined by extraction of DNA and amplification of a 241 base pair fragment of the amelogenin gene (AML-X) or both a 241 and a 178 base pair fragment for AML-X and Y, respectively. Overall sex ratio (M:F) among in vitro blastocysts was 61.2:38.8 (M:F), and was not affected by day (Day 7: 61.2:38.8; Day 8: 65.4:34.6; p=0.685).
CONCLUSIONS
In conclusion, the bias towards male foetuses on Day 62-65 in recipient cows was mirrored by a similar sex bias in IVP blastocysts on Day 7 and 8. These results indicate similar survival of male and female embryos after transfer to recipients on Day 7.

M28
EFFECT OF 400 UI OF ECG-LIKE GLYCOPROTEIN OR NATIVE ECG ON THE FTAI PREGNANCY RATE IN SUCKLED BEEF COWS IN ARGENTINA IN A DROUGHT PERIOD

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BACKGROUND-AIM
The objective of this experiment was to compare an eCG-like glycoprotein (reCG) with native eCG on the pregnancy rate in a progesterone and estradiol-based TAI program in suckled beef cows in Argentina in a drought period.
METHODS
The experiment was conducted in two beef farms in Argentina where 367 suckled beef cows (Farm A: primiparous Aberdeen Angus, n=186; Farm B: multiparous Brahaman and Angus crossbred, n=181) with a body condition score (BCS) of 2.4±0.3 (average ± standard deviation, scale 1-5) in a negative nutritional plane due to drought. All cows were ultrasound on Day 0 to determined ovarian structures and were treated with the same FTAI progesterone–estradiol protocol: Day 0: 2 mg im of estradiol benzoate (Gonadiol, Zoetis, Argentina) and 0.5 gr intravaginal device insert (DIB 0.5, Zoetis); Day 8: 500 µg of sodium cloprostenol (Ciclase DL, Zoetis), 1 mg of estradiol cypionate (Cipiosyn, Zoetis), and were painted in the sacrocaudal region to identify cows that displayed estrus; Day 10: fixed time insemination and tail paint check (estrous: >75% of paint loss). On Day 8, cows were randomly assigned to received 400 IU of native eCG (eCG; Novormon, Zoetis; n=134), 400 IU of reCG produced by Syntex (reCG; n=133; PCT/EP2019/073277) or remain untreated (control; n=100). Pregnancy diagnosis was performed by ultrasound at Day 40. Response variable was pregnancy rate (PR); explanatory variables were treatment, BCS, estrous manifestation, ovarian structure, location and their interactions. Statistical analysis was performed by logistic regression (PROC GENMOD, SAS).
RESULTS
The percentage of cows with CL on Day 0 was 9.1% and 1.1% for Farm A and B respectively. The overall PR was 19.1% and tended to be affected by the treatment (P=0.086) and was affected the estrous expression (P<0.0001). PR was 22.6%, 20.9%, and 12.0%, for reCG, eCG and control respectively (reCG vs control: P=0.048; eCG vs control: P=0.097; reCG vs eCG: P=0.70). Estrous manifestation 10 was 55.0%, cows that did show estrous had higher pregnancy (27.2% vs 9.1%).
CONCLUSIONS
In an extreme drought scenario, reCG and native eCG tended to increase PR in a FTAI synchronization protocol in suckled beef cows compared with control. Cows that display estrous had greater PR than cows that did not.

M29
EFFECT OF THE ENVIRONMENT AND NUTRITIONAL TREATMENT ON INSULIN AND PROGESTERONE CONCENTRATIONS DURING EARLY LACTATION IN HOLSTEIN COWS

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BACKGROUND-AIM
Thisstudy evaluated the combined effect of different nutritional strategies and environment upon milk production, insulin, and progesterone (P4) concentrations.
METHODS
After calving, Holstein cows (n=31) were randomly assigned to different treatments: 1) High environmental control and total mixed ration in compost barn (HEC-TMR), 2) High environmental control and partial mixed ration in compost barn plus grazing (HEC-PMR), and 3) Low environmental control and partial mixed ration in open sky confinement plus grazing (LEC-PMR). At 50 days pos-partum animals were synchronized with synthetic prostaglandin analog and blood was collected daily until day 10 of estrus cycle to determine insulin and P4 plasmatic concentrations by radioimmunoassay.
RESULTS
The nutritional strategies affected milk production (p=0.01) and insulin concentrations (p<0.001): HEC-TMR group presented greater daily milk production and greater insulin concentrations respect to HEC-PMR and LEC-PMR groups. All cows responded to the synchronization protocol as determined by P4 concentrations. Progesterone concentrations tended to be affected by nutritional treatment (p=0.08), and the interaction between days and treatment was significant (p=0.04): HEC-TMR (1.4±0.2 ng/mL) and HEC-PMR (1.3±0.2 ng/mL) presented greater plasmatic concentrations than animals managed in open sky confinement (LEC-PMR; 0.7±0.2 ng/mL).
CONCLUSIONS
The greater milk production in HEC-TMR has been previously reported [1] and is consistent with the higher nutrient density of the diet. The finding that the management – but not the nutritional treatment- affected P4 concentration is novel. As others [2] showed that insulin stimulates P4 synthesis in luteal cells, a greater P4 concentration could be expected in HEC-TMR although the greater milk production could be associated with greater liver flux and P4 clearance [3]. As P4 modulates uterine environment and promotes embryo development, alterations in P4 concentrations are associated with early embryo mortality in dairy cows [4]. Further studies are currently being performed investigating uterine functionality in this experiment to test this hypothesis.

M30
PREGNANCY RATES IN BEEF HEIFERS SYNCHRONIZED WITH ESTRADIOL-BASED PROTOCOL AND INSEMINATED WITH UNSORTED OR SORTED SEMEN.

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BACKGROUND-AIM
The aim of the study was to determine pregnancy rates in beef heifers synchronized with an estradiol-based protocol, named J-Synch, and inseminated with two different concentrations of sexed-sorted semen of 65% purity.
METHODS
The experiment was done in 7 locations using cycling, 18 to 24-month Angus heifers (n=465) in La Pampa, Argentina. On random days (Day 0), all heifers received a progesterone device releasing (Pluselar 0.6 g, Calier Argentina) and 2 mg of estradiol benzoate (Calier Argentina). On Day 6, devices were removed and also received 150 µg D-cloprostenol (PGF, Calier Argentina), 300 IU eCG (Novormon, Zoetis Argentina) and tail-painted for estrus detection before artificial insemination (AI). Heifers with >50% the tail-paint rubbed off by 72 h after device removal were AI at the same time with either non-sexed conventional semen (25 X 10 6 sperm per dose) or sex-sorted semen with 65% purity, with either of two concentrations: 6 X 10 6 or 8 X 10 6 live cells per dose, form one of four Angus Bulls that were collected and processed by ST Genetics Argentina. All heifers not in estrus by 72 h received 0.01 mg buserelin acetate (GnRH, Pluserelina, Calier Argentina) and were inseminated at 80 h with the same three types of semen. Pregnancy rate was diagnosed by ultrasonography (Exago, IMV Imaging) at Day 35. Data were analyzed using general mixed model for a binomial distribution and a logit link.
RESULTS
Expression of estrus was 80.4% and pregnancy rate was greater (P<0.05) in heifers in estrus at the time of AI (62.0%, 232/374) than those not in estrus at 72 h nor 80 h (47.2%, 43/91). Furthermore, pregnancy rate was greater (P<0.01) in heifers AI with non-sexed semen (69.6%, 110/158) than those sexed sorted semen (6 X 10 6: 50%, 76/152 and 8 X 10 6: 57.4%, 89/155, respectively). Finally, fertility among bulls ranged 42.9% to 65.1% (P=0.01), but there was no bull type of semen interaction (P=0.1).
CONCLUSIONS
Although pregnancy rates were greater with non-sorted semen, with the use of sexed sorted semen resulted pregnancy rates were greater than 50%, which may be considered acceptable for commercial use.

M31
EARLY RESYNCHRONIZATION OF OVULATION AND TIMED INSEMINATION IN NON-PREGNANT BEEF CATTLE

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BACKGROUND-AIM
To assess the combination of GnRH and Doppler color to re-inseminate non pregnant beef cows.
METHODS
At d-10, heifers (HE; n=202) and cows (CO; n=125) with a CL or a follicle >10 mm were estrus synchronized with a CIDR insert (750 mg P4) and estradiol benzoate (EB, 2mg, IM). On d-2 the CIDR insert was removed, and animals received PGF2a (PGF; 150 ug Cloprostenol, IM) and estradiol cypionate (EC; 1 mg, IM) and fixed timed insemination (TAI) was performed at d0 (52 h). On d14, animals were randomly assigned to one of two treatments (TRT). Heifers (n=92) and cows (n=61) in TRT1 were resynchronized at d14 with a buserelin (GnRH, 8ug). On d21 (CO) and d22 (HE), a non-pregnancy diagnosis (NPD) was performed with Doppler color ultrasound. Animals with a CL with <25% blood flow were diagnosed as non-pregnant (NPREG) and received PGF, EC, and TAI (52 h). Animals in TRT1 diagnosed NPREG at d21-22 were diagnosed pregnant (PD) at d35 (HE) and d32 (CO) by b-mode US (PD1). In TRT2, on d35, HE (n=109) and on d32 CO (n=64) were PD by US (PD1; B-mode). HE and CO diagnosed open in TRT2 were resynchronized with the same protocol used at the beginning of the study. In both TRT, at d85 (HE), and d75 (CO) were PD by B-mode US (PD2). The percent of non-pregnant animals (NPREG) at d21-22 and the pregnancy rate (PR) at PD1-PD2; and potential pregnancy loss (PPL1; NPD vs. PD1), and PPL2 (PD1 vs. PD2) were analyzed by SAS.
RESULTS
In TRT1, the rate of NPREG was 45.2% (42/93) in HE and 60.6% (37/61) in CO. In HE, the PR at PD1 in TRT1 was similar to TRT2 (39.8 [37/93] vs. 28.4 [31/109], P>0.08); and the PPL1 was 11.9% (5/42). In CO, the PR at PD1 in TRT1 was similar to TRT2 (55.7 [34/61] vs. 68.7 [44/64], P>0.13); and the PPL1 was 8.1% (3/37). Lastly, in HE, the PR at PD2 in TRT1 was higher to TRT2 (76.5 [39/51] vs. 38.5 [30/78], P<0.01); but the PPL2 was similar in both TRT (13.5% [5/37] vs. 3.2% [1/31], P>08). In CO, the PR at PD2 in TRT1 was similar to TRT2 (79.2 [19/24] vs. 70.0 [14/20], P>0.48); and the PPL2 was similar in both TRT (5.9% [2/34] vs. 2.3% [1/44], P>41).
CONCLUSIONS
The combination of GnRH at d14 and doppler US at d21-22 post AI allowed to re-inseminate 13 and 11 d earlier non pregnant HE and CO with similar fertility to the control group

M32
EARLY DETECTION OF NON-PREGNANT DAIRY COWS BY DOPPLER AND B-MODE ULTRASONOGRAPHY

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BACKGROUND-AIM
Early identification of non-pregnant cows after AI improves the reproductive efficiency of dairy herds, reducing the interval between consecutive inseminations. The objectives of this study were to i) evaluate the corpus luteum blood perfusion (CL-BP) by Doppler-US to detect non-pregnant cows at 19-20 d post-AI and ii) assess if uterine echotexture by B-mode US evaluation could add valuable information to improve the detection of non-pregnant cows.
METHODS
A prospective cohort study was performed on a commercial dairy farm in Buenos Aires province, Argentina. After the voluntary waiting period of 45 DIM, cows were synchronized and AI. At 19-20 d post-AI, CL-BP of all study cows (n=131) was examined by Color mode and Power mode of Doppler-US and by using an image processing software. The endometrium thickness and echotexture were evaluated at the bifurcation of uterine horns by B-mode US at the same visit. The agreement between the on-farm CL-BP assessment and the image processing software (ImageJ 1.42q) was determined by kappa coefficient. The CL-BP was considered positive when was scored as ≥2 (scale 0-4) or when the software evaluation indicated >25% of CL-BP. The characterization and concordance among CL-BP Doppler diagnosis and the uterine echostructure at 19-20 d (uterine lumen, stratum vasculare, thickness rate) and the pregnancy status determined by the B-mode US at 33-34 d were assessed by Proc GLIMMIX (SAS 9.4) and by Proc FREQ (SAS 9.4).
RESULTS
The CL-BP measurements analyzed by image processing software showed a higher agreement with Color than Power mode (K= 0.70 vs.0.42). The CL-BP by Color doppler-US using ≤1 score showed high accuracy (74.8 %) and high negative predictive value (98.2%) to detect non-pregnancy by simple visualization. The presence of a thick endometrial layer (endometrium:myometrium, relation 3:1) and the visualization of the uterine stratum vasculare (OR= 2.79 IC 95% 1.31- 5.93 P< 0.01) at 19-20 d were correlated with non-pregnancy diagnosis by Color doppler and B-mode US at 33-34 d (P<0.001).
CONCLUSIONS
Color doppler-US showed to be a good on-farm option to quickly detect non-pregnant cows in order to rebreed them earlier. Nevertheless, we suggest evaluating the stratum vasculare and endometrium thickness as an additional assessment to improve the detection of non-pregnant cows.

M33
ESTIMATION OF EMBRYO LOSSES IN GRAZING DAIRY COWS

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BACKGROUND-AIM
In dairy cows, pregnancy losses have a high impact on the farm's reproductive and economic performance. Little is known about embryo mortality estimations in grazing dairy cows. The objective was to assess the proportion of potential pregnancy losses between 19 to 34 d post-AI.
METHODS
A prospective cohort study was performed on a commercial dairy farm in Buenos Aires province, Argentina. After the voluntary waiting period (45 DIM), cows were synchronized and AI. A total of 59 healthy Holstein cows were enrolled. At 19-20 d post-AI, the ovaries were examined by Color mode of Doppler-US (ESAOTE MyLab OneVET, Genova, Italy) to evaluate the corpus luteum (CL), considering the amount of colored area within the luteal tissue as an indicator of CL functionality. The CL blood perfusion (CL-BP) was deemed to be positive when was scored as ≥2 (>25% of CL-BP, scale 0-4). Blood samples were also obtained for progesterone (P4) measurement by chemiluminescence and to determine the mRNA expression of interferon-stimulating genes (ISG) in leukocytes by real-time PCR. Pregnancy diagnosis based on embryo visualization was performed at 33-34 d post-AI by B-mode US. ROC analyses were used to determine critical cutoff values for the gene expression that provided evidence of an embryo's presence (Proc LOGISTIC, SAS 9.4). To improve predictive capacity, the genes selected as biomarkers (ISG15, MX2 and OAS1; AUC>80%) were used in several combined ways (parallel and series tests; Proc FREQ, SAS 9.4).
RESULTS
In parallel interpretation, ISG15 and MX2 mRNA expression (cutoff values 0.22 and 3.70, respectively) were found as suitable biomarkers for early pregnancy (100% of SE and 70.5% of SP). At 19-20 d post-AI, 50.8% (30/59) of the cows were considered possible pregnant (positives to ISG, positives to CL-BP and with P4 ≥1.0 ng/mL). From them, 30% (9/30) were non-pregnant at 33-34 d and classified as probable embryo mortality, EM (pregnancy rate 38.9%; 23/59). Potential pregnancy loss was calculated as cows classified as EM (9) divided by the cows pregnant at 33-34 d (23) plus EM cows (9), resulting 28.1%.
CONCLUSIONS
We found that 28.1% of the pregnant cows could have lost their pregnancy between 19 to 34 d post-AI. Further research is needed to confirm these results with a larger number of cows and farms.

M34
CAUSES OF PREGNANCY LOSSES BETWEEN 30 AND 210 DAYS OF GESTATION IN GRAZING DAIRY COWS

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BACKGROUND-AIM
The objective of this study was to assess the risk of pregnancy losses (PL) in grazing dairy cows between 30 and 210 days (d) of gestation in a large commercial dairy farm in Argentina.
METHODS
A retrospective study including 24,698 records of 1st, 2nd, and 3rd pregnancies within the same lactation from 1st, 2nd, 3rd or ≥4th lactating cows (LACT) calving during 2010-2018 was used. Pregnancy diagnosis (PD) was performed every two weeks between 30-44 d post artificial insemination (AI). A total of 6,548 PL were classified as: 1) cows that had a dead embryo at PD with ultrasonography 30-60 d after AI, 2) PL after PD detected by visual observation, 3) cows that returned to estrus and were diagnosed not pregnant at the subsequent examination after detected in heat, and 4) cows diagnosed as pregnant and returned to estrus 30 d after PD and were inseminated. Cow health events (HE) were classified as uterine diseases (UDIS, diagnosed before the end of voluntary waiting period (VWP); retained placenta, metritis, endometritis, twins, stillbirth), nonuterine diseases (NUDIS, diagnosed in a window of ±90 d of AI that became pregnant; mastitis, lameness), both (BOTH) and healthy (HTH). Additional records included days in milk (DIM) to PL, test-day milk yield (TD) at AI, number of AI per pregnancy (NAI), season (SEA) of pregnancy. Cox hazard models were fit in STATA that included HE, LACT number, NAI number, SEA, TD and DIM. Significance was set at P≤0.05.
RESULTS
Prevalence of PL during the study was 26.5%. From all PL, 80% were 1st, 17% were2nd, and 3% were 3rd pregnancies. Median days of gestation to 1st, 2nd, and 3rd PL were 79, 96 and 118 d, respectively. The risk of PL was different between HE (HTH base level [BL]-UDIS HR=1.12, p<0.01, NUDIS HR=1.02, p>0.05, BOTH HR=1.06 0.98-1.14 p>0.05); NLACT (1 BL; 2 HR=1.15, p<0.001; 3 HR=1.24, p<0.001; 4 HR=1.33, p<0.001); NAI (1 BL; 2 HR=1.29, p<0.001; 3 HR=2.49, p<0.001); SEA (SU BL; FA HR=0.91, p<0.05; WI HR=0.84, p<0.001; SP HR=0.93, p>0.05); TD HR=0.98, p<0.001; and DIM HR=0.98, p<0.001).
CONCLUSIONS
In conclusion, management practice that improve postpartum uterine health may reduce pregnancy losses, but high milk production at time of AI does not affect pregnancy losses.

M35
FACTORS ASSOCIATED WITH EMBRYO FERTILIZATION AND QUALITY IN DAIRY COWS

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BACKGROUND-AIM
This study aimed to evaluate parameters that affected fertilization and embryo development up to the morula stage.
METHODS
Artificial insemination (AI) were performed on cows between 46 and 60 days in milk, following a synchronization protocol (progesterone (P4) supplemented presynchronization followed by Ovsynch), and flushed for embryo collection 5 or 6 days later, according to each study protocol. The recovered structures were graded (1 = excellent, 2 = fair, 3 = poor, 4 = degenerated, and 5 = not fertilized). Parity, body condition score on the day of AI, estrus cycle during presynchronization, size of the ovulatory follicle, circulating hormone concentrations before and on the day of AI, and the number of accessory spermatozoa were recorded and assessed as potential risk factors. Odds ratio (OR) and 95% confidence intervals (CI) of grading were obtained using cumulative link mixed models, including confounders identified using a directed acyclic graph.
RESULTS
A total of 418 structures (embryos and oocytes) from 389 lactating Holstein cows (34% primiparous and 66% multiparous) were recovered. Recovered structures were excellent or good quality embryos (35%), fair quality embryos (21%), poor quality embryos (11%), degenerated embryos (16%), and not fertilized oocytes (17%). Structures from cows with P4 ≥ 0.5 ng/mL at insemination were more likely to be oocytes or embryos of lower quality than structures from cows with P4 < 0.5 ng/mL (OR = 1.96, 95% CI = 1.20; 3.22). Structures from multiparous cows were less likely to be oocytes or embryos of lower quality than structures from primiparous cows (OR = 0.66, 95% CI = 0.45; 0.95). Finally, structures with > 7 accessory spermatozoa were less likely to be oocytes or embryos of lower quality than structures with ≤ 7 accessory spermatozoa (OR = 0.41, 95% CI = 0.28; 0.60).
CONCLUSIONS
The results of this analysis highlight the importance of low P4 concentration on the day of insemination for the fertilization and development of dairy cow embryos. This highlights the necessity of a complete regression of the corpus luteum. Additionally, the number of accessory spermatozoa was associated with better fertilization and embryo quality, suggesting the mechanisms surrounding uterine motility are also important.

M36
DEVELOPMENT OF AN EFFICIENT AND EFFECTIVE PROTOCOL FOR THE ISOLATION AND CULTURE OF BOVINE PRIMORDIAL FOLLICLES

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BACKGROUND-AIM
The ability to grow undifferentiated oocytes in vitro from primordial follicles (PMF) would increase the supply of fully grown oocytes, to be destined to downstream applications in the livestock industry and fertility preservation programs. To date, the production of living offspring using in vitro development of oocytes from the PMF reserve has only been achieved in mice, providing the proof of principle of the potential value of follicle culture as a source of fully grown oocytes. However, culture systems to produce mature oocytes from PMF are still experimental.
One of the main limiting factor in PMFs in vitro culture's efficiency is follicle death occurring shortly after isolation from surrounding tissue. Therefore, we hypothesize that counteracting the cell-death signaling network(s) triggered upon isolation from the surrounding ovarian cortex should improve the outcome of PMF in vitro culture. To pursue this objective, we started by developing a reliable and efficient protocol for the isolation of viable PMF from the bovine ovarian cortex.
METHODS
Fragments of ovarian cortex of about 2 cm2 and 1 mm thick removed from slaughter-derived heifers (14-22 months) and adult cows (48-60 months) were chopped up into small fragments with a blade, dispersed in manipulation medium and homogenized. The resultant homogenate was then passed through a serial sieves system up to the lower limit of 30 µm. In the first set of experiments, homogenization speed and time were optimized to reduce follicle damage and foam formation. The second set of experiments was conducted to evaluate the viability after 16 hrs of culture by live/death staining and TUNEL and Caspases 3/7 assay.
RESULTS
The obtained results indicate that the number of follicles isolated from 2 cm2 and 1 mm thick ovarian cortex is inversely related to the age (mean of 100 vs. 40 from heifers and adult cows, respectively). Moreover, PMFs revealed a 30% decline in viability after culture compared to the freshly isolated ones. Finally, live/death staining showed two different patterns, suggestive of different modes of PMF cell death.
CONCLUSIONS
Our protocol can allow isolation of a high number of PMF per animal, higher than previously reported, and poses the basis for a morphological and functional characterization of isolated PMFs.

M37
EVALUATION OF THE IN VITRO CULTURE OF BOVINE OOCYTES (BOS TAURUS L.) USING THE HANGING DROP TECHNIQUE ON EMBRYONIC DEVELOPMENT

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BACKGROUND-AIM
The aim of this research was to evaluate the in vitro culture of bovine oocytes (Bos taurus L.) using the Hanging Drop technique on embryonic development
METHODS
Holstein cow's ovaries were used, obtained from a slaughterhouse and transported within 4hrs of slaughter. The oocyte cumulus complex was obtained by aspiration of small antral follicles (2-8mm) with 18-gauge needle. After 15 mins, the supernatant was discarded and sediment diluted in TCM199 with 0.2mM Sodium Pyruvate, 4.2mM NaHCO3, 2.64g/ L HEPES, 50mg/ml gentamicin for selection. Oocytes with multilayered compact cumulus cells and evenly granulated cytoplasm were selected. The COCs were divided into two groups: for group 1, 10 COCs were placed per 40ul drops of medium in petri dishes and covered by mineral oil. Group 2 used the Hanging Drop technique, consisted of placing 10 COCs in suspended drops of 40ul medium in petri dish. For both groups, TCM199 with 20mg/ml FSH, 1mg/ml 17b estradiol, 10% FBS, 50mg/ml gentamicin, 2.2mg/ml sodium pyruvate, 0.22g/l NaHCO3 was used, incubated at 38.5°C, 5% CO2, 99% humidity for 24hrs. Straws of frozen semen from a bull certified were used. Fertilization medium was HTF® with 0.01mg/ml sodium heparin, 20mM caffeine, 6mg/ml BSA. The thawed sperm was washed with fertilization medium at 500g for 5 minutes. The final concentration was adjusted to 1x106 sperm/ml. Aliquots of 15ul sperm suspension were used per well, incubating at 38.5°C, 5% CO2, 99% humidity for 18 hrs. After 18hrs oocytes were washed with HTF-HEPES® and remaining cumulus and sperm cells removed by mechanical pipetting until oocytes were denuded. The presumptive zygotes were placed in G-TL® and culture plates were placed in hermetic bags and kept in the mixture: 5% CO2, 5% O2 and 90% N2 at 38.5°C and 99% humidity for 7 days.
RESULTS
The division rate of group 2 was 67.08%, while for group 1 it was 59.82%. An average percentage of morulas and blastocysts of 39.24% and 27% was obtained for the group 2 compared to 34.37% and 21.42% of group 1
CONCLUSIONS
There's a difference in the rate of division, in obtaining morulas, and blastocysts with group 2 respect to group 1. However, there's no significant difference in embryonic development patterns with Hanging Drop treatment even when there's a percentage difference ($p < 0.05$).

M38
DEVELOPMENT OF AN IN VITRO MODEL TO STUDY THE EXPOSURE TO NUTRIENT IMBALANCE IN FEMALE BLASTOCYSTS

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BACKGROUND-AIM
According to the Developmental Origins of Health and Disease (DOHaD), an imbalanced maternal diet has long-term effects on the offspring's health and epigenetic events are involved. Major epigenetic remodeling, such as DNA de-methylation/re-methylation waves, occurs during preimplantation embryo development in a sex-specific manner. Here we describe the set up of experimental conditions to investigate the impact of energetic imbalance on the epigenetic remodeling of bovine embryos. We conducted experiments aimed at: 1) reducing the sex-related variability in DNA methylation patterns by using X-sorted semen for in vitro fertilization (IVF); 2) defining a serum-free culture medium to manipulate the concentration of energetic substrates without affecting the overall medium composition.
METHODS
COCs were recovered from 2-8 mm follicles of abattoir-derived bovine ovaries, in vitro matured, fertilized and the presumptive zygotes were fixed or cultured according to the experimental design. Embryos were cultured in synthetic oviductal fluid (SOF) with 5% serum or bovine serum albumin (BSA) plus glucose. Two discontinuous Percoll gradients, G1: 90%-45% and G2: 78.7%-67.5%, were compared to isolate of motile sperm from commercial X-sorted semen. Experimental endpoints were: spermatozoa concentration, amount, motility, and pronuclei (PN) formation. Finally, different IVF timing were tested, from 10 to 18 hours. Data were analyzed with Fisher's exact test or T-test. $P<0.05$ was considered statistically significant.
RESULTS
Replacing serum with BSA plus glucose did not affect blastocyst yield (43±7.56 and 35±9.51%, respectively, N=6). G1 and G2 sperm concentration, amount, and motility were similar. However PN formation was higher for G2 (G1: 9/34 (26%), G2: 27/31 (87%); $p<0.0001$). Reducing the IVF timing to 10 h improved the blastocyst rate, although not significantly (10 h: 12/46 (26%); 14 h: 5/46 (11%); 18 h: 5/47 (11%), $P=0.06$).
CONCLUSIONS
We reached the goal of successfully producing in vitro female bovine embryos with a serum-free medium, needed for investigating the effects of energetic imbalance during preimplantation embryo development. The limited span of IVF might prove crucial to improving the developmental competence of embryos fertilized with X-sorted semen.

M39

INFLUENCE OF INTRAFOLLICULAR TRANSFER OF IMMATURE OOCYTES (IFIOT) ON OVULATION OF THE INJECTED FOLLICLE

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BACKGROUND-AIM
The intrafollicular transfer of immature oocytes (IFIOT) surge as a new alternative to produce bovine embryos. As a developing technique, IFIOT still has some limiting factors, which reduce its efficient. Among them, the low recovery of the injected structures is the most relevant. Therefore, this study aimed to evaluated whether the injection into a preovulatory follicle would affects its ovulation.
METHODS
To do that we monitored the time of ovulation and size of the follicle of thirty-eight Nellore (Bos Taurus indicus) heifers. All females received a progesterone device (P4) and an application of estradiol benzoate (2mg; i.m.) on the first day (D0). On D8, P4 device was removed and 0.5 mg of Cloprostenol was administered (i.m). Twenty-four hours later (D9), ovulation was induced with 25µg of lecirelina (GnRH). Six hours after ovulation induction (D9.5), the animals were distributed into three groups: Control, Injection and IFIOT. Control group did not receive any treatment; Injection, animals were submitted to IFIOT but only with 60µl of Follicular Fluid (FF); and IFIOT, they were injected with 25 immature oocytes in 60µl of FF. All animals were evaluated by ultrasonography every 6 hours, for the first 12 hours from the injection, after that, every 4 hours until the moment of ovulation. Data were analyzed by ANOVA ($P > 0.05$).
RESULTS
The injection did not affect ($P > 0.05$) ovulation time, since mean ovulation time was 30 hours after induction in the all groups. However, when size of follicle was compared among groups, we observed a smaller diameter ($P < 0.05$) of the preovulatory follicle in the Injection and IFIOT groups, (9.7mm ± 2.1ab and 8.7mm ± 1.2b, respectively) when compare to the Control group (10.7mm± 1.7a).
CONCLUSIONS
Possibly, a lower recovery of structures and embryos, in IFIOT, is due to a greater decrease in the preovulatory follicle close to ovulation, something inherent to the technique that needs to be improved.
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M41

THE USE OF ECG DILUTED IN D-CLOPROSTENOL REDUCES THE NUMBER OF INJECTIONS REQUIRED FOR TAI PROTOCOLS IN POSTPARTUM BOS INDICUS BEEF COWS WITH SAME PREGNANCY EFFICIENCY AS THE CONVENTION TREATMENT

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BACKGROUND-AIM
Using eCG in TAI protocols is an essential strategy to improve P/AI in females with low body condition score (BCS), short postpartum period, and in anestrous. We assessed if the dilution of eCG in PGF would enable administration of both drugs in a single injection, reducing the number of shots to accomplish TAI protocols with same reproduction efficiency.
METHODS
The study was done in 2020 and assigned 642 Bos indicus (Nelore) cows (346 primiparous, 296 multiparous) with 2.60±0.03 BCS and ranging 30-60d postpartum from 3 farms in MT State, Brazil. Cows were kept in pasture. At the onset of the protocol (D0) they received an intravaginal device with 0.5g P4 (Repro one, Globalgen) and 2mg estradiol benzoate (Bioestrogen, Biogénesis Bagó) IM. On D8, device was removed and 1mg estradiol cypionate (Croni-Cip, Biogénesis Bagó) was given IM. At that time, cows were randomly allocated in 3 groups. Cows in Control Group (CG) received only 150µg D-cloprostenol (PGF; Croniben, Biogénesis Bagó) IM. Cows in Traditional Group (Trad) were treated with 150µg PGF and 300IU eCG (Ecegon, Biogénesis Bagó) IM, given in two injections apart. Cows in Group Combined eCG+PGF received eCG diluted in PGF and given in a single injection. The eCG+PGF was set by diluting 3 vials of lyophilized eCG (total 15,000IU) in 100mL PGF and the dose used was 2mL/cow (equivalent to 300IU eCG+150µg PGF). TAI was done 48h after device removal. Pregnancy was checked 30d after TAI. The occurrence of estrus was evaluated, and the diameter of the dominant follicle (DDF) was measured on D8 and 10 in the ovaries of all cows to access follicular growth rate (FGR). Data was analyzed with SAS.
RESULTS
The DDF on D8 was similar for groups (CG:10.3±0.13; Trad:10.2±0.13; eCG+PGF:10.5±0.15; P=0.73), yet, it was greater on D10 in cows receiving eCG (CG:11.7±0.16b; Trad:12.4±0.12a; eCG+PGF:12.5±0.15a; P<0.0001) with greater FGR in those cows (CG:0.7±0.04b; Trad:1.1±0.05a; eCG+PGF:1.0±0.04amm/d; P=0.005). eCG treated cows also had greater estrus rate (CG:56.2%b; Trad:66.7%a; eCG+PGF:66.7%a; P=0.05) and P/AI (CG:50,5%b; Trad:66.7%a; eCG+PGF:66.2%a; P=0.0005), regardless of form of administration.
CONCLUSIONS
In Conclusion, eCG diluted in PGF was efficient in TAI protocols of B. indicus cows, reducing the number of injection and keeping pregnancy outcomes.

M42
MATERNAL EXPOSURE TO HIGH ENVIRONMENTAL TEMPERATURES IN EARLY PREGNANCY IS NOT ASSOCIATED WITH THE DURATION OF PRODUCTIVE LIFE OF THE OFFSPRING

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BACKGROUND-AIM
Evidence indicates that the total number of healthy follicles and oocytes (ovarian reserve) is positively associated with fertility in dairy cattle and that individuals with low serum anti-Müllerian hormone concentrations (AMH) as heifers have a shorter productive life compared to age-matched herdmates with high AMH serum concentrations. Heifers born to mothers exposed to high environmental temperatures in early gestation had smaller ovarian reserve compared to herd-mates conceived in winter. We hypothesized that dairy heifers conceived in winter had a longer productive life (from first calving to culling), greater age at culling and parity compared to heifers conceived in summer.
METHODS
To estimate the size of the ovarian reserve, peripheral AMH concentrations were previously evaluated in Holstein Friesian heifers (n=69; 16.5±1.1 mo. of age; mean±SD) born between February and January 2016 in a commercial dairy farm. Based on the season from conception to the end of the first trimester of their fetal life, heifers were placed into 2 groups: heifers born to mothers that spent the first trimester of pregnancy during summer or winter (Succu et al, 2020). Dates at first calving, culling, and parity were retrieved from farm records 4.8 years after the birth of each heifer. Data were analyzed with MiniTab. Differences between groups in culling rate were assessed with chi-squared test; AMH, parity, productive life were analyzed with ANOVA and Tukey test. Results are expressed as mean±SD.
RESULTS
Mean circulating AMH concentrations at 16 mo. of age were 384.2±310.4 pg/ml in heifers in the summer group (n=31) and 552.7±654.8 in the winter group (n=38; P=0.19). Within 4.8 years after birth, culling rate and age at culling were similar between groups (summer 39%, 50.0±6.8 mo.; winter 39%, 49.8±7.5). No difference was detected in productive life between groups (summer 28.8±6.4; winter 28.3±6.6), but parity was greater in summer compared to winter heifers (summer 2.9±0.9; winter 2.6±0.7; P=0.05).
CONCLUSIONS
Maternal exposure to high temperatures during the first trimester of gestation may impair the size of the ovarian reserve, but does not influence the length of productive life in offspring.
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M43
THE COMPARISON OF CPAG AND BPAG CONCENTRATIONS IN MILK OF DAIRY COWS USING RIA METHOD

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BACKGROUND-AIM
Pregnancy-associated glycoproteins (PAG) are secreted by the binucleate giant cells of the ruminant placenta and then enter maternal circulation. Their presence in maternal serum has long been recognized since 22. day post conception. Pregnancy diagnosis based on concentration of this glycoproteins is currently being considered by many research groups. By using biochemical procedures, some molecules of the PAG family were isolated from cotyledons of cow, ewe, goat, buffalo, bison, moose and elk. In cow's serum and milk two kinds of PAGs may be found: bovine (bPAG) and caprine (cPAG). PAGs levels may be assessed also in milk, usually in lower concentrations, however collection is much easier than in blood and may be performed during usual milking. Purified and semi-purified preparations were used to immunize rabbits and the antisera obtained allowed the development of homologous and heterologous radioimmunoassay (RIA) for PAGs levels determination.
METHODS
76 milk samples taken from cows in different stage of gestation were analyzed. All samples were tested by the RIAII and RIAIII method using specific antibodies (RIA II for bPAG67 and RIA III for cPAG55+62). RIAIII is characterized by the highest sensitivity towards the PAG molecules secreted by the placenta during the first weeks of pregnancy. A modification of the method RIAIII developed for the testing of milk with larger volumes of the test material was used.
RESULTS
Both bPAG and cPAG levels in milk are increasing in association to the length of pregnancy. Correlation of pregnancy length and cPAG ($r=0.31$; $p<0.05$) was slightly higher than between length of pregnancy and bPAG ($r=0.25$; $p<0.05$). Mean concentrations of bPAG ranged from 0.06 ng/ml in day 42, 0.21 ng/ml in day 119, 1.29 ng/ml in day 168 to 4.84 ng/ml in day 201. At the same time mean concentrations of cPAG increased in a similar way from 0.68 ng/ml, 1.02 ng/ ml, 1.58 ng/ml to 2.07 ng/ml, respectively. Strong correlations between bPAG and cPAG concentrations were found in all crucial days ($r\#<0.80$; $0.86>$; $p<0.05$).
CONCLUSIONS
It is concluded, that for proper evaluation of PAGs levels in milk, RIA test not only for bPAG, but also for cPAG should be performed, in order to increase sensitivity of milk testing, in order to increase sensitivity of test, if milk samples are used.

M44
NEW OOCYTE IN VITRO CULTURE STRATEGIES TO ENHANCE THE OUTCOME OF ASSISTED REPRODUCTIVE TECHNOLOGIES

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BACKGROUND-AIM
In cattle, early antral ovarian follicles (EAF;0.5-2 mm in diameter) typically contain growing oocytes, most of which present filamentous chromatin within the germinal vesicle (GV), are still transcriptionally active and have not acquired meiotic competence. This limits the exploitation of the follicle population for assisted reproduction purposes. Our study is aimed at improving the outcome of in vitro culture of growing oocytes (IVCO) to increase the number of exploitable oocytes for embryo production.
METHODS
Based on previous proof-of-principle studies, a culture system based on physiological approach has been developed to allow growing oocytes to become competent for embryonic development. The efficiency of this system ("Long IVCO", L-IVCO) was assessed by evaluating oocyte diameter and chromatin remodeling process after L-IVCO, and cumulus oophorus expansion and capability to reach Metaphase II (MII) after in vitro maturation (IVM). Finally, blastocyst rate and cell number were assessed as embryonic development capacity parameters. Data were analyzed by Student's t-test or ANOVA followed by Holm-Sidak post-hoc test, where appropriate. Significance was set at $p<0.05$.
RESULTS
Culturing cumulus-oocyte complexes (COCs) for 5 days in L-IVCO significantly stimulated oocyte growth (increased oocyte diameter) and improved the proportion of oocytes with advanced stages of chromatin compaction within the GV. Accordingly, L-IVCO promoted a significant increase of both the percentage of COCs showing full cumulus expansion and the percentage of oocytes reaching MII stage after IVM. Finally, COCs cultured in L-IVCO prior to standard in vitro embryo production (IVP) acquired competence to reach the blastocyst stage, while the same population submitted directly to IVP could not reach this stage. Compared to control (fully-grown oocytes), no differences were observed in terms of blastocyst quality (cell number, expanded and hatched blastocyst).
CONCLUSIONS
L-IVCO expands the exploitation of female reproductive potential by allowing the utilization of gametes isolated from EAF and provides a valuable model to study cellular and molecular processes regulating acquisition of meiotic and developmental competence during oocyte growth.Funded by Regione Lombardia PSR INNOVA n.201801061529 and UNIMI n.PSR2019_DIP_027_ALUCI_01

M45
EFFICIENCY OF LACTATION INDUCTION PROTOCOL WITH REDUCED DOSES AND TREATMENTS IN HOLSTEIN COWS

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BACKGROUND-AIM
The aim of the present study was to evaluate reduced doses of progesterone (P4; 300mg per day vs. 450mg each three days), dexamethasone (DEX; 40mg per day vs. 20mg per day) and the change of estradiol benzoate (EB; 30mg per day) to estradiol cypionate (EC; 10 mg each three days) at the induction lactation protocol on success of protocol and milk production in Holstein cows.
METHODS
Twenty-six non lactating and non-pregnant Holstein cows, from two farms were assigned in one of the treatments according to BCS (3.58 ± 0.08), age (7.1 ± 1.1 years) and weight (498.1 ± 20.4 kg): 1) Conventional group (n=13): Cows were induced into lactation with daily i.m injections of P4 (300mg per day; Sincrogest Injetável ® Ourofino Saúde Animal, Brazil) and EB (30 mg per day; Sincrodiol® Ourofino Saúde Animal) on experimental d 1 to 8; EB (20 mg per day) on d 9 to 15; sodium cloprostenol (0,530 mg; PGF2a; Sincrocio® Ourofino Saúde Animal) on d 16; DEX (40mg per day; Cortiflan® Ourofino Saúde Animal) on d 19, 20, 21. They also received bovine somatotropin (bST; 500mg; Boostin® MSD, Brazil) on d 1, 8, 15 and 21. 2) Reduced group (n=13): Cows were induced into lactation with i.m injections each three days of P4 (450mg each three days) and EC (10 mg each three days; Sincrocp® Ourofino Saúde Animal) on experimental d 1, 4, 7 and 10; EC (5mg each three days) on d 13 and 16; PGF2a (0,530 mg) on d 16; DEX (20 mg per day) on d 20, 21, 22. They also received bST (500mg) on d 1, 10, 16 and 22. Milking began on experimental d 22 and milk production was evaluated during until 60 DIM. Statistical analysis was performed by GLIMMIX procedure of SAS.
RESULTS
Both groups efficiently induced lactation [1: 84.6% (11/13) vs. 2: 76.9% (10/13); P=0.97]. There was no difference on milk production (P=0.94) between groups (1: 16.23 kg/d vs. 2: 16.03 kg/d). Also, no interaction between group and time (P= 0.81) was observed.
CONCLUSIONS
In conclusion, both lactation induction protocols induced Holstein cows into lactation.

M46
LACTATION OF BEEF COWS DURING PREGNANCY AFFECTS OFFSPRING'S FOLLICULAR RESERVE

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BACKGROUND-AIM
Maternal environment plays a crucial role in programming fetuses for their future reproductive performance, however, the effect of lactation during pregnancy on fetal programming in cows has not been evaluated yet.
METHODS
The objective of this study was to evaluate the effect of the lactation of the mothers on F1 offspring Bos taurus heifers' follicular reserve. The experiment was performed on 87 Angus heifers, in which their mothers were (Lactating group, n = 38) or were not subjected to lactation (Non-lactating group, n = 49) during early gestation. Their mothers, being Bos taurus beef multiparous cows of 4-5 years old with 60 to 75 days postpartum, were pregnant by natural mating with bulls in a 40-day length period. Day 0 of the experiment was defined as the beginning of mating period, and at this moment cows in Non-lactating group were weaned, while cows of Lactating group remained with their calves until Day 114 (i.e., 74-114 days of pregnancy). The F1 female offspring was evaluated at 13-14-month-old. Number of heifers that reached puberty was determined by the presence of corpus luteum. Antral follicular count (follicles ≥ 2 mm) and number of small follicles (≤ 5 mm) were determined by transrectal ultrasonography (Estoate MyLab, 10MHz). Results were analysed by LGMM.
RESULTS
The increase (mean ± SEM) in body weight and body condition score (BCS, 1-8 scale) from Day 0 to Day 114 was lower in Lactating (19.3 ± 2.3 kg and 0.7 ± 0.1 BCS) than in Non-lactating cows (61.4 ± 1.6 kg and 1.2 ± 0.1 BCS) ($P < 0.05$). Body weight at 13-14-month-old showed no difference between experimental groups (308.4 ± 4.7 kg vs. 310.3 ± 3.1 kg for Lactating and Non-lactating group, respectively) (P = NS). Proportion of heifers with corpus luteum at time of evaluation was not different for Lactating and Non-lactating group, 71% (27/38) vs. 71% (35/49), respectively (P = NS). Antral follicular count and number of small follicles was lower in in those heifers that were born from Lactating than from Non-lactating cows (14.7 ± 1.1 vs. 18.1 ± 0.9 and 12.3 ± 1.1 vs. 15.4 ± 0.9, respectively) ($P < 0.05$).
CONCLUSIONS
In conclusion, we suggest that lactation of the mothers during early gestation affects Bos taurus beef cattle offspring's follicular reserve.

M47
EFFECT OF PG600 DOSE ON ESTROUS EXPRESSION, CORPUS LUTEUM FUNCTION AND PREGNANCY IN BEEF COWS SUBMITTED TO FIXED TIME ARTIFICIAL INSEMINATION

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BACKGROUND-AIM
Equine chorionic gonadotropin (eCG) administered at time of progesterone (P4) withdrawal during a fixed time artificial insemination (FTAI) protocol increases fertility as a result of FTAI in beef cattle. In the USA eCG as a single component is not commercially available, however, the swine industry utilizes PG600 which contains both eCG and human chorionic gonadotropin (hCG). The objective of this study was to assess effects of PG600 dose on estrous expression, corpus luteum (CL) function and fertility in beef cows.
METHODS
Non-lactating cows (n=197) were randomly assigned to one the following treatments: Control (untreated); 2.5 mL PG600 (200 IU eCG + 100 IU hCG); 4 mL PG600 (320 IU eCG + 160 IU hCG); 5 mL PG600 (400 IU eCG + 200 IU hCG). On d-9 all cows were administered an intravaginal P4 device (CIDR) and 100 µg of gonadorelin (GnRH). Six days later (d-3) CIDRs were removed concurrently with administration of 1000 µg of cloprostenol sodium, an estrous detection patch and the assigned treatment. Cows with an activated patch 48 h after CIDR removal were inseminated at 60 h (d0) while remaining cows were inseminated at 72 h. All cows were administered GnRH at the time of AI. A subset of cows (n=86) was examined using ultrasonography on d7 to assess CL and blood samples were collected to quantify P4. Pregnancy was assessed using ultrasonography on d30. Data were analyzed using generalized linear mixed models.
RESULTS
A lesser ($P<0.05$) percentage of cows administered 4 (47.9%; 23/48) and 5 (40.4%; 19/47) mL of PG600 expressed estrus compared to Control cows (76.5%; 39/51) and those administered 2.5 mL of PG600 (71.4%; 35/49). There were no differences ($P>0.3$) between treatment groups in percentage of cows with multiple CL (13.9%; 12/86), total luteal tissue volume (4667 ± 282 mm3) nor circulating P4 (3.2 ± 0.2 ng/ml) on d7. Cows in the Control group had greater ($P<0.05$) pregnancies per AI (71.4%; 35/49) than cows treated with 4 (43.7%; 21/48) and 5 (36.2%; 17/47) mL PG600, while cows treated with 2.5 mL were intermediate (58.8%; 30/51).
CONCLUSIONS
In conclusion, the combination treatment with eCG and hCG at time of P4 device withdrawal did not affect CL function and failed to improve fertility to FTAI. Furthermore, administration of PG600 resulted in a dose dependent decrease in fertility.

M48
DEVELOPMENT OF LUTEAL TISSUE FOLLOWING FOLLICULAR DRAINAGE OF SUBORDINATE FOLLICLES FOR TWIN PREGNANCY PREVENTION IN BI-OVULAR DAIRY COWS

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BACKGROUND-AIM
Twin pregnancies are undesirable in dairy cattle because their effects on the herd economy. A follicular transvaginal drainage technique without ultrasound was recently validated for follicular drainage of subordinate follicles with no suction at artificial insemination (AI) for twin pregnancy prevention in bi-ovular cows. All cows showed as a luteal structure in the drained ovary. The objective of this study was to evaluate size and development of the induced luteal structures after follicular drainage at AI in bi-ovular dairy cows.
METHODS
Bi-ovular cows (a pre-ovulatory follicle over 10 mm in each ovary) diagnosed by ultrasonography were selected from cows synchronized for fixed-time AI. The subordinate (smaller) follicle of all cows was punctured and drained with a steel transvaginal cannula designed for follicular cyst puncture (Minitub Ibérica S.L., Spain). Cows were then AI and received hCG (3000 IU hCG i.m;Veterin Corion 750 UI/ml, Divasa-Farmavic, Spain). Corpus luteum (CL) size in ovulating ovaries and the luteal structure as a CL in drained ovaries were recorded seven days post-AI as the mean of the greatest and lowest measurements. Pregnancy diagnosis was performed by ultrasound at 28 days post-AI. Luteal structures were also recorded.
RESULTS
All cows (n=30) showed a CL in the drained ovary seven days after follicular drainage, whereas the corresponding dominant follicle failed to ovulate in 5 (16.6%) cows. The mean size of CL was 10.2±4.0 and 22.5±5.1 cm for the drained and ovulating ovary, respectively (P<0.0001). None of the 12 pregnant cows (40%) showed the induced by drainage CL at pregnancy diagnosis.
CONCLUSIONS
induced CL coming from follicular drainage were significantly smaller than CL derived from ovulation. The induced CL did not last until pregnancy diagnosis.

M49
LYCOPENE SUPPLEMENTATION IN SERUM-FREE EMBRYO CULTURE MEDIUM AND ITS EFFECT ON DEVELOPMENT AND QUALITY OF BOVINE BLASTOCYSTS PRODUCED IN VITRO

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BACKGROUND-AIM
Reactive oxygen species (ROS) production is a physiological, dynamic process in all cells. However, ROS production is exacerbated during in vitro conditions mainly due to its high oxygen tension. This study aimed to evaluate the effect of lycopene supplementation into a serum-free culture medium on blastocyst development and quality.
METHODS
Ovaries were gathered from a local abattoir, and collected cumulus-oocyte complexes were matured and fertilized in groups of 60 over 5 replicates. After fertilization, presumed zygotes (n = 967) were cultured in 50 µL droplets of synthetic oviductal fluid (covered with 900 µL parafilm oil). Culture medium supplementation was done forming four experimental groups: insulin, transferrin, selenium (ITS; control), ITS + DMSO (diluent control), ITS + DMSO-Lycopene 0.1 µM (ITSL), and IT + DMSO-Lycopene 0.1 µM (ITL). DMSO was used as a diluent for lycopene, which is a water-insoluble carotenoid antioxidant. Development (cleavage and day 8 blastocyst rates) among experimental groups were fitted in mixed-effects models, and blastocyst quality parameters (assessed via differential apoptotic staining) were evaluated in mixed linear regression models.
RESULTS
The cleavage (85.3 ± 0.2, 82.6 ± 0.2, 86 ± 0.2, and 86.4 ± 0.2% for control, diluent control, ITSL, and ITL, respectively) and day 8 blastocyst rates (37.4 ± 0.3, 36.9 ± 0.3, 39.7 ± 0.3, 46.2 ± 0.3% for control, diluent control, ITSL, and ITL, respectively) were not different (P < 0.1) among experimental groups. Embryos produced in the ITL group resulted in bigger blastocysts with higher total cell numbers (TCN; 141 ±19.2), inner cell mass (ICM; 65.3 ±11.6), and trophectoderm cells (TE; 75.2 ±8.8) compared to the control (129 ± 19.2, 56.3 ± 11.6, 72.7 ± 8.8, for TCN, ICM, and TE; P > 0.01, respectively). Lycopene supplemented groups (ITSL and ITL) resulted in blastocyst with similar TCN, ICM, and TE (P < 0.2). The number of apoptotic cells was not different among experimental groups (P < 0.1).
CONCLUSIONS
Lycopene supplementation to the culture medium did not increase the blastocyst development, but replacing selenium with lycopene in a serum-free culture medium resulted in larger blastocysts.

M50
FACTORS THAT AFFECTS THE EFFICIENCY OF AN EXTRA DOSE OF PGF AT THE ONSET OF THE SUPEROVULATION PROTOCOL IN BEEF CATTLE

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BACKGROUND-AIM
The aim was to evaluate the effect of antral follicle count (AFC), category, genetic group and body condition score (BCS) on efficiency of an extra dose of prostaglandin (PGF) at the onset the protocol in beef cattle submitted to an eCG-based superovulation protocol.
METHODS
A total of 546 beef heifers (214 Bos indicus and 332 Bos indicus-taurus) with BCS = 3.04±0.3 (1-5 scale) from three different commercial farms were used. At a random stage of the estrus cycle (D0), heifers received 2mg IM of EB (Sincrodiol®, Ourofino, Brazil), 500µg IM of PGF (Sincrocio®, Ourofino, Brazil) and 1.0g progesterone (P4) intravaginal device (Sincrogest®, Ourofino, Brazil). In this moment, all animals were evaluated by ultrasonography to detect the presence of corpus luteum (CL) and AFC was performed of both ovaries. Only animals with CL were used in this study. On D5, heifers received 1000IU IM of equine chorionic gonadotropin (Sincro eCG®, Ourofino, Brazil). On D8, P4 device was removed and the presence of CL was verified by ultrasound. Statistical analysis was performed by GLIMMIX procedure of SAS.
RESULTS
Only 9.9% (54/546) had CL on D8 of the protocol. From the AFC on D0 frequency distribution, three classes were established: low AFC (12.8±2.7 follicles, n=182), intermediate AFC (19.3±1.6 follicles, n=182) and high AFC (27.5± follicles, n=182). In addition, heifers were divided into three groups according to BCS: low BCS (2.72±0.1; n=182), intermediate BCS (2.97±0.1; n=182) and high BCS (3.43±0.2; n=182). There was no interaction between category and genetic group (P=0.12) for presence of corpus luteum on D8. The AFC did not differ between classes (P=0.32) and there was no difference according to genetic group (P=0.42). The presence of corpus luteum was lower in heifers [Cows – 15.4% (19/124)a and heifers – 8.3% (30/442)b; P=0.02]. Furthermore, there was a difference for presence of corpus luteum among BCS groups [low BCS – 11.0% (20/182) a; intermediate BCS – 11.5% (21/182)a; high BCS – 7.1% (13/182)b; P=0.01).
CONCLUSIONS
In conclusion, AFC and genetic group on D0 of the superovulation protocol does not influences the presence of corpus luteum on D8. However, category and BCS influences the efficiency of an extra PGF application at the onset the superovulation protocol in beef cattle.

M51
EMBRYO TRANSFER IN TROPICAL ENVIRONMENTS: EFFECTS OF PARITY, CORPUS LUTEUM SIZE AND SERUM PROGESTERONE CONCENTRATION ON PREGNANCY RATE IN BOS TAURUS RECIPIENTS

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BACKGROUND-AIM
Pregnancy rates (PR) derived from embryo transfer (ET) in tropical environments could be compromised due to environmental factors. This fact is even more important in Bos taurus compared to Bos indicus because of adaptation differences related to genetic factors. The objective was to study the effects of parity, corpus luteum size (CLS) and serum progesterone concentration (PGC) on PRs in Bos taurus recipients submitted to ET under tropical conditions.
METHODS
Charolais breed heifers/cows (BW:~550± 51.1 kg ; BCS: 3-4) were maintained under the same nutritional, management and environmental conditions [Köppen-Geiger (Af), humid tropical climate, Ecuador; Coord.:S~01°29´8"; W~078°02´7"; Prec.:~2,000 mm; R.H.:~80%; M.T.:~26.1°C; Alt.:~1,000 m.a.s.l.]. Three experimental groups [Nulliparous heifers (N; n=20); Primiparous cows (P; n=20); Multiparous cows (M; n=20)] were synchronized by a combined protocol: Day 0 [P4 (3 mg/cow subc.)+ P4 (50 mg/cow i.m.)+ Estradiol Benzoate (2 mg/cow i.m.)]; Day 8 [PF2a (50 mg/cow i.m.)+ eCG (500 U.I./cow i.m.); Day 10 [GnRH (250 µg/cow i.m.)]. PGC (ng/mL) serological analyses were carried out from samples obtained on Day 7 by competitive indirect ELISA. Ultrasonography was carried out to determine follicle size (FS) (mm; before ovulation), CLS (mm; before ET) and embryonic loss/absorption or pregnancy confirmation (Day 45 after ET). Kolmogorov-Smirnov/Shapiro-Wilk test were used to determine data normality. Chi-Square, Mann-Whitney and Kruskal Wallis test were used to compare variables (anat. struct./pregnancy/categories).
RESULTS
From 60 animals, 65% (39/60) showed pregnancy 45 days after ET. Regarding N, P and M groups, 55% (11/20), 65% (13/20) and 75% (15/20) reached pregnancy status although no differences were detected among groups (p>0.05). There were no significant differences regarding FS, CLS or PGC among parity groups (p>0.05). When pregnant vs. nonpregnant individuals were compared statistical significant differences were observed in CLS (20.28± 1.96 vs. 18.86± 2.29; p= 0.009) and PGC (12.23± 4.86 vs. 8.07± 2.95; p< 0.001) but no differences were observed in FS (p> 0.05).
CONCLUSIONS
In conclusion, similarly than in other environmental conditions the tropical environment was not relevant in affecting FS, CLS, PGC and PR after ET in Bos taurus. (ANID: 2020–21201280)

M52
EVALUATION OF TWO APPROACHES FOR IN VIVO OOCYTE COLLECTION AND IN VITRO EMBRYO PRODUCTION IN HOLSTEIN COWS AND HEIFERS

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BACKGROUND-AIM
In dairy cattle, follicular development and oocyte quality depends on age, production performance, reproductive status and other factors affecting the success of ovum pick up (OPU) and subsequently in vitro embryo production, thus it is mandatory to standardize protocols for oocyte recovery considering particular characteristics of donors. In this work, different approaches for OPU were evaluated in cows or heifers on a milk production system, with the aim of producing quality oocytes for in vitro maturation and fertilization (IVM/IVF).
METHODS
Cows and heifers of Holstein breed were kept under same managing conditions. First approach: multiparous lactating cows (n=5), second approach: nulliparous heifers (n=23). Cows were subjected once to ovary super stimulation with CIDR (1.38g/ cow) + progesterone (100 mg/cow i.m.) + estradiol beta (2 mg/cow i.m.) at day 0, followed 24h later with eCG (2500 IU/cow i.m) and follicular aspiration on day 4. After that, OPU was performed once a week for 30d without further stimulation. Heifers were not super ovulated, instead after estrous synchronization, underwent OPU on a 15 days interval. Follicular size (FS) was determined by ultrasound at the moment of eCG administration and every day thereafter until OPU (for cows) or at every OPU for heifers.
RESULTS
In cows, after treatment, high number of follicles was observed (9±1) but low recovery rate (25%). OCC subjected to IVM/IVF yielded (5/13) 38% of grade I blastocysts. In the subsequent OPUs both follicular number and oocyte recovery decreased and no blastocysts were produced. The heifers showed a unceasing response with an average of 7.2 follicle per animal with a FS range of 2 to 8mm, the recovery rate was 82% (5.9 OCC/heifer) and the development rate 10.9%, of grades I and II blastocysts also there was a correlation between the number of aspirated follicle and the number of oocytes collected ($r = 0.72$, $p < 0.01$). However, there was no correlation between the number of OCO and blastoscyts ($r = 0.11$, $p = 0.54$).
CONCLUSIONS
In spite of an initial increase in embryo yield after superovulation, it did not sustain oocyte and embryo production overtime. The use of non-stimulated heifers seems to be more useful approach for continuous embryo production in milking animals.
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M53
ANTRAL FOLLICULAR COUNTS, ANTI-MÜLLERIAN HORMONE CONTENT AND OVARIAN DYNAMIC OF SENESCENT BOS INDICUS BEEF COWS

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BACKGROUND-AIM
This study characterizes the AFC, AMH profile and ovarian dynamics of senescent cows, close to the estimated period of depletion of the follicular reserve.
METHODS
Lactating Nellore cows (B. taurus indicus) were used in two experiments. In the first experiment, 16 senescent (age of 14 to 22 years) and 9 young (age of 4 to 8 years) cows were treated with a progesterone vaginal devise (PVD) and injected with EB and PGF2a. Five days later the PVD was removed and ovaries were scanned by Doppler ultrasound to register the ovarian volume, AFC (AFC1) and punctured the visible follicles. One second AFC (AFC2) was done five days later to count only the antral growing follicles. Blood samples were collected at the time of ACF1 to evaluate AMH concentration in serum. In a second experiment, 6 senescent and 3 young cows were submitted to daily ovarian scanning ultrasound during two consecutive ovulations. Data were submitted to regression analysis and Student t-test.
RESULTS
In Experiment 1, ovarian volume of young and senescent cows was 8.0 ± 1.2 and 13.5 ± 0.8 cm2, respectively ($P < 0.01$). AFC1 and AFC2 were no different in senescent (12.6 ± 3.8 and 11.7 ± 2.8) and young (14.4 ± 4.8 and 10.4 ± 3.5) cows ($P > 0.05$). AMH concentration was related to AFC ($P < 0.01$), but not to the age of cows. In experiment 2, young cows, as well as senescent cows younger than 20 years showed a pattern of two and three waves of follicular growth, while senescent cows older than 22 years showed some small antral follicles, but ovulation did not occur. There was no difference in the length of the first, second and third follicular waves of young and senescent cows. The duration of the estrous cycle ranged from 18 to 22 days in the two-wave pattern and 23 days in the three-wave pattern. The size of the preovulatory follicle (12.2 ± 0.3 mm) was similar for young and senescent cows.
CONCLUSIONS
In conclusion, senescent and young cows present similar AFC, AMH levels and ovarian dynamics. On the other hand, senescent cows older than 22 years appear to have lower AFC and significant changes in the follicular wave pattern.
Financial support was provided by FAPESP (proc. 2018/07450-5).

M55
THE USE OF ECG DILUTED IN D-CLOPROSTENOL REDUCES THE NUMBER OF INJECTIONS REQUIRED FOR TAI PROTOCOLS IN POSTPARTUM BOS TAURUS BEEF COWS WITH SAME PREGNANCY EFFICIENCY AS THE CONVENTION TREATMENT

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BACKGROUND-AIM
It was previously proved that adding eCG in TAI protocols of cows with reduced postpartum period, anestrous cows, and females with low body condition score (BCS) is a crucial approach to improve P/AI. The objective of this study was to assess if the dilution of eCG in D-cloprostenol would enable the administration of both drugs in a single injection, reducing the number of shots to accomplish TAI protocols without reducing reproduction efficiency of Bos taurus (Red Angus) cows.
METHODS
The study was done in 2021 at Establecimiento El Fortin, Partido de Rauch, Buenos Aires, Argentina and allocated 316 Bos taurus multiparous cows with average BCS 2.75±0.3 and ranging 45-60 days postpartum. All cows were kept in pasture with water ad libitum and were evaluated by ultrasonography for uterine condition and cyclicity at the onset of the protocol (D0), more of the 80% of the cows were in anestrous (absence of CL). Subsequently, cows received an intravaginal device with 0.5g P4 (Cronipres 0,5g, Biogénesis Bagó S.A.) and 2mg estradiol benzoate (Bioestrogen, Biogénesis Bagó S.A.) IM. On D8, device was removed and 0.5mg estradiol cypionate (Croni-Cip, Biogénesis Bagó S.A.) was given IM. At that time, cows were randomly distributed in one of three groups. Cows in Control Group (CG) received only 150µg D-cloprostenol (PGF; Enzaprost DC; Biogénesis Bagó S.A.) IM. Cows in Traditional Group (Trad) were treated with 150µg D-cloprostenol and 400IU eCG (Ecegon, Biogénesis Bagó S.A.) IM, in two injections apart. Cows in Group Combined eCG+PGF received eCG diluted in PGF and administered in a single injection. The eCG+PGF was prepared by diluting 4 vials of lyophilized eCG (total 20,000IU) in 100mL of PGF and the dose used was 2mL/cow. TAI was done 50h after device removal (D10) using semen of a single bull. Pregnancy diagnosis was 35d after TAI. Data was analyzed using SAS for Windows.
RESULTS
Pregnancy at AI was greater for eCG treated cows (CG: 41.6%b; Trad: 63.9%a; eCG+PGF: 62.6%a; P=0.00017), regardless of the form of administration (isolated or combined with PGF).
CONCLUSIONS
In conclusion, the use of eCG diluted with PGF was efficient in TAI protocols of B. taurus cows, reducing the number of injection and keeping pregnancy outcomes.

M56
NANOTECHNOLOGY AND ARTIFICIAL INSEMINATION: VITAMIN E NANOEMULSIONS PREVENT LOSS OF RAM (OVIS ARIES) SPERM MOTILITY CAUSED BY OXIDATIVE STRESS FOR UP TO 96H AT 22 °C

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BACKGROUND-AIM
Artificial insemination (AI) is a routine breeding technology which has been extensively used in ovine reproduction. However, factors such as temperature and time elapsed from ejaculate collection to insemination limit its efficiency. Ram sperm are particularly sensitive to temperature changes, being motility one of the most affected parameters. In the present work we have proposed to evaluate the efficacy of drug delivery systems (DDS), specifically nanoemulsions as a tool to improve sperm motility. We hypothesized that controlled release of vitamin E by nanoemulsions (E NE) could preserve sperm motility during sperm transport at 22 °C and avoid the detrimental effects of oxidative stress.
METHODS
For this purpose, the ejaculates of five mature rams of Manchega breed were collected by artificial vagina and extended to 60 x 106 spz/mL in Andromed®. Once aliquoted, the samples were incubated as follows: Control and Vitamin E nanoemulsion (12mM), with and without oxidative stress (100 µM Fe2+/ascorbate). Total (TM, %) and progressive motility (PM, %), straight velocity (VSL, µm/s) and linearity (LIN, %) were analyzed with a CASA® after 0, 24, 48, 72 and 96 h of incubation at 22°C. For statistical analysis, we used a one-way ANOVA. The results are presented as mean ± SEM, and statistical significance was accepted for $p < 0.05$.
RESULTS
Our results show that the deleterious effects of oxidative stress on ram spermatozoa were prevented by the vitamin E NE, preserving TM (72 h, 73.86 ± 9.91; 96 h, 67.15 ± 15.22) with respect to Control (72 h, 0.60 ± 0.53; 96 h, motionless) $p < 0.05$). The same effect was observed for PM ($p<0.05$). Also, vitamin E NE enhanced VSL (46.45 ± 4.4) and LIN (37.20 ± 3.62) with respect to Control (motionless) for up to 96 h ($p = 0.00$). However, without oxidative stress, no significant differences were observed for any of the parameters analyzed, although samples supplemented with vitamin E NE showed a major percentage of total motility (72.16 ± 11.27) with respect to Control (55.52 ± 10.82) after 96 h.
CONCLUSIONS
Our work shows that vitamin E nanoemulsion is a new innovative tool for the preservation of sperm motility and opens new horizons in the transport of sperm samples for artificial insemination.

M57
SUBSTANTIAL CONTROL OF REPEAT BREEDING SYNDROME USING RAD PROTOCOLS IN DAIRY COWS

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BACKGROUND-AIM
Repeat breeding syndrome (RBS) is a substantial problem in cattle breeding leading to huge economic loss for the dairy producer. There seems to be paucity of reports on risk factors such as epidemiological and patho-biological factors associated with repeat breeder (RB) cows and their treatment in Bangladesh. Therefore, the aim of the study was to investigate the patho-biological factors that influence RBS and to develop proper strategies for the treatment and improvement of fertility in repeat breeder dairy cows and to formulate proper strategies for the control of RBS in dairy cattle.
METHODS
A total of 412 RB cows were identified using a survey. Occurrence of RB cows were analyzed according to different causes identified by hemato-biochemical analysis of blood serum, microbiological study of vaginal and uterine swabs and ovarian dynamics with trans-rectal ultrasonography. Findings of different investigation were also compared with normal cyclic (NC) cows. RB cows were divided into three groups for the treatment: Group 1- malnutrition (n=80), Group 2 -improper heat detection and AI (n=120), Group 3 - having history of clinical endometritis (n=200). Non-pregnant RB cows of Groups 1, 2 and 3 were assigned for single time AI after estrous induction (Group 4, n=120) and embryo transfer Group 5 (n=22). Farmers were provided with a designed reproduction clock to maintain their pregnant RB cows from 210 days of pregnancy through RAD protocol ie. monitoring RB cow's ration (R) and farmer's attention (A) during transient period and double-timed artificial insemination (D) (8-10 hours apart) after 40 days of post- partum estrus.
RESULTS
Observed pregnancy rates were 48.75% (39/80) in Experimental Group 1, 58.33% (70/120) in Experimental Group 2, 55.50% (111/200) in Experimental Group 3, 85.00% (102/120) in Experimental Group 4 and 45.45% (10/22) in Experimental Group 5. Overall pregnancy rate of RB cows obtained during the study period was 83.00% (332/400) and calving rate was 81.25% (325/332) in RB cows, later considered as normal cows. Following RAD protocols, 303 (93.23%) were pregnant among the 325 post-partum normal cows.
CONCLUSIONS
This study recommends RAD protocols for the prevention of repeat breeding syndrome in dairy cows.

M58
IMPACT OF DROUGHT ON THE REPRODUCTIVE PERFORMANCE OF EXTENSIVE BEEF CATTLE IN NAMIBIA

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BACKGROUND-AIM
Namibia prides itself in exporting beef of high quality and taste that is sourced from cattle that are mainly reared extensively on semi-arid vegetation. However, recurrent drought can have devastating impacts on the health and welfare of cattle. It can also affect reproductive performance, particularly in the communal areas, which can prolong the impact of drought far beyond the return of rains. In 2019, Namibia experienced its worst drought in over 90 years after several years of reduced rainfall; the aim of the current paper was to assess the impact of the drought on beef cattle reproductive performance in Namibia.
METHODS
During an animal welfare assessment visit to 55 Namibia beef farming herds (17 commercial farms, 20 semi-commercial village farms and 18 communal village farms) in autumn 2019 (March - April), the reproduction performance indicators (pregnancy, calving, and weaning rates and rates of reproductive conditions) were captured in the yards and in a questionnaire, compared to the previous year. A representative number of bulls were also tested for sheath washing, semen evaluation and physical condition. A follow-up visit in winter (July - August) evaluated changes in animal health, welfare, cattle management, and reproductive performance.
RESULTS
The impact of drought was evident from the increased mortality rate (20.7% average cf. 7.5% $p < 0.05$) due to poor nutrition, predators, poor body condition, and long-distance walking to grazing and water. Pregnancy rates were much lower than normal (36.2% average cf. 60.3% $p < 0.05$) with commercial herds only achieving 60% average (cf. 68%), semi-commercial 27.5% (cf. 36.8%) and communal 20.7% (cf. 27%). High incidences of reproductive conditions (10.2% cf. 9.6% vs. 2.9% cf. 1.9% $p < 0.05$), especially abortions, retained foetal membranes, dystocia and vaginal prolapse was evident. All bulls tested negative for venereal diseases but more than 40% of bulls had poor semen quality and reduced libido.
CONCLUSIONS
This drought has resulted in poor beef cattle welfare and poor reproductive performance. It is hoped that this research can assist Namibian beef farmers and countries at large to adopt appropriate management practices in order to achieve and maintain an increase in reproductive efficiency, despite any stresses such as drought.

M62
A NOVEL BULL SPERM STORAGE MEDIUM FOR USE WITH FIXED-TIME ARTIFICIAL INSEMINATION

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BACKGROUND-AIM
Historically, sperm storage methods have favoured the use of reduced temperatures to suppress sperm metabolism, which causes damage to the cells. Cryopreserved spermatozoa have a limited lifespan post-thaw, necessitating precise insemination timing. Although chilled storage (5°C) is less damaging, this method has a relatively short storage window (approx. 3 days), and is not widely used by the beef cattle industry. We hypothesised that by storing bull spermatozoa above the phase transition temperature (15°C), membrane integrity would improve, thereby increasing longevity in the female tract and improving fertility following fixed-time artificial insemination (FTAI). As such, we developed a novel storage medium capable of supporting bull sperm metabolism for at least 7 days at room temperature (RT; 22°C).
METHODS
Bull semen was collected by electroejaculation (n=10) and high-quality spermatozoa were isolated using density gradient centrifugation. Samples were then chilled in INRA96 or incubated at 22°C in the novel medium (Australian Provisional Patent No. 2021903289). Semen was also cryopreserved using Andromed. After 3 and 7 days, sperm motility was assessed using computer assisted sperm analysis (CASA) and fertilising capacity was confirmed via in vitro and in vivo fertility trials.
RESULTS
Compared to chilled, spermatozoa stored in the novel RT medium had higher total (TM) and progressive (PM) motilities after 3 days (TM: 71.6±3.9% vs. 58.8±6.5%; PM: 38.0±4.1% vs. 11.5±2.7%; PM P=0.0002) and 7 days (TM: 65.9±4.8% vs. 51.3±6.2%; PM: 27.3±5.2% vs. 9.5±3.4%; PM P=0.0142). The TM of 7-day RT-stored spermatozoa was also superior to that of cryopreserved spermatozoa (90.6±1.5% vs. 55.7±5.0%; P=0.0005) prior to IVF, and no loss of fertilising capacity was observed based on the rate of zygote formation as determined by the presence of two pronuclei (2PN) (%2PN: 44.1±4.8% vs 34.1±4.0%; P>0.05). In vivo fertility was assessed using FTAI performed on 2-year-old virgin Bos taurus heifers (n=18) following oestrus synchronisation, with a higher pregnancy rate observed than the industry average using cryopreserved semen (77.8% vs. 60%).
CONCLUSIONS
These results indicate that RT storage is a viable alternative for samples that will be used for FTAI within 7 days of collection.

M63
EMBRYO TRANSFER PROGRAM IN PARAGUAY BY USING OPU-DERIVED COCS EMBRYOS: EMBRYONIC AND FETAL MORTALITY RATES

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BACKGROUND-AIM
In vitro embryo production (IVP) is a feasible biotechnological tool that can be used in domestic animal species of economic interest, being an interesting alternative for the acceleration of animal reproductive process and the genetic improvement. Despite the considerable development of IVP techniques, its efficiency still remains low compared to other biotechnologies, in part due to the high rates of embryonic and fetal losses. The objective of this study was to assess the loss outcomes of a commercial embryo transfer program in several bovine breeds.
METHODS
Cumulus-Oocyte-Complexes (COCs) were obtained by ovum-pick up (OPU) from Bos indicus (Nelore, n=1,569; Gyr, n=1,086), Bos taurus (Holstein, n=401; Aberdeen Angus, n=1,276) and Bos taurus x Bos indicus (Brangus, n=182; Girolando, n=297) cattle and transported to the lab located in Asunción (Paraguay). The IVP protocol used was the same for all breeds. Embryos were cultured for 7 days post-fertilization and transferred to synchronized recipients (developmental stages 4 to 7, IETS) 9 days after CIDR removal. Pregnancy rates (PR; %) were scored by ultrasonography 23 days post-transfer (Day 30, 45 and 60) to determine embryonic (EMR; %) and fetal mortality (FMR; %) rates. The comparative analysis was performed by ANOVA.
RESULTS
The PR was 33.9±9.6 (19,195 transfers/6,503 pregnancies). The EMR at Day 45 was 5.2±7.1 (343 pregnancies) and the FMR at Day 60 was 1.6±2.8 (105 pregnancies). There was no effect of breed on PR (p=0.473). Significant differences were observed in EMR (p=0.008), being higher in the Holstein breed (18/147, 12.2±1.9). No significant differences were observed in pregnancy losses at Day 60 irrespective of the breed (p>0.05). The PR in 4th to 7th stage transferred embryos was 33.4, 30.5, 37.2, and 40.6%, respectively, being higher in 6th and 7th stage blastocysts compared to any other developmental stage (p<0.0001). EMR at Day 45

was higher in 4th stage embryos (p=0.0026), being 7.9, 5.4, 4.5, and 5.2% respectively. FMR at Day 60 was 0.0, 1.6, 1.6, and 2.0% respectively, for each embryo developmental stage (p=0.596).
CONCLUSIONS
In conclusion, PR, EMR and FMR after embryo transfer by using in vitro produced embryos obtained from OPU-derived COCs were similar to those reported in other South American countries

M64

EFFECTS OF POSTPARTUM HEALTH ON ESTRUS DETECTION AND SUBSEQUENT REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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BACKGROUND-AIM
Nearly half of dairy cows develop a health disorder in the early postpartum period which may impair reproductive performance. However, the link between health and reproductive function is not fully understood, particularly regarding estrus expression. The objectives were to investigate associations of postpartum health with the probability of detection of estrus by automated activity monitors (AAM) and subsequent reproductive performance in dairy cows.
METHODS
Holstein cows (n = 1,309) from 2 farms in Ontario, Canada were examined from 3 weeks before to 9 weeks after parturition. At d 2 and 6 (±1), total calcium, haptoglobin, and non-esterified fatty acids (NEFA) were measured in serum. At d 4, 8, 11 and 15 (±1), blood ß-hydroxybutyrate (BHB) concentration was measured and metritis assessed using a Metricheck device. Purulent vaginal discharge (PVD) and endometritis (by endometrial cytobrush) were assessed at week 5. Body condition and lameness were measured throughout, and clinical disease data obtained from farm records. Herds primarily used detection of estrus by AAM (Afimilk or SCR Engineers Ltd.) with minimal intervention for first breeding. Outcomes were estrus detected between 50 and 75 d and pregnancy rate to 150 d. Data were analyzed using multivariable logistic regression or Cox proportional hazard models with farm as a random effect.
RESULTS
Seventy-one percent of cows were detected in estrus, 42% were pregnant at first breeding, and 79% pregnant by 150 d. Estrus detection was less likely in cows with haptoglobin ≥ 0.5 g/L at d 6 (64 vs. 74%), BHB ≥ 0.7 mmol/L at d 15 (65 vs. 73%), PVD (66 vs. 73%), endometritis (64 vs. 74%), or body condition loss of ≥ 0.5-point (1 to 5 scale) (63 vs. 75%). Pregnancy rate was reduced in cows with NEFA ≥ 0.6 mmol/L at d 2 [hazard ratio and 95% confidence interval (HR): 0.8; 0.7-1.0], haptoglobin ≥ 0.5 g/L at d 6 (HR: 0.8; 0.7-0.9), endometritis (HR: 0.8; 0.6-0.9), or body condition ≤ 2.75 at week 9 (HR: 0.7; 0.6-0.9).
CONCLUSIONS
Markers of systemic inflammation and maladaptation to lactation can help to identify cows that may benefit from reproductive intervention, whereas cows without these risk factors are candidates for non-intervention and reproductive management based on estrus detection.

M65
EFFECT OF PATTERN OF P-FSH TREATMENT PRIOR TO OVUM PICK-UP ON OVARIAN RESPONSE AND IN VITRO EMBRYO PRODUCTION IN PREGNANT HOLSTEIN HEIFERS

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BACKGROUND-AIM
Administration of porcine FSH (p-FSH) prior to ovum pick-up (OPU) in Bos taurus cattle improves in vitro embryo production (IVEP). Even though, p-FSH is typically administered following a decreasing dose schedule, other dose schedules have not been evaluated. Thus, the aim of the present study was to evaluate the effect of p-FSH treatment pattern on ovarian response and IVEP.
METHODS
Pregnant Holstein heifers (n = 22), at 73.1 ± 1.7 d of gestation, were enrolled in the study. Heifers were randomly assigned to be administered p-FSH in a decreasing, constant, or increasing dose schedule, in a crossover design with a 14-day washout between sessions. Follicular wave emergence was synchronized using follicle ablation (FA) followed by p-FSH treatment initiated 36 h later, consisting of 6 administrations 12 h apart. The total dose of p-FSH (350 IU) was distributed among days as follows: decreasing (50%, 30% and 20%), constant (33.3%, 33.3% and 33.3%), and increasing (20%, 30%, and 50%). Forty-four hours after the last p-FSH injection OPU was performed, and cumulus-oocyte complexes (COCs) were classified and subjected to IVEP. Total number of follicles were evaluated by ultrasonography at OPU and follicles were classified into small (<6 mm), medium (6-10 mm), or large (>10 mm). Data were evaluated using generalized linear mixed models (SAS 9.4).
RESULTS
Total number of follicles did not differ (p = 0.54) between decreasing (28.8 ± 1.8), constant (27.5 ± 2.1), and increasing (29.3 ± 2.1) groups. Similarly, the proportion of small, medium, and large follicles did not differ (P > 0.10) between groups. Despite of a tendency for greater total number of COCs (P = 0.07) for the increasing group (19.6 ± 1.8) compared to decreasing (17.0 ± 1.7) and constant (17.1 ± 1.3), no differences (P > 0.10) were observed for number of viable COCs, number of COCs grade I, nor COC recovery rate. Cleaved oocytes, cleavage rate, and blastocyst rate did not differ between groups (P > 0.10), resulting in a similar (P > 0.10) number of blastocysts per heifer for the decreasing (6.0 ± 1.0), constant (5.7 ± 0.9) and increasing (6.0 ± 0.7) groups.
CONCLUSIONS
In conclusion, the pattern of administration for p-FSH prior to IVEP does not affect ovarian response, oocyte developmental competence nor embryo production.

M66
OVARIAN DYNAMICS, ESTROUS EXPRESSION AND FERTILITY IN HEIFERS SUBMITTED TO A 5- OR 6-DAY CO-SYNCH FOR FIXED TIME EMBRYO TRANSFER

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BACKGROUND-AIM
Length of progesterone (P4) treatment during a CO-Synch protocol affects fertility to AI, however, with embryo transfer (ET) effects are unknown. The aim of the study, therefore, was to evaluate the effect of duration of P4 treatment on ovarian dynamics, behavioral estrus, and fertility as a result of ET.
METHODS
Holstein heifers (n=207) at 15.2 ± 0.2 months of age, were randomly assigned to a 5- or 6-day CO-Synch protocol for fixed time ET. Heifers were administered an intravaginal P4 device (CIDR) and 100 µg of gonadorelin (GnRH) on D-9 or D-8, for the 6- and 5-day group, respectively. On D-3 CIDRs were removed, 500 µg of cloprostenol (PGF) was administered and an estrous detection patch (Estrotect) was applied. On D-2 there was a second PGF treatment. At 72 hours after CIDR removal (D0) GnRH was administered and embryo transfer performed on D7±1. There was estrous detection from D-2 to D0 every 6 h and ultrasonography on D-3 and every 6 h from D-2 to D2 to detect ovulation, on D5 for corpus luteum (CL) size, and on D32 and D60 for pregnancy diagnosis. Data were analyzed using generalized linear mixed models.
RESULTS
Follicle size on D-3 (10.0 ± 0.2 mm) and preovulatory follicle size (15.3 ± 0.2 mm) were not different (P > 0.5) between groups. Percentage of heifers expressing estrus (80.5%; 153/190) was similar between groups (P > 0.5), however, heifers in the 6-day group expressed estrus earlier (56.4 ± 1.5 h) than with the 5-day group (62.4 ± 1.5 h; P < 0.01). Ovulation as a result of GnRH administration (96.3%; 183/190) and time of ovulation (82.5 ± 1.0 h) were similar (P > 0.5) between groups. At D5, CL volume (4044 ± 130 mm3) did not differ between groups. Utilization rate (93.7%; 194/207) and pregnancies per ET (P/ET) at D32 (37.6%; 73/194) were not affected (P > 0.3) by treatment. The P/ET at D60, however, tended to be greater (P = 0.09) for the 6-day group (35.1%; 34/97 vs 27.8%; 27/97) and pregnancy loss tended (P = 0.08) to be less for the 6-day compared with the 5-day group (10.5%; 38/102 vs 22.9%; 8/35).
CONCLUSIONS
Even though, duration of P4 treatment during a CO-Synch did not affect ovarian dynamics nor behavioral estrus, increasing treatment duration by one day may improve fertility as a result of ET by reducing pregnancy loss.

M67
FEED EFFICIENCY AND REPRODUCTIVE PERFORMANCE RELATIONSHIP IN NELORE (BOS INDICUS) HEIFERS SUBMITTED TO TAI.

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BACKGROUND-AIM
The objective of this study was to evaluate relationships between fertility traits and feed efficiency in Nelore (Bos indicus) heifers submitted to TAI.
METHODS
A total of 285 Nelore heifers [10.6±0.1 months of age, body weight (BW)= 261.3±1.7 kg and body condition score (BCS)= 2.70 ± 0.02 (1-5 scale)] from commercial farm (HoRa, Brazil) during the year 2019 and 2020 were used. Individual feed intake was monitored by Intergado efficiency automated feeding system (Intergado®, Brazil) for 90 days to estimate the residual feed intake (RFI). Heifers were synchronized to receive TAI 70 days after starting feedlot. At random day of the estrous cycle (D0), heifers received an intravaginal device with 0.6g P4 (Fertilcare 600®, MSD) associated with 2mg EB (Fertilcare Sincronização®, MSD). At the same time, BW and BCS were evaluated, and the presence of CL was detected by US. Also, longissimus Muscle Area (LMU) and subcutaneous backfat thickness (RFAT) were estimated by US. On D8, device was removed and heifers received 0.5mg PGF (Ciosin®, MSD), 0.5mg of EC (Fertilcare Ovulação®, MSD) and 200IU of eCG (Folligon®, MSD). TAI was performed 48h after device removal. Pregnancy diagnosis was done 30 days after TAI, and non-pregnant heifers were assigned to a second TAI. Statistical analysis was performed by proc GLIMMIX of SAS® 9.4.
RESULTS
Heifers were classified as low RFI (good feed efficiency; -1.01±0.09 kg DM/d, n=143) and high RFI (1.20±0.06 kg DM/d, n=142). On D0, heifers with low RFI had lower BCS than high RFI (2.95±0.02 vs. 3.03±0.02; P=0.003), but similar BW (P=0.71) and cyclicity rate (P=0.14). The LMU did not differ among groups (Low RFI= 57.2±0.63 vs. High RFI= 56.6±0.69 cm2; P=0.11]. However, RFAT was lower in heifers with good feed efficiency (Low RFI) than High RFI (6.00±0.14 vs. 6.28±0.14 mm; P=0.02). No difference was observed at 1st pregnancy per IA (P/AI) [Low RFI= 46.9% (67/143) vs. High RFI= 52.1% (74/142); P=0.17]. Nevertheless, heifers with Low RFI had lower 2nd P/AI [Low RFI= 27.6% (21/76) vs. High RFI= 36.8% (25/68); P=0.05] and lower cumulative P/AI (1st + 2nd TAI) [Low RFI= 62.2% (89/143) vs. High RFI= 69.7% (99/142); P=0.04].
CONCLUSIONS
The results showed a negative relationship between reproductive performance and feed efficiency in Nelore heifers

M68
EXTRACELLULAR VESICLES MIRNAS FROM OVIDUCT AND UTERUS MAY AFFECT LIPID METABOLISM IN BOVINE EMBRYOS

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BACKGROUND-AIM
Extracellular vesicles (EV) in oviductal (OF) and uterine fluid (UF) improve bovine embryo quality during in vitro culture by reducing lipid contents and modulating lipid metabolism-related genes (LMG). We evaluated miRNA cargo in EV from OF and UF and its possible influence on LGM.
METHODS
Bovine reproductive tracts were selected based on corpus luteum morphology and fluids collected from oviducts (early luteal phase) and uterine horns (mid luteal phase). EV were isolated by size exclusion chromatography and characterized. Levels of 383 miRNAs in OF-EV (n=3) and UF-EV (n=3) were determined by qRT-PCR (miSCRIPT II RT kit, Qiagen). Ct values were normalized by the geometric mean of bta-miR-99b, Hm/Ms/Rt U1 snRNA and RNT43 snoRNA, and statistical differences assessed by t-test. miRNAs with Ct < 37 and detected in two of three samples were considered present. Bioinformatics analyses were performed (miRWalk 2.0 database) to identify predicted genes regulated by miRNAs differentially detected between groups.
RESULTS
EV in both fluids had typical morphology, modal size <200nm, 3 to 8x1010 particles/ml, and were positive for sEV proteins (CD9, HSP70 and ALIX), and negative for CANX (negative control). miRNAs (333) were detected in both groups, 11 exclusive to OF, 59 to UF, and 263 were common. From differentially expressed miRNA (20), 19 upregulated in UF-EV and one in OF-EV. Only miRNAs upregulated in UF-EV (11) were predicted to regulate LMG (LDLR, CD36, FABP3, PPARGC1B, ACACA, PLIN2). For exclusive miRNAs, 10 LGM were predicted as targets of 28 UF-EV miRNAs, while in OF-EV on 2 miRNAs were predicted to modulate 3 LGM. PPARGC1B was the gene with highest number of related miRNAs (15).
CONCLUSIONS
In conclusion, differences in miRNAs in EV from early luteal phase in OF and mid luteal phase in UF, may reflect different environments to meet changing needs of the embryo and miRNAs may be involved, particularly in uterus, with regulation of embryo lipid metabolism. PPARGC1B may be an interesting target to understand the influence of miRNA in UF-EV on embryo metabolism. Funding: MINECO-Spain PID2019-111641RB-I00; SENESCYT-Ecuador; FAPESP 2014/22887-0, 2015/21829-9, 2017/20339-3 and CNPq-Brazil 304276/2018-9.

M69
SPERM GSTM3 IS ASSOCIATED TO IN VIVO CATTLE FERTILITY

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BACKGROUND-AIM
Glutathione S-transferase Mu3 (GSTM3) has already been reported to be associated to sperm quality and fertility in humans and pigs. However, GSTM3 has not been investigated as a potential sperm biomarker in cattle. Thus, the present study seeks to determine the relationship between sperm GSTM3 and in vivo fertility in this species.
METHODS
Sperm samples from eight Holstein bulls were collected and cryopreserved, and 90-day non-return rates (90-NRR) after artificial insemination were calculated (in vivo fertility). Flow cytometry and computer-assisted sperm analysis were used to evaluate post-thaw sperm quality (morphology, viability, total and progressive motility) and chromatin (de) condensation. Immunoblotting and immunofluorescence were performed to determine the presence, localization, and relative content of GSTM3 in bovine sperm. Pearson correlation coefficients between sperm quality and functionality parameters, GSTM3 relative content and 90-NRR were calculated.
RESULTS
A single GSTM3-specific band of 48 kDa was present in all immunoblots. Moreover, GSTM3 was found to be localized along the principal, mid and end pieces of the tail. 90-NRR rates were correlated to the percentage of morphologically normal sperm (R=-0.80; P<0.05) and progressive motility (R=0.77; P<0.05), but not to total motility, viability or chromatin (de)condensation (P>0.05). Interestingly, the relative content of GSTM3 in sperm was negatively correlated to 90-NRR (R=-0.86; P<0.01).
CONCLUSIONS
In conclusion, the present study showed a relationship between in vivo cattle fertility and GSTM3 content in sperm. These results suggest that sperm GSTM3 could be an in vivo fertility biomarker in bovine. This work was supported by MICIU, Spain (AGL2017-88329-R, FPU18/00666 and PID2020-113320RB-I00) and Regional Government of Catalonia (2017-SGR-1229).

M70
DAYS IN MILK CORRELATES NEGATIVELY WITH PREGNANCY FOLLOWING EMBRYO TRANSFER OF IN VITRO PRODUCED EMBRYOS IN MULTIPAROUS DAIRY COWS

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BACKGROUND-AIM
One strategy for improving the low fertility of dairy cows or reducing twin pregnancies is embryo transfer. The objective of this study was to determine the conception rate of slaughterhouse in vitro produced Angus embryos in multiparous high producing dairy cows. Moreover, fertility of fresh and vitrified embryos was compared.
METHODS
Ovaries were collected from slaughtered cows from Angus breed and transported in a zyploc bag at 35ºC. Cumulus−oocyte complexes (COCs) were IVM in 500 µL of IVF-Bioscience commercial media under Nidoil. COCs matured during 24 h at 38.7°C, 5% CO2, and high humidity. In vitro fertilization (IVF) was performed using commercial frozen/thawed sperm dose from Angus breed. Oocytes and sperm were incubated for 18−20 h at 38.7°C and 5% CO2 humidified atmosphere. After IVF, COCs were denuded using 125µM capillary and in vitro culture (IVC). Presumptive zygotes were cultured in 100 µL of pre-equilibrated IVC-Bioscience media under mineral oil at 38.7°C, 5% CO2,5%O2, 90% N2, and saturated humidity. Embryos were cultured in groups until Day-7. Excellent and good-quality morulae and early blastocysts were selected, and transferred either fresh or were submitted to vitrification. Multiparous cows (>3 lactations) that underwent a synchronization protocol, 15 – 20 at the beginning protocol, were submitted to embryo transfer (ET) under epidural anaesthesia. The pregnancy diagnosis was made 21 days after ET and 14 days later, the pregnancy was confirmed.
RESULTS
The final study population resulted in 75 ETs of a single embryo. None of the 11 fresh embryos resulted in pregnancy, compared to 25 of 64 vitrified embryos (39.0%) (P=0.03). Cows with more than 90 DIM were less likely to get pregnant (P=0.03) than the others. Fourteen out of 25 cows with less than 90 DIM get pregnant (56.0%), compared to 11 out of 50 cows with 90 DIM or more (22.0%).
CONCLUSIONS
Multiparous cows with less than 90 DIM are the best candidates to receive an in vitro Angus produced embryo.

M71
HUMAN CHORIONIC GONADOTROPHIN IN CYCLIC ANIMALS AT THE BEGINNING OF SYNCHRONIZATION PROTOCOL IMPROVES FERTILITY IN DAIRY CATTLE SUBMITTED TO FOLLICULAR DRAINAGE.

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BACKGROUND-AIM
A follicular transvaginal drainage technique without ultrasound at artificial insemination (AI) for reduce twin pregnancies has been proposed in cows with two follicles. This technique was demonstrated to improve fertility, eliminate twin pregnancies but increases ovulation failure. Several factors can modify ovulation failure, but an inductor of ovulation is needed. The objective of this study was to determine whether cyclicity at the beginning of fixed time AI (FTAI) affect fertility in cows with two codominant follicles submitted to a simplified follicular drainage treated with GnRH or hCG.
METHODS
Cows were classified as cyclic or non-cyclic at beginning of the FTAI, when a corpus luteum (CL), or a persistent follicle or a cystic ovarian follicle, was detected, respectively. Briefly, FTAI consisted a controlled intravaginal progesterone-releasing device (CIDR) (Zoetis SL, Spain). The CIDR was left for 5 days, and these animals were also given cloprostenol (PGF Veyx Forte, Ecuphar, Spain) on CIDR removal. Twenty-four h and 60 h later, the cows received a second cloprostenol dose and a GnRH analogue dose (dephereline i.m; Gonavet Veyx, Ecuphar, Spain), respectively, and were FTAI 70-72 h after CIDR removal. Bi-ovular cows at AI were selected. The small follicle of all cows was drained with a steel transvaginal cannula. Cows were then AI and received randomly hCG (3000 IU hCG i.m; Veterin Corion, Divasa-Farmavic, Spain) (n= 47) or Dephereline (n= 41). Pregnancy diagnosis was performed by ultrasound at 28 days post-AI.
RESULTS
The final study population resulted in 88 bi-ovular cows. Of the 29 pregnant cows, 14 were cyclic (48,2 %). Eleven out of 20 cows treated with hCG (55%) and 3 out of 22 cows with Dephereline (13,6 %) get pregnant, respectively. Thus, cyclical cows treated with hCG were more likely to get pregnant (p = 0,043).
CONCLUSIONS
Cyclical animals treated with hCG submitted to follicular drainage are more likely to become pregnant than cyclical cows treated with GnRH agonist.

M72
RELATIONSHIP BETWEEN FOLLICULAR SIZE AND OVULATORY FAILURE IN BI-OVULAR DAIRY CATTLE SUBMITED TO A MANUAL FOLLICULAR DRAINAGE

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BACKGROUND-AIM
Recently, a follicular transvaginal drainage technique without ultrasound at the moment of artificial insemination (AI) to reduce twin pregnancy in bi-ovular cows was described. Although this technique was demonstrated to improve fertility, it Increased ovulation failure. The objective of this study was to evaluate size of the follicular structures at AI to determine its relationship with ovulation failure in drained cows.
METHODS
This study was performed over the study period of April to November 2018 in a commercial dairy herd of 200 Holstein-Friesian cows. Bi-ovular bilateral cows diagnosed by ultrasonography were selected after fixed time AI (FTAI) synchronization program. Briefly, FTAI consisted to a controlled intravaginal progesterone-releasing device (CIDR) (containing 1,38 g of progesterone; Zoetis SL, Spain). The CIDR was left for 5 days, and these animals were also given cloprostenol (500 µg i.m.; PGF Veyx Forte, Spain) on CIDR removal. Twenty-four h and 60 h later, the cows received a second cloprostenol dose and a GnRH analogue dose (dephereline: 100 µg gonadorelin acetate i.m; Gonavet Veyx, Spain), respectively, and were FTAI 70-72 h after CIDR removal. The smallest follicle (n= 27) was punctured and drained with a steel transvaginal cannula designed for follicular cyst puncture (Minitub Ibérica S.L., Spain). Thirty-four cows with two bilateral follicles were not drained (control group). Cows were then AI and received dephereline. Pregnancy diagnosis was performed by ultrasound at 28 days post-AI.
RESULTS
The mean milk production and the number of lactations were 10.800 ± 961 kg and 2,39 ± 1,24, respectively (mean ± SD). None of the cows in the control group and those punctured with a follicle <18 mm (n= 6) suffered ovulation failure. Ten of 21 punctured cows with a follicle ≥18 mm (47,6 %) suffered ovulation failure (p= 0,002).
CONCLUSIONS
Candidate cows for follicular drainage are animals with follicles <18 mm at the time of AI.

M73
CELLULAR PROLIFERATION, CROSS-SECTIONAL MORPHOLOGY, AND LUMINAL SURFACE MORPHOLOGY OF THE MID-PREGNANT BOVINE UTERUS.

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BACKGROUND-AIM
Cattle are inseminated approximately 2 months after calving. Before first insemination, the uterus must undergo involution, a process that includes the reestablishment of the luminal epithelium (LE), and the restoration of uterine gland function. We studied the morphology of the mid-pregnant uterus in an effort to better understand the changes in the endometrium that must occur during the transition from the pregnant to non-pregnant state postpartum.
METHODS
Holstein cows (n=14) were slaughtered at 157±17 d of pregnancy and intercaruncular endometrium was collected, sectioned, and stained for Ki67 proliferation marker. An adjacent site was processed for scanning electron microscopy (SEM) of the luminal surface of the uterus. The morphology of the endometrium in cross-section and the percentage of Ki67 positive cells in the LE and glandular endometrium (GE) were determined.
RESULTS
In all cows, the morphology of the endometrium in cross-section consisted of an undulating surface with a thick band of densely packed subepithelial stroma (453±106 µm width) that separated the LE from uterine glands. Glands were numerous but had distended lumen (175±67 µm diameter) and failed to penetrate the subepithelial stroma to reach the luminal surface. The percentage of Ki67 positive (proliferating) cells was >10 times (P<0.001) for LE 15.7±5.0% compared with GE (1.2±0.4%). Under SEM, the luminal surface possessed deep folds. The apical surface of cells was covered with microvilli. Ciliated cells or gland openings that are typically found on the surface of non-pregnant intercaruncular endometrium were not present. There were numerous (9.4±4.9 per 65,536 µm2) openings in the LE (approximately 10 µm diameter) some of which had intact cells migrating through them.
CONCLUSIONS
The morphology of the intercaruncular endometrium of the mid-pregnant uterus differed from what is typically described for the nonpregnant uterus where gland openings and ciliated cells are found. There is a proliferating LE that accommodates the growing pregnancy. Openings were found in the LE and these may provide a conduit for immune cells migrating into the uterine lumen. Supported by the National Institute of Child Health and Human Development of the National Institutes of Health under award number R01HD092254.

M74
THE PROTEOMIC LANDSCAPE OF BOVINE OVIDUCT FLUID AROUND THE TIME OF FERTILIZATION

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BACKGROUND-AIM
The oviduct and its secretions offer optimized conditions for sperm survival (in the isthmus), fertilization (in the ampulla) and early embryo development (in the isthmus) during the peri-ovulatory period. In order to better understand the spatio-temporal regulation of this periconception environment, the aim of this study was to assess the effect of the (1) oviduct region (ampulla vs. isthmus), (2) time relative to ovulation (pre-ovulatory vs. post-ovulatory) and (3) proximity of the ovulating ovary (POF; ipsilateral vs. contralateral) on the proteomic composition of the oviductal fluid (OF).
METHODS
Oviducts from adult cows at pre- and post-ovulatory stages of cycle were collected at a local slaughterhouse. Isthmus and ampulla from both sides were flushed separately (4 pools of 4 cows per region x stage x side condition, 32 samples). Proteins of samples were Lys-C/trypsin-digested and purified using the iST kit (Preomics) and then analyzed by nano liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS). Proteins were identified using the Uniprot Bos taurus database and considering at least 2 unique peptides per protein. Protein abundance was quantified by label-free spectral counting and differences between conditions were analyzed after normalization by t-tests (p-value ≤ 0,05). Prediction of secretory pathways and functions were assessed by Outcyte, SignalP and DAVID bioinformatics resources.
RESULTS
In total, 3760 proteins were identified in the OF, of which 37% were predicted to be secreted and 41% previously reported in oviduct extracellular vesicles. Principal component analysis of protein abundance evidenced the oviduct region as the greatest source of variation between conditions. A total of 8 to 12% of proteins varied in abundance according to the region, 3-8% according to the side and 2% according to the stage. Enrichment analysis of differentially abundant proteins revealed a wide range of biological functions among which protein binding, response to stress, cell-to-cell adhesion, and calcium and redox homeostasis were predominant.
CONCLUSIONS
This work presents for the first time the region-specific landscape of the oviduct lumen proteome and provides new protein candidates for sperm and embryo protein interactions.

M75
SPATIOTEMPORAL REGULATION OF SPERM-INTERACTING PROTEINS IN THE BOVINE OVIDUCT FLUID

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BACKGROUND-AIM
Interactions between proteins from the oviduct fluid (OF) and spermatozoa (spz) are crucial for the progressive acquisition of sperm fertilizing ability in mammals. However, only few sperm-interacting proteins (SIP) were identified and their regulation during sperm migration toward the fertilization site around ovulation time is currently unknown. Our aim was to identify OF SIP according to the (1) oviduct region (isthmus vs. ampulla) and (2) time relative to ovulation (pre- vs. post-ovulatory).
METHODS
The ipsilateral isthmus and ampulla from adult cows at both stages collected at local slaughterhouse were flushed separately (4 pools of 2-3 cows per region x time condition). Frozen-thawed Percoll-washed bull spz were co-incubated (40.106 spz/mL) with phosphate-buffered saline (controls) or OF flushes (spz-OF) at 3 mg/mL of proteins at 38.5°C for 1h. Sperm and OF proteins were extracted, Lys-C/trypsin-digested (iST kit, PreOmics) and analyzed by nano liquid chromatography coupled with tandem MS (nanoLC-MS/MS). Protein abundance was evaluated by spectral counting and normalized using the Scaffold software. Proteins were considered as SIP when metting the following criteria: (i) identification in the OF and (ii) specific detection in spz-OF (no detection in controls) or higher detection in spz-OF than in controls (p-value ≤ 0,05) with a minimum fold-change ratio of 3. Prediction of secretion pathways and functions were assessed by Outcyte, SignalP and Metascape online tools.
RESULTS
In total, 2220 proteins were identified among which 228 SIP were detected, with 28% predicted to be secreted and 78% previously reported in oviduct extracellular vesicles. SIP included MYH9, OVGP1 and and GRP78 as the most abundant ones. The highest number of SIP (170) and specific SIP (30) were found in the pre-ovulatory isthmus, i.e. time and place of the sperm reservoir. Among the 68 SIP shared between conditions, 9 were differentially abundant between regions at a given stage and 7 between stages in a given region. Only 11 SIP have previously reported roles in fertilization and establishment of pregnancy.
CONCLUSIONS
This study provides for the first time an exhaustive list of SIP and highlights important region- and time- specific interactions between bull spz and OF proteins

M76
EVALUATION OF OXIDATIVE STRESS AND PERIPHERAL CONCENTRATIONS OF INFLAMMATORY CYTOKINES IN DAIRY COWS WITH POSTPARTUM METRITIS

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BACKGROUND-AIM
Postpartum (pp) dairy cows suffering from metritis may experience greater oxidative stress (OS) in comparison to healthy herdmates. This OS might occur due to the consequences of dysregulation of systemic inflammation in the early postpartum. This study aims to assess OS indicators and inflammatory cytokines in the serum of pp dairy cows and their use as potential markers for metritis.
METHODS
Twenty-five Holstein cows were weekly blood sampled from 7 ± 2 to 35 ± 2 dpp. Eleven cows were diagnosed with metritis (abnormal vaginal discharge, enlarged uterus, and >39.5°C within 21 dpp). Oxidative stress markers like reactive oxygen metabolites (d-ROM), antioxidants (OXY), and oxidative status index (OSI) (evaluated via photometric determination of plasma thiols) and inflammatory cytokines like pro-inflammatory cytokines (TNF-a and IL-6) and the anti-inflammatory cytokine (IL-10) (all evaluated via ELISA) were analyzed in the serum samples. Statistical analyses were done via ANOVA, accounting for repeated measurements.
RESULTS
Serum concentrations of d-ROMs and OSI were greater in metritis than healthy at day 7 and 14 pp (106 ± 6.26 and 111 ± 6.26 Carratelli Units (UCarr) in metritis vs 81 ± 4.75 and 84 ± 4.69 UCarr in healthy; $P < 0.001$ and 0.35 ± 0.03 and 0.34 ± 0.03 UCarr in metritis vs 0.20 ± 0.02 and 0.18 ± 0.02 UCarr in healthy; $P < 0.001$, respectively). The concentration of OXY was lower in metritis than healthy at days 7, 14, 21, 28, and 35 pp ($P < 0.05$). Serum concentrations of IL-10 were greater in metritis than healthy cows at 21 dpp (53 ± 4.5 and 35 ±3 .4 ng/L; $P < 0.001$, respectively), whereas no differences ($P > 0.05$) in TNF-a and IL-6 concentrations could be detected.
CONCLUSIONS
This study showed that cows with metritis experience a greater degree of OS in comparison to healthy cows. However, the serum concentrations of pro-inflammatory cytokines were not different between groups. Metritis cows had greater IL-10 (anti-inflammatory) than healthy at 21 dpp, which may be associated with the uterine healing process. These findings provide new avenues for research for prevention and potential supportive treatments for metritis via the utilization of antioxidants in the feed and immunomodulating agent-based dietary adjuvant therapy.

M77
IMPACT OF DIFFERENT CONCENTRATIONS OF PROGESTERONE DURING THE FOLLICULAR GROWTH FOLLOWING SUPEROVULATION IN HOLSTEIN HEIFERS.

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BACKGROUND-AIM
Estrus intensity has been associated with greater embryo viability and production in dairy heifers. However, the association between progesterone (P4) and estrus intensity and embryo viability is unclear. Therefore, the aim of this study was to evaluate the association between P4 concentration during follicular growth, and intensity of estrous expression and embryo viability in Holstein heifers.
METHODS
A total of 63 Holsteins heifers were randomly assigned into two experimental groups; Low P4 exposure (LP4 = 31) and High P4 exposure (HP4 = 32). Animals received a pre-synch protocol followed by the P4 treatment, with a protocol of superovulation. Activity was monitored continuously by an automated activity monitor (AAM, AfiMilk, Israel). Ovarian ultrasonography and blood samples were performed throughout experimental time to monitor ovarian structures and analysis of progesterone levels. Heifers were flushed 7d post artificial insemination (AI) for embryo collection. A total of 105 embryos were collected and evaluated for fertilization, stage of development and grades of quality from (1) to degenerated (4, IETS 2013). A subsample of embryos was analyzed for RNA sequencing (Illumina). Peak of activity was defined as the maximum index activity (heat indicator; AfiMilk) during estrus.
RESULTS
High P4 heifers had greater peak of activity (HP4 = 309.6 ± 135.8 index vs. LP4 = 215.6 ± 91.6 index; P = 0.007) and tended to have longer duration of estrus (HP4 = 19.3 ± 6.6 h vs. LP4 = 15.6 ± 6.5 h; P = 0.06) in comparison to Low P4 heifers. Embryo quality was not affected by peak of activity (OR = 0.9; CI = 0.9 – 1.0; P = 0.7) or duration of estrus (OR = 1.0; CI = 0.9 – 1.0; P = 0.9). However, High P4 heifers tended to have greater embryo quality in comparison to Low P4 heifers (OR = 1.9; CI = 0.9 – 4.3; P = 0.08). Furthermore, High P4 heifers had - 0.41 difference in the log of expected embryo counts in comparison with Low P4 heifers (P = 0.02).
CONCLUSIONS
In conclusion High P4 treatment had greater peak of activity and tended to have greater duration of estrus in comparison to Low P4 treatment. Peak of activity and duration of estrus did not affect embryo quality. However, High P4 heifers tended to have greater quality of embryos and Low P4 heifers had greater number of embryos 7d post-AI.

M78
IMPACT OF FREQUENCY OF MILKING ON MILK YIELD AND FERTILITY OF HOLSTEIN COWS UNDERGOING EXTENDED LACTATIONS DUE TO FAILURE TO CONCEIVE

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BACKGROUND-AIM
In zones where dairy cows undergo thermal stress for most of the year, heat stress negatively impacts the ability of a cow to become pregnant through both internal and external controlled pathways, and this infertility leads to involuntary extended lactations. The objective of this study was to determine the effect of two (2x; n= 214) compared to three times a day milking (3x; n=245) in Holstein cows undergoing lactations ≥600 d because of failure to conceive, on milk production and reproductive performance.
METHODS
Data were obtained from two geographically adjacent intensive dairy herds (>2500 milking cows) in northern Mexico (25° N). Cows received two injections of PGF2□ eleven days apart and were subsequently submitted to the Ovsynch protocol at 81 ± 3 DIM and then inseminated at 90 ± 2 DIM. Cows not pregnant were observed for estrus and were artificially inseminated with frozen/thawed semen. Pregnancy diagnoses were performed at 45±3 days after the last AI. High thermal stress caused that many cows got pregnant >300 days postpartum, which lead to extended lactations (669 ± 117 days for cows included in the present study; mean ± SD).
RESULTS
For first lactation 3x cows, there was no difference in total milk yield compared to 2x cows (19796 ± 3354 vs. 19269 ± 3652 kg; P>0.10) in lactations with an average days in milk of 696 and 650 days, respectively. Multiparous 3x cows produced more total milk yield than 2x cows (20942 ± 3920 vs. 18910 ± 2632 kg; P<0.01) with greater (P<0.01) days in milk for 3x (685 ± 117 days) than 2x (631 ± 88 days) cows. First service conception rate was affected (P<0.01) by frequency of milking being 7 percentage points lower in 3x (12.2%) compared to 2x cows (19.2%). Overall conception rate did not differ between 2x and 3x cows (53.3% vs. 49.8%) but 3x cows required one more service (5.9 ± 3.7 vs. 4.8 ± 3.4; P<0.01) to get pregnant than 2x animals.
CONCLUSIONS
Given that average milk yield throughout the complete lactation did not differ between 2x (29.8 ± 2.0 kg) and 3x (29.3 ± 2.9 kg), it was concluded that 2 × milking is equally effective than 3 × milking to attain acceptable milk yield in lactations >600 days, although reproductive performance based on first-service conception rate and services per pregnancy was negatively affected by 3x milking.

M79
EFFECT OF HEAT STRESS DURING THE DRY PERIOD ON MILK YIELD AND REPRODUCTIVE PERFORMANCE OF HOLSTEIN COWS

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BACKGROUND-AIM
Hyperthermia constitutes one of the most significant factors limiting milk production and fertility in high-yielding dairy cows. The objective was to determine the influence of heat stress during the dry period (DP) on milk yield and reproductive performance of Holstein cows not exposed to cooling systems during the DP.
METHODS
Breeding and milk production records, as well as meteorological data for cows between 2017 and 2020 in a commercial dairy herd (n=12102), were used to determine the relationship between the temperature-humidity index (THI) during the DP [average of the THI at the beginning, middle, and end of the DP] and breeding efficiency and milk yield traits. THI was divided into >70, 70-80, and >80. Milk traits were analyzed with the GLM procedure of SAS using parity and body condition score at calving as covariates. Pregnancy rates were compared with PROC GENMOD of SAS.
RESULTS
First-service pregnancy rate of lactating cows decreased (P<0.05) with increasing hyperthermia during the DP (8.9, 10.5, and 5.0% for THI of >70, 70-80, and >80, respectively). All-services conception rate was highest (P<0.05) for cows not undergoing heat stress during the DP (61.6%) and lowest (46.7%) for cows with severe heat stress (THI >80) during the DP. Cows not suffering heat stress during the DP required a mean ± SD of 4.4 ± 2.8 services per pregnancy compared with 5.9 ± 3.9 (P<0.05) for cows subjected to THI >80 during the DP. Cows with the absence of heat stress (THI ≤ 70) during the DP produced more (P<0.05) 305-d milk (10986 ± 1200 kg) than cows subjected to moderate (10880 ± 1218 kg) or severe (10757 ± 1269 kg) heat stress during the DP. Increased average THI from ≤70 to ≥80 units during the DP was associated with 15 more days in milk, but total milk yield did not differ (P>0.10) between cows not undergoing heat stress during the DP (13449 ± 3472 kg) and cows subjected to severe heat stress during the DP (13536 ± 3575 kg).
CONCLUSIONS
It was concluded that environmental management of dry cows during hot months is warranted to maximize reproductive performance and milk yield.

M80
ASSOCIATION BETWEEN DAUGHTER PREGNANCY RATE AND REPRODUCTIVE PARAMETERS IN HOLSTEIN DAIRY CATTLE.

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BACKGROUND-AIM
Genomic daughter pregnancy rates (GDPR) predict the genetic in pregnancy rates for a future daughter of a bull. Recent studies have shown that GDPR is associated with fertility in heifers and lactating dairy cows, but there is no information about its association with pregnancy loss (PL). The aim of this study was to determine the relationship between GDPR with reproduction parameters such as pregnancy at first insemination (P1AI), pregnancy per artificial insemination (P/AI), and PL. .
METHODS
A total of 12,949 AI events from 3,499 Holstein cows (nulliparous [n=1,220)]; primiparous [n=1,314]; or multiparous [n=965]) were included. Cows were bred either after a timed AI protocol, embryo transfer (ET), or spontaneous estrus. Hair samples were collected from the tail switch and cows were genotyped using a single nucleotide polymorphism platform. Tail chalk was applied on the day of the CIDR removal, and it was evaluated at AI (No Estrus:100% or >50% of chalk remaining; Estrus: <50% of chalk). Pregnancy diagnosis was performed at 32 and 60d post-AI using ultrasonography. Pregnancy loss was defined as the proportion of pregnant cows on d32 that were found non-pregnant on d60.
RESULTS
Cows with greater GDPR were more likely to become pregnant at 1stAI compared to cows that had lower GPDR (OR=1.28). Similarly, cows with higher GDPR were more likely to become pregnant overall, as they had higher pregnancy per AI over all inseminations (OR=1.31). The odds of PL decreased as GDPR increased (OR=0.66). Most cows that were bred on the day of the timed AI demonstrated estrus (n=6.075; 92.9%), however, cows with higher GDPR were also more likely to demonstrate estrus on the day of timed AI than cows with lower GDPR (OR=1.31). There was no interaction between GDPR and parity or breeding management (e.g., spontaneous estrus, timed AI, and ET) for P1AI, P/AI, and PL.
CONCLUSIONS
In conclusion, P1AI and P/AI was positively associated with greater GDPR and the odds of PL were greater for cows with low GDPR compared to cows with high GDPR. Greater GDPR was also associated with greater occurrence of estrus on the day of AI. The addition of genomic selection for GDPR was associated with better reproduction outcomes, which suggests selecting for higher GDPR could result in better reproduction performance.

M81
INCUBATION WITH 5.5 % COMPARED TO 16.0 % OXYGEN INCREASES METABOLIC CELL ACTIVITY OF ENDOMETRIAL EXPLANTS IN BOVINE SPECIES

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BACKGROUND-AIM
High-yielding dairy cows are prone to suffer from reproductive diseases such as metritis and endometritis, which impact bovine fertility and profitability of dairy farms. To generate a better understanding of cellular mechanisms in the endometrium, a bovine endometrial explant model was developed. Reports from cell culture models have previously shown cell viability to be dependent on oxygen supply, but no such information is available for explant tissue. To test the effect of the oxygen concentration on metabolic cell activity in bovine endometrial explants, incubation conditions were compared between two different oxygen concentrations.
METHODS
The concentration of 5.5 % oxygen reflects reported physiological tissue concentration in the uterus, whereas 16.0 % oxygen is regularly used in routine cell culture protocols. Endometrial tissue explants (n = 144 from 4 uteri) were collected with a 5 mm biopsy punch at the local abattoir and incubated at either 5.5 % or 16.0 % oxygen for 3 or 24 h. Water soluble tetrazolium salt 8 (WST 8) was added 3 h before the end of incubation in order to determine metabolic cell activity. Using the photometer 'Microplate Reader CLARIOstar' (BMG Labtech, Ortenberg, Germany), the optical densities (OD) of the explant supernatants were determined at the end of each incubation period. For statistical analysis, a fit linear mixed effect model was performed with R Studio, method REML and repeated in factor 'cow' (fixed effect 'oxygen concentration', random effect 'cow').
RESULTS
Explants incubated for 24 h at 5.5% oxygen showed significantly higher ODs (OD 1.74 ± 0.67) compared to explants incubated at 16.0 % oxygen (OD 0.89 ± 0.35; $P < 0.001$).
CONCLUSIONS
In conclusion, incubation with 5.5 % oxygen increases cell functionality in bovine endometrial tissue and might have an impact on the cells' pathogen-specific immune response. In future studies, this optimized model will be used to investigate mechanisms linking cellular antimicrobial activity with endometrial receptivity.

M83
THE EFFECT OF HIGH AND LOW PROGESTERONE EXPOSURE TREATMENTS IN A CROSSOVER TRIAL ON ESTROUS EXPRESSION AND OVULATION TIMING IN HOLSTEIN HEIFERS

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BACKGROUND-AIM
The aim of this study was to evaluate the associations between progesterone (P4) concentrations during diestrus with intensity of estrous expression and time from estrus to ovulation in nulliparous Holstein cows.
METHODS
In a randomized cross-over design experiment, post-pubertal heifers (n = 31) were presynchronized and fitted with a leg-mounted automated activity monitor (AAM). On d -17 relative to estrus, the animals received GnRH, P4 implant for 7 days and GnRH again on d -8. From d -7 to -1, heifers in the high P4 group (HP4) received a new CIDR while the heifers in the low P4 group (LP4) received a second use CIDR. Additionally, heifers in LP4 received multiple PGF2a injection during the diestrus. Heifers in both treatment group received PGF2a on d -1, and estradiol cypionate on d 0. Upon estrus activity alert, and every 4 h after until ovulation, the ovaries of the heifers were scanned by ultrasonography for the presence and subsequent disappearance of a dominant follicle (ovulation). Blood samples were taken at estrus and 7 d later for P4 analysis. After a washout period of 13±1 d, the heifers began pre-synchronization followed by the next treatment. Ovulation timing was defined as the number of hours between estrus alert time, provided by the AAM, and the time of ovulation. The effect of treatment on P4 on the day of estrus, estrous expression was tested with mixed-effect linear regression models (animal as random effect).
RESULTS
The effect of treatment on ovulation timing was tested by a Cox proportional hazard frailty model. The HP4 treatment had significantly lower P4 concentrations on d 0 than the LP4 treatment ($P = 0.001$) and a tendency for higher P4 on d 7 after the HP4 treatment ($P = 0.07$). There was no effect of treatment on ovulation timing (HR = 1.17 ± 0.27, $P = 0.56$). Estrous expression was not affected by treatment when measured by Duration (HP4:17.8 ± 0.9 vs LP4: 17.3 ± 0.6; $P = 0.64$) or Relative Increase of activity (HP4: 363.4 ± 31.9 vs LP4: 332.8 ± 19.9; $P = 0.42$).
CONCLUSIONS
In conclusion, heifers with low P4 during diestrus had higher P4 on the day of estrus and a tendency for lower P4 7 d after than heifers with high P4 during the diestrus, but no associations were found for ovulation timing or estrous expression.

M84
EFFECT OF 400 UI OF ECG-LIKE GLYCOPROTEIN OR NATIVE ECG ON THE REPRODUCTIVE PERFORMANCE IN SUCKLED BEEF COWS IN ARGENTINA

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BACKGROUND-AIM
The objective of this experiment was to compare an eCG-like glycoprotein (reCG) with native eCG on the reproductive performance in a TAI program in suckled beef cows in Argentina
METHODS
461 suckled Braford multiparous (n=388) and primiparous (n=73) cows with an AVG±SD of body condition score (BCS) was 2.45±0.32 (scale 1-5) and 90 days after calving were ultrasound on Day 0 to determined anestrous and were treated with the same TAI progesterone–estradiol protocol: Day 0: 2mg im of estradiol benzoate (Gonadiol, Zoetis, Argentina) and 0.5gr intravaginal device insert (DIB 0.5, Zoetis); Day 8: 500µg of sodium cloprostenol (Ciclase DL, Zoetis), 0.5mg of estradiol cypionate (Cipiosyn, Zoetis); Day 10: fixed time insemination. On Day 8, cows were randomly assigned to received 400IU of reCG produced by Syntex (PCT/EP2019/073277; n=145), 400IU of native eCG (NAT; Novormon, Zoetis; n=167), or remain untreated (CON; n=149). On Day 25, 3% of bulls were introduced. Pregnancy diagnosis was performed by ultrasound at Day 80. Response variable was FTAI pregnancy rate (PR); first 42 days' pregnancy rate (P42) explanatory variables were treatment, parity, anestrous, BCS at Day 0 and 80, BCS change and their interactions. Statistical analysis was performed by logistic regression (Infostat)
RESULTS
PR was 38.2% and was affected by the interaction of anestrous and treatment (P<0.05) and the interaction of parity and treatment (P<0.05). reCG and native cows has higher PR than control (41.4%, 40.1% and 32.9%; P<0.05). The percentage of cyclic cows was 51.8%. While the in cyclic cows the PR was similar reCG, NAT and CON: 43.1%, 41.6%, and 41.0% respectively, in anestrous cows reCG and NAT treatment increase the PR than CON: 39.7 %, 38.5% and 23.9%. In multiparous cows reCG, NAT and CON cows had similar PR: 40.5%, 40.1% and 35.2%; nevertheless, in primiparous cows reCG and NAT cows had higher PR than CON: 47.4%, 40.0% and 20.8%. P42 was affected by the interaction of anestrous and treatment (P=0.03). The treatment with reCG and NAT increase the P42 than CON in anestrous cows (60.2%, 67.9% and 50.7%), however in cyclic cows the P42 was similar (70.8%, 62.9% and 65.4%)
CONCLUSIONS
reCG and native eCG increased the reproductive performance in primiparous or anestrous suckled beef cow

M85
CERVICOVAGINAL CYTOLOGY ASSESSMENT OF DAIRY COWS SUBMITTED TO AN ESTRUS AND OVULATION SYNCHRONIZATION PROTOCOL

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BACKGROUND-AIM
FTAI was developed because of the difficulty in estrus detection and usually relies on the implantation of an intravaginal P4 device. But, the use of this device can cause health problems, such as vaginitis. The objectives of this study were to assess the inflammatory process caused by the intravaginal P4 device through cervicovaginal cytology testing of cows submitted to an estrus synchronization protocol, and to compare the results with those of cows submitted to conventional artificial insemination.
METHODS
16 Jersey and Girolanda cows at 3 to 6 years of age, with a body condition score of between 2.5 and 3.5 (0 to 5) were divided into two groups. For G1 (n=8), the cows were submitted to the synchronization protocol and an intravaginal P4 implant was used on D0 (1g, single use) and 2mg of estradiol benzoate, IM. On D8, the P4 implant was removed and 0.5mg of PGF2a and 1mg of estradiol cypionate, IM, and then insemination was performed on D10 (inseminators in training). For G2 (n=8), the cows were inseminated after estrus detection. Using a cytology device and a gynecological brush, cervicovaginal cytology samples were collected on D0, D8, and D10 for G1 and on the day of insemination for G2. Smears were prepared on slides and then stained with the panoptic rapid staining method. The slides were analyzed under an optical microscope at M400× to characterize the morphological and tinctorial properties of the cells, 200 cells were counted per slide. The data were analyzed using the T test, p<0.05.
RESULTS
The mean percentages and standard deviations of the epithelial and inflammatory cells for G1 were 99.63±0.47% and 0.38±0.47% on D0, 81.75±15.44% and 18.25±15.44% on D8, and 89.63±8.06% and 10.38±8.06% on the day of insemination, respectively. For G2, the values were 98.50±0.02% and 1.50±0.02% on the day of artificial insemination, respectively (p>0.05). Mucus was present in 50% (4/8) of the G1 cows on D8 and in 100% (8/8) of them on the day of insemination, whereas it was detected in 100% (8/8) of the G2 animals on the day of insemination. Pregnancy rates were 0% and 75% for groups G1 and G2, respectively.
CONCLUSIONS
Thus, it can be concluded that a mild inflammatory process was observed on D8 and D10 in all of the G1 cows, confirming an irritation of the vaginal mucosa by P4 device.
Financial Support: Fundação Araucária.

M86
PREGNANCY RATE OF ¾ TABAPUÃ × ¼ CHAROLÊS COWS SUBMITTED TO AN ESTRUS AND OVULATION SYNCHRONIZATION PROTOCOL

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BACKGROUND-AIM
To better understand and control the reproductive processes in domestic animals, researchers have developed several reproductive biotechniques, all of which have great importance in the different species. In this context, artificial insemination is an excellent tool for the genetic improvement of cattle, but the detection of estrus remains challenging. Therefore, ovulation synchronization protocols have been developed with the aim to facilitate both the exogenous administration of drugs and artificial insemination without the need for estrus detection, thus allowing for the optimization of reproductive management and planning of the delivery for an advantageous time period. As literature data on Tabapuã crossbred animals are lacking, the objective of this study was to evaluate the pregnancy rate of ¾ Tabapuã × ¼ Charolês cows submitted to an estrus and ovulation synchronization protocol.
METHODS
In total, 130 pluriparous cows at 2.5 to 5 years of age and more than 40 days postpartum, with a body condition score of between 2.5 and 3.5 (0 to 5), were submitted to a fixed-time artificial insemination protocol, followed by natural mating for 25 days. For estrus and ovulation synchronization, a vaginal progesterone implant (1200mg, Fertilcare implante®, MSD), was used on day 0 (D0) together with the administration of estradiol benzoate (2mL, 100mg/100mL, IM, Fertilcare sincronização®, MSD). On D8, the implant was removed and prostaglandin (2mL, 0.15mg/2mL, IM, Prolise®, MSD) was administered together with equine chorionic gonadotropin (1.5mL, 5000UI/10mL, IM, Folligon®, MSD) and estradiol cypionate (0.5mL, 50mg/100mL, IM, Fertilcare ovulação®, MSD). On D10, artificial insemination was carried out using Tabapuã bull semen from a certified company.
RESULTS
The pregnancy diagnosis was performed by ultrasonography and the total pregnancy rate from the synchronization protocol was 58.46% (76/130). The females that were not pregnant were naturally mated with bulls, with a resultant pregnancy rate of 62.96% (34/54). Thus, the total pregnancy rate of the batch was 84.61% (110/130).
CONCLUSIONS
In conclusion, the pregnancy rates of the Tabapuã crossbred cows submitted to the synchronization protocol were similar to those described in the literature for other breeds of beef cattle.
Financial Support: Fundação Araucária.

M87
A DEFECTIVE REDOX SCAVENGER SYSTEM NEGATIVELY AFFECTS THE IN VITRO EMBRYO DEVELOPMENT OF COW OOCYTES

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BACKGROUND-AIM
Mitochondria produce most of the ATP necessary for the cell via oxidative phosphorylation, and despite being constantly exposed to the generation of oxidant species, they remain functional thanks to the mitochondrial antioxidant defense system, comprising glutathione (GSH), glutaredoxin, and thioredoxin. Since mitochondrial dysfunctions are associated with low fertility, we carried preliminary investigations of mitochondria amount, activity and distribution, and redox buffering systems in bovine oocytes with low developmental competence.
METHODS
Cumulus–oocyte complexes (COCs) were retrieved from 2-8 mm follicles of abattoir-derived bovine ovaries with ≤ 10 (low developmental competence) or > 10 middle antral follicles (control) and processed either immediately as immature (GV) or upon in vitro maturation as metaphase II (MII). Total and active mitochondria were stained using MitoTracker Green and Orange, respectively, and GSH concentration was assessed using a spectrophotometric assay. Upon manipulation of the GSH content, some oocytes were in vitro fertilized and cultured for 8 days. Data were analyzed by Fisher's exact test, Mann–Whitney test, T-test, or one-way ANOVA.
RESULTS
Oocytes with low developmental competence failed to allocate the mitochondria to the cortex and in accumulate redox scavengers during oocyte maturation. Notably, blastocyst formation significantly improved when the GSH content was experimentally increased in low developmental competence oocytes. Conversely, the ovarian type did not affect mitochondrial mass and activity, while we observed a general decrease in mitochondrial activity at the MII stage.
CONCLUSIONS
These preliminary findings indicate that defective redox scavengers negatively affect in vitro embryo development of cow oocytes, seemingly without perturbing mitochondrial amount and activity. Furthermore, in agreement with previous reports of polysomal-bound mRNAs, showing that energy production and oxidative phosphorylation are among the most down-regulated cellular functions in bovine MII oocytes, these findings seem to convey that ATP generation is not the primary function of mitochondria at this stage. Whether anomalies in mitochondria localization may impact embryo development remains to be assessed.

M88
BREEDING AND OTHER MANAGEMENT PRACTICES IN COSTA RICAN CATTLE FARMS AND THEIR POTENTIAL IMPACT ON THE HERD´S REPRODUCTIVE EFFICIENCY

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BACKGROUND-AIM
Productivity in tropical livestock is historically characterized by a low outcome yield. Subfertility, nutrition and environmental conditions account as major causes of this scenery. Further, social and management practices may also contribute to impair the profitability of cattle herds. Thus, characterization of these systems allows to know their status in order to guide decisions to improve their productive efficiency.
METHODS
157 cattle farms being 55.4% dairy (D), 24.2% dual purpose (DP) and 20.4% beef (B) from the two most important livestock areas in tropical Costa Rica were surveyed to determine reproductive and other management practices.
RESULTS
Natural mating as the unique breeding system was reported by 54.0%, 73.7% and 62.5% in D, DP and B cattle farms respectively. Continuous mating was registered by 74.4% of farms being single siring more frequent than multiple (61.8% vs. 36.4%). The ratio cow/bull was 33.2. Data recording was reported by 95.4%, 86.8% and 81.3% in D, DP and B respectively (P<0.05). Although, the use of weighing scale was stated only by 5.8%, 26.3% and 65.6% respectively (P<0.0001). Reproductive pathologies (irregular cycles, abortions, repeat breeders, metritis and dystocia were reported less frequently in B compared to D and DP (37.4%, 70.4% and 62.4% respectively, P<0.0001). Monthly veterinary assistance was indicated by 37.9%, 18.4% and 25% in D, DP and B respectively (P<0.0001). Evaluation of bull breeding soundness (BSE) was reported by 15.9% of farms, being more common in B (46.9%) compare with D (4.7%) and DP (16.2%) (P<0.0001). BSE was also rarely reported to be requested when purchasing a sire (49.7%). Sharing sires between farms was still reported by 8.3% of farms. Purchasing replacement breeders in commercial meat auctions was confirmed by 11.5% of farms surveyed.
CONCLUSIONS
These data sadly support that basic approaches like testing regularly the bull breeding soundness still overlooked in tropical farms. The uncommon use of scales to monitoring growth in replacement breeders impairs also their adult reproductive performance. Furthermore, the scarce use of veterinary assistance affects the prevention of reproductive problems in cattle farms. Herd health and educational programs are still pending issues to improve in tropical cattle livestock.

M89
TRANSFORMING GROWTH FACTOR (TGF)-β1 PROMOTES STRUCTURAL LUTEOLYSIS IN BOVINE CORPUS LUTEUM

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BACKGROUND-AIM
Luteal regression is characterized by a decrease in progesterone (P4) production (functional luteolysis), followed by a decrease in luteal size (structural luteolysis), during which cells of the corpus luteum (CL) undergo apoptosis. Transforming growth factor (TGF)-β has been shown to reduce progesterone (P4) production in bovine luteal cells (Miyamoto et. al. J Endcrinol 1992) and to contribute to prostaglandin F2a-induced regression of CL in cattle (Hou et. al. Mol Endocrinol 2008). Here, to test the hypothesis that TGF-β1 contributes to structural luteolysis in bovine CL, we examined the mRNA expressions of TGF-β receptors (TGFBR1, R2) in bovine CL throughout the estrous cycle, and the effect of TGF-β1 on bovine luteal cell death and the mRNA expressions of apoptosis-regulating factors.
METHODS
1) The expressions of TGFBR1 and R2 mRNA in CL of five luteal stages (Days 2-3: early, 5-6: developing, 8-12: mid, 15-17: late, 19-21: regressed) were measured by RT-qPCR. 2) Bovine mid luteal cells were cultured with TGF-β1 (0.0001-10 ng/ml) with or without hCG (1.0 U/ml) for 24 h. P4 concentration in culture supernatant was determined by enzyme immunoassay, and the expressions of apoptosis-regulating factors (FAS, BAX, BCL2, CASP3, CASP8) were assessed by RT-qPCR. 3) Luteal cells were cultured with or without TGF-β1 (10 ng/ml) for 24 h, followed by culture with or without FAS ligand (FASL; 200 ng/ml) for 48 h. Cell viability was measured by WST-8 assay. The statistical significance of differences was assessed by ANOVA followed by a multiple comparison with Bonferroni correction.
RESULTS
TGFBR1 and R2 mRNAs were expressed in bovine CL throughout the estrous cycle, and TGFBR2 mRNA was higher at the late and regressed luteal stages than at the early luteal stage. TGF-β1 dose-dependently reduced P4 production in hCG-treated luteal cells. Luteal cell death was induced by TGF-β1 significantly, and the treatment with FASL promoted TGF-β1-induced cell death in both hCG-treated and -untreated cells. Furthermore, TGF-β1 induced the expressions of FAS, BAX and CASP3 mRNAs.
CONCLUSIONS
The present results in vitro strongly support the idea that TGF-β1 contributes to luteal cell death by activating FAS/FASL-related pathways, resulting in structural luteolysis in cattle.

M91
AGENT-BASED SIMULATION MODEL TO EVALUATE THE ECONOMIC PERFORMANCE OF REPRODUCTIVE PROGRAMS IN BEEF CATTLE.

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BACKGROUND-AIM
The objective of this study was to create a stochastic, agent-based simulation model and then use it to compare the economic performance of different reproductive strategies in beef cattle.
METHODS
The model was parameterized using data from a real herd and the peer-reviewed scientific literature and was implemented using AnyLogic® simulation tool. The scenarios evaluated were: natural mating (NM) only (ONM); one timed artificial insemination (TAI) plus NM (1TAI+NM); two TAI plus NM, with 24, 32, and 40 days of interval between TAI (2TAI/24+NM, 2TAI/32+NM, and 2TAI/40+NM, respectively); three TAI without NM, with 24, 32, and 40 days (3TAI/24, 3TAI/32, and 3TAI/40, respectively), and three TAI plus NM, with 24 and 32 days (3TAI/24+NM and 3TAI/32+NM, respectively). The initial female herd was 400 and remained constant. The bull population varies from 0 to 15, depending on the scenario. The outcomes for each scenario are annual means ± S.E. assessed on 32 farms, using a 5000-day time horizon at one-day time intervals and an animal-by-animal basis.
RESULTS
The 3TAI/24+NM scenario resulted in the highest incomes (US$ 96,479.2 ± 709.8), while ONM had the least value (US$ 79,753.4 ± 741.9). The total operating cost was highest for 3TAI/24+NM (US$ 101,720.6 ± 79.2) and lowest for ONM (US$ 90,898.6 ± 59.2). However, when the total operating cost was evaluated per kg of weaned calf, the highest and lowest costs were for ONM (US$ 2.8 ± 0.0/kg) and 2TAI/24+NM (US$ 2.17 ± 0.0/kg), respectively. The 2TAI/24+NM (US$ -4,651.3 ± 630.7) scenario presented the best net margin, while the lowest result was for 3TAI/40 (US$ -12,590.0 ± 746.3). About profit, the 2TAI/24+NM scenario obtained the best result with US$ -79,867.2 ± 631.1, and the lowest profit was to 3TAI/40, with a value of US$ -87,239.1 ± 744.3. Our model suggests that reproductive strategies that use TAI have better economic performance than those under NM. However, when three TAI were performed with an interval of 40 days, the benefit was lower, and even for some analyzes, it was worse than the ONM.
CONCLUSIONS
Combining TAI with early pregnancy diagnosis resulted in better economic performance than other TAI programs and NM. The 2TAI/24+NM scenario outperformed the others because of the contrast between its high income with moderate costs.

M92
ASSOCIATION OF THE CHARACTERISTICS OF LOCHIA WITH UTERINE INFLAMMATION AND FERTILITY IN DAIRY COWS

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BACKGROUND-AIM
Observation of the color of lochia is useful for the clinical diagnosis of uterine diseases in postpartum cows. However, information on inflammatory response in the very early postpartum period and characteristics of lochia in the cow is scarce. Aim of this study was to clarify association of the characteristics of lochia (color and cytokine level), with development of endometritis and fertility in dairy cows.
METHODS
Lochia samples were collected from 74 Holstein cows at days 2, 5, 9, and 16 postpartum (pp), and endometrial samples were collected at week 5 pp (W5). Pictures of lochia were taken and the color of the samples was quantified by RGB analysis. The R, G, and B components were plotted in three-dimensional space, and the square root value of the sum of three (R, G, and B) squares was named as a "white index". Likewise, the square root value of the sum of three (R − 255, G, and B) squares was named as a "red index". A median of the red indices was set as the threshold. The cows were divided into two groups depending on whether the red index was lower than the threshold (Red group) or higher than the threshold (Non-red group). Expression of cytokine mRNA in lochia and endometrial samples were determined. Uterine cytology was carried out to calculate polymorphonuclear (PMN) cells in endometrial smear samples and endometritis was defined as the presence of >6% PMNs at W5. Fertility at first service was recorded.
RESULTS
White index at day 16 pp and PMN% at W5 showed a positive correlation ($r = 0.518$, $P < 0.05$). PMN% in Red group (5.7 ± 1.3) was higher than that in Non-red group (2.5 ± 0.4) ($P < 0.05$). Cows with endometritis had higher expression of interleukin (IL)-1α, IL-1β, and IL-8 than that without endometritis at W5 ($P < 0.05$) and that of IL-1α and IL-8 tended to increase ($P < 0.1$) from day 5 to 9 pp in cows that conceived at first service.
CONCLUSIONS
White index of lochia and inflammatory cytokine profile from day 5 to 9 pp may serve as an indicator for evaluating the risk of subsequent development of endometritis and fertility in dairy cows.

M93
COMPARISON OF TWO GNRH TREATMENTS (GONADORELIN VERSUS DEPHERELINE) SEVEN DAYS POST-ARTIFICIAL INSEMINATION ON INTERFERON-STIMULATED GENE EXPRESSION DURING THE IMPLANTATION PERIOD IN LACTATING DAIRY COWS.

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BACKGROUND-AIM
Early embryo losses may be a main limiting factor for the efficiency of dairy herds. Several therapeutic approaches have demonstrated that GnRH treatment in the early pregnancy favors pregnancy maintenance. Dephereline is a synthetic analogue of GnRH that has shown a high efficiency in improving luteal function. This study compared the effects of gonadorelin (natural GnRH) or dephereline applied 7 days post-artificial insemination (AI) on interferon stimulated gene (ISG) expression in peripheral blood on Day 21 post-AI.
METHODS
Cows were selected from groups synchronized for fixed-time AI and randomly allocated on Day 7 post-AI in three treatment groups: CONTROL group (C: n=10), gonadorelin group (GON; Cystoreline; 100 µg im; CEVA Salud Animal, Barcelona, Spain) or dephereline group (DEP, gonadorelin acetate [6-D-Phe]; 100 µg im; Gonavet Veyx, Ecuphar, Barcelona, Spain). Pregnancy diagnosis was performed by ultrasound 28 days post-AI. Blood samples were drawn in Tempus Blood RNA tubes (Applied Biosystems, Foster City, CA) on Day 21 post-AI. Gene expression of ISG15 and OAS1 was analysed by real time PCR. The Student's t-test or one-way ANOVA test were used to compare relative ISG gene expression for fixed effects and their interactions. When significant differences were detected, the Bonferroni test was used to examine all possible pairwise comparisons.
RESULTS
No significant effects were observed for treatments on pregnant status. A higher ISG15 and OAS1 expression was observed in pregnant than non-pregnant cows ($P < 0.001$). No effects of GnRH treatments on ISG expression were observed. However, a significant ($P < 0.05$) interaction was detected between treatment and pregnancy. A higher OAS1 expression was observed in pregnant GON and DEP cows respect to non-pregnant GON and DEP cows. These differences were not observed in C group suggesting that there were cows suffering pregnancy loss in this group.
CONCLUSIONS
Although GnRH treatment (gonadorelin or dephereline) on Day 7 post-AI did not increase ISG expression during peri-implantation period, our results suggest that GnRH treatment improves embryo survival.

M94
FOLLICULAR DRAINING OF SUBORDINATE FOLLICLES FOR TWIN PREGNANCY PREVENTION IN BI-OVULAR DAIRY COWS DOES NOT ALTER ANTIOXIDANT MECHANISMS DURING PREGNANCY ESTABLISHMENT.

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BACKGROUND-AIM
Twinning has negative effects on reproductive performance of dairy cows. Follicular transvaginal drainage without ultrasound of subordinate follicles at artificial insemination (AI) prevents the risk of twin pregnancies maintaining the fertility. The aim of this study was to determine gene expression of superoxide dismutase 1 and 2 (SOD1, SOD2), and catalase (CAT) in peripheral blood during the peri-implantation period after follicular ovarian drainage at AI in bi-ovular cows. The effects of GnRH treatment on Day 7 post-AI were also assessed.
METHODS
Bi-ovular cows, determined by ultrasonography and selected from groups synchronized for fixed-time AI, were randomly assigned to control (C: n=8) or follicle drainage (FD: n=11) group. In FD group, the subordinate follicle was punctured and drained with a steel transvaginal cannula designed for follicular cyst puncture (Minitub Ibérica S.L., Spain). Ovulation was recorded as the presence of a corpus luteum (CL)on Day 7 post-AI and cows were then randomly assigned to a no-treatment (NT: n=10), or GnRH group (GnRH: n=9; 100 µg gonadorelin acetate [6-D-Phe] i.m; Gonavet Veyx, Ecuphar, Barcelona, Spain). Pregnancy diagnosis was performed by ultrasonography 28 days post-AI. Blood samples were drawn in Tempus Blood RNA tubes (Applied Biosystems, Foster City, CA) on Day 21 post-AI. Gene expression of SOD1, SOD2, and CAT was analysed by real time PCR. The Student's t-test was used to compare relative gene expression of SOD1, SOD2, and CAT for fixed effects using the SPSS computer package (SPSS Inc., Chicago, IL).
RESULTS
Eight of the 19 cows enrolled became pregnant: 5 (62.5%) C vs. 3 (27.3%) FD cows of which 5 (55.5%) in the GnRH vs. 3 (30%) in the NT group. Follicular drainage, GnRH treatment or pregnancy status had not significant effect on expression levels of SOD1, SOD2, and CAT.
CONCLUSIONS
Our results suggest that follicular drainage at AI does not alter antioxidant mechanisms during pregnancy establishment in dairy cattle.

M96
PERSISTENT SUBCLINICAL ENDOMETRITIS DIFFERENTIALLY AFFECTS GENE EXPRESSION PROFILES OF STROMAL, GLANDULAR AND LUMINAL EPITHELIAL CELLS IN POSTPARTUM DAIRY COWS

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BACKGROUND-AIM
Subclinical endometritis (SCE), impairs the fertility of postpartum dairy cows. Transcriptomic studies related to this condition were mainly based on biopsies from whole endometrium, thus neglecting variations in gene expression in the different cell types.
METHODS
Heathy cows (H, n = 6) and Persistent endometritis cows (PE, n = 10) were identified by endometrial cytology. Biopsies were taken at 44 days postpartum and endometrial cell types (luminal epithelium - LE; glandular epithelium - GE, stroma - ST) were isolated by Laser capture microdissection. Transcriptomic profiles of H and PE cows were determined by RNAseq and differentially expressed genes (DEGs) identified by DESeq2 package in R (adjusted p-value of 0.05).
RESULTS
In PE cows, LE cells over-expressed genes related to leukocyte chemoattraction and protection against inflammatory responses such as members of the chemokine signalling pathway (CCR10 and CCL21), responsible for the recruitment of circulating immune cells into local tissues, and proteins involved in Eph/ephrin signalling (EPHA4, EFNA1) playing an important role in inflammation.
In GE cells, genes preventing epithelial damage and inducing anti-inflammatory and tolerogenic responses were under-expressed in PE cows, thus contributing to a delayed resolution of inflamation. These included genes encoding TGF-β receptors, IL10RA and two interleukin-1 receptor-associated kinases (IRAK3, IRAK4).
In ST cells, genes involved in the resolution of inflammation and mucosal barrier regeneration were over-expressed in PE cows when compared to H cows. These included genes of the interleukin 17 family (IL17B, IL17D), possibly contributing to the recruitment of innate immune cells, and protein members of the R-spondin family of Wnt modulators (RSPO1 and LGR6).
CONCLUSIONS
These results illustrate specific differences between endometrial cell types associated to persistence of inflammation. They stand the cell compartment, rather than the whole tissue, as a physiological unit and provide new insights on possible roles of DEGs on establishment of pregnancy through deregulation of immune-tolerance mechanisms.
This work was supported by FCT (Project UIDB/00276/2020 and PTDC/CVT-CVT/6932/2020) and FORMAS (Grant No 2015-00888).

M97
EFFECT OF 400 IU OF ECG-LIKE GLYCOPROTEIN OR NATIVE ECG IN LACTATING DAIRY COWS ON TAI PREGNANCY RATE

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BACKGROUND-AIM
The aim of this experiment was to compare the effect of the application of 400 IU of eCG-like glycoprotein with native eCG on the pregnancy rate in dairy cows treated with progesterone and estradiol-based TAI program in dairy cows in Argentina.
METHODS
The experiment was conducted in three commercial dairies in Cordoba Province (Argentina) where 472 Hostein cows with a body condition score of 2.9±0.3 (avg± SD, scale 1-5), 2.0±1.3 lactations, 124±100 days after calving, number of previous breeds 0.8±1.7 and a production of 27.3±8.3 litres/cow/day were used. All cows were treated with the same FTAI progesterone and estradiol-protocol: Day 0: 2 mg im of estradiol benzoate (Gonadiol, Syntex, Argentina) and 1 gr intravaginal device insert (DIB, Syntex); Day 8: intravaginal device removal, 500 µg of sodium cloprostenol (Ciclase DL, Syntex), 1 mg of estradiol cypionate (Cipiosyn, Syntex), and were painted in the sacrocaudal region to identify cows that displayed estrus; Day 10: cows that show estrous (>75% paint loss) were inseminated (48 h after intravaginal removal; n=188) and cows that did not display estrous received 100 µg of gonadorelin acetate and were inseminated 12 h later (Day 10.5; n=284). On Day 8, cows were assigned to received 400 IU of eCG like-glycoprotein (reCG; n=187) or 400 IU of native eCG (native; Novormon, Syntex; n=194) or remain untreated (control; n=91). Pregnancy diagnosis was performed by ultrasound at Day 40. Response variable was pregnancy rate (PR) and explanatory variables were treatment, days in milk, body condition score, milk production, ovarian structure on Day 0, lactation, breed number, milk production, estrous manifestation at 48 h (or GnRH treatment) and their interactions. Statistical analysis was performed by logistic regression (PROC GENMOD, SAS).
RESULTS
The overall PR was 38.6% (182/472) and was affected by the treatment (P=0.0332). PR was 29.7% (27/91), 36.6% (71/194) and 44.9% (84/187) for control, native and reCG respectively. reCG treated cows had higher PR than control cows (P=0.0099) and Native treated cows tended to have higher than control (P=0.071). No difference was found in reCG and native (P=0.2757). No other significant effect of the variables on the PR was found.
CONCLUSIONS
eCG like-glycoprotein and native eCG increase equally the PR in a progesterone and estradiol-based synchronization protocol.

M98
IDENTIFICATION OF SPERM PROTEINS AS BIOMARKERS OF FERTILITY IN HOLSTEIN BULLS

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BACKGROUND-AIM
Despite passing stringent quality controls, bulls used in artificial insemination (AI) can vary significantly in their fertility, emphasizing the need for markers of sperm quality related to this trait. This study aimed to identify sperm proteins acting as biomarkers of fertility in two different populations of dairy bulls classified based on their field fertility.
METHODS
Frozen-thawed sperm was collected from: 54 Holstein bulls located in Ireland, classified according to their fertility indexes (FI) as: low-fertility (LF; FI: -0.268 to -0.016, n=23); medium-fertility: (MF; FI: 0.023 to 0.029, n=14) or high-fertility (HF; FI: 0.058 to 0.072, n=17); and 18 Holstein bulls located in Denmark, classified as LF (FI: -0.068 to -0.052, n=8) or HF (FI: 0.062 to 0.114, n=10). The mean FI of both populations of bulls was zero and was based on a minimum of 500 AI per bull. The proteome was measured through LC-MS/MS, and the resulting data were analyzed using R software. After data normalization and filtering, the coefficients of differentially abundant proteins (false discovery rate; FDR<0.1) between HF and LF Irish bulls were obtained from a principal component analysis. Biomarker proteins were defined as those with the highest absolute coefficient values separating HF and MF from LF samples in hierarchical clustering. Their predictive ability was evaluated using a support vector machine as the classifier, using their abundance levels in the Irish bulls to train the model and in the Danish bulls to test it.
RESULTS
Thirty-five biomarker proteins were identified. The prediction accuracy was 89% (p=0.002), with only two HF bulls misclassified, corresponding to the lowest FI bulls in the HF group. Fifteen of the biomarkers were more abundant in sperm of HF bulls, which mapped to seven known genes, three involved with the axoneme and sperm motility (CFAP43, CCDC40 and DNAH17, FDR<0.05). The 20 proteins more abundant in sperm from LF bulls mapped to 12 known genes and were associated with proteasome and protein folding (FDR<0.05).
CONCLUSIONS
A robust model coupled with the application of appropriate bioinformatic tools allowed the identification of functionally relevant sperm proteins predictive of the fertility of Holstein bulls used in AI.
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M99
SPERM TELOMERE LENGTH IN CATTLE IS RELATED TO REACTIVE OXYGEN SPECIES BUT NOT TO REPRODUCTIVE OUTCOMES

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BACKGROUND-AIM
Telomere integrity is known to be important to maintain genomic stability, as telomeres impede the recognition of chromosome ends as DNA breaks by DNA-damage repair mechanisms. The measurement of sperm telomere length (STL) has recently been tested as a novel biomarker for fertility in humans, with studies supporting different conclusions regarding its utility. In animal reproduction, however, this biomarker is much less explored, so that research is required to determine the link between STL, sperm quality, embryo development and fertility outcomes. The aim of this work is to evaluate the relation between bull STL and sperm quality, the intracellular levels of reactive oxygen species (ROS) and fertility rates.
METHODS
For this purpose, pools of three samples from each of the 29 bulls included in this study were used. The STL was analyzed through a quantitative Fluorescent In Situ Hybridization conducted on chromatin-decondensed sperm cells. Motility was assessed using a CASA system, and viability and intracellular ROS levels were determined through flow cytometry. Fertility outcomes were determined as 90-day non-return to estrus rates.
RESULTS
An average telomere length and a standard deviation of 12.13 kb ± 2.75 was observed. No correlation between STL and sperm motility or viability was found (P>0.05), nor between STL and fertility rates (P=0.579). In contrast, a negative correlation between STL and non-viable sperm with increased ROS (H2+/PI+)(Rs=-0.538; P=0.002) was detected. Furthermore, no differences when comparing the bulls with the highest fertility rates (>75th percentile) to those with the lowest fertility rates (<25th percentile) (P=0.161) were found, despite differences between these groups regarding total motility (P=0.003), the percentage of rapid sperm (P=0.007) and the percentage of sperm with high ROS levels (P=0.015) being observed.
CONCLUSIONS
Our data indicate that while bull STL is related to ROS levels in sperm, it is not linked to reproductive outcomes. The strong genetic selection performed in the recent decades in this species could be the reason of obtaining such a homogeneous cohort regarding telomere length and fertility rates.

M100
EFFECTS OF REPEATED OVUM PICK UP (OPU) PRACTICE ON OOCYTE RECOVERY RATES AND MILK PRODUCTION PERFORMANCE DURING THE EARLY-LACTATION STAGE IN HIGH PRODUCING DAIRY COWS

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BACKGROUND-AIM
Although the oocyte collection by ovum pick up (OPU) is performed with minimal stress to the donor the repeated OPU practice may influence cattle productivity. The present study evaluated the effects of repeated OPU practice on oocyte recovery rates (ORRs) and milk production performance in dairy cows of different parity status.
METHODS
Early-lactation (21 DIM) Holstein cows (1st-4th calving; BCS~3-3.5; n=10) were divided into 2 groups; primiparous (P; 1st calving; n=5) and multiparous (M; 2nd-4th calving; n=5). All recipients were superovulated using: Day 0 [CIDR (1.38 g/cow) + P4 (50 mg/cow im) +EB (2 mg/cow im)]; Day 1 [eCG (2,500 IU/cow im)]; Day 4 [OPU]. ORRs were scored in both groups for 3 weeks in a row (from 3rd to 5th lactation week, once OPU per week). The quantitative [milk yield (MY; kg/day)] and qualitative milk production performance [fat (F;%) and protein (Pr;%)] were scored weekly during the 3 OPU weeks. Somatic Cell Count (SCC;x103cell/mL-1) was recorded as an indicator of milk quality/udder health and milk urea (MU; mg 100 mL-1) as a diet balance indicator. Q-Q plots and Shapiro-Wilk test were carried out to determine data normality and Mann-Whitney and Kruskal Wallis test to compare different score means among groups/variables.
RESULTS
ORRs differed statistically between groups in favour of M during the first 2 OPU weeks ($p<0.05$). However, no differences were observed in the 3rd OPU week between groups ($p>0.05$). A significant decrease was observed regarding MY when 1st and 3rd OPU week were compared irrespective of the group (P: 41.2±2.1 vs 31.8±3.2 kg/day; M: 67.7±5.6 vs 51.1±4.3 kg/day; $p<0.05$). Similarly, a significant decrease was detected in F when 1st and 2nd OPU week were compared in both groups ($p<0.05$). A significant increase of SCC was detected in both groups when 1st and 3rd OPU week were compared ($p<0.05$). No significant differences were observed in Pr and MU irrespective of the week/group assessed ($p>0.05$).
CONCLUSIONS
The repeated weekly OPU exerted a negative influence on milk production performance (mainly in MY, F and SCC) in high producing dairy cows. ANID:2020/21201280;Fondef:ID18i10082.

M101
INSIGHTS INTO THE MIRNAS EXPRESSION IN SPERMATOZOA FROM BULLS OF KNOWN FERTILITY

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BACKGROUND-AIM
MicroRNAs (miRNAs) are functional RNA molecules of 22-24 nucleotides that regulate gene expression. In the last decade, miRNAs have been described in spermatozoa from several mammals, including bulls. It is known that miRNAs can act as key gene regulators for early embryogenesis in mice and humans; little, however, is known about the content, expression, and function of sperm miRNAs in bulls.
METHODS
Frozen ejaculated samples from 29 bulls were obtained. Total sperm RNA was isolated using RNeasy kit and treated with DNase I. RNA concentration and purity were determined using the Epoch spectrophotometer (BioTek). To check the lack of ribosomal RNA, we ran the Nano-RNA chip in the Agilent Bioanalyzer. The evaluation of sperm expression of ten bull candidate miRNAs (previously described in bull testis and/or epididymis) was performed according to the manufacturer's protocol and using the thermal cycler 7900 (Life Technologies). The C.elegans miR-39-3p was used as a spike-in exogenous control to normalize expression values. The 2–ΔΔCt method was applied to explore the differential expression (DE) in miRNAs between two groups of bulls with known total fertility potential: high fertile (HF) group (total fertility range -TFR-=43.5 to 39.5) and low fertile (LF) group (TFR=39.3 to 33.3). To identify the DE-miRNAs, the normalized mean Ct value of every single miRNA was compared in both groups using the nonparametric paired Wilcoxon test.
RESULTS
RNA concentration and purity (mean±SD) from the 29 samples were 99.3 ng/µl (±84.6) and 1.97 (±0.72), respectively. Bioanalyzer results showed no ribosomal RNA in the samples, confirming the lack of RNA from somatic cells. In terms of miRNA presence or absence, we consistently detected eight out of ten miRNAs (bta-miR-10b, -138, -146b, -19b, -26a, -449a, -495, -7) and we confirmed the absence of two miRNAs in bull sperm (bta-miR-10a, and -34a). We identified a total of one miRNA down-expressed (bta-miR-138) out of the eight miRNAs present in bull sperm (p-value=0.038) in the LF group compared with those of the HF group (median DeltaCt HF=22.78; median DeltaCt LF=23.42).
CONCLUSIONS
Eight of the miRNA candidates were expressed in bull sperm, and the bta-miR-138 was differentially expressed between HF and LF groups.

M102
CARBENOXOLONE BLOCKS THE GAPJUNCTIONAL TRANSFER IN BOVINE CUMULUS-OOCYTE COMPLEXES

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BACKGROUND-AIM
Carbenoxolone sodium (CBX), a derivative of glycyrrhetinic acid, is generally used as a gap junction inhibitor to investigate intercellular communication in cumulus-oocyte complexes (COCs). However, the most optimal concentration of CBX to block COCs intercellular communication in maturation medium is unknown. We aimed to assess the optimal concentration of CBX supplementation (0, 50 or 100 µM) to the maturation medium by using lucifer yellow (LY) as a fluorescent marker to visualize gap junction blocking in COCs.
METHODS
Twenty-four bovine COCs were held, in vitro, for 1 h in serum-free maturation medium supplemented with 0, 50, or 100 µM CBX. Then, a 2% LY solution was injected directly into the oocyte using a piezo-actuated micromanipulator. Twenty to 30 minutes after injection, the LY flow-through was evaluated by fluorescence microscopy. COCs were categorized as C0 (no flow-through), C1 (flow-through to ≤3 inner cell layers of cumulus cells) or C2 (flow-through to >3 inner cell layers of cumulus cells). These experiments were performed in triplicate resulting in 24 oocytes for each group (0, 50, and 100 µM CBX), including failed injections.
RESULTS
The dye transfer was greater ($P < 0.05$) in the non-CBX supplemented group (0, 4, and 18 COCs in C0, C1, and C2, respectively) in comparison to 50 (7, 9, and 4 COCs in C0, C1, and C2, respectively) or 100 µM (15, 4, and 2COCs in C0, C1, and C2, respectively). No differences were found between 50 and 100 µM supplemented groups ($P > 0.05$).
CONCLUSIONS
LY injection is a suitable method to demonstrate the flow-through from the oocyte across the gap junctions to the cumulus cell. Using this method, we demonstrated that CBX can block gap junctions in the bovine COC, with a numerically higher efficiency reached at a concentration of 100 µM within the maturation medium. In this way, we can gain insight in the components playing a role in the intercellular communication in the oocyte for the purpose of improved oocyte culture.

M103
BULL BREEDING SOUNDNESS EVALUATION (BBSE) OF ITALIAN DUAL BREEDING YOUNG BULLS: A 12-YEAR ANALYSIS

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BACKGROUND-AIM
Bull selection is important for introducing desired genetic traits and enhancing herd reproductive performance; and whether both aims are achieved depends on fertility of the bull. Approximately 65–85% of beef bulls will be classified as "satisfactory potential breeders" when subjected to a BBSE. The objectives of the present research were to define the values of the reproductive characteristics of dual-purpose breed's young bulls and the thresholds of semen characteristics at the end of the performance test.
METHODS
Bulls were classified as a satisfactory, deferred or unsatisfactory potential breeder in accordance with 1993 SFT guidelines. One thousand and eighty-one young bulls were subjected to BBSE for period of over10 years (2009-2021).
RESULTS
The mean ages of bulls were 14.5±1.1, 13.2±018, 13.4±0.8, 14.1±1.7, 1 and 12.4±0.7, for Simmental (S; n = 880), Grigio Alpina (GA; n = 86), Pinzgauer (P; n = 11), Reggiana (R; n = 18) and Rendena (RN; n = 86), respectively. Twenty bulls were eliminated due to physical abnormalities, 198 were deferred for unsatisfactory seminal characteristics and re-evaluated. Of the 198 deferred bulls, 78 were approved and 120 eliminated. Seminal characteristics were as follow: volume (mL) 3.4±1.4, 3.0±1.5, 2.6±0.9, 3,2±1.2 and 3.4±1.3; progressive motility (%) 39.7±20.2, 37.4±20.4, 35.7±20.8, 49.8±18.7 and 29.5±21.7; concentration (x 106) 650.7±387.1, 608.7±345.9, 555.2±268.0, 657.4±437.5 and 594.9±369.6; normal spermatozoa (%) 76.5±14.7, 82.1±11.3, 70.2±17.5, 83.5±10.1 and 73.4±13.3; 1ry abnormalities (%) 8.3±8.1, 6.4±5.9, 11.9±7.1, 5.3±3.2 and 10.4±8.6 and 2ri abnormalities (%) 15.2±10.8, 11.6±8.7, 17.8±12.0, 11.2±7.5 and 16.2±9.5; for S, GA, P, R and RN, respectively. In addition, 263 subjects, 132 S, 39 GA and 42 RN bulls, with approximately the same age, were compared for semen features. RN bulls had higher spermatozoa abnormalities (29%) and lower progressive motility (37%) than S (25.2% and 47%) and GA (19.7% and 46%) ones; while GA bulls presented the lowest percentage of abnormalities (19.7%).
CONCLUSIONS
Major factors influencing spermatozoa morphology were age, breed and influence of heat stress (season of collection). BBSE is an efficient procedure to avoid reproductive shortages due to the bull and may improve reproductive efficiency of daughters.

M105
SAFETY OF DIFLUBENZURON TREATMENT IN BOVINE OOCYTE DONORS

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BACKGROUND-AIM
There is an increasing concern about the potential negative effects on fertility of chemicals used in agriculture and livestock. The diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluoro- benzoyl) urea, DFB) is an insect growth regulator used in the control of ectoparasites in both dairy and beef herds. The toxicity of DFB, however, remains controversial. In this regard, in vitro embryo production (IVEP) offers an excellent experimental model for toxicity studies, both considering the sensitivity of gamete and embryo culture systems, and the scale used. The aim of this study was to evaluate the safety of DFB in bovine oocyte donors and subsequent impact on IVEP.
METHODS
Sound, nulliparous Nelore (Bos taurus indicus) heifers (n=16) were allocated into a control (CG) or treatment (DFB) groups. The heifers were confined and received 100g/head/day of a mineral mix supplement containing (DFB) or not (CG) 1.2 g diflubenzuron 3% (Difly, Champion, Brazil). Heifers were weighed and blood samples were collected weekly, during nine weeks, for analysis of hematological and biochemical endpoints. Heifers were subject to five ovum pick-up sessions performed every other week, beginning just before DFB treatment (week 0). The COC recovered were morphologically evaluated and those classified as viable were send to an IVEP laboratory. Data were analyzed considering the effects of treatment, time, and their interaction, using the Proc Glimmix of the SAS.
RESULTS
There was no effect of DFB treatment on average body weight (P=0.9396), hematocrit (P=0.1632), or in plasma protein (P=0.6144), alkaline phosphatase (P=0.7731), creatinine (P=0.7605) or urea (P=0.1199). In both groups, all blood endpoints remained within the physiological range throughout the experimental period. There were no differences between CG and DFB groups in the number of total (P=0.3694), viable (P=0.3947) or grade I oocytes (P=0.3242), as well as in cleavage (P=0.2893) or blastocyst rates (P=0.5301). Similarly, the proportion of expanded blastocysts (P=0.7913) or the number of embryos with more than 100 cells (P=0.9452) were similar between groups.
CONCLUSIONS
The oral administration of diflubenzuron, within the recommended dose, has no short-term negative effects on oocyte quality or in vitro developmental competence in cattle.

M106
USE OF HUMAN RECOMBINANT FSH FOR IN VITRO EMBRYO PRODUCTION IN CATTLE

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BACKGROUND-AIM
The recombinant human FSH (rhFSH) has been used during in vitro maturation (IVM) of cattle oocytes as an alternative to replace FSH obtained from porcine pituitary (pFSH) and available for veterinary use. Among the advantages of the rhFSH are the lower variation in biological activity and the lack of sanitary risks associated with the use of protein extracts from other species. However, few studies have directly compared the efficiency of rhFSH and pFSH for IVM in cattle. In this study we evaluated in vitro embryo production outcomes using both sources of FSH.
METHODS
Bovine cumulus-oocyte complexes (COC, n=945) recovered from slaughterhouse ovaries and morphologically classified as grades I or II were used. The COC were allocated into three groups, which were IVM in TCM 199 medium: (1) without FSH (-FSH n=315), (2) with 0.5 µg/mL pFSH (Folltropin-V, Vetoquinol, n=315), or (3) with 0.1 UI/mL rhFSH (Gonal, Merck, n=315), under the same culture conditions (38.5°C, 5% CO2). Cumulus expansion was addressed after 22 h of IVM and subjectively graded as poor, average, or good. Semen from a sire with known fertility was used for in vitro fertilization. The presumptive zygotes were cultured under low oxygen tension (5% CO2 and 5% O2, 38,5°C). Cleavage, blastocyst, and hatching rates were evaluated at days 3, 7, and 10 of culture, respectively. Embryo production data were analyzed using the Proc GLIMMIX of the SAS (SAS Institute), and cumulus expansion using the Kruskal-Wallis test.
RESULTS
As expected, cumulus expansion grade was greater (P<0.0001) when FSH was used, regardless the source. There was a significant effect of treatment, but not of replica or treatment*replica, on cleavage and blastocyst rates. The cleavage (day 3) rate was similar (P=0.7339) between groups -FSH and pFSH, but in both groups rates were lower (P<0.05) when compared with rhFSH (71.5% and 70.0% vs. 80.4; respectively. The blastocyst rate was affected (P<0.05) by the source of FSH (34.6%a, 38.0%ab and 46.0%b for -FSH, pFSH and rhFSH, respectively). However, there was no difference (P>0.05) in hatching rates among groups, which only varied (P=0.0024) according to replica.
CONCLUSIONS
The rhFSH improve blastocyst rates and therefore is an alternative to pFSH for IVM of cattle COC.

M107
SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF THE BOVINE ADULT OVARIAN CORTEX REVEALS REMODELING CHARACTERISTICS OF STROMAL CELLS

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BACKGROUND-AIM
The ovary is a dynamic organ comprised of diverse cell populations. Although not often examined, the stroma of the ovarian cortex plays roles in ovarian remodeling that are critical for fertility. In this study, we used single cell RNA sequencing (scRNAseq) to characterize the cell populations of the bovine ovarian cortex and particularly, the cells comprising the stroma.
METHODS
Ovaries of two nulliparous heifers at 49-52 days of pregnancy were harvested and the outer cortex was dissected. Following single cell dissociation, viability >75% was confirmed by trypan blue staining. Cell suspensions were processed for individual barcoding and cDNA synthesis before undergoing scRNAseq using the 10x Genomics platform. Initial analysis using the CellRanger pipeline indicated that 7326 and 8419 cells were sequenced from each sample, and alignment to the bovine genome was 95 and 96%, respectively. Following normalization, downstream analyses were performed using the Seurat package of R. An elbow plot was used to determine the fit of the data before mapping into clusters to create a UMAP.
RESULTS
We observed eight cell clusters in the ovarian cortex samples. Using the 10 most differentially expressed genes in each cluster based on adjusted P-value, populations were identified as stromal, epithelial, endothelial, immune, and follicular cells. The cluster classified as stromal cells had 3 subpopulations that were further examined using the Ingenuity Pathway Analysis software. The top biological functions upregulated in the stromal subgroup 1 were angiogenesis, cell contact and apoptosis; in subgroup 2, organ development and migration of connective tissue and fibroblasts; and in subgroup 3, cellular movement, immune cell trafficking and cell viability. Transcripts involved in remodeling of the extracellular matrix such as COL1A1, COL1A2, COL3A1, COL5A1, MMP2, MMP14 and MMP28 were upregulated in subgroup 2, indicating that this cell population may play a role in ovarian remodeling.
CONCLUSIONS
In conclusion, scRNAseq of the bovine ovarian cortex showed the presence of 8 distinct cell clusters, among which subgroups of stromal cells could be identified. Gene expression patterns indicate that these subgroups might have specific roles in the remodeling necessary for proper ovarian function and fertility.

TOPIC Canine and feline reproduction

M109
THE EFFECTS OF GLUCOCORTICOID TREATMENT ON OVARIAN AND TESTICULAR FUNCTION OF DOMESTIC CATS

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BACKGROUND-AIM
The reproductive success of many felids in captivity is often poor. While this may be partly attributed to captivity-related stress, direct physiology evidence is lacking. Glucocorticoids (GC) mediate many of the negative effects of stress on reproduction. This study aimed to examine the effects of GC treatment on the ovarian and testicular function of domestic cats.
METHODS
Post-pubertal queens (n=12) and tom cats (n=16) were allocated evenly into treatment (T) and control (C) groups. Treatment cats were given 1 mg/kg/day oral prednisolone for 45 days (queens; duration of ovarian stimulation regime) or 50 days (toms; duration of sperm cycle). All queens were given 0.088 mg/kg/day altrenogest from Day 0-37 to suppress follicular growth. On Day 40, 75 IU eCG was given to stimulate follicular growth, followed by 50 IU hCG 80 h later to induce ovulation. Queens were spayed 30 h after the hCG treatment, after which the number of ovulations per cat was assessed, the oviducts flushed, and oocytes collected for morphological evaluation. Toms were neutered at the end of the 50-day treatment period and cauda epididymal sperm retrieved for assessment. The testes were fixed for histological assessment.
RESULTS
The number of ovulations per cat (10.5±1.1) and oocyte recovery (47.6±9.7%) did not differ between T and C cats. Oocyte diameter (54.4±0.8 µm) did not differ between the groups, but the zona pellucida was thinner in T cats (3.1±0.3 µm vs. 4.1±0.3 µm; P=0.03). Based on morphology, a greater percentage of poor quality oocytes were retrieved from T cats (26.7%) than C cats (13.6%; P=0.04). In toms, sperm motility was similar between T and C groups, but T cats had higher proportions of primary (18.1±2.2% vs 8.2±1.2%; P<0.001) and secondary (38.6±1.7% vs 27.6±1.0%; P<0.001) sperm abnormalities. This may be related to the higher Sertoli cell load in T cats (11.5±0.8 germ cells per Sertoli cell) than C cats (9.4±1.2 germ cells per Sertoli cell; P<0.001) or abnormal epididymal function.
CONCLUSIONS
In conclusion, we produced strong evidence that GC treatment can adversely affect gonadal function, as evidenced by alterations of both oocyte and sperm morphology. Whether the observed changes in gamete morphology would affect fertility warrants further investigation.

M110
INFLUENCE OF SPERM CRYOPRESERVATION ON DNA INTEGRITY AND FERTILIZATION POTENTIAL IN DOGS

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BACKGROUND-AIM
Sperm cryopreservation in dogs has a primordial role in maintaining breeding lineages during long periods, aiming to propagate genetic material even post mortem. On the other hand, cryopreservation may reduce fertility rates and the impact on sperm DNA is still not completely known in dogs. Thus, the aim of this study was to identify the influence of sperm cryopreservation on DNA, with simultaneous assessment of plasmatic, acrossomal, and mitochondrial membranes in dogs.
METHODS
For such purpose, 13 dogs were assigned to two experimental groups: Fresh-semen group and Frozen-semen group. Initially, a simultaneous assessment of sperm plasmatic, acrossomal and mitochondrial membranes was standardized for dogs with a triple stain technique (PI, FITC-PSA, JC-1 fluorescent probes, respectively), with further analysis of computer-assisted analysis of sperm motility (CASA). Sperm samples were either kept at 5°C (intact sperm) or submitted to snap-frozen (damaged sperm) and then mixed in order to obtain a known and progressive proportion of intact sperm (0%, 50% and 100%). Subsequently, sperm samples of both groups were analyzed using the referred triple stain technique, as well as the analysis of CASA and sperm DNA integrity, by means of TUNEL (Terminal dUTP Nick End Labelling) and sperm chromatin protamination (CMA3) assays. Linear regression was performed with Guided Data Analysis by SAS for the triple stain standardization and differences between groups were analyzed using T test (P<0.05).
RESULTS
A high linear regression coefficient between the expected proportion of intact plasma membrane, intact acrosome and high mitochondrial function (IPIAH) was achieved for dogs (P<0.01; R2=0.9236). Fresh-semen (78.8±3.9%) had higher IPIAH than frozen-semen (40.1±3.6%). Moreover, post-thaw semen (4.1±0.7% and 3±0.5%) had higher DNA damage analyzed by TUNEL and CMA3 technique, respectively, in comparison to fresh-semen (0.8±0.2% and 0.8±0.2%).
CONCLUSIONS
In conclusion, triple stain technique is a feasible methodology for sperm analysis in dogs. In addition, sperm cryopreservation has an important negative effect on overall membrane integrity, as well as DNA integrity, which may impact canine fertilization potential of dogs.

M111
ULTRASOUND-GUIDED FUNICULAR BLOCK: ROPIVACAINE INJECTION INTO THE SPERMATIC CORD TO IMPROVE ANALGESIA IN CATS UNDERGOING ORCHIECTOMY

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BACKGROUND-AIM
Cat orchiectomy is a common procedure carried out in general practice and requires general anaesthesia and effective analgesia. Surgical analgesia is very important because pain causes various negative effects that prevent patient recovery. The aim of this study was to compare intraoperative analgesia in two groups of cats undergoing orchiectomy.
METHODS
This was an assessor-blinded, randomised, clinical research study. Forty healthy cats were operated on by the same team, with a scrotal approach for all animals. All cats were assigned in two groups (n=20) to receive pre surgery ropivacaine hydrochloride (0.2 mL/kg at 0.5% concentrate) into the spermatic cord (R Group), or an equal volume of NaCl 0.9% at the same sites (C group) in general anesthesia. A fentanyl bolus (2 µg/kg) was administered intraoperatively in response to an increase in blood pressure, heart rate or respiratory rate during surgery (> 30% compared with the pre-incisional values). The intraoperative evaluation was carried out using the hemodynamic stability parameters and eventually administration of rescue analgesia.
RESULTS
Repeated-measures ANOVA showed significant differences in the comparison of intraoperative heart rate among different times (p < 0.0001), groups (p<0.0001) and interaction between time and group (p=0.025). ANOVA test showed significant differences in respiratory rate among various times (p = 0.001), between groups (p<0.0001), but not in the interaction between time and group (p=0.267). In addition, ANOVA test showed a significant difference also in blood pressure values among the various times (p < 0.0001), between groups (p<0.0001) and in the interaction between time and group (p<0.0001). Intraoperative rescue analgesia was administered 6 times in C group and 1 time in R group. Multivariate analysis showed a statistically significant association between rescue analgesia and group; no further associations were observed between outcomes and determinants (p>0.05). As result cats in R group showed better intraoperative hemodynamic stability compared to the C group.
CONCLUSIONS
The ultrasound-guided funicular block used in this study, as already demonstrated in dogs, is a good method to protect the cats from surgical pain and ensure a good level of surgical analgesia. Considering the cost, availability, restrictions, and possible side effects, routine use of this block is considered desirable in in daily clinical practice.

M112
CAN OBESITY AFFECT THE GLYCOALYX OF DOG SPERM?

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BACKGROUND-AIM
The role of overweight and obesity in the development of subfertility in males has generated a considerable amount of interest in recent years. However, there is no consensus on whether overweight or obesity impaired sperm quality. Sperm glycocalyx is the first interface between sperm and the environment, therefore it is involved in the acquisition of fertilizing ability. This study was designed to investigate the effect of overweight on sperm quality parameters and glycan pattern of sperm glycocalyx from normal and obese dog ejaculates.
METHODS
Ejaculates (sperm-rich fraction) were collected by digital manipulation from ten adult dogs of different breeds (n=3 normal and n=7 obese dogs). They were divided in two aliquots: one used for assessing sperm physical and kinematic properties (sperm concentration, vitality, motility and morphology) as well as percentages of sperm DNA fragmentation using Halosperm assay and one for analysis of sperm surface glycome. The latter aliquot was fixed in 4% (v/v) buffered paraformaldehyde, washed, smeared on slides and incubated with appropriate dilutions of 15 fluorescent lectins. Lectins are currently used for deciphering the composition of sperm glycocalyx.
RESULTS
Compared to normal sample, sperm from obese dogs did not show significant impairment of motility and morphological properties as well as DNA integrity. Lectin histochemistry revealed a light changes in the staining intensity of spermatozoa from obese dogs. Specifically, acrosomal cap showed a RCA120, PHA-E, HPA, and LCA binding sites decrease and Con A, SBA, and PNA increase. In addition, the tail displayed highest SNA, RCA120, Con A, PHA-E, PNA, GSA I-B4, and LTA binding intensity and disappearance of DBA reactivity.
CONCLUSIONS
These results indicate that obesity could affect the glycosylation status of ejaculated spermatozoa surface of dog. Surface of the plasma membrane of mammalian sperm becomes coated with various glycoproteins secreted by the epididymal epithelium during sperm transit through the epididymis. Since increased levels of chronic inflammatory factors occur in male reproductive system of obese mammalian males, including epididymis, the observed changes in sperm glycocalyx pattern could be related with the obesity status.

M113
GENITAL MYCOPLASMA PREVALENCE IN THE HEALTHY AND SUB-FERTILE BREEDING DOG

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BACKGROUND-AIM
Canine reproductive failure can be the result of poor breeding timing, genetic incompatibility, inflammation, or infectious disease. While bacteria such as Brucella canis are known to cause reproductive failure, mollicutes such as Mycoplasma have a more controversial role in canine infertility. While at least 17 mycoplasma spp have been isolated from dogs, M. canis, considered an opportunistic pathogen, and M. cynos, a proven respiratory pathogen, are most common. We hypothesized that a similar pattern would be noted in breeding dogs, with M. cynos having a more significant effect on fertility than M. canis. The objectives of this study were to determine Mycoplasma prevalence in dogs presenting for breeding management to the University of Georgia Veterinary Teaching Hospital, and to determine pregnancy rates in Mycoplasma-positive dogs.
METHODS
13 dogs with a history of infertility, and 56 dogs presenting for routine breeding management were enrolled in the study. 100 uL of semen (n=26), or a vaginal cytology swab (n=41) were collected during routine breeding management. Nucleic acids were extracted from samples using QIAamp cador Pathogen Kit (Qiagen, Hilden, Germany) and a QIAcube nucleic acid extraction system (Qiagen). Samples were stored at -20˚C until analysis. Samples were analyzed for M. Cynos and M. Canis using a previously validated multiplex real-time PCR assay.
RESULTS
Three of 13 dogs presenting with a history of infertility were positive for M. cynos, and 3/13 were positive for M. canis. Four out of 5 females bred to males positive for M. cynos failed to conceive, while 0/2 females conceived when bred to males positive for M. canis. The pregnancy rate for females bred to males negative for Mycoplasma spp was 89% (8/9). Seven out of 41 females tested positive for Mycoplasma (M.cynos = 4, M.canis =3), and all six that were bred did not conceive. Of Mycoplasma- negative dogs that were subsequently bred, 63% (12/19) conceived.
CONCLUSIONS
Collectively, this suggests that Mycoplasma sp. may contribute to canine infertility. The presence of M. cynos in semen samples decreased conception rate but didn't prevent all pregnancies. While no females positive for mycoplasma sp. conceived, the low prevalence in the breeding population suggests the need for follow up studies with positive females.

M114
DISACCHARIDES AND VITRIFICATION OF FELINE OOCYTES

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BACKGROUND-AIM
Today, vitrification is considered the election method for create oocyte and embryo banks to the conservation of threatened species, both domestic and wild animals. However, a reliable protocol for feline species oocyte cryopreservation has not yet been obtained. Determine the influence of sucrose and trehalose as cryoprotective agents in the vitrification of queen oocytes by direct drops on liquid nitrogen, is the objective of this study
METHODS
222 immature oocytes were obtained by slicing of 17 ovaries from sterilized cats. Using Colombo et al. (2021) modified vitrification protocol, in each experiment two groups were established: oocytes vitrified with 0.5 M sucrose or with 0.5 M trehalose. The oocytes were transferred to the holding medium (HM: TCM 199-H supplemented with 20% fetal bovine serum) for been maintained until be processed, and then pass in groups of five to the equilibration medium (EM: HM with 7.5% EG and 7.5% DMSO) for 15 minutes. Finally, the oocytes remained for less than 90 seconds in the vitrification medium (VM: HM with 15% EG and 15% DMSO), and they were transferred directly to sterile liquid nitrogen in a 5 µl microdroplet, each drop containing 5 oocytes. The spheres formed were stored in cryotubes until their use. Warming was performed in decreasing concentrations of the corresponding disaccharide, and the survival degree was established using "Brilliant Cresyl Blue" staining (BCB). Oocytes were incubated in 26 µM of BCB diluted in PBS for 90 min at 38.5°C in humidified air and assigned to two groups: BCB+ (blue cytoplasm) and BCB- (colourless cytoplasm). Differences between treatments were analysed using Pearson's Chi-square test and were considered significant when $p < 0.05$.
RESULTS
Although no significant differences were found between the two treatments ($p = 0.234$), the survival rate of oocytes vitrified with sucrose was higher than that of oocytes vitrified with trehalose (72.73% vs 61.22%).
CONCLUSIONS
The results have been positive in both cases, and although sucrose is the most widely sugar used in conservation process, either of the two disaccharides would be a good option in the vitrification of feline oocytes.

M115
PRELIMINARY STUDY ON THE OCCURRENCE OF MAMMARY GLAND TUMORS IN BITCHES IN POLAND

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BACKGROUND-AIM
Canine mammary tumor (CMT) is the most frequently diagnosed neoplasia in bitches (1). CMT occurs mainly in bitches aged 9-11 (4). It is estimated that about 50% of all CMTs are malignant. The most frequently diagnosed malignant tumor (MT) is adenocarcinoma, while among benign tumors (BT) the benign mixed tumor is the most common (1,5). According to the available CMT literature, they are most often found in poodles, English springer spaniels, cocker spaniels, English setters, poiners, German Shepherds, Maltese, Yorkshire terriers and dachshunds (1). However, the occurrence of CMT is different in different geographical areas. In order to compare the data, the occurrence of CMT among bitches in Poland in the years 2016-2018 was examined.
METHODS
158 CMT from 111 bitches (mostly intact) were evaluated histopathologically (HE staining). The tumor type, grade of malignancy and mitotic index were determined in accordance with the WHO histological classification guidelines from 1999. Statistical analysis of the results was carried out.
RESULTS
The average age of dogs with CMT was 9-12 years. MT constituted 70%, BT-24%, and non-neoplastic lesions-6%. The most common malignancies were: adenocarcinoma (16% of all tumors), simple carcinoma-15%, complex carcinoma-13%, mixed carcinoma-9%. Among BT complex adenoma-10% and benign mixed tumor-7% predominated. Among non-neoplastic lesions, connective tissue growth-3% predominated. II Grade of malignancy predominated- 45%, grade I-29% and grade III-26%. The most common CMTs occurred in: crossbreed-32%, Yorkshire Terrier 22%, German Shepherds-6% and Dachshunds-6%.
CONCLUSIONS
The results are mostly consistent with the literature data, however, it seems that malignant CMTs occur much more often than described so far. This indicates a growing clinical problem. The large diversity of neoplastic lesions and the diversity of the group (different breeds and ages of dogs) means that further research needs to be done.
References:
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M116
RARE CONGENITAL DISORDERS OF REPRODUCTIVE TRACT IN THE BITCH

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BACKGROUND-AIM
In female mammals, reproductive tract develops from Müllerian ducts [1]. Segmental aplasia of the paramesonephric ducts results in anomalies of anterior vagina, cervix, uterus and uterine tube. Disorders of ontogenic differenciation are rare in dogs, but among them septa and strictures are the most common [2]. A case of blind vagina has only been described in mare and in queen [3,4].
METHODS
A two-years old Bull terrier bitch was referred to clinic due to lack of heat symptoms. The bitch was clinically healthy but an ultrasound examination revealed mucometra and the bitch was treated aglepriston for 3 weeks (5 dosis of 10 mg/kg). No discharge was noticed and another ultrasound examination showed further enlargement of the uterus, especially visible in the area of uterine body. Vaginal endoscopy was performed and showed no external opening of the cervix was visible. An ovariohysterectomy was performed. During surgery, a massive enlargement of uterine body, consisting of two ampoules and significant stricture between them was seen. The ampoules were filled with serous fluid. Both uterine horns looked normal and both ovaries had multiple corpora lutea. Histopathological examination (HP) of the whole reproductive tract was performed. Karyotype and the presence of SRY gene were also assessed.
RESULTS
The HP revealed general aplasia of the mucous membrane of the reproductive tract (uterine horns, uterine body and uterine ampullas). Epithelial metaplasia was seen (in both ampullas stratified squamous epithelium was present The stricture visible during the surgery histopathologically corresponded to the cervix. Karyotype of this bitch was XX and the SRY gene was absent, so disorder of sex development was excluded.
CONCLUSIONS
This is the first described case of co-existence of this rare congenital anomalies: cervix stricture, blind vagina, mucous membrane aplasia and epithelial metaplasia together with the presence of mucometra. The cause of these disorders is unknown, however genetic mutations are the most probable.
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M117
DYSURIA AS A SYMPTOM OF ATYPICAL LOCALIZATION OF A VAGINAL TUMOR

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BACKGROUND-AIM
Tumors of vagina in bitches are rare and represent around 3% of all canine neoplasias [1]. However, after mammary gland tumors, they are the second most common neoplasias of reproductive tract in bitches [2]. Most of those tumors are benign, being leiomyoma the most common type. Sexual hormones are considered a great risk factor for this neoplasia. They usually occur in older bitches. The symptoms may include hemorrhagic vulvar discharge, perineal enlargement, tenesmus and usually vaginal masses are protruding from vaginal lips. Surgical excision is the recommended treatment.
METHODS
A 7-year-old intact bitch was referred to the clinic due to dysuria. The bitch had no other symptoms. Catheterization of the urethra was impossible. Vaginal examination showed no abnormalities. An ultrasound examination revealed a large vaginal mass about 7 cm x 3.5 cm in abdominal cavity and had irregular and solid echostructure. An X-ray showed no visible metastasis to the lungs or other in abdominal cavity. Blood test results were normal, apart from slight elevation of the urea (46,9 mg/dl, normal range: 20-45 mg/dl). A laparotomy was performed. Surgery revealed multiple adhesions of the mass to the rectum and to the urethra. All adhesions were removed, vaginal mass was removed and ovariohysterectomy was performed. Antibiotic therapy (enrofloxacin 5 mg/kg/daily during 7 days) together with anti-inflammatory drugs (meloxicam 0,2 mg/kg/daily during 5 days) was prescribed to the bitch. The bitch recovered soon after the surgery. The mass was sent for histopathological examination.
RESULTS
The histopathological examination showed that the vaginal tumor was fibroma.
CONCLUSIONS
A case of large benign vaginal tumor that protruded into pelvic cavity and caused pressure on the urethra and dysuria is a rare finding.
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M118
EFFECT OF TREHALOSE IN CANINE OOCYTE VITRIFICATION

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BACKGROUND-AIM
The domestic dog serves as an animal model for assisted reproductive studies of endangered canine species. The success of in vitro embryo production is not very high, moreover only few studies have achieved successfully results with regard to oocyte preservation. The aim of this study was to evaluate the effect of sucrose and trehalose on vitrified canine oocytes survive rate.
METHODS
A total of 111 immature oocyte cumulus complexes (COCs) were obtained from 24 ovaries of ovariectomized bitches in private clinics. In each experiment, two groups were established: oocytes vitrified with 0.5 M sucrose or with 0.5 M trehalose. All COCs were maintained in holding medium (HM: TCM 199 supplemented with 20% foetal bovine serum) until be processed. In groups of 5 oocytes pass to equilibration media (EM: HM+4% EG) for 5 min. Then they were exposed to first vitrification solution (VM1: HM+10% EG+10% DMSO) for 1 min and finally placed in sucrose or trehalose second vitrification solution (VSII: HM+20% EG+20% DMSO+0.5 mM sucrose and HM+20% EG+20% DMSO+ 0.5 Mm trehalose) for 30 sec. Finally, COCs were vitrified directly plunged into the liquid nitrogen. The spheres formed were stored in cryotubes. Warming was performed with decreasing concentrations of the corresponding disaccharide, then survival degree was established using "Brilliant Cresyl Blue" staining (BCB). Oocytes were incubated in 26 µM of BCB diluted in PBS for 90 min at 38.5°C in humidified air and assigned to two groups: BCB+ (blue cytoplasm) and BCB- (colourless cytoplasm). Differences between treatments were analysed using Pearson's Chi-square test and were considered significant when $p < 0.05$.
RESULTS
The percentages of oocytes BCB + vitrified with sucrose (65.38%) did not show significant differences (p= 0.382) in comparison to oocytes BCB + vitrified with trehalose (63.63%).
CONCLUSIONS
Although the most common sugar in vitrification protocols is sucrose, with these results we can conclude that trehalose also offers protection to canine oocytes in the vitrification protocol through droplet.

M119
IMMUNODETECTION OF LEPTIN RECEPTOR IN CANINE OOCYTES IN TWO DIFFERENT STAGES OF THE OESTRUS CYCLE.

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BACKGROUND-AIM
Leptin (Ob) has been shown to play a role in the regulation of the female reproductive system acting on the gonadotropic axis and on the ovaries. Ob exerts its biological effects by interacting with its receptor (Ob-R) which has been detected in the ovarian cells, including thecal cells, granulosa cells, and oocytes of several species. In the canine species, the expression and localization of Ob-R in oocytes has not been previously documented. In this study we explored the Ob-R protein expression in canine oocytes in two different stages of the oestrus cycle.
METHODS
Canine cumulus-oocyte complexes (COCs) were recovered from ovaries of adult bitches (n=15) undergoing routine ovariohysterectomy in two different stages of oestrus cycle (anestrus and estrus). After fixation and permeabilization, COCs (n=30 for each stage) were incubated with the primary antibody (1:50, mouse anti Ob-R) overnight at 4°C, followed by incubation with an anti-mouse fluorescein isothiocyanate secondary antibody (1:100). Nuclei were counterstained with Hoechst 33342. COCs were mounted on glass slides and observed using a confocal laser-scanning microscope. The fluorescence intensity of the cytoplasm area of the oocytes was measured at the equatorial plane. The statistical significance of the results was evaluated by the Analysis of Variance (ANOVA).
RESULTS
Ob-R was detected in cumulus cells (CC) and in the cytoplasm of all oocytes analyzed. Ob-R was localized within all layers of CC. Ob-R was homogeneously distributed throughout the cytoplasm of the oocytes in both stage of the oestrus cycle. The analysis of fluorescence levels revealed that the oocytes collected from bitches at the estrus stage exhibited a significantly higher protein content compared to those collected in the anestrus stage($P<0,001$).
CONCLUSIONS
Leptin receptors are expressed in canine COCs. The presence of an increased expression of Ob-R in oocytes recovered from bitches in estrus indicates that Ob could be involved in oocyte growth and maturation. Further investigations are needed to understand the role of Ob in oocyte in vitro maturation and its potential in enhancing meiotic competence of bitch oocytes.

M120
UPTAKE OF EXOGENOUS OESTRADIOL BY FEMALE DOGS: REPORT OF TWO CASES

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BACKGROUND-AIM
Vaginitis and ovarian remnant syndrome (ORS) are often associated with clinical signs of heat in young bitches and in bitches that have been previously spayed, respectively. The inclusion of exogenous estrogens as a differential diagnosis is often disregarded and may lead to misinterpretation of the case.
METHODS
Clinical Case: Bitch 1: 1.4 years-old Teckel, OVH by laparoscopy at 9 months of age. The bitch was presented because of suspected ORS with oedema of the vulva, licking of the vulva, muco-sanguinolent vulvar discharge, turgid nipples and moderate ventral hyperpigmention. Hair clipped before the OVH was still not growing. Bitch 2: 3 months-old Teckel with oedema of the vulva and turgid nipples, that has been with the owner for one month. Vaginoscopy, vaginal cytology, ultrasound and P4 measurement were performed when possible.
RESULTS
Bitch 1: Vaginal mucosa crenulation and a cytology with 60% of keratinized epithelial cells and 40% of intermediated cells with signs of keratinization were observed. Progesterone concentration was 0.54 ng/mL. On ultrasound (US), no ovarian remnant was observed but the uterine stump was hypertrophied and E. coli was isolated from the vaginal swab. Bitch 2: Vaginal cytology showed intermediate and parabasal cells in high number. Progesterone was 0.2 ng/mL. Vaginoscopy ans US were not performed. In both cases, female owners were on hormonal therapy by means of a transdermal estrogen spray applied to the skin of the forearm (oestradiol -1.53 mg/spray) because of menopausal symptoms. In the bitch 1, vaginal cytology revealed intermediate and parabasal cells in moderate number 2 weeks after the owner stopped using the spray and the female stopped the liking behaviour. Four weeks later, the vulva was less swollen and the hyperpigmentation disappeared. In the bitch 2, vaginal cytology revealed intermediated and parabasal cells in low number and vulva was less swolen, 4 weeks after the owner stopped the treatment. At the moment both females are still being followed.
CONCLUSIONS
The uptake of exogenous estrogens should be taken into consideration as a differential diagnosis for heat symptoms in juvenile and spayed bitches, as females can come into direct contact with oestradiol when petted by the owners. A thorough anamnesis is crucial to identify the source of estrogen of the affected females pet animals. Also, women´s gynecologist should be aware of this problem.

M121
TOTAL PROTEIN QUANTIFICATION BY BRADFORD'S PROTOCOL IN FROZEN CANINE SEMEN SAMPLES

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BACKGROUND-AIM
The total protein quantification is great importance in different samples and tissues, about various aspects. For example, the enzymatic activity is calculated based on the amount of protein in the sample where the result is expressed in U enzyme/mg protein. Moreover, the sperm concentration may affect the amount of protein in sample, due to variation in cell numbers. The aim of this study is report total protein quantification in frozen canine semen using Bradford's protocol, and your correlation with sperm concentration.
METHODS
The semen of six dogs, males, frenchie bulldog, between 2 and 5 years, was collected by digital manipulation and sperm concentration was analyzed with Neubauer chamber. The semen was frozen at -196°C in liquid nitrogen, using Trisegg yolk, ethylene glycol 5%, and five different concentrations and one control group of melatonin (0 mM, 1 mM, 1,5 mM, 2,0 mM, 2,5 mM e 3,0 mM) for evalution of your antioxidant function. Six samples of each treatment was thawed at room temperature for protein analyses, and 3µL of each sample and 297µL of Bradford reagent (Sigma-Aldrich) were used (1:300 v/v) in duplicate, for absorbance measurement in spectrophotometer at 595nm. Eight protein standard of bovine serum albumin (BSA) were used, in duplicate, and calibration curve it was established. Therefore, the protein in each sample was calculated using calibration curve equation y = 1,723x – 0,053 (r2=0,986) being that X is sample absorbance and Y is amount of protein, in milligram.
RESULTS
The data has been evaluated in statistic software R, with Shapiro-Wilk test and F Test for media testing and there was no difference (p>0,05) between different treatments using melatonin. A correlation between total protein and sperm concentration has been evaluated by Pearson's correlation test in Software R, and there is no correlation between sample protein and sperm concentration.
CONCLUSIONS
Based on this study, the Bradford's protocol was a excellent test for total protein quantification in frozen canine semen samples. Moreover, the sperm concentration has no influence on the amount protein in semen.

M122
SEX REGULATION OF THYROID GENE EXPRESSION

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BACKGROUND-AIM
Gender effect in thyroid physiology has been observed in other species as well as in canines. In the present study, we aimed to investigate the effect of gender on thyroid gene expression of Thyrotropin receptor (TSH-R), thyroglobulin (Tg), thyroperoxidase (TPO), and androgen receptor (AR) by real-time PCR in healthy thyroid canine glands.
METHODS
Male (n=7) and female (n=8) adult healthy dogs were used for this study. Thyroids were obtained from dogs that were euthanized after suffering severe trauma, and after receiving the signed consent of the owners for the participation of their animals in this study. Total RNA from thyroid was extracted using TRIZOL followed by DNase-treatment and after determining RNA concentration and purity, cDNA was synthesized by reverse transcription using the SuperScript III First-Strand Synthesis System Kit with random primers. Samples were analyzed in duplicate in a 72- disk Rotor-GeneTM Corbett 6000. Gene expression was measured by relative quantification to the exogenous control and normalized to the endogenous control genes (HPRT and Beta actin). Transcript data were analyzed using the mixed procedure and model included the effect of gender as fixed effect.
RESULTS
Male dogs presented greater mRNA expression of TSH receptors (2.2 vs 0.43, P=0.018) and Tg (3.2 vs 1.8, P=0.0021) than female dogs. On the other hand, gender did not affect the concentration of TPO mRNA (P=0.27) and AR mRNA (P=0.20).
CONCLUSIONS
These preliminary results suggest that gender may affect the capacity to synthesize thyroid hormones due to a differential status of gene expression.

M123
DOG SEMEN EXPRESSES AQUAPORINS 3 AND 11

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BACKGROUND-AIM
Aquaporins (AQP) are transmembrane proteins which main function is water trafficking to maintain cell homeostasis. The presence of AQP has been demonstrated in human, mouse, mice, bull, donkey and horse semen (1). However, studies in dog semen are scarce, being AQP 1 and 8 the only ones described. Thus, the aim of the present study was to evaluate the presence of AQP3 and 11 in dog semen.
METHODS
Six male dogs from 1 to 9.5 years were included. Ejaculates were collected by manual stimulation and only the rich fraction was kept. Rich fractions were centrifuged at 13000 xg during 5 minutes. The supernatant was discarded and the pellet was snap-frozen in liquid nitrogen and kept at -80ºC. Pellets were homogenized in cold-homogenization buffer and further centrifuged at 13000 xg 4ºC for 10 minutes. Total protein concentration of supernatants was determined by the Bradford method. Further, the expression of AQP3 and 11 were evaluated by Western blotting.
RESULTS
Both AQP3 and 11 were expressed in canine spermatozoa. These AQPs have been widely described in sperm cells of different mammal species. AQP3 is an aquaglycerolporin, meaning it is also permeable to glycerol besides to water (2), and has been related to cryotolerance in boar semen (3,4). AQP11 is a superaquaporin and has been suggested to be permeable also to glycerol in human adipocytes (5) and has been correlated with sperm motility and membrane integrity (3,4), but not to cryotolerance (3,4).
CONCLUSIONS
Canine sperm cells express AQP3 and 11. However, further research in canine semen is warranted to establish a potential role of AQP3 and 11 since neither motility nor cryotolerance were evaluated.

M124
RESPIRATORY AND ACID-BASE FEATURES OF PREGNANT BRACHYCEPHALIC BITCHES

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BACKGROUND-AIM
During pregnancy, the respiratory adaptation is one of the most notorious physiological changes. However, gestational adaptations can be jeopardized by preexisting disorders, such as breathing anomalies, negatively influencing maternal health and fetal development. Dogs with brachycephalic conformation are predisposed to airway obstruction (brachycephalic obstructive airway syndrome), leading to wide range of clinical signs and even life-threatening risk during pregnancy. Thus, the present study aimed to analyze respiratory and metabolites features of brachycephalic bitches throughout gestation compared to non-brachycephalic.
METHODS
Twenty bitches were assigned to 2 experimental groups: brachycephalic syndrome (n=10) and non-brachycephalic syndrome (control, n=10). Females were evaluated at pregnancy diagnosis (33-36 days after ovulation), mid-to-late pregnancy (43-46 days) and late-pregnancy (56-59 days). Bitches were clinically evaluated, from which a severity score of the brachycephalic syndrome was established. Mean arterial pressure (MAP), systolic (SBP) and diastolic (DBP) blood pressure and peripheral oxygen saturation were analyzed throughout pregnancy. Venous and arterial blood samples were collected in order to perform glycemia, lactatemia and acid-base analysis, respectively. Variables were analyzed by an interaction group x time, and Student's T and LSD tests (P≤0.05).
RESULTS
Non-brachycephalic group showed decrease in HCO3, TCO2, heart rate (HR) and base-excess (BE), and an increase in capillary refill time (CRT) throughout gestation. Conversely, brachycephalic group had lower HR, HCO3, TCO2 and BE at pregnancy diagnosis, and lower CRT at the late-pregnancy compared to non-brachycephalic. Brachycephalic bitches had higher respiratory rate, mucous color score, glycemia, lactatemia and SBP, compared to the non-brachycephalic, regardless of the experimental moment. In addition, there was an increase in severity score of the brachycephalic syndrome, mucous color score and oxygen saturation throughout gestation, regardless of the experimental groups.
CONCLUSIONS
In conclusion, brachycephalic syndrome alters respiratory and acid-base balance throughout pregnancy, with additional maternal distress by metabolites and pressure changes.
Ethical approval CEUA-FMVZ-USP number 5264070317.

M125
DOG SPERM MEMBRANE STATUS ON FRESH, COOLED AND FROZEN/THAWED SEMEN

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BACKGROUND-AIM
Understanding of the cryoinjury process is very import to achieve better cryopreservation and pregnancy results [1]. The use of fluorescent dyes as merocyanine 540 (M540) can be used to monitor the level of disorder of the phospholipids of the plasma membrane lipid bilayer, which indicate membrane destabilization that also occurs during the first steps of sperm capacitation [2]. The combination of M540 with Yo-Pro 1 (YP) permit simultaneously analyzes of sperm cell viability and membrane integrity. YP penetrates the cell upon plasma membrane destabilization and an increase in the permeability of pannexin-gated channels, before complete loss of membrane integrity occurs [3]. The aim of this study was to analyze the effects of freezing on dog sperm.
METHODS
A total of 15 ejaculates were analyzed. Semen was diluted (80 x 106 sperm/ml) and freeze as previously described [4]. At each step (fresh, cooled and frozen/thawed semen) sperm total motility (TM), progressive motility (PM) and % of rapids (RAP) were accessed by CASA, the membrane fluidity (M540) and cell permeability (YP) by flow cytometer as previously reported [5]. Statistics were performed using Kolmogorov–Smirnov, variance and Dunn's tests.
RESULTS
The results were as following: Fresh semen: TM 85% ± 7, PM 67% ± 9, RAP 80% ± 9; YP-/M540- 78,29 ± 6,22; YP-/M540+ 18,33 ± 5,54. Cooled semen: TM 86% ± 5, PM 68% ± 9, RAP 78% ± 8; YP-/M540- 75,76 ± 6,60; YP-/M540+ 22,55 ± 6,49. Frozen/thawed semen: TM 72% ± 12, PM 55% ± 11, RAP 63% ± 13; YP-/M540- 23,02 ± 9,12; YP-/M540+ 71,78 ± 9,85. An increase in sperm membrane fluidity and permeability was noted on frozen/thawed. In the population that was not permeable to YP ($P < 0.01$) and in samples showing an increase in YP uptake, there was a common M540-fluorescent population among frozen/thawed samples, indicating an increase in cell membrane permeability and compromised membranes after cryopreservation. Although a significant decline ($P < 0.01$) on sperm motility parameters after freezing, percentages are considered sufficient for AI.
CONCLUSIONS
We conclude that despite acceptable motility cryopreservation cause membrane damage to most of the sperm population and this would impair spermatozoa life spam and could be one of the causes for low pregnancy results using frozen/thawed dog semen.

M126
TOTAL PROTEIN MEASUREMENT OF THE UTERINE TISSUE AND FLUID FROM THE BITCHES WITH PYOMETRA

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BACKGROUND-AIM
The cystic endometrial hyperplasia-pyometra complex is the uterine disease most common in bitches, caused by progesterone and estrogens influence. This study aimed to measure total proteins in uterine tissue and fluid of healthy bitches and bitches with pyometra.
METHODS
Sixteen bitches attended in the Smal Animal Reproduction Service from the Veterinary Hospital, at Sao Paulo State University (UNESP, Botucatu, São Paulo, Brazil) were included in the study. The bitches formed two groups, healthy (n = 8) and with pyometra (n = 8) and aged between 1 and 10 years old, weighing 6 to 30 kg. All bitches were clinically examined, blood count and ultrasonography were performed to identify healthy bitches and with pyometra. Vaginal cytology and blood samples were collected to determine the stages of the reproductive cycles and identify leukocytosis and neutrophilia, respectively. The bitches were anesthetized and ovariohysterectomy was conducted. The uterine horns were isolated and flushing of the lumen was performed with 2 mL of 50 mmol TRIS pH 7.2 containing protease inhibitors (1 µg/mL aprotinin, 1 µg/mL leupeptin, 35 µg/mL PMSF, and 0.8 mmol of EDTA). Furthermore, a uterus fragment was taken and added a protein extraction buffer (30 mmol TRIS HCl pH 8.5, 2% Triton X-100, 8 mol urea, and 30 mmol DTT) containing protease inhibitors. Tissue samples were sonicated in an ice bath in a range of 20% for 30 s, repeated 10X with 1-minute intervals between each series. Then, to remove cellular debris, the samples were twice centrifuged at 15.000xg for 30 min at 4 °C. The supernatant remaining of uterine tissue preparation and uterine lumen flushing were used to measure total protein concentration by spectrophotometry (Pierce™ BCA Protein®, Thermo Scientific, Rockford, IL, USA).
RESULTS
There was no difference between groups to total protein from uterine tissue (9.64 ± 2.78 mg/mL vs. 10.65 ± 3.28 mg/mL). However, differences were observed between healthy bitches and with pyometra in flushing samples (1.15 ± 0.05 mg/mL vs. 5.19 ± 1.42 mg/mL), respectively.
CONCLUSIONS
The study of the protein by mass spectrometry could show the differences between samples studied for identifying biomarkers for better understanding the pathophysiological mechanisms involved in pyometra.

M127
SOY LECITHIN PRODUCES SUPERIOR FERTILITY COMPARED TO EGG YOLK FOLLOWING SEMEN CRYOPRESERVATION AND LAPAROSCOPIC OVIDUCTAL ARTIFICIAL INSEMINATION (LO-AI) IN DOMESTIC CATS

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BACKGROUND-AIM
Replacement of animal proteins with plant proteins in semen cryomedia reduces microbial contamination risk, batch-to-batch variation and regulatory concerns. In our earlier research in domestic cats, semen cryopreservation in a soy lecithin-based medium (SOY) produced superior longevity of sperm motility post-thaw compared to egg yolk (TEY), but no differences were seen in IVF success (Vick et al., 2012; Therio. 78:2120-2128). Our objective in this study was to assess in vivo fertility of cat sperm frozen in SOY vs. TEY using laparoscopic oviductal artificial insemination (LO-AI).
METHODS
Semen samples collected from two males via artificial vagina were divided, extended in SOY or TEY and frozen in straws over LN2 vapor. Domestic cats (n = 13), treated with exogenous gonadotropins (100 IU eCG, 1000 IU porcine LH), were inseminated laparoscopically at 31-33 h post-pLH. For each female, one oviduct received thawed sperm (2-4 million motile) from one male frozen in SOY while the opposite oviduct received thawed semen from the second male frozen in TEY. At 20-21 days post-AI, pregnant females were spayed and fetuses recovered for short tandem repeat (n=38) analysis to determine paternity.
RESULTS
Percent progressively motile sperm post-thaw was slightly higher (P<0.05) for samples frozen in TEY (76.9+1.7%) vs. SOY (71.9+1.4%), but rate of progressive motility (on scale of 0-5) did not differ (P>0.05; TEY, 3.2±0.1; SOY, 3.2±0.1). Overall, 7 of 13 females (56%) conceived, averaging 7.1±1.2 fetuses/queen. Paternity analysis found equal pregnancy percentages (5/13, 38%) for sperm frozen in each cryomedia following unilateral LO-AI, and mean fetal mass was similar (P>0.05) between treatments (SOY, 162.2±6.2 mg; TEY, 198.4±22.1 mg). However, more than twice the number of fetuses were generated from sperm frozen in SOY (68%, 34/50) compared to TEY (32%, 16/50). In pregnant females, fertilization success (no. fetuses/no. CL) also was higher (P<0.01) for SOY (34/64, 53%) versus TEY (16/75, 21%).
CONCLUSIONS
These results confirm that cryopreservation of cat semen in SOY can produce high pregnancy percentages and fetal numbers following LO-AI, with greater fertility in vivo than for sperm frozen in TEY. These findings further validate the use of SOY as a superior alternative to TEY for felid semen cryopreservation.

TOPIC Control of estrous cycle

M128
7 & 7 SYNCH AND 7-DAY PROGESTERONE-BASED PROTOCOLS FOR ESTRUS SYNCHRONIZATION PRIOR FIXED-TIME ARTIFICIAL INSEMINATION IN MULTIPAROUS BEEF SUCKED COWS

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BACKGROUND-AIM
Estrogen-based protocols combined with a progestin-releasing intravaginal device (P4RID) and prostaglandin (PG) synchronize follicle wave emergence prior to artificial insemination (AI) in Bos taurus, with pregnancies per AI (P/AI) ranging from 40 to 60%. Gonadotropin-releasing hormone (GnRH) based protocols + P4RID + PG rely on the presence of a physiologically mature dominant follicle at initial GnRH. Failure to induce ovulation represents a major obstacle to improve P/AI. Pre-synchronization by PG + P4RID 7 days in advance of GnRH increase likelihood of ovulation following GnRH. The aim of this trial was to evaluate fertility in suckled beef cows using GnRH or estrogen treatments with or without pre-synchronization.
METHODS
Cyclicity status was determined in multiparous Red Angus suckled cows by ultrasound (US). Animals were randomly assigned based on cyclicity status, body condition, days of postpartum and weight. All reagents were supplied by Biogégesis-Bagó (Buenos Aires, Argentina). Group 7-Day Estradiol (n = 59): 2 mg estradiol benzoate-EB on Day -9 + 1 mg estradiol cypionate-EC on Day -2. Group 7-Day GnRH (n = 59): 10.5 µg GnRH on Day -10 and at the time of FTAI. Group 7 & 7 Estradiol (n = 60): 150 µg PG on Day -16 + 2 mg EB on Day -9 + 1 mg EC on Day -2. Group 7 & 7 GnRH (n = 57): 150 µg PG on Day -17 + 10.5 µg GnRH on Day -10 and at the time of FTAI. All animals received 1.0 g P4 P4RID and 150 µg PG + 300 IU eCG at the time of P4RID withdrawal. A single technician performed AI at 52±2 h post device removal for estradiol and 66±2 h for GnRH groups using semen from a proven sire. Pregnancy diagnoses were performed 35 days after FTAI by US. Data were analyzed as a 2×2 factorial using the MIXED or GLIMMIX procedures of SAS for continuous or binomial data, respectively.
RESULTS
CL presence at P4RID withdrawal was greater in GnRH-based protocols compared to estradiol groups ($P < 0.01$). Presynchronized cows showed greater CL presence and number of CL (single or double) at the time of P4RID withdrawal in comparison to non-presynchronized groups ($P < 0.01$). 7 & 7 GnRH showed greater P/AI (72%, $P < 0.01$) in comparison to 7-Day GnRH (61%), 7-Day Estradiol (49%) and 7 & 7 Estradiol (33%).
CONCLUSIONS
7 & 7 Synch (GnRH-based) treatment involving PG administration and P4RID treatment seven days prior to GnRH resulted in enhanced P/AI.

M129
NANODELIVERY SYSTEM FOR OVSYNCH PROTOCOL IMPROVES OVARIAN RESPONSE, DOPPLER SONOGRAPHIC ANALYSIS, AND HORMONAL PROFILE OF GOATS

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BACKGROUND-AIM
The success of an estrous synchronization protocol is mainly ascribed to the pharmacokinetics and bioavailability of hormones. Nanotechnology allows improving physicochemical properties of hormones, thus the efficiency of common Ovsynch protocol or Ovsynch protocol using nanodelivery system (hormones at a nanoscale range) was evaluated.
METHODS
Four equal treatment groups of twenty goats including: control, GPG (common Ovsynch protocol :50 mg gonadorelin followed by 125 mg cloprostenol 7 days later and a further 50 mg gonadorelin 2 days later), NGPG (nanodelivered hormones with common doses), and HNGPG half dose (nanodelivered hormones with half common doses) were evaluated for ovarian response and ovarian hemodynamic pattern, steroid and nitric oxide concentrations.
RESULTS
All Ovsynch treatments resulted in higher number of total follicles compared to control. The highest significant ($P < 0.05$) diameter of primary, secondary, and tertiary dominant follicles was observed in HNGPG treatment. All Ovsynch treatments reduced ($P < 0.05$) time required for ovulation than control, being the lowest for the HNGPG treatment. The luteal diameter was significantly increased in the HNGPG treatment. The HNGPG treatment had the highest significant ovarian artery homodynamic indices (lower resistance and plasticity and higher peak velocity) at the follicular phase. All Ovsynch treatment resulted in better ($P < 0.05$) luteal artery homodynamic indices than control at the luteal phase, but the highest luteal artery homodynamic indices was for the HNGPG treatment. All Ovsynch treatments showed higher significant levels of estradiol and nitric oxide at the follicular phase, and a marked increase in progestrone and nitric oxide at the luteal phase than control. Compared to GPG and NGPG treatments, HNGPG treatment significantly improved all previous variables.
CONCLUSIONS
The HNGPG treatment enhanced the ovarian and luteal arteries blood flow of the synchronized estrous cycle through improving nitric oxide concentrations, characteristics of ovulatory wave and estradiol synthesis at follicular phase, and enhanced luteal functions through improving progesterone synthesis during luteal phase. Nanodelivery system for Ovsynch protocol could be a new strategy for improving estrous synchronization outcomes of goats with lower hormone doses.

M130
PLASMA PROGESTERONE PROFILES IN NON-LACTATING OVARIECTOMIZED BEEF COWS TREATED WITH DIB® (SYNTEX) OR CIDR 1.38 (ZOETIS)

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BACKGROUND-AIM
This was a two-treatment, two-period pharmacokinetic crossover study comparing the pharmacokinetic profile of progesterone (P4) after application of an intravaginal device (IVD) DIB (1g P4, Syntex; Argentina) or CIDR (1.38g P4, Zoetis) in ovariectomized beef cows.
METHODS
Eighteen healthy, non-lactating, ovariectomized, adult Angus and Hereford cows in two groups of 9 cows each were used. Animals were treated twice at an interval of 16 days. Blood samples were collected over a period of 7 days (at 0 h, 1h, 3 h, 6 h, 9 h, and then every 12 h until D7, and at 1 h; 2 h; 12 and 24 h after device removal), blood samples were processed to obtain plasma and were analyzed for the concentration of progesterone using (Elecsys P4 III Roche assay in the Cobas 8000 e602 Roche equipment). Plasma drug concentration data were used to determine individual and mean time concentration profiles. Comparisons between the formulations were established in relation to key parameters such as AUC and Cmax in order to evaluate the bioequivalence (BE) of DIB and CIDR. The arithmetic means and standard deviations of all pharmacokinetic parameters were summarized by treatment. AUC0-LOQ and CMAX data for the individual animals were transformed by the natural logarithm prior to the analysis, to stabilize the variability. A mixed model analysis was used to estimate the upper and lower bounds of the two pivotal BE parameters log AUC and Cmax. The model included the following as fixed effects: sequence, period and formulation. Animal within sequence was included as a random effect. The estimated error of variance, obtained in the ANOVA, was used for the calculation of the 90% confidence interval (CI) for the ratio of the two treatment means. BE was achieved when the 90% CI for AUC and for Cmax fell into the interval (0.80-1.25).
RESULTS
Confidence intervals (lower and upper 90%) for the log transformed AUC and Cmax were 0.73 and 0.93 and 0.87 and 1.26, respectively. The 90% CI for the pivotal parameters is outside the pre-defined BE interval, Cmax falls marginally outside the upper limit of the confidence interval at 1.26 and AUC falls below the lower limit at 0.73. BE was not demonstrated as the 90% confidence intervals for AUC and Cmax.
CONCLUSIONS
The differences found were marginal, so DIB could have the same effectiveness of use as CIDR in FTAI protocols.

M132
EVALUATION OF SYNCHRONIZATION EFFICIENCY BASED ON PROGESTERONE MODELS IN PRIMIPAROUS BEEF COWS

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BACKGROUND-AIM
Young cows are generally more prone to prolonged postpartum anestrus and reproductive failure in synchronization, which is mainly caused by inadequate levels of hormones. Progesterone is used as an indicator to assess reproductive functions due to its multiple effects on the estrous cycle. The aim of this study was to evaluate the impact of progesterone concentration (P4) before starting synchronization on the pregnancy in primiparous beef cows.
METHODS
Primiparous purebred postpartum (108.66±28.19 days) Angus and Limousine beef cows (n=50) were selected. Cows were inserted with intravaginal progesterone (PRID delta, Ceva) for 7 days and injected with GnRH (Receptal, Intervet) on D 0. At PRID removal PGF2a (Dinolytic, Zoetis) was injected. Cows were inseminated on observed heat 56 h after PRID removal. Blood samples were collected 7 days (D -7) before synchronization and on D 0. Pregnancy was diagnosed with an ultrasound device (iScan Draminski, 7,5 MHz) at 32 days post insemination.
Three different P4 range models were designed: Model I was divided into P4 values <1 ng/ml (L), 1-3.99 ng/ml (M) and ≥4 ng/ml (H); Model II into <1 ng/ml and ≥1 ng/ml; Model III into <1 ng/ml and ≥1 ng/ml. Each Model was grouped differently depending on the P4 values on D -7 and D 0: Model I formed 9 groups (L (D -7) L (D 0), LM, LH, ML, MM, MH, HL, HM, HH); Model II formed 4 groups (ED (early diestrus), LD (late diestrus), PEM (proestrus-estrus-metestrus) and A (anestrus); Model III formed 2 groups (C (cyclic) and N (non-cyclic).
RESULTS
Synchronization efficiency was 66.0% (P<0.05) and no significant effect of the breed was detected. Pregnancy per TAI (P/TAI) was improved when the postpartum period was longer than 108 d (P<0.05). The highest P/TAI (from the total number) in Model I (P<0.05) were found in the HH (20.52%) and LH (15.39%) groups and in Model II (P<0.05) in the LD (25.65%) and ED (17.95%) groups, and in C cows (Model III) was 48.72% compared to that of N being 17.95% (P<0.001), respectively.
CONCLUSIONS
In conclusion, the P/TAI was significantly increased in all analyzed models when P4 at the onset of the synchronization was high (≥4 ng/ml) or increasing (≥1 ng/ml).

M133
THE USE OF TWO-LEVEL ECG IN A J-SYNCH PROTOCOL IN BEEF HEIFERS DOES NOT MODIFY THE PRESENCE OF ESTRUS AND THE GESTATION RATE DURING THE SUMMER.

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BACKGROUND-AIM
The success of FTAI depends on the manipulation of different physiological processes, such as the recruitment of a new wave and follicular dominance, as well as the moment of ovulation; for this reason, the FTAI protocols have been developed in reproductive programs in a cow-calf production system. Therefore, the objective of the present work was to evaluate the J-Synch protocol with two doses of eCG in beef heifers during the summer in Northeast Mexico.
METHODS
In this experiment, 218 heifers of the Machona breed (109/treatment) with a live weight of 350 ±12.1 kg were used. On Day 0, all heifers received an intravaginal device (1.2 g P4) plus 2 mg, i.m., EB; On day 5, it was withdrawn and 0.15 mg of D-Cloprostenol was applied; where treatment 1 (T1) consisted of the application of 250 IU of eCG; in treatment 2 (T2), 300 IU of eCG was applied. Subsequently, the total number of heifers from both treatments were inseminated at a fixed time (FTAI) at 72 hours after the removal of the device with frozen bull semen; likewise, at the time of the FTAI, 10.5 mcg of GnRH was applied to each heifer.
RESULTS
There was no significant differences (p > 0.05) in the percentage of estrus at the first service (T1 = 91%; T2 = 96%; as well as for the percentage of repeaters (T1 = 25.2% vs T2 = 19.2%). Estrus appeared on average at 42.5 ± 6.2 h for T1 and T2 = 37.4 ±5.8 h, with no differences (p > 0.05). For the percentage of pregnancy at the first service, it was 64.8% for T1 and 70.3% for T2 (p > 0.05).
CONCLUSIONS
According to the conditions that the present investigation was developed, the indistinct application of 250 or 300 IU of eCG can be used with the same results of the presence of estrus and gestation during the summer season in beef heifers.

TOPIC Cryobiology of gametes and embryos

M134
EFFECTS OF TREHALOSE CONCENTRATION AND PACKAGE ON RAM SPERMATOZOA VITRIFICATION

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BACKGROUND-AIM
Sperm vitrification is based on the rapid cooling of cells by direct immersion in liquid nitrogen, reducing the big ice crystals formation. This is a simple and low cost technique, and presents great possibilities of its implementation in field conditions. However, studies carried out with ram semen have not yet achieved the good results obtained in other species, included human.
METHODS
Ejaculates from five Merino rams were pooled and diluted in a Tris based extender. After a temperature decrease (30° to 5 °C), the samples were mixed with three different trehalose (T) concentrations (50, 100 or 200 mosm/L), and then packaged in 0.25 mL straws into a 0.5 ml straw (PP) or in 30 µL droplets (D). Vitrification was developed by plunging the samples directly into liquid nitrogen. Separately, an aliquot of semen was frozen in liquid nitrogen vapors as a control. At thawing, samples were evaluated by sperm motility (SM), live cells (LC), acrosome integrity (AI), HOST, chromatin status (CS) and area of sperm head (ASH). The parameters were analyzed by ANOVA, with Fisher–LSD post hoc test.
RESULTS
For PP package, most of the post-thawing parameters showed higher values ($p<0.05$) when semen samples were vitrified with T 200 mosm/L, compared with 100 and 50 mosm/L (SM = 27.50 ± 5.95%; LC = 24.33 ± 0.88%; AI = 28.00 ± 4.62%; HOST = 18.50 ± 6.70%; CS = 24.50 ± 7.50%).
For D package, only AI, HOST and CS showed significant differences when vitrification was performed with T 200 mosm/L, compared with 100 and 50 mosm/L (SM = 21.83 ± 7.05%; LC = 24.17 ± 5.13%; AI = 36.67 ± 7.40%; HOST = 27.25 ± 4.31%; CS = 7.50 ± 0.50%).
When packaging comparison was performed, it was observed better results for D than PP package using T 50 mosm/L, showed by SM, LC, AI and HOST. The other T concentrations did not show significant differences.
Trehalose concentration on ASH showed the lower value for T 200 mosm/l (32.22 ± 0.42 µm2), whereas the control achieved the highest value for this parameter (34.37 ± 0.26 µm2). That might indicate an important dehydration and an incomplete recovery of the cell volume after the vitrification process.
CONCLUSIONS
It may be concluded that a high concentration of trehalose applied on ram semen vitrification would represent a way to improve this sperm conservation technique.

M135
EFFECT OF THE OPIOID ANTAGONIST NALOXONE ON BOVINE IN VITRO EMBRYO PRODUCTION

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BACKGROUND-AIM
The reduced quality of bovine in vitro produced embryos is due to suboptimal culture conditions, that may induce oxidative stress. Naloxone (Nx) is an opioid receptor antagonist that has been shown to have beneficial effects on bovine in vitro maturation rate (Dell'Aquila et al., 2002). Aim of this work was to evaluate the effects of Nx during in vitro embryo production (IVEP) on embryo development and oxidative stress.
METHODS
Abattoir-derived bovine cumulus-oocytes complexes (COCs; n=444, over 4 replicates) were in vitro matured (IVM), fertilized (IVF) and cultured (IVC) according to standard procedures. Oocytes were randomly divided in two groups: Control (n=225) and supplemented with 10-8 M Nx (n= 219). On Day 7, cleavage and embryo yields were assessed. In addition, spent media were recovered and stored at -20° C. Nitric oxide levels were evaluated indirectly by the dosage of nitrate that were converted by nitrate-reductase and NADPH to nitrite, colorimetrically at 550 nm after addition of Griess reagent [1% sulfanilamide, 5% phosphoric acid and 0.1% N-(1-naphtyl)ethylenediamin]. Data were analyzed by Student's t-test and expressed as means ± SEM.
RESULTS
The supplementation of Nx during in vitro embryo production improved cleavage rate compared to the control (79.4 ± 1.5 vs 72.1 ± 2.1, respectively; $P<0.05$). However, no differences were reported in blastocyst yields between the two groups (26.5 ± 3.5 and 30.4 ± 2.9 in the control and treated groups, respectively). Similarly, Nx did not affect IVM, IVF and IVC nitric oxide levels compared to the control (25.5 ± 1.6, 8.8 ± 1.1 and 13.8 ± 3.1 vs 29.5 ± 1.6, 11.1 ± 0.9 and 11.8 ± 1.1. µM respectively).
CONCLUSIONS
The results of this study demonstrated that the supplementation of Naloxone improved cleavage rate, this is in line with the effect of Nx in pig embryo production (Dang-Nguyen et al., 2013), however it needs more experiment to deep understand the effects of opioid system on embryo production oxidative stress. Funding: This work was supported by a joint project between Federico II University of Naples-Italy, University of Bari-Italy and National University of Asuncion, Paraguay-PINV 15-484.

M136
VITRIFICATION AFFECTS GENE EXPRESSION PROFILE OF IN VIVO PORCINE EMBRYOS

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BACKGROUND-AIM
Pig embryo cryopreservation is needed for international exchanges of genetic progress and conservation of genetic diversity in commercial and local breeds as well as in genome-edited strains. The use of vitrification has provided interesting results for pig embryo cryopreservation, but the success rate remains low, and a better understanding of the vitrification damages would help to improve the technique. With this aim, vitrification-induced gene expression alterations in porcine blastocysts were analyzed.
METHODS
Large White sows were synchronized, superovulated, and inseminated twice within 12h. Embryos were collected surgically at Day 6 post insemination. Embryos at the blastocyst stage were randomly allocated to two groups: (i) in vitro culture embryos (IVC) and (ii) vitrified embryos (VIT). The IVC embryos were cultured for 24h in 500 µL of NCSU23 medium with 0.4% BSA and 10% FCS at 39°C.The VIT embryos were thawed and cultured under the same conditions. After culture, both groups of embryos were snap frozen in pools of 5 embryos and used for RNA-sequencing. Briefly, vitrification consisted in washing 5 min in TALP-HEPES containing 0.1 g/L PVA, 3 min in the same medium with 7.5% ethylene glycol (EG) and 7.5% dimethyilsulfoxide (DMSO), and 1 min with 16% EG, 16% DMSO and 0.4M sucrose. Embryos were loaded into a superfine open pulled straw and plunged into liquid nitrogen. For thawing, straws ends were plunged in 1 mL TALP-HEPES-PVA containing 0.1 g/L PVA with 0.13 M sucrose at 39°C for 5 min, washed and placed in culture.
RESULTS
RNA-sequencing identified 9,325 expressed genes and PCA analysis showed a clear separation of the two groups. Comparative statistical analysis (false discovery rate <0.006) between VIT and IVC embryos revealed 321 differentially expressed genes (DEGs) with 198 up- and 123 down-regulated by vitrification. Among these, 208 had a fold change >2, most of them being upregulated by vitrification (164 genes). Gene ontology analysis revealed that these DEGs were related to reactive oxygen species metabolism, endocrine system development, and ageing.
CONCLUSIONS
To conclude, our data showed that vitrification induces deep changes on embryonic gene expression that may reflect the heal of freezing damages and provided with potential biomarkers for vitrification success.

M137
CHONDRICHTHYAN SPERM CRYOPRESERVATION AS A NOVEL TOOL FOR THE EX-SITU CONSERVATION OF SHARKS, RAYS AND CHIMAERAS.

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BACKGROUND-AIM
Chondrichthyans are one of the most threatened groups of vertebrates. Given this situation, ex situ conservation strategies could be used for multiple species. However, to assure the success and sustainability of these strategies, breeding programs, and reproductive techniques should be implemented. Among these reproductive techniques, sperm preservation is a potential tool scarcely used in chondrichthyans. In fact, there were no widespread preservation protocols for chondrichthyans sperm, and shark sperm cryopreservation had never been achieved before.
METHODS
Here we present a series of successful cryopreservation protocols for chondrichthyan sperm, tested in 13 species (Scyliorhinus canicula, S. stellaris, Prionace glauca, Hexanchus griseus, Centrophorus granulosus, Raja radula, R. montagui, R. asterias, R. undulata, R. clavata, Dasyatis pastinaca, Aetomylaeus bovinus, Dipturus oxyrinchus, and Chimaera monstrosa). We have formulated a sperm extender useful for different species, where sperm kept its motility for up to 36 days at 4 °C. The cryopreservation of sperm was achieved by supplementing our extender with different combinations of cryoprotectants: methanol, dimethyl sulfoxide (DMSO), and fresh egg yolk. Samples were frozen in cryotubes inside a styrofoam box using liquid nitrogen vapor. Pre-freezing and post-thawing sperm quality was assessed by analyzing spermatozoa motility and membrane integrity. All the samples used showed mean pre-freezing quality values of 70%.
RESULTS
In rays, the use of 10% DMSO or 10% methanol resulted in post-thawing motility values higher than 40%. In sharks and chimaeras, the combination of 5% DMSO, 5% methanol, and 10% egg yolk produced mean values close to 35%, with the notable exception of H. griseus with values over 70%. Overall, the addition of egg yolk increased the post-thawing motility values, by up to 42.1%.
CONCLUSIONS
For the first time, shark, skate and chimaera sperm cryopreservation has been reported, including species considered Critically Endangered according to IUCN criteria, such are the blue shark P. glauca and the bull ray A. bovinus. This expands our knowledge of the reproductive techniques that can be applied to chondrichthyans, laying the foundations of the first cryobanks for the long-term storage of their sperm.

M138
CULTURING BOVINE OVARIAN CORTEX MAY RESCUE DAMAGES CAUSED BY CRYOPRESERVATION ON THE EXTRACELLULAR MATRIX, FOLLICLE, AND STROMAL MORPHOLOGY

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BACKGROUND-AIM
Slow freezing (SF) and vitrification (VIT) are ovarian tissue cryopreservation (OTC) techniques used to preserve preantral follicles in situ; however, few studies have examined effects of SF and VIT on the extracellular matrix (ECM) or tissue and follicle viability post-thawing. Our aim was to investigate the impact of SF, VIT, and post-thaw culture on follicle viability, development, stromal cell density, apoptosis, and ECM composition in bovine ovarian cortical tissue.
METHODS
Ovaries (n=3) were collected from an abattoir on separate occasions and the cortex cut into 3 x 5 x 1mm fragments that were assigned to 1 of 6 treatments: fresh no culture (FN); fresh culture (FC); SF-thaw (ST); VIT-thaw (VT); SF-culture (SC); and VIT-culture (VC). SF and VT protocols were from publications using human and/or bovine ovary. Fragments were stored in liquid nitrogen for 20 days before thawing. Proportion of normal and abnormal follicles at each stage of development, stromal cell density, proportion of TUNEL-positive cells, and Masson's trichrome stain intensity (collagen abundance in ECM) were analyzed by ANOVA with treatment and replicate as main effects.
RESULTS
Culturing fragments (FC, SC, and VC) for 5 days decreased the proportion of primordial follicles and increased primary follicles compared to non-cultured tissue (FN, ST, VT; P<0.05). The proportion of normal follicles was higher in FN (79%) compared to ST (45%) and VT (35%; P<0.05), suggesting both techniques damaged follicles. After culture, the proportion of normal follicles in FC and SC was similar (55% and 39%; P=0.34), however VC had a lower (P<0.05) proportion of normal follicles (29%) compared to FC. Stromal cell density was similar among treatments. Collagen abundance in FN tissue was different between biological replicates; however, treatment effects were similar between replicates where VT had more (P<0.05) collagen compared to cultured tissue (FC, SC, VC). The proportion of TUNEL-positive cells was higher (P<0.05) in VC when compared to FN, but proportions in VC were not different than FC or SC.
CONCLUSIONS
These data suggest that OTC by SF and VIT leads to changes in ECM abundance and damage to follicles post-thawing, but culturing tissue may partially rescue the negative effects of cryopreservation on the ovary and follicles.

M139
EVALUATION OF THE COMBINED USE OF LUTEIN AND BLUE LED LIGHT RADIATION ON EPIDIDYMAL EQUINE SPERM CRYOPRESERVATION

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BACKGROUND-AIM
Nowadays, different alternatives to antibiotics in seminal doses are evaluated due to the increase in the antimicrobial resistances. Blue LED light could be an effective bacterial growth inhibition method, although this light radiation produces ROS that affect sperm quality. The objective of this study was to evaluate the effect of lutein (10 µM) as an antioxidant and protective barrier against to ROS generated during blue LED light radiation (30 minutes, 5.06 mW/cm2 and 380-475nm), on frozen equine sperm quality.
METHODS
The epididymal sperm samples were obtained from 9 stallions by retrograde flushing. Subsequently, sperm samples were centrifuged and divided into 4 groups according to the treatment: INRA (INRA96®), INRAR (INRA96® with radiation), INRAL (INRA96® with lutein) and INRARL (INRA96® with lutein and radiation). The sperm samples were frozen in nitrogen vapors and thawing during 21 seconds at 37°C. Sperm kinetics (CASA system), plasma and acrosome membrane integrity (PI/PNA-FITCT), mitochondrial activity (Mitotracker Deep Red) and DNA integrity (orange acridine) were evaluated after 10 min and 120 min after thawed. Data were analyzed by GLM test, evaluating 3 independent variables: stallion, lutein and radiation.
RESULTS
After thawing, stallion variable showed significant differences (p<0,001) in all parameters evaluated, except in DNA and acrosome integrity. Blue LED light radiation had no effect on nearly sperm quality parameters, although increased the percentages of sperm with high mitochondria activity (p<0.05). The incorporation of lutein on freezing extenders decreased the percentages sperm motility, viability and mitochondrial activity (p<0,001). No significant differences were found on sperm motility results 120 min after thawing between experimental extenders.
CONCLUSIONS
The addition of 10 µM of lutein did not affect sperm quality parameters; however blue LED light radiation before cryopreservation process increased significantly the mitochondrial activity on equine sperm samples.

M140
INFLUENCE OF PROCESSING TIME AND LUTEIN ADDITION ON EPIDIDYMAL EQUINE SPERM VITRIFICATION

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BACKGROUND-AIM
Epididymal sperm preservation in the equine specie is a feasible option for those stallions that have to be sacrificed or have died from a pathology. Knowing the time´s window we have to process the testicles is interesting, and the incorporation of lutein to vitrification media as well because it´s carotenoid that protects sperm against lipid peroxidation, so will improve semen parameters after devitrification. The objective of this work has been to assess if it is possible to keep the testicles for 24 hours in refrigeration before processing, and what is the optimal lutein concentration in vitrification media.
METHODS
14 epididymis of testicles from healthy stallions were processed after their slaughter. 7 were processed immediately and the other 7 after 24 hours at 4°C. The control extender (C) was INRA 96® supplemented with 1% BSA and 0.15M trehalose. With this base the different vitrification media were prepared, supplemented with lutein: 5 µM (LT5), 10 µM (LT10) or 15 µM (LT15). The sperm were obtained by retrograde flushing using INRA 96®. The final concentration for the different media was 50x106 sperm/ ml. For vitrification, 50 µl of each suspension were dropped on a cryotube containing 300 µl of N2L. After samples were warmed, sperm motility (ISAS® Proiser), viability and integrity of the acrosome (PNA-FITC staining) and DNA fragmentation (Acridine orange stain) were assessed. The statistical analysis of the data obtained was performed with the SPSS statistical package, version 22.0 for Windows, using a general univariate linear model (GLM).
RESULTS
Progressive motility were significantly better when sperm was vitrified at 0h compared to those at 24h in the case of media C, LT 5 and LT 10. For viability and DNA fragmentation, there are no significant differences between the values. Nevertheless, the percentage of intact acrosomes was higher at 0h compared to 24h.
CONCLUSIONS
With these results we can conclude that the processing of the testicles and the vitrification of their spermatozoa is better immediately after slaughter and that there is no significant protective effect of the addition of lutein in the vitrification media in the equine specie.

M141
VITRIFICATION OF PORCINE EMBRYOS AT THE ZYGOTE STAGE MARKEDLY DELAYS DEVELOPMENT AT THE 4- TO 8-CELL TRANSITION

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BACKGROUND-AIM
Livestock embryos are today routinely vitrified as part of advanced breeding programs, and in the treatment of human infertility, embryo vitrification has become indispensable. While the post-warming rates of embryo survival and development to term are considered acceptable, the impacts of vitrification on the embryo and resultant offspring are still poorly understood. Evidence indicates that vitrification alters developmental kinetics and gene expression profiles, possibly due to aberrant epigenetic reprogramming. Using time-lapse imaging, the aim of this study was to assess the effect of vitrification at the zygote stage on the morphokinetic parameters of porcine embryos.
METHODS
Porcine oocytes were matured and activated as described previously (Lowe et al. 2019, Reprod Fertil Dev, 31:557-569). About 16 h after activation, zygotes were either vitrified using a solid surface vitrification procedure or not vitrified (control embryos). Vitrified zygotes were immediately warmed and washed. Cohorts of zygotes from each group were cultured in 16-microwell dishes and monitored using the Primo Vision time-lapse system (Vitrolife) with images recorded every 10 min for 6 d. The intervals of progression to the 2-/3-/4-/5-/8-cell, early/compacted morula and early/expanding/hatching blastocyst stages were noted for each embryo. Three replicates were performed, and data were analysed using t-tests.
RESULTS
In vitrified embryos, the 4- to 5-cell and 5- to 8-cell transition times were 26.1 ± 5.2 h and 6.0 ± 1.4 h, respectively, which were both significantly longer than those in control embryos (13.8 ± 3.4 h and 1.9 ± 0.5 h, respectively). The transition times for all the other stages did not differ ($P > 0.05$). The expanding blastocyst stage was attained in both groups at a similar time post-activation (140.8 ± 2.7 h vs 138.6 ± 5.1 h).
CONCLUSIONS
In conclusion, morphokinetic assessment revealed that embryos vitrified at the zygote stage remained at the 4- to 8-cell transition a staggering 17 h longer than the non-vitrified control embryos. A delay during this period, which corresponds with zygotic genome activation in the pig, suggests vitrification at the zygote stage disrupts the epigenetic reprogramming process. Such a disruption has worrying implications for resultant offspring and warrants further investigation.

M142
USE OF SUCROSE IN THE EQUINE SEMEN FREEZING EXTENDER

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BACKGROUND-AIM
Despite the advantages of cryoprotection for semen transport and storage, low fertility rates are achieved with cryopreserving equine semen. Therefore, several studies seek to reduce the deleterious effects of this technique. Sugars are used as non-penetrating cryoprotectants and play an important during freezing, reducing osmotic stress, inducing cell dehydration, and minimizing ice crystal formation. Studies in other species have demonstrated that sucrose induces an increase of the transition temperature during the formation of ice crystals, resulting in the presence of a more stable glassy matrix. Thus, the objective of this study was to investigate the effects of sucrose addition to the equine semen freezer.
METHODS
Twenty-four ejaculates from six stallions (Mangalarga Marchador and Campolina) were used. The samples were obtained by artificial vagina, evaluated, diluted (1:1) and transported (5 °C) to the laboratory, where they were centrifuged and diluted in freezing medium based on skim milk and egg yolk plus 4% dimethylformamide (0; 25;50 and 100 mM sucrose) and Botu-Crio® (positive control) at a concentration of 200x106 / mL, packed in 0.5 mL straws and frozen. Immediately after thawing (37 ° C, 30s), semen samples were evaluated for kinematic parameters (CASA), plasma and acrosomal membrane integrity, membrane stability and mitochondrial membrane potential (flow cytometry). Analysis of variance (ANOVA) was used. Results were considered significant when P <0.05.
RESULTS
Supplementation of sucrose at 50 and 100 mM concentrations to the freezing diluent increased (P <0.05) the MT, MP, VCL, VSL and VAP parameters when compared to the control (0 mM). The WOB parameter of the 100 mM sucrose supplemented group was higher than the control (0 mM). Higher ALH and BCF values were observed in 25, 50 and 100 mM sucrose compared to Botu-Crio®. Frozen semen in the presence of 100 mM sucrose presented higher percentages
of sperm with intact plasma and acrosomal membranes, and high mitochondrial membrane potential compared to the other groups.
CONCLUSIONS
The addition of sucrose (50 and 100 mM) to the freezer, based on skim milk and egg yolk, proved to be an efficient cryoprotectant for equine semen freezing.

M143
EFFECTS OF MELATONIN SUPPLEMENTATION IN MILK EXTENDER ON THE QUALITY OF FROZEN SEMEN OF GOATS

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BACKGROUND-AIM
Semen cryopreservation is a reproductive biotechnology widely used to transport and store genetic material, once it allows the preservation of sperm viability. Despite the advantages, the freeze-thaw process causes structural, biochemical and functional damages to the sperm, affecting its fertilization potential. Oxidative stress is one for the most common cause of injury, for this reason, antioxidant molecules are added to extender for the purpose of reducing the impact of oxidative damage and also improving sperm quality. Melatonin is considered to be a potent antioxidant, protecting cells from apoptosis, inhibiting lipid peroxidation, stimulating antioxidant enzymes and sequestering free radicals. The objective of this study was to evaluate the antioxidant effect of melatonin on the post-thawing quality of goat semen.
METHODS
Six semen pools were diluted in skim milk based extender supplemented with melatonin (0 nM, 0.1 nM, 1 nM, 10 nM and 100 nM) and cryopreserved using an automatic system. After thawing, the evaluation of spermatic kinetics was performed in the CASA system. Flow cytometer was used to analyse plasma and acrosomal membrane integrity (iPAM), high mitochondrial membrane potential (hPMM) and intracellular ROS production (iROS).
RESULTS
Total motility (MT) results show that the control mean 56.10 ± 6,39 and melatonin supplements at concentrations 0.1, 1, 10, 100 were 48.22 ± 7,90, 49.06 ± 8,84, 50.12 ± 14,07 and 43.72 ± 11,94, respectively. As the other parameters analyzed, there was no significant difference between the control group and the groups with melatonin supplementation. Except for CASA's variable rapidity (RAP%), which demonstrated that the higher concentration of melatonin supplementation decreased the sperm velocity.
CONCLUSIONS
In conclusion, melatonin does not improve the post-thawing quality of goat semen cryopreserved and also may decrease sperm velocity at higher concentrations. Despite de addition of melatonin there was no difference in ROS, which may be explained due to the unknown presence of melatonin receptors in goat semen.The study has shown importance in highlighting the lack of interaction of melatonin in the goat semen freezing process, this may indicate a new research opportunity on goat sperm melatonin receptors.

M146
MATHEMATICAL SIMULATIONS FOR THE VITRIFICATION AND DIRECT TRANSFER OF BOVINE EMBRYOS

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BACKGROUND-AIM
In cattle embryo transfer, direct transfer (DT) of cryopreserved embryos offers a huge practical advantage. For biopsied IVP embryos (genomic testing), vitrification renders good results in non-DT, but not in DT protocols. Our aim was to explore possibilities to decrease risk of swelling after DT vitrification.
METHODS
We mathematically modelled swelling of embryos after DT vitrification when using different temperatures (T), or different concentrations of membrane-permeant and non-permeant cryoprotective agents (CPAs), with a 2P formalism (Kleinhans, Cryobiology, 37,271-289), and using membrane permeability characteristics obtained from the literature. In the 'standard' protocol, embryos were in VS1 (with 7.5% EG and 7,.5% DMSO (v/v) for 5 min, then 1 min in VS2 (15% EG and 15% DMSO + 0.7 mol/l sucrose; total osmolality 12 Osm./kg water).
RESULTS
After DT-'warming', these embryos swelled to 180% of isotonic volume. Increase of temperature from 22 to 37°C did not reduce post-DT swelling, as the ratio of membrane permeabilities for water and CPA (Pf/Ps) was hardly affected by T at the assumed parameterization. However, the embryos regained their initial volume much faster (9 vs 19 min). The simulations showed that post-DT swelling could be reduced dramatically by limiting CPA loading, either by short exposure to VS1, or by lowering permeant [CPA] in VS1 and VS2. In the latter approach, embryos were fully equilibrated in VS1 (4%-7% permeant CPA) for 10 minutes and incubated in VS2 (22-27% permeant CPA - combined with elevated [sucrose] (1.39-0.95 mol/l)) for 1 min. In all VS1-VS2 combinations, the ratio of permeant and non-permeant solutes in VS2 was made equal to that of VS1, and total osmolality of VS2 was 12 Osmol/kg, which should be sufficient for vitrification. Post-DT swelling of the embryos (110-140% of isotonic volume) was much lower than in the standard protocol. However, embryos were more shrunken after CPA loading, i.e. to 32-38% of isotonic volume (versus 46% in the standard protocol).
CONCLUSIONS
The simulations showed that the swelling is strongly depended on the assumed parameterization and the concentration of CPAs. The results of empirical tests, weighing the effects of prevention of post-DT swelling versus increased embryo shrinking after CPA loading, will be presented.

M147
ROSMARINIC ACID IN THE VITRIFICATION OF PREPUBERAL EWE OOCYTES

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BACKGROUND-AIM
The main objective in animal genetic resources cryopreservation is to obtain competence oocytes after vitrification process. The embryonic development of vitrified oocytes is lower than fresh oocytes, so improving the quality of these oocytes improving embryo production. The addition of antioxidants to the vitrification solutions may be the key to achieving this goal. This study evaluated the effect of the incorporation of different rosmarinic acid (RA) concentrations on the vitrification media of prepuberal ewe oocytes
METHODS
A total of 559 oocytes (COCs) from 381 ovaries of slaughtered ewe were used. COCs were selected and divided into three experimental (vitrified) and one control (fresh) groups. Experimental groups were treated with different concentrations of RA: 0 µM (VO), 100 µM (V100) and 150 Mm (V150). For this propose, COCs were equilibrated in base medium (BM) (TCM 199 supplemented with 20% foetal bovine serum) with 7.5% EG + 7.5% DMSO for 5 min. Then, they were placed in vitrification solution BM+7.5% EG + 7.5% DMSO supplemented with the different rosmarinic concentration (0, 100 and 150 µM) for 30 sec. Finally, COCs were vitrified directly plunged into the liquid nitrogen. After warming, oocytes were in vitro matured and nuclear maturation was assessed using Hoescht 33342 fluorescence staining. Differences between treatments were analysed using Pearson's Chi-square test and were considered significant when $p < 0.05$.
RESULTS
The maturation percentages were significantly lower in vitrified oocytes with 100 and 150 µM RA than oocytes vitrified without RA (16.0 and 20.0% vs 31.20%) ($P < 0.05$). Degeneration rates did not show differences between experimental groups with RA supplementation, however were significantly lower in VO group than V100 and V150 groups.
CONCLUSIONS
So, it can be concluded that the addition of rosmarinic acid on the vitrification media in prepuberal ewe oocytes did not protect them during preservation process.

M148
VITFARM®: A VITRIFICATION DEVICE WITH A LARGE STORAGE CAPACITY USING MINIMUM ESSENTIAL VOLUME METHOD

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BACKGROUND-AIM
Different commercial vitrification devices have been produced during the two last decades. However, for prolific species, the principal limitation of these devices is the low number of embryos that they can hold. Moreover, with superovulation, the use of vitrification in rabbits, mouse and porcine is limited due to being time-consuming and costly. Therefore, these prolific species need to develop a system that allows the simultaneous vitrification of the number of embryos necessary to obtain a physiological pregnancy after transfer. This fact would suppose an important impulse to the commercial application of this technology since the vitrification and warming protocols would be significantly simplified. Therefore, this study aimed to evaluate the efficiency of a new vitrification large storage capacity device.
METHODS
A two steps vitrification procedure was used to cryopreserve rabbit morulae embryos. In the process, vitrification solution (20% DMSO and 20% EG) and the embryos were loaded into VITFarm or French mini-straw; then, they were cryopreserved. In each device, a total of 25 embryos were stored. After thawing, a total of 284 embryos (102 for VITFarm, 86 for French mini-straw and 96 for a fresh group) were transferred to foster mothers. Implanted embryos were determined on day 14. At birth, total offspring were recorded, and embryonic and fetal losses were calculated.
RESULTS
The rate of implantation and development to term was similar between both vitrification devices (72±5.4% and 67±5.1% for implantation rate and 52±5.4% and 44±4.9% for offspring rate, for VITFarm and Frech straw); but significantly lower than in the fresh group (88±3.4% for implantation rate and 74±4.5% for offspring rate). Likewise, embryonic and fetal losses were similar between vitrification devices (28±4.5% and 33±5.1%) but significantly higher than in the fresh group (12±3.4%). However, fetal losses were similar between groups (18±5.7%, 22±5.5% and 15±3.9%, for VITFarm, Frech straw and fresh, respectively).
CONCLUSIONS
These results indicate that the VITFarm® is a device suitable for vitrification of many embryos using the minimum essential volume. It was funded by MCIN/AEI/10.13039/501100011033 and by the European Union "NextGenerationEU"/PRTR (PDC2021-120767-I00).

M149
GOAT BUCK SPERM FREEZABILITY IN SPRING AND SUMMER IN THE CENTRE OF MEXICO

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BACKGROUND-AIM
Goat bucks are seasonal breeders showing variations in sperm quality throughout the year, and this variation may affect sperm freezability. To see whether seasonal variation in sperm quality is responsible for sperm freezability, spermatozoa from 7 Saanen goat bucks were cryopreserved during spring (29 ejaculates) and summer (30 ejaculates) in the central valley of Mexico (19°N).
METHODS
Semen was collected by artificial vagina, centrifuged to removed seminal plasma, diluted in a Tris-egg yolk medium (4% final concentration of glycerol, 200x106 sperm/ml), packaged in 0.25 ml plastic straws, and cooled to 5˚C in 3.5 hours. Straws were then exposed to nitrogen vapours for 15 min, plunged in liquid nitrogen, and stored in this condition until required. Thawing was done by putting the straws in a water bath at 37°C for 30 sec. Sperm motility (visual), viability (eosin/nigrosine, NucleoCounter™), capacitation status (CTC assay), acrosome integrity (phase contrast microscopy), and plasma membrane fluidity (MC540) were assessed before and after cryopreservation. Data was arcsine transformed to normalize it before ANOVA, possible differences between males, and between fresh and frozen-thawed spermatozoa in the two different seasons were analysed.
RESULTS
Regarding fresh semen, in spring there were differences ($P<0.05$) in viability and acrosome reacted sperm between some males; in summer there were differences ($P<0.05$) in progressive motility, viability, and acrosome integrity between some males. There were differences ($P<0.05$) in progressive motility (75.0±1.69% vs. 81.5±1.05%), non-capacitated (76.1±1.59% vs. 70.1±1.69%), capacitated (14.2±1.31% vs. 20.1±1.31%), hyper-fluid membranes (3.5±0.58% vs. 7.2 ±0.85%), and acrosome integrity (87.5±0.98% vs. 81.1±1.71%) between sperm collected in spring vs. summer. However, there were ($P<0.05$) differences in non-capacitated (14.4±0.99% vs. 6.3±0.56%), and hyper-fluid membranes (71.0±1.94% vs. 58.8±2.44%) between sperm cryopreserved in spring vs. summer.
CONCLUSIONS
In conclusion, quality of fresh sperm was better in spring than in summer; however, quality of frozen-thawed sperm was similar in spring and summer, except in two variables: non-capacitated (CTC F pattern), and hyper-fluid membranes.
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M150
THE DIGITCOOL ALPHA: A NEW PERFORMANT AND ERGONOMIC DEEP-FREEZER

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BACKGROUND-AIM
Programmable deep-freezers for semen cryopreservation were developed to allow controlled freezing curves and precise adjustment of temperature according to cryoprotectant level in the media. The Digitcool Alpha, a novel deep-freezer developed by IMV Technologies, has been primarily designed for user comfort and security. The main objective of the study was to compare the freezing performance of the Digitcool Alpha with the historical Digitcool (control, ref. 007262).
METHODS
Five bull ejaculates were diluted at 100 million sperm cells/mL in egg yolk based medium, pooled, and split equally into two parts: one part frozen in Digitcool Alpha and one part in the control freezer. A total of 432 straws were frozen in 4 replicates per position, for each of the 4 freezing cycle per freezer. Straws were placed at the bottom, middle and top levels, and in left and right positions of the freezers (n = 12 positions). Semen total and progressive motility was randomly assessed using a CASA system.
RESULTS
Total motility of sperm cells frozen in Digitcool Alpha and control was significantly different. For all positions, total motility was 58.1± 5.06 % and 52.5 ± 10.7 % ($p<0.0001$) and progressive (STR>80%, VAP>50µm/s) motility was 33.6 ± 4.05 % and 29.7 ± 7.4 % ($p<0.0001$), for Digitcool Alpha and control, respectively. According to the positions of straws, Digitcool Alpha showed a lower coefficient of variation between straws than the control. Digitcool Alpha showed CV% of 8.7 % and 12.1 % for motile and progressive, respectively. The control freezer showed a CV% of 20.4 % and 24.9 % for motile and progressive, respectively.
CONCLUSIONS
Digitcool Alpha ensures the quality of semen post-thawing, with additional security and ergonomic advantages added according to users' needs of the machine relative to the historical Digitcool.

M151
SUCCESSFUL THAWING OF MOUSE EMBRYOS FROZEN 35 YEARS AGO

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BACKGROUND-AIM
It is commonly believed that storage length of frozen embryos is unlimited. In spite of it, the longest, well documented periods of storage are noted and acknowledged. As far as we know the longest are almost 20 years period in case of human (Dowling-Lacey D. et al. 2011, Fertil Steril) and 22 years of cattle embryo storage (Detterer J. et al. 2013, Reprod Fertil Dev). In Poland we described 5 cases of childbirths after 10-years long period of frozen embryos storage (Papis K. et al. 2013, Gin Pol).
METHODS
Last year we thawed mouse embryos frozen at an Institute of Genetics and Animal Breeding in Jastrzebiec. Freezing was performed in years 1985-1986, according to the method described by Rall W. et al. (1984, Reproduction). Embryos at a blastocyst stage were slow frozen in small thin wall glass tubes using 0.1 ml of 1.5 M DMSO and PROH or 3.0 M methanol solutions. Fast thawing was employed (10 sec in air followed with agitation in warm, 37°C water until last ice crystals disappeared). Contents of tubes was evacuated on Petri dish and embryos were moved to two subsequent trehaloze solutions (0.25M and 0.125M) for 5 and 3 min respectively. After microscopic evaluation embryos were cultured in 50µL droplets of BlastGen (Origio) medium under mineral oil at 37°C, 6%CO2, 6%O2 for 48h.
RESULTS
Out of unknown original number of embryos frozen (detailed documentation was lost) 73 embryos were recovered, and 63 of them (86.3%) were assessed viable. Thirty-four (46.6%) and 27 (37%) embryos developed to the expanding and/or hatching/hatched blastocyst stage after 24 and 48h culture, respectively.
CONCLUSIONS
The results shown here are similar to those described by Rall at al. (1984). It means that we obtained comparable ratio of viable mouse embryos capable of in vitro development after 35 years storage period. This is the longest period of successful mammalian embryo storage reported in literature. However in vivo trial should be performed to ascertain developmental capability of those embryos.

M152
EFFECT OF FREEZING PROCEDURE ON POST-THAW SEMEN QUALITY IN FIVE ENDANGERED SPANISH CHICKEN BREEDS

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BACKGROUND-AIM
The aim of the study was to test two freezing methodologies (straws vs. pellets) on the ejaculates freezeability of five endangered Spanish chicken breeds: Castellana negra, Utrerana perdiz, Utrerana franciscana, Prat Leonada and Penedesenca negra.
METHODS
Pooled semen samples for each breed (n=85) were collected twice a week by dorso-abdominal massage from 6 roosters/breed. Samples were diluted (1:2; v:v) with freezing media without cryoprotector (Lake extender) and were subjected to a cooling rate to 4°C in 30 min in a programmable biofreezer. Extender was then added at 4°C up to 6% DMA and samples were equilibrated for 1 min at 4°C. Samples were then divided into 2 aliquots, one was packed into 0.25 mL straws that were immediately plunged into LN2, and the other aliquot was dropped (0.25 mL each drop) directly in LN2 and resulted pellets stored in cryotubes in LN2. Straws were thawed in a water bath whereas pellets were thawed on thermoregulated hotplate, both at 60°C. In thawed samples subjective sperm motility (MI%), movement quality (Q), normal sperm morphology (NSM%), membrane integrity (HOSt%) and viability (V%; SYBR14/IP) were assessed and the results were analysed by three-way ANOVA including the breed, the photoperiod (decreasing vs. increasing), due to the marked semen production seasonality of these breeds (1), and the freezing protocol-used.
RESULTS
Breed effect was significant for HOSt% and NSM% (P<0.0001); photoperiod was significant for MI% (29.2±12.04 vs. 33.5± 10.7% for increasing and decreasing, respectively; P=0.035) and the freezing method was significant for all quality post-thaw parameters except Q. Thus, MI% was 27.5±10.9 vs. 32.9±12.2% (P=0.002); HOSt% was 40.3±17.2 vs. 61.3±16.2% (P<0.0001) and V% was 18.5±9.4 vs.41.97±11% (P<0.0001) for straws and pellets, respectively; but NSM% was, however, higher in straws (67.3±15.8 vs. 61.4±18.5%; P=0.004).
CONCLUSIONS
In conclusion, freezing semen with DMA-based samples provides better post-thaw quality results when pellets packaging are used than straws in these five endangered Spanish chicken breeds, despite the fact that straw packaging is more efficient for safety reasons and for identification of ejaculates. Freezing samples in the decreasing photoperiod to optimize post-thaw MI% is recommended too.
(1) https://doi.org/10.5209/RCCV.55176
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M153
EFFECT OF FREEZING PROTOCOL ON POST-THAW CLASSICAL SEMEN PARAMETERS IN WAGYU BULLS

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BACKGROUND-AIM
The aim was to assess the efficacy of Andromed® extender, without the use of cooling ramp before freezing, on the freezability of Wagyu bull ejaculates, because it is a technique widely used by veterinarians in field conditions with this extender, assessing the post-thaw cryosurvival percentages (CPs) of sperm quality classical parameters.
METHODS
Ejaculates from 4 Wagyu males were obtained by electroejaculation in 10 sessions and divided into 2 aliquots that were diluted with Andromed® extender, to reach 120x106 sperm/mL. In diluted samples subjective sperm motility (MI%), movement quality (Q), acrosome (NAR%) and membrane (HOSt%) integrity, viability (V%), sperm morphology (SM%) and percentage of live-acrosome intact spermatozoa (LAI%) were assessed. An aliquot (A-5°C) was subjected to a slow cooling rate (-0.2°C/min) to 5°C and a stabilization period of 2 hours at 5°C, while the other aliquot (A-RT), was kept at room temperature. Samples were packed in 0.25 mL straws and frozen in LN2 vapor for 10 min and then plunged into LN2. Doses were thawed in a water bath (37°C, 30 sec), and sperm quality was re-assessed. Cryosurvival percentage for each seminal parameter was calculated as [(final value/initial value)*100](1), and the results were analysed by two-way ANOVA including the bull and the freezing protocol used.
RESULTS
Bull effect was no significant for CPs of seminal quality parameters, but trended toward significance for MI% CP (P=0.056). According to the freezing method used, MI%, V% and LAI% CPs were significantly lower in the A-RT treatment compared to A-5°C (26.62±23.33 vs. 37.95±23.39, P=0.028; 34.81±21.62 vs. 55.24±30, P= 0.008 and 24.73±22.13 vs. 41.03±30.87, P=0.025; for MI%, V% and LAI% CPs, respectively). CPs with A-RT for other parameters analysed, were numerically lower than A-5°C treatment, but not significant.
CONCLUSIONS
In conclusion, freezing protocol has a clear impact on post-thaw MI%, V% and LAI% cryosurvival percentages, and A-RT method is discouraged for freezing seminal doses in Wagyu bulls under field conditions.
(1) Cryobiology 1986, 23(6): 518-24
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M154
SUCCESSFUL RECOVERY OF BOAR SPERM AFTER VITRIFICATION WITH DIFFERENT METHODS (PEARLS AND MINI STRAWS) USING SUCROSE AS A CRYOPROTECTANT

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BACKGROUND-AIM
Vitrification of sperm by direct contact with liquid nitrogen (LN2) is an increasingly popular alternative to conventional freezing. Although slow freezing is very challenging in boar sperm cryopreservation, this is currently the standard method used. Here we present the successful recovery of boar sperm after two vitrification methods, showing that it also suits boar sperm qualities.
METHODS
This study compared vitrification in pearls (VP) with vitrification in mini straws (VMS) using in vitro fertilization media Porcine Gamete Media (PGM) supplemented with 0.3M sucrose. Prior to vitrification, samples were selected using two-layer density gradient centrifugation and tested (Pre-test, PT), then incubated in media for 1 h at 5ºC. All samples were then vitrified using both methods. VMS: 50µL were packed in a 0.25mL straw and plunged into LN2; VP: droplets were form using a Kitazato device by direct contact with LN2 and funnelled through the device into a cryovial. All samples were stored in a LN2 tank, and then thawed by immersion in PGM at 37ºC for 30 secs prior to centrifugation at 600G for 7 mins.
RESULTS
Compared to PT both vitrification methods significantly reduce the proportion of live sperm, with the VMS group showing less viability (81.56%a vs. 30.38%b/11.85%c for VP/VMS respectively). VP did not increase DNA fragmentation compared to PT (1.21%a/1.57%a, respectively) but was drastically increased in VMS (5.18%b). Tail abnormalities were significantly higher in both VP and VMS compared to PT (19.52%b/20.10%b vs. 5.72%a, respectively). Head abnormalities were significantly higher in VMS but not significantly higher in VP compared to PT (5.89%a/9.17%ab/12.27%b respectively). For total and progressive motility, all groups were significantly different to one another (80.20%a/26.15%b/9.39%c for PT/VP/VMS and 60.10%a/8.31%b/1.44%c for PT/VP/VMS, respectively. abc= GLM and Tukey test when p <0.5.
CONCLUSIONS
As with other methods, vitrification significantly reduces sperm viability. Yet, the small volume of the pearls has a positive effect on the survivability of the samples since they are less damaged during the freezing process, reducing DNA sperm damage and preserving motility after thawing, making this method the more suitable for boar sperm.

M155
VITRIFICATION OF PORCINE IMMATURE OOCYTES AND ZYGOTES RESULTS IN DIFFERENT LEVELS OF DNA DAMAGE IN SUBSEQUENTLY DEVELOPING EMBRYOS

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BACKGROUND-AIM
Oocyte cryopreservation is a potent tool for female germplasm preservation. Immature porcine oocytes at the germinal vesicle (GV) stage survive vitrification at high rates and retain their ability for maturation and fertilization. However, their competence for embryo development is severely compromised unlike that of vitrified zygotes. The reason for this phenomenon remained unknown. Previously, a negative correlation between the extent of DNA double-strand breaks (DSBs) in porcine embryos at the cleavage-stage and their developmental competence of was reported (Bohrer et al., 2015 Biol Reprod 93: 59, 1-8). The aim of our study was to clarify if vitrification of porcine oocytes at the GV or zygote stage affects the extent of DSBs in the nuclei of subsequently developing embryos at the cleavage-stage.
METHODS
Porcine cumulus-oocyte complexes (COCs) of the same batch were vitrified/warmed in microdrops (Somfai et al., 2014 PloS One 9(5):e97731) either at the immature stage (GV-vit) or after maturation and fertilization (Zyg-vit) or processed without vitrification (Control). The COCs of all groups were subjected to in vitro maturation, fertilization (Day 0) and embryo culture. Cleaved embryos were harvested on Day 2 to analyse cell numbers and DSB levels by immunostaining of γH2AX and counterstaining of DNA with Hoechst 33342. The γH2AX fluorescence in nuclear area was quantified to arbitrary units from fluorescent images by the ImageJ software. Five replications were performed.
RESULTS
ANOVA revealed significantly higher (P<0.05) relative DSB levels in the GV-vit group compared to the Control group (1.2±0.06 and 1.0±0.02, respectively) which was associated with significantly lower (P<0.05) blastomere numbers in cleaved embryos (2.33±0.17 and 3.45±0.16, respectively). The relative DSB levels and cell numbers in the Zyg-vit group (1.05±0.03 and 4.18±0.46, respectively) did not differ significantly from those of the Control group.
CONCLUSIONS
Vitrification at the GV stage but not at the zygote stage caused DSBs in subsequently developing embryos which was associated with a delay of early embryo development. The increase of DSBs may be a possible cause for the compromised developmental ability of vitrified porcine oocytes. The study was supported by JSPS Kakenhi 21K05912.

TOPIC Reproductive pathologies

M157
HEAT SHOCK TRANSCRIPTION FACTOR IMMUNOEXPRESSION IN MAMMARY GLAND TUMOURS OF FEMALE DOGS.

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BACKGROUND-AIM
Canine mammary gland carcinomas are the most frequent pathologies in the veterinary clinic practice. In breast cancer in women the heat shock transcription factor HSF1 is associated with a poor prognosis and considered a factor of tumor aggressiveness. However, the expression of HSF1 in the mammary gland tumors of female dogs are not yet elucidated.
METHODS
Mammary gland tumours (n=30) were obtained from surgery of female dogs at Faculty of Veterinary Hospital Centre. Tumour samples were formalin fixed and paraffin embedded to perform immunohistochemistry technique. Tumor were classified according to the histopathological characteristics and percentage of immunostaining area of heat shock factor HSF1 in mammary gland tumours were measured. Data were described as mean ± s.e.m. and were compared by ANOVA between the ovariectomized/ non ovariectomized status with the level of significance at $P< 0.05$.
RESULTS
The HSF1 factor was localized in the cytoplasm and nuclear region of glandular epithelial and mesenchymal cells. Malignant tumour had the highest HSF1 immunoexpression ($p <0.0001$). A myoepithelial cell hyperplasia was add as control slide showing sligh immunoexpression. The order of immunoexpression of HSF1 from lowest to the highest expression was as follows: solid carcinoma, comedo carcinoma, simple carcinoma, cystic carcinoma, adenocarcinoma, inflammatory carcinoma, mixed papillary carcinoma, infiltrating ductal carcinoma and the highest expression of HSF1 were achieve in sarcoma.
CONCLUSIONS
In conclusion, HSF1 factor was immunoexpressed in mammary gland tumours from females dogs. Given the role of HSF1 factor in the prognosis of malignancy, we suggest adding it in the histopathological analysis panel of tumours, in order to determine aggressiveness, metastatic and poor prognosis. Moreover, the results achieved could be the basis for possible treatments with anti-HSF1 vaccines.

M158
EFFECT OF PUERPERAL UTERINE DISEASE ON UTERINE AND OVARIAN STRUCTURES OF PRIMIPAROUS HOLSTEIN COWS

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BACKGROUND-AIM
The effect of metritis on the ovarian structures is not fully understood. The objective of this study was to evaluate the effect of metritis on the ovarian and uterine structures during the first 42 days postpartum (DPP).
METHODS
Primiparous Holstein cows (n=34) were monitored during calving period to detect signs of dystocia. The parameters: percentage of endometrial polymorphonuclear cells (%PMN), uterine horn, endometrium, and artery diameter, total number of antral follicles (AFC), diameter of the preovulatory follicle (POF) and corpus luteum (CL) of the first ovulation, and blood flow of the POF and CL were recorded at 8, 15, 22, 29, 36, and 42 DPP. Data were retrospectively analyzed after 45 DPP and animals were grouped as: healthy (HC) and metritis cows (MC; retained placenta, presence of purulent discharge, fever, and/or uterine content). Statistix v.9 was used for statistical analyses using the ANOVA and Tukey's test for comparing groups A p-value <0.05 was considered statistically significant.
RESULTS
A total of 13 animals were classified as MC. Overall, HC had lower %PMN than MC (6.9 ±1.2 vs. 19.4±1.8%) and at 22DPP the lowest percentage of %PMN in HC compared to MC (3.0±3.0vs.32.2±4.4%). The uterine horn diameter (mm) was smaller in HC (left=29.1±0.5; right=31.0±0.9; gestation horn=29.9±0.4) compared to MC (left=45.6±1.6; right=37.6±1.3; gestation horn=32.3±0.6) at 8 (38.7±1.2vs.43.8±1.5) and 15DPP (32.0±1.1vs.38.5±1.5). Conversely, the endometrium diameter (mm) of the gestation side was thicker in HC (5.7±0.2) than MC (4.9±0.2) at 8DPP (6.6±0.5 vs. 4.2±0.6; P=0.07). The uterine artery diameter did not differ between groups. The interval calving to first ovulation (days) was shorter in HC (21.2±0.4) than MC (23.5±0.6). However, the total AFC prior to first ovulation did not differ between HC (18.6±0.7) and MC (19.6±1.0). Moreover, the diameter of POF and the subsequent CL, and the CL blood flow did not differ between groups.
CONCLUSIONS
Therefore, metritis leads to thicker uterine horn and thinner endometrium diameter up to 15DPP, delaying the first ovulation with no effects on the number, diameter, and blood flow of POF and CL within 42DPP.

M159
ANTIMICROBIAL CHOICE FOR TREATMENT OF PYOMETRA IN THE BITCH: WHAT DOES THE BACTERIA SUSCEPTIBILITY TEST SAY?

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BACKGROUND-AIM
Ovariohisterectomy is the elective treatment for pyometra. Culture of the uterine content is performed to adjust the initial therapy. The present study aimed to critically assess the antimicrobial therapy by comparing it with cultural and bacterial susceptibility results.
METHODS
Bitches (N=26) with pyometra undergoing OVH at the Veterinary Teaching Hospital (University of Torino, Italy) were included. Uterine swabs were collected from the organ for culture and susceptibility testing. Samples were processed within 48 hours (IZSVe). Cephazolin (20 mg/kg iv q12h) was administered during hospitalization, unless peritonitis or abdominal fluid were present. Amoxicillin-clavulanate was prescribed (12.5 mg/kg os q12h) at discharge.
RESULTS
Bitches (mean age 9 ±3.2 years) presented either with open (62%) or closed (38%) cervix. A single bacterium was isolated from 17 bitches, two or more bacteria from 8 bitches (N=7 and N=1, respectively), no bacteria was found in the uterus of one bitch. Escherichia coli was the most frequently detected bacterium (N=18, 77% of hemolytic strains), followed by Gram+ cocci (N= 4). Other isolated bacteria were Pseudomonas spp., Proteus spp., Acinetobacter spp. (N=2), Haemophilus haemoglobinophilus (N=1). Majority of bitches hosted bacteria resistant to amoxicillin-clavulanic acid (69%) and to ampicillin (65%). Bacteria from 11 bitches (42.3%) showed resistance to class I cephalosporins and four of them also showed resistance to class III cephalosporins (15.4%). Resistance to tetracyclines, aminoglycosides and trimethoprim-sulfamethoxazole was found in 42.3%, 38.4%, and 30.7% (N=8) swabs, respectively. In one case E. coli was resistant to fluoroquinolones (3.8%).
Overall, resistance to the antimicrobial administered post-surgery was found in 69.2% of cases. However, the antibiotic was not changed in consideration of the quickly improving clinical conditions.
CONCLUSIONS
Our data suggest that in uncomplicated cases, healing can take place even when bacteria within the uterus are resistant to the post-surgical antimicrobial. Moreover, in vitro susceptibility can differ from in vivo results. Changing the antibiotic treatment should be carefully considered when clinical conditions are quickly improving, in order to reduce the risk of bacterial resistance.

M160
VALIDATION OF A DEEP LEARNING-BASED IMAGE ANALYSIS SYSTEM TO DIAGNOSE SUBCLINICAL ENDOMETRITIS IN DAIRY COWS

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BACKGROUND-AIM
Subclinical endometritis (SCE) is the most prevalent uterine disease in dairy cows. The assessment of polymorphonuclear leukocyte (PMN) proportions (%) of endometrial samples is the hallmark for SCE diagnosis. However, observing cytology slides under a light microscope is a time-consuming and subjective procedure. Yet, a non-biased, automated diagnostic method for assessing PMN% in endometrial cytology slides, has not been validated so far. We aimed to validate a computer vision algorithm based on deep machine learning to quantify the PMN% in endometrial cytology slides.
METHODS
Uterine cytobrush samples were collected from 116 postpartum Holstein cows. After sampling, each cytobrush was rolled onto three different slides. One slide was stained using Diff-Quick, while a second was stained using Naphthol (golden standard to stain PMN). One single observer evaluated the slides twice at different days under light microscopy. The last slide was stained with a fluorescent dye, and the PMN% were assessed twice by using a fluorescence microscope connected to a smartphone. Fluorescent images were analyzed via the Oculyze Monitoring Uterine Health (MUH) system, which uses a deep learning-based algorithm to identify PMN. The Spearman correlation test was calculated to assess intra-method repeatability. Lin's concordance correlation coefficient (CCC) was calculated to measure the agreement of the PMN% between evaluation methods. Samples were categorized as SCE positive using different PMN cut-offs (≥1, >5, and >10%), and Kappa values (κ) were calculated within and among evaluation methods.
RESULTS
The intra-method repeatabilities were 0.67, 0.73, and 0.76 for Diff-Quick, Naphthol, and Oculyze MUH, respectively. The intra-method agreements at ≥1% PMN (κ = 0.44 to 0.47) were lower than at >5 (κ = 0.69 to 0.78) or >10% (κ = 0.67 to 0.85) PMN cut-offs. The CCC between Diff-Quick and Oculyze MUH, Naphthol and Diff-Quick, and Naphthol and Oculyze MUH were 0.68, 0.69, and 0.77, respectively. The agreements among evaluation methods at ≥1% PMN were weak (κ = 0.06 to 0.28), while it increased at >5 (κ = 0.48 to 0.81) or >10% (κ = 0.50 to 0.65) PMN cut-offs.
CONCLUSIONS
Deep learning-based algorithms in endometrial cytology are reliable and useful for simplifying and reducing the diagnosis bias of SCE in dairy cows.

M161
HIPPO PATHWAY EFFECTORS: NOVEL PLAYERS IN THE PATHOGENESIS OF CYSTIC OVARIAN DISEASE IN CATTLE

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BACKGROUND-AIM
Among the causes of infertility in high-producing dairy cows, cystic ovarian disease (COD) represents a major problem and causes important economic losses to the dairy industry worldwide. Treatments to cope with COD present highly variable success and, for this reason, it is still critical to better identify, at the cellular level, the key components involved in the mechanism of cyst formation and persistence. Interestingly, the Hippo pathway effector yes-associated protein (YAP) has been described in humans and mice as a key player of anovulatory cystic disorders. Thus, we hypothesized that YAP deregulation in bovine granulosa cells can be also involved in the pathogenesis of COD in dairy cows.
METHODS
Bovine ovaries were obtained from a local abattoir and separated in control group (healthy large follicles >10mm) and COD group (follicular cysts of at least 20 mm in diameter). Following granulosa cells (GCs) isolation for RT-qPCR and immunoblot (WB) or entire follicle preparation for immunohistochemistry (IHC), we assessed Hippo pathway effectors and their target markers.
RESULTS
The results showed that mRNA and protein levels for total YAP (and its transcriptional co-activator TAZ) are significantly higher ($P<0.05$) in GCs from follicle cysts in comparison to GCs from healthy large follicles. In addition, IHC subcellular localization showed that staining for total YAP and TAZ proteins was more intense not only in GCs but also in theca cells from COD in comparison to control group. Considering that when accumulated in the nucleus, YAP/TAZ form complexes notably with TEAD family of transcriptional factors, we then decided to assess mRNA levels for the classic YAP/TAZ-TEADs transcriptional target genes CTGF, CYR61, BIRC5 and ANKRD1. The results indicated that the mRNA abundance for CTGF and BIRC5 genes is significantly increased in GCs from COD in comparison to control group ($P<0.05$).
CONCLUSIONS
Together, these results provide considerable insight of a completely novel signaling pathway as a critical player of the cystic ovarian disease pathogenesis in dairy cattle. Future studies will be conducted to determine if YAP/TAZ can be considered potential target proteins for the pharmacological treatment of COD.

M162
ASSOCIATION OF ENDOMETRIAL INFLAMMATORY AND DEGENERATIVE LESIONS IN DAIRY COWS WITH ENDOMETROSIS

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BACKGROUND-AIM
Uterine diseases decrease reproductive efficiency in dairy cattle. Bovine endometrosis (BE) is defined as endometrial, periglandular and/ or stromal fibrosis with alterations of the affected glands. Endometritis and angiosclerosis are considered by some authors to be causally related to the development of equine endometrosis, however in cattle this is not clear. The aim of this study was to determine if there is an association between endometritis and angiosclerosis with endometrosis in (i) clinic healthy, and (ii) sub/infertile dairy cows.
METHODS
Uterine tissue of 105 cows were assigned to two groups: Group I - endometrial biopsies (n=36) from clinically-gynecologically healthy dairy cows (age 3.89 ± 1.86 years; parity 2.33 ± 1.4; open days 94 ± 28.4), and Group II - uterine tissue sections (n=69) from sub/infertile cows (age 4.38 ± 1.56 years; parity 2.01 ± 1.19; open days 252.82 ± 163.83). Samples were fixed in 10% neutral buffered formalin, paraffin-embedded and cut in 5 µm thick sections; the slides were stained with Hematoxylin/ Eosin. Significant differences between variables were tested by the Mann and Whitney U-Test, and chi²-crosstabs; correlation analysis was performed by Spearman´s rank correlation.
RESULTS
In the group I, in addition to endometrosis, 28% of animals had endometritis, and 26% had angiosclerosis; in the group II, 78% had endometritis and 80% had angiosclerosis. Histopathologically, group II showed a higher degree of endometrial changes than group I. Group II showed endometritis (78%) more frequently ($p<0.001$) than group I (27%); regarding open days, value of group I (94 ± 28.4 days) was different ($p=0.015$) from that of group II (252.82 ± 163.83 days).
CONCLUSIONS
Longer open days from group II may be the result of more severely damaged endometrium (in character and degree). There was no correlation between (i) endometrosis and endometritis, and (ii) endometrosis and angiosclerosis in the present work, however all alterations had a negative effect on the bovine fertility. Supported by PAPIIT IN219620.

M163
ASSOCIATION OF ENDOMETRIAL INFLAMMATORY AND DEGENERATIVE LESIONS IN DAIRY COWS WITH ENDOMETROSIS

M.d.C. Espejel Del Moral [2], S. Merbach [1], H. Schoon [1]
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METHODS
Uterine (body) tissue, one sample per each uterus, from 105 cows were assigned to two groups: Group I - endometrial biopsies (n=36) from clinically gynecologically healthy dairy cows (age 3.89 ± 1.86 years; parity 2.33 ± 1.4; open days 94 ± 28.4), and Group II - uterine tissue sections (n=69) from sub/infertile cows (age 4.38 ± 1.56 years; parity 2.01 ± 1.19; open days 252.82 ± 163.83). Samples were fixed in 10% neutral buffered formalin, paraffin-embedded and cut in 5 µm thick sections; the slides were stained with Hematoxylin/ Eosin. Significant differences between variables were tested by the Mann and Whitney U-Test, and chi²-crosstabs; correlation analysis was performed by Spearman´s rank correlation.
RESULTS
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CONCLUSIONS
Longer open days from group II may be the result of more severely damaged endometrium (in character and degree). There was no correlation between (i) endometrosis and endometritis, and (ii) endometrosis and angiosclerosis in the present work, however all alterations had a negative effect on the bovine fertility. Supported by PAPIIT IN219620.

M165
KISSPEPTIN AND GNIH IN BOVINE FOLLICULAR CYSTS

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BACKGROUND-AIM
Recently, two different molecules have been discovered to play an important role in reproduction: kisspeptin (Kp) and gonadotropin inhibiting hormone (GnIH) (Wang et al., 2018).
Kp stimulates LH release through its effect on GnRH neurons (De Roux et al., 2003), while GnIH inhibits gonadotropin release in vitro and in vivo (Kadokawa et al., 2009). Based on these data, the aim of this study was to investigate Kp, GnIH, LH, cortisol, oestradiol 17β (E2) and progesterone (P4) concentrations in healthy dairy cows and in cows diagnosed with ovarian follicular cysts, to understand the aetiopathogenesis of endocrine imbalances of the hypothalamic-hypophyseal-ovarian axis.
METHODS
Thirty cows diagnosed with follicular cysts (Group FC) and 30 healthy cows on heat (Group H) were enrolled in the study. Blood samples were taken at the second examination in which ovarian follicular cysts were confirmed (Group FC) and on the day of heat (Group H).
GnIH, LH, E2, P4 and cortisol levels were determined using a ELISA kit, while Kp-10 levels were determined using an RIA kit, as previously described and validated (Rizzo et al., 2018; Rizzo et al., 2019). The results are expressed as the mean ± standard deviation (SD). Statistical analyses were carried out using SPSS version 19 (IBM, New York, USA). A value of $P<0.05$ was considered significant.
RESULTS
GnIH, Kp-10, LH, E2, P4 and cortisol levels were significantly higher in Group FC than in Group H. The Pearson's test showed a positive correlation between E2 and Kp-10 ($P<0.05$), in both Group FC ($r=0.76$) and Group H ($r=0.67$).
CONCLUSIONS
To the best of our knowledge, this is the first study evaluating GnIH and Kp-10 levels to assess endocrine imbalances of the hypothalamic-hypophyseal-ovarian axis, in both physiological (healthy cows on heat) and pathological (cows with Follicular Cysts) conditions of dairy cows.
We surmise that the higher levels of Kp-10 in group FC were induced by E2 secreted from cysts, and Kp-10 contributed to the maintenance of the cysts, because it stimulated LH release.
It is plausible that the GnIH increase may be due to stress. In fact, a close link between stress and the development of bovine follicular cysts has been shown (Mimoune et al., 2019).

TOPIC Buffalo reproduction

T01
PHOTOPERIODICITY AFFECTS THE RATE OF OOCYTE RECOVERY USING ULTRASOUND GUIDED OVUM PICK-UP IN NILI-RAVI BUFFALOES

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BACKGROUND-AIM
Nili-Ravi buffalo has potential to produce 30 liters of milk per day and its genetic merit needs to be exploited using assisted reproductive technologies. However, the standardization of these technologies is still lacking in Nili-Ravi buffalo. We hypothesized that photoperiodicity would influence the rate of oocyte recovery (OR) and quality (OQ) using ovum pick-up (OPU) in buffaloes. Therefore, the objective of the present study was to determine the impact of photoperiodicity on the OR and OQ using OPU in buffaloes. An additional objective was to evaluate the effect of cyclicity (cyclic vs. acyclic), and gauge (G) of needle (17G vs. 18G) for the optimization of OPU technique in buffaloes, and whether the efficiency of technique equivalent to Bos taurus or Bos indicus cows.
METHODS
In 1st experiment, repeated OPU sessions once a week (n = 64) either using 17G or 18G of needles were conducted during peak (Sep–Nov), transition (Dec–Feb), and low (Mar–May) breeding seasons in buffaloes (n = 12). The cyclicity was determined based on the presence or absence of corpus luteum between two consecutive OPU sessions. In 2nd experiment, recurrent OPU sessions once a week (n = 24) were performed in Bos taurus (n = 5) and Bos indicus (n = 5) cows. Data on the OR and OQ were analyzed using the PROC GLIMMIX procedures of SAS.
RESULTS
Results (LSM ± SEM) revealed that rate of OR was higher ($P < 0.05$) during peak (0.61 ± 0.09) and transition (0.54 ± 0.10) as compared to low (0.31 ± 0.09) breeding seasons in buffaloes. The OR rate was greater ($P < 0.05$) using 17G (0.62 ± 0.09) as opposed to 18G needle (0.35 ± 0.08) in buffaloes. However, OR did not differ ($P > 0.05$) between cyclic (0.42 ± 0.11) and acyclic (0.55 ± 0.08) buffaloes. The OQ was not influenced ($P > 0.05$) during peak (0.04 ± 0.04), transition (0.06 ± 0.07), and low (0.13 ± 0.12) breeding seasons in buffaloes. Similarly, the OQ did not differ ($P > 0.05$) between 17 (0.05 ± 0.05) or 18 (0.09 ± 0.08) G of needle in buffaloes. Interestingly, OR (0.57 ± 0.16 vs. 0.24 ± 0.12) and OQ (0.45 ± 0.15 vs. 0.40 ± 0.22) did not differ ($P > 0.05$) between Bos taurus or Bos indicus cows.
CONCLUSIONS
Taken together, it is concluded that peak or transition breeding seasons are suitable to perform OPU using 17 G needle for maximum OR in Nili-Ravi buffaloes.

T02
EFFECT OF STEM CELLS INJECTION IN THE OVARY ON THE OOCYTE AND EMBRYO PRODUCTION IN BUFFALO

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BACKGROUND-AIM
The aim of this study was to evaluate the follicular population, oocytes recovery per OPU and embryo production after the injection of allogeneic mesenchymal stem cells (MSCs) in the ovarian cortical layer.
METHODS
Buffalo (Bubalus bubalis) were synchronized for follicular growth wave [D-42: insertion of an P4 device (1.0g) and administrations of EB (2.0mg i.m.) and PGF2a (0.53mg i.m., sodium cloprostenol)]. Five days later (D-37), the P4 were removed and the animals submitted to ultrasonographic evaluations and ovum pick up (OPU) followed by in vitro embryo production (IVEP). These procedures were repeated at 21days intervals (D-21 and D0). On D1, buffaloes were distributed according to the age, BCS, PPP, parity and follicular population (verified in 3 OPU section) in one of three experimental groups: C (no stem cells application; n=8), MSC1 (MSCs application in one ovary; 5x106 cells per ovary; n=8) and MSC2 (MSCs application in two ovaries; 5x106 cells per ovary; n=8). Allogeneic MSCs from adipogenic origin were isolated and cultivated in IMDM culture medium with 20% FBS and 1% P/S, at 37°C in 5% of CO2 for cellular expansion from third passage. Posteriorly, MSCs were frozen in DMSO and maintained in liquid nitrogen until the day of application in the ovaries. On the injection day (D1), the stem cells were thawed and DMSO removed. Followed, were maintained in IMDM culture medium until the application moment. After MSCs application, the buffaloes of all groups were again submitted to follicular synchronizations, ultrasonographic evaluations and OPU-IVEP procedures at 21, 42, 63, 84, 105 and 126 days. The data were analyzed as time-repeated measures using the GLIMMIX procedure of SAS.
RESULTS
It was not observed interaction between treatment and time for any variables evaluated. There were no significant differences among groups in the total follicles aspirated (C=11.1±0.6, MSC1=10.3±0.6 and MSC2=11.0±0.6; P=0.83), oocytes retrieved (C=7.1±0.6, MSC1=5.7±0.4 and MSC2=6.6±0.6; P=0.59), viable oocytes (C=3.9±0.4, MSC1=2.9±0.3 and MSC2=3.0±0.3; P=0.40), oocytes cleaved (C=2.4±0.3, MSC1=1.6±0.2 and MSC2=2.0±0.3; P=0.17) and embryos/OPU (C=1.6±0.2, MSC1=1.0±0.1 and MSC2=1.4±0.2; P=0.30).
CONCLUSIONS
MSCs application did not increase the OPU-IVEP efficiency in buffalo.

T03
RELATIONSHIP OF BLOOD PLASMA, URINE, UTERINE FLUID AND EMBRYO PROTEOMIC FROM PREGNANT BUFFALOES

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BACKGROUND-AIM
Early diagnosis of pregnancy is a tool that can contribute to increase reproductive rates, allowing estrus
resynchronization in nonpregnant females. Then, the aim of this study was to describe the relationship of the proteins found in the serum, urine, uterine fluid and embryo of the pregnant buffaloes.
METHODS
Ten crossbred Murrah females had estrous cycle synchronized. Eighteen days after artificial insemination, the females
were slaughtered and samples of urine, blood and reproductive tract was collected. In the laboratory, the blood samples
were centrifuged at 800g for 10 min to obtain plasma. Embryos were collection by the washing of uterus with 10 mL of
PBS/horn simultaneously and the embryos retrieved in Petri plates. Embryos were stored in protein extraction buffer
(RIPA) containing protease inhibitors (0.8 mM EDTA; 1 µg/mL aprotinin; 1 µg/mL leupeptin; 35 µg/mL PMSF; 0.7 µg/mL pepstatin), and the proteins were extracted by sonication. Recovered uterine fluid was centrifuged at 15,000g for 30 min at 4° C. The urine was collected by bladder puncture and also centrifuged. The urine supernatant was concentrated
by ultrafiltration (cutoff 3 kDa). In all samples, an aliquot containing 50 µg of protein was submitted to a 12% SDSPAGE and running stopped when the samples reached the separation gel. Bands were stained and cut out from the gel.
Protein in gel digestion was performed and the peptide mixture was analyzed by ESI-Q TOF mass spectrometry.
RESULTS
Descriptive analysis found 205 proteins. The major proteins were serum albumin, serotransferrin, hemopexin, serpin A3 and alpha-2-macroglobulin. In the gene ontology analysis the main molecular functions of the proteins were catalytic activity and binding, in the biological process, adhesion and regulation, and in the cellular component, cell junction. These proteins have already been reported in other studies to be involved in metabolic and energy pathways in the stages of embryonic development, acting in the endometrial modulation necessary for the establishment of pregnancy and maternal-fetal communication.
CONCLUSIONS
Based on our results, we conclude that the proteins found can be used as possible biomarkers in the early diagnosis of pregnancy as they are involved in crucial events during embryonic implantation, establishment and maintenance of pregnancy. Since this is an experiment with partial results, it must be completed so that we can have a larger sample size and reach more precise conclusions regarding the functions of these proteins during the analyzed period.

T04
EFFECT OF PROSTAGLANDIN ADMINISTRATION AT THE MOMENT OF TAI IN BUFFALO HEIFERS

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BACKGROUND-AIM
The hypothesis was that PGF2a administration at the moment of TAI increases the synchronization of ovulation, as well as ovulation and pregnancy rates.
METHODS
At random stage of the estrous cycle (D0), 374 buffalo heifers were subjected to ultrasonographic examinations (Mindray DP2200Vet) to check ovarian activity, and received an intravaginal progesterone device (P4; Sincrogest) more 2mg im of estradiol benzoate (EB, Benzoato HC). In D9, the animals received 0.53mg im of PGF2a (sodium cloprostenol, Sincrocio), 400IU im of eCG (Folligon), and the P4 were removed. After 24h (D10), the heifer's ovulation was induced by the application of 1mg im of EB (Benzoato HC) and, at this moment, only animals with follicles larger than 9mm, verified by ultrasound, remained in this study (n=267). 32h later, the heifers were again subjected to the ultrasound examination and those who ovulated (n=13) were removed from the experiment. The remaining females were submitted to TAI (56h after P4 remove) and were divided according to age, weight, body condition score, ovarian activity and the diameter of largest follicle verified in D10, into one of two groups: Control (n=126) and PGF (n=128). The heifers of the PGF group received 0.53mg im of PGF2a (Sincrocio) at the moment of TAI. The pregnancy was acceced by ultrasound examination on D41. In a subgroup of animals (Control, n=18 and PGF, n=22), the ultrasonund examination was carried out from D9 to D11 (24/24h) to verify the follicle diameter and growth rate, from D11 to D14 (12/12h for 60h) to check the time of ovulation and the diameter of ovulatory follicle (OF) and on D19 to measure the diameter of CL. The statistical analysis was performed by GLIMMIX of the SAS.
RESULTS
There was no difference between the experimental groups (Control and PGF) for the analyzed variables: follicle growth rate (1.7±0.2 and 1.7±0.1 mm/day; P=0.94); time of ovulation (67.4±1.0 and 70.4±1.5 h, P=0.13); OF (13.1±0.3 and 13.0±0.4 mm; P=0.77); ovulation rate [94.4% (17/18) and 100.0% (22/22); P=0.98]; diameter of CL (17.6±0.5 vs. 17.2±0.5 mm; P=0.53) and pregnancy rate [37.3% (47/126) vs. 46.9% (60/128); P=0.11].
CONCLUSIONS
The PGF2a administration at the moment of TAI does not promote any increase in ovulation synchronization or in ovulation and pregnancy rates, rejecting the initial hypothesis.

T07

SERUM AND RECOMBINANT ECG STUMULATION TREATMENT PRIOR TO OVUM PICK-UP AND IVP IN WATER BUFFALO

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BACKGROUND-AIM

The low quantity and quality of oocytes collected via ovum pick-up is one of the main limitations to IVP in water buffaloes. For this reason, hormonal stimulation prior to folicular aspiration could provide an alternative to improve the number and quality of oocytes obtained. The objective of this study was to evaluate two sources of eCG (serum and recombinant) prior to OPU.

METHODS

A total of 30 OPUs were performed in two aspiration sessions, using 15 Murrah (n=10) and Mediterranean (n=5) buffalo cows (age: 4,07±2,2 and weight: 502,7±78,2 kg). Treatments prior to OPU were as follows: TRT1 (n=10): day 0 intravaginal progesterone device + 2 mg estradiol benzoate / day 4 1050 IU recombinant eCG (foli-rec®) / OPU 72 hs post eCG. TRT2 (n=10): day 0 intravaginal progesterone device + 2 mg estradiol benzoate / day 4 2500 IU serum eCG (ECEGON®) / OPU 72 hs post eCG. TRT3 (n=10): control, day 0 intravaginal progesterone device + 2 mg estradiol benzoate and no eCG stimulation prior to OPU. Follicular response (number of follicles) was recorded prior to OPU as small (≤3mm), medium (4 to 8 mm) and large (≥9 mm) diameter. Recovered oocytes were classified according to IETS guidelines as grades 1 to 4 and placed in IVM at 38.5°C and humidified atmosphere of 5% CO2 in air; IVF was conducted using bulls of proven fertility. Embryos were cultured for 6.5 days to blastocyst stage and were then vitrified. Results were analyzed using ANOVA.

RESULTS

Small follicle count prior to OPU was 3.3 for TRT1; 2.1 for TRT2; and 5.6 for TRT3, the last being significantly higher (P<0,05). Medium follicle count was 5.7 for TRT1; 8.3 for TRT2; and 4.8 for TRT3, being TRT2 statistically significant (P<0,05). Large follicle count did not differ among treatments (0.2; 0.8; and 0.3 for small, medium and large, respectively). Oocyte quality differed significantly among treatment groups; the highest oocyte quality was observed in TRT2. The number of embryos produced per OPU/animal was not statistically different among groups (1 for TRT1; 1.11 en TRT2; and 0.8 for TRT3; P>0,05).

CONCLUSIONS

Results indicate serum eCG stimulation prior to OPU could improve medium size follicular count and oocyte quality, although no effect was detected on embryo production.

T09

RETROSPECTIVE STUDY ON INNATE IMMUNITY RESPONSE DURING DRY PERIOD IN PLURIPAROUS BUFFALOES WITH HEALT DISORDERS

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BACKGROUND-AIM

The transition period (TP) represents a critical life phase in buffaloes. In this period, the intensification of milk production associated with metabolic-nutritional disorders, and unfavourable environmental conditions produce stress, changes in the innate-immunity status and increase the risk of diseases in buffaloes.

The haptoglobin (HP) concentrations were identified as the most sensitive parameters able to detect stress conditions in buffaloes. Furthermore, changes in the HP concentrations were observed in buffaloes during TP.

The aim of this retrospective study was to evaluate the innate-immunity status of pluriparous buffaloes and to assess the differences in HP levels between healthy and sick animals during TP.

METHODS

15 pluriparous Buffaloes (milk yield = 2833.50 ± 231.60 kg) were enrolled in an intensive buffalo dairy farm. The blood samples were collected weekly, from -45 days before calving, until 30 days in milk.

The samples were centrifuged in field (2.500 rpm/15') and the serum was stocked at -80°C in the laboratory. On serum samples, the concentrations of hemolytic complement, lysozyme, bactericidal activity and HP were assessed. Uric acid, amylase, bilirubin, ALP, cholesterol, creatinine, γGT, glucose, AST, ALT, ALP, LDH, Mg, P, triglycerides and urea were evaluated. Body condition score (BCS) was recorded monthly. After parturition, buffaloes were classified according to the incidence of diseases (dystocia, uterine or vaginal prolapse, retained placenta) during the first 7 days after calving:

healthy animals (Group H, n=10) and sick animals (Group NH, n=5).

Statistical analysis was performed by Student's t-test.

RESULTS

HP showed higher value in sick animals starting to 7 days (P<0.05) before calving to 7 days (P<0.01) after parturition. No differences were observed between two groups in other parameters.

CONCLUSIONS

Analysis of these data suggests that HP values could be used as a predictive index for healthy disorders risk during the TP. Further studies are required to define values and

cut-off of HP during the transition period and the related correlations with diseases after calving.

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TOPIC Male reproductive physiology

T10
ANABOLIC-ANDROGENIC STEROIDS INHIBIT TESTOSTERONE AND LUTEINIZING HORMONE BIOSYNTHESES TO CAUSE HYPOGONADISM IN MALE RATS

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BACKGROUND-AIM
Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone (T), possess both anabolic and androgenic properties. However, the mechanisms through which AAS disrupt reproductive system are largely unknown. We studied the adverse effects of nandrolone decanoate (ND) on reproductive system in male rats, and compared with testosterone enanthate (TE) as positive control. ND is metabolized to nandrolone with higher affinity to AR compared to T that is metabolized from TE. The administration doses were determined based on previous studies with consideration to aromatization to estradiol.
METHODS
Adult male Wistar rats (8 weeks old) were treated with intramuscular injection of ND (1, 3, and 9 mg/kg/week) and TE (1 and 3 mg/kg/week). One week after the 4th and 8th injection, gonadosomatic index (GSI = gonad weight / total tissue weight × 100) and reproductive hormones levels were measured using ELISA.
RESULTS
There were no changes in the progesterone and 17beta-estradiol (E2) levels during the period of the experiment. After the 4th injection, GSI was decreased in both ND and TE treated rats ($P<0.05$). T levels and T/E2 ratio were decreased in ND treated rats ($P<0.05$). Trends toward decreases in T and T/E2 ratio were observed in rats treated with TE ($P>0.05$). Dose-dependent decreases in luteinizing hormone (LH) levels were observed in ND treated rats, which was significant at 9 mg/kg. In TE treated rats, LH levels were decreased at 1 mg/kg ($P<0.05$). After the 8th injection, GSI was decreased in rats treated with 1 and 3 mg/kg ND and 3 mg/kg TE ($P<0.05$). Except for 3 mg/kg TE, T levels were decreased in all treatments ($P<0.05$). The T/E2 ratio showed trends toward decreases, which were significant in rats treated with 3 and 9 mg/kg ND. LH levels were increased in rats treated with 1 mg/kg ND ($P<0.05$), but remained unchanged in all other treatments.
CONCLUSIONS
Results indicate AAS-inhibited T biosynthesis causes hypogonadism in male rats. This was associated with decrease in LH levels after 4th injection suggesting that AAS are capable of acting on pituitary to inhibit LH release. However, inhibition of T biosynthesis after 8th injection was not accompanied by decrease in LH levels suggesting that feedback regulation of T on LH release was not probably disrupted. Taken together, disruption of hormonal functions of the pituitary and testes depends on duration of administration of AAS to cause hypogonadism.

T11
THE EFFECTS OF CONSUMING RED CLOVER PASTURE AND BENEFICIAL FATTY ACID SUPPLEMENTS ON RAM LAMB REPRODUCTIVE HORMONES

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BACKGROUND-AIM
Components of the diet, such as fatty acids and phytoestrogens, can affect current and future reproductive performance. Fish oil (FO) is a good source of omega-3 polyunsaturated fatty acids (n-3 PUFAs) and has been shown to affect reproductive events. There is an increase in n-3 PUFAs and conjugated linoleic acid (CLA) in lambs that consume red clover (RC) pasture. Both fatty acids have been shown to reduce body fat. Also, phytoestrogens are found in RC. The objective of this study was to determine the effects of consuming red clover pasture and beneficial fatty acid supplements on ram lamb reproductive hormones.
METHODS
Thirty-two 2-3 month old crossbred ram lambs were randomly divided into four groups (n=8 for each group) and balanced by body weight and dam parity. Two groups grazed tall fescue (TF)/RC and two groups grazed TF pasture for 10 weeks. These groups were then further randomly subdivided to receive a fat supplement (soybean oil (SBO; n=8), SBO+CLA (n=8), or FO (n=8)) or no fat supplement (CON; n=8) during a 5-week finishing diet. Blood samples were collected every 2 weeks on pasture, and before the, mid-, and at the end of the finishing diet. Serum LH, FSH and testosterone (T) concentrations ([]; ng/mL) were determined in the blood samples by radioimmunoassay.
RESULTS
Serum [T] increased from Week 0 to 2 and Week 6 to 10 over the pasturing period (P<0.05). Serum [FSH] decreased from Week 0 to 2 and increased from Week 4 to 6 and Week 8 to 10 over the pasturing period (P<0.05); [LH] did not change (P>0.05). Serum [T] decreased (P<0.05) over the finishing diet period. Serum [LH] and [FSH] decreased (P<0.005) from before the to mid-finishing diet period. Lambs that grazed TF/RC had lower (P<0.001) [FSH] than lambs that grazed TF alone both before (TF/RC=0.19±0.02; TF=0.29±0.02) and over (TF/RC=0.15±0.01; TF=0.20±0.01) the finishing diet period. For CON and SBO+CLA lambs, [FSH] decreased (P<0.0005) from before the to mid-finishing diet period. For SBO lambs, [FSH] decreased (P<0.05) from before to the end of the finishing diet period; [FSH] did not change (P>0.05) for FO lambs.
CONCLUSIONS
Grazing RC reduced [FSH], but had no effect on [LH] or [T]. Over the finishing diet period, [FSH] decreased regardless of pasture or fat supplement consumed; FO had no effect.

T12
ASSOCIATIONS OF SPERM HEAD MORPHOMETRICS WITH SEGMENTAL DEFECTS IN FRESH AND FROZEN-THAWED RAM SEMEN

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BACKGROUND-AIM
There is a known association between sperm head morphometrics (SHM) and overall viability/fertilizing ability, but no previous studies have analyzed associations of SHM with sperm segmental defects (SD) in different breeds of sheep. The objectives of the present experiment were to determine if SHM were correlated with SD of fresh and frozen-thawed ram spermatozoa, and if SHM and/or SD ratios differed between natural (N) and synthetic (S) genotypes of rams.
METHODS
Ejaculates (n=2/animal) were obtained from eleven clinically healthy and fertile rams aged 4 to 12 years: PLS (Polish Lowland Sheep; n=3 (N)); SCPLS (Suffolk x Charolaise x PLS; n=4 (S)) and BCPLS (Berrichon x Charolaise x PLS; n=4 (S)). Semen was collected into a pre-warmed (~39 degrees C) artificial vagina during the breeding season. All ejaculates were diluted in a commercial semen extender Triladyl® and frozen in 0.25-cc straws. Microscopic images of semen smears were evaluated for sperm segmental defects (Nikon Eclipse 80i microscope, Nikon Corp., Tokyo, Japan), and sperm head morphometrics (length (L), width (W), area (A), perimeter (P) and roundness (R)) were determined using ImageProPlus® analytical software.
RESULTS
Mean L and W were less (P<0.05) while mean P and R were greater (P<0.05) in frozen-thawed compared with fresh semen in all breeds of sheep studied. In addition, W was greatest (P<0.05) in BCPLS before freezing. Although there was a numerical increase for all types of SD after freezing, the significant rise in percentages of all sperm head and mid-piece defects was only noted for BCPLS. Significant correlations were found among L (r=-0.44, P=0.04), A (r=-0.42, P=0.05), P (r=-0.45, P=0.04), and the percentage of tail defects in fresh sperm. In frozen-thawed inseminates, L was inversely related to the percentage of head defects (r=-0.56, P=0.007) and A to the percentage of thin mid-pieces (r=-0.60, P=0.003).
CONCLUSIONS
Sperm head dimensions were significantly greater (L and W)/smaller (P and R) in fresh than in frozen-thawed ram semen, while the percentage of sperm head and mid-piece defects was significantly greater only in frozen-thawed compared with fresh sperm of BCPLS (synthetic breed). Dissimilar correlations were recorded between SHM and SD in freshly collected and frozen-thawed ram semen across all three breeds.

T13
EFFECT OF BULL OR RAM SEMINAL PLASMA ON BOVINE EPIDIDYMAL SPERM FUNCTION: AN INTERSPECIES MODEL TO DETERMINE THE ROLE OF ACCESSORY GLANDS IN CATTLE FERTILITY

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BACKGROUND-AIM
In the male reproductive tract, sperm are bathed in secretions from the testes, epididymides, and accessory sex glands (AGs), which collectively constitute seminal plasma (SP). The role of epididymal fluid in sperm function is clear, as it induces progressive acquisition of motility and fertilizing ability. However, although artificial insemination with cauda epididymal sperm leads to pregnancies in cattle, the role of AG secretions in bull fertility is not as clear. SP composition varies between species, including bull and ram. Thus, we used a model in which bull epididymal sperm was exposed to bull or ram SP, to determine the role of AG secretions in sperm motility and in vitro fertility.
METHODS
To obtain bull and ram SP, ejaculates were collected using an artificial vagina (n=6 males/species), pooled within species, centrifuged, filtered, and stored at -80°C. Epididymal sperm were collected from the cauda epididymis of bulls (n=3) slaughtered in a commercial abattoir and pooled (n=3 replicates). Samples were then washed by centrifugation in Tyrode's medium, resuspended in bull SP, ram SP, or PBS (control); and incubated for 10 min at 38.5°C. Then, each treatment was washed through a Bovipure gradient to select motile sperm and remove the supernatant. Finally, sperm concentration was adjusted to 2x106 sperm/ml. Total motility and progressive motility were determined by CASA at 0, 2 and 24 h. In addition, in vitro fertilization (Day 0) of bovine oocytes was performed.
RESULTS
At 0 and 24h, no differences in total and progressive motility between treatments were detected. However, at 2h, epididymal sperm exposed to bull SP, but not ram SP, had lower total motility than the control (48±11.09% vs. 71±10.08% and 71±3.17%, respectively; $P<0.05$), while both bull and ram SP reduced the percentage of progressively motile sperm vs. the control (38±11.09% and 55±7,69% vs. 64±7.69%, respectively; $P<0.05$). No differences were observed in the percentage of cleaved embryos at Day 2, nor blastocyst rates or hatched blastocyst rates on Days 7, 8, or 9 ($P>0.05$).
CONCLUSIONS
In conclusion, AG secretions do not seem regulate bovine sperm-oocyte interaction, fertilization and early embryo development; however, these results suggest that they can influence sperm motility.

T14
A NOVEL APPROACH FOR OBJECTIVE MEASUREMENT OF LIBIDO IN BOARS

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BACKGROUND-AIM
Libido can be defined as the sexual drive of boars before and during semen collection. Sexual behaviour and different environmental factors affecting libido have been previously reported. However, previous studies included subjective measurements for libido. The interval between arrival at the dummy and ejaculate collection (IADEC) is an objective parameter that can be easily and massively measured during semen production by means of automatic detection systems and it could be used as an objective trait for libido evaluation. The aim of this study was to stablish an objective measurement of libido in boars.
METHODS
The study was performed in one AI center (AIM Ibérica, Spain) between Dec 30th, 2020 and Oct 31st, 2021. The collection area included 11 identical collection dummies. All boars were kept under similar controlled conditions (feed, light, temperature) and were managed by skilled technicians. Each boar was equipped with an electronic RFID ear tag that was manually registered when boar entered the collection pen, and then again, few seconds after semen collection.
RESULTS
Records of a total of 15,620 visits to the dummy and ejaculate collections from 423 boars (Duroc, Pietrain, Hybrid lines) were recorded for this study. The IADEC averaged 14.2 ± 8.5 min and was analysed with the assumed model using the software ASREML. Least square means were calculated. The significance of the effects was assessed using a Wald F test. There was a significant effect of breed, day of the week, week of the year, dummy and rest days from previous collection ($p<.001$) on IADEC. No significant effect was found for age of the boars. Correlations between IADEC and semen quality and quantity traits (total number of sperm per ejaculate, motility at 0 and 96h and sperm abnormalities) were all negligible in this study.
CONCLUSIONS
In conclusion, the IADEC can be monitored in AI centers. The relationship found between the variables under study and the interval between arrival at the dummy and ejaculate collection may help AI centers to design optimal workflows and to optimize productivity during sperm collection. Further studies on genetic influence on this trait are required to evaluate if this trait could be considered as part of a selection index.

T15
EXPRESSION OF ANGIOGENIC GENES DECREASES IN THE TESTICLES OF SPONTANEOUSLY HYPERTENSIVE RATS

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BACKGROUND-AIM
Hypertension is a cause of low fertility in men and exercises are indicated to decrease blood pressure and improve overall health. The objective of this study is to verify the relative expression of angiogenic genes in the testicles of spontaneously hypertensive rats (SHR) submitted to high intensity interval training (HIIT).
METHODS
Wistar-Kyoto rats, male, adult, with and without spontaneous hypertension were distributed in 3 groups: WKY (control of Wistar-Kyoto rats without hypertension, n=5); SHR (group of SHR rats, n=9); and SHR-HIIT (group of SHR rats submitted to HIIT, n=9). The treadmill HIIT training was realized for 5 days/week for 8 weeks, for 50 minutes approximately, based on the maximum exhaust speed, with active rest intervals. The lactate threshold analysis was performed to determine the training speeds. The animals initially underwent an adaptation to the HIIT training, which consisted of running on the treadmill for 2 minutes at 0.5 km/h, followed by 5 minutes of rest and 3 minutes at the speed of 0.7 km/h, increasing 0.2 km/ha every 3 minutes until it reached 1 mmol/L of lactate above the initial test. After the adaptation period HIIT was performed with 5 minutes of heating in at 40% of the lactate threshold. After warming up, the training was started with 3 minutes at 60% of the lactate threshold followed by 4 minutes of 85% of the lactate threshold, which was repeated seven times each session. The testicles were collected immediately at the time of euthanasia and analyzed by RT-qPCR for the angiogenic genes (Vegf, Kdr and Flt1). The reference gene used was Hprt1. The results were analyzed by analysis of variance (ANOVA), followed by the Tukey test ($P<0.05$).
RESULTS
SHR (Vegf = 0.45 ± 0.03; Kdr = 0.15 ± 0.03; and Flt1 = 0.61 ± 0.05) and SHR-HIIT (Vegf = 0.41 ± 0.04; Kdr = 0.40 ± 0.22; and Flt1 = 0.53 ± 0.05) had lower ($P<0.05$) relative expression of angiogenic genes than normotensive rats, WKY (Vegf = 0.90 ± 0.14; Kdr = 0.87 ± 0.20; and Flt1 = 0.99 ± 0.09).
CONCLUSIONS
It is concluded that hypertension decreased the relative gene expression of angiogenic factors and HIIT do not alter the expression of these genes. Financial support by FAPESP (process number: 2018/22682-0) and PIBIC-EM (CNPq).

T16
ROLE OF ALDOSE REDUCTASE B1 ON POST-THAW QUALITY AND FUNCTION OF BOVINE SPERM

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BACKGROUND-AIM
Aldose reductase B1 (AKR1B1) is a multi-function enzyme involved in both male and female reproductive success. In bull sperm, AKR1B1 has been found to increase alongside epididymal transit and to be involved in sperm epididymal maturation. However, no studies have been conducted evaluating the relevance of AKR1B1 on ejaculated sperm quality and function in bulls. Against this background, the present work aimed to evaluate the potential relationship between sperm AKR1B1 levels and sperm quality and functionality parameters after thawing.
METHODS
A total of 45 ejaculates (three per bull) were cryopreserved. Straws from three ejaculates from the same bull were thawed, pooled and split into three aliquots for: i) sperm morphology assessment; ii) post-thaw sperm quality/functionality evaluation through computer-assisted sperm analysis [CASA] and flow cytometry (intracellular calcium, peroxide and superoxide levels, acrosome integrity, membrane destabilisation); and iii) AKR1B1 analysis with immunoblotting.
RESULTS
The western blot revealed a specific two-band pattern, at 36 kDa (monomeric AKR1B1) and ~80 kDa (dimeric AKR1B1), in all sperm samples. Two different ratios were calculated: 36 kDa form/total AKR1B1 and ~80 kDa form/total AKR1B1, the total amount of AKR1B1 resulting from the sum of the two bands. Next, Pearson correlations between these ratios and post-thaw quality and functionality parameters were calculated. Only a weak correlation between AKR1B1 levels and sperm function was found ($P < 0.05$); specifically, both ratios correlated to the percentage of viable sperm with an intact acrosome (PNA-/PI-; for 36 kDa form/total AKR1B1 $R = -0.592$, and for ~80 kDa form/total AKR1B1, $R = 0.592$).
CONCLUSIONS
Considering the post-thaw sperm quality and functionality parameters assessed in the present work, AKR1B1 does not seem to influence sperm quality/function of cryopreserved sperm. However, because of the positive influence of this protein on in vivo fertility in pigs, one cannot discard that AKR1B1 could have a positive influence in oocyte fertilisation and even embryo development in cattle. Funded by: EC (H2020-MSCA-IF-2019-891382), MICINN, Spain (AGL2017-88329-R) and AGAUR, Catalonia (2017-SGR-1229; 2020-FI-B-00412).

T17
EFFECT OF RECOMBINANT RABBIT β-NGF ON VIABILITY AND MOTILITY OF RAW RABBIT SEMEN OVER TIME

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BACKGROUND-AIM
The β-NGF is present in seminal plasma and has different roles on the sperm in terms of viability and motility depending on the species and doses used. In this study, we investigated the effects of recombinant rabbit β-NGF (rrβ-NGF) produced in our laboratory (Sanchez-Rodriguez et al., PLOS ONE 2019, 14(7):e0219780) on the viability and motility of ejaculated rabbit sperm.
METHODS
Semen was collected from 6 rabbits according to the Animal Ethics Committee (PROEX 302/15). Different concentrations of rrβ-NGF (0, 2, 20 and 100ng/ml) were added to the pooled semen at room temperature. Sperm viability was evaluated with eosin-nigrosin staining and motility with Computer Assisted Semen Analysis (CASA) at 0, 1 and 2h post-addition. The parameters studied with CASA were static (STAT) and progressive (PMOT) sperm percentages, curve-linear (VCL), straight-line (VSL), and average path velocities (VAP), linearity (LIN), straightness (STR), and wobble (WOB). Two-way ANOVA with repeated measures was used, considering the experimental concentrations, the time and the interaction between them as fixed effects.
RESULTS
The sperm viability, STAT and PMOT were similar over concentrations and times studied.
Regarding the time of challenge, sperm cells had similar values of VCL, VSL, VAP, LIN, STR and WOB in all concentrations tested at time 0, suggesting that rrβ-NGF does not alter those parameters at the moment of its addition. VCL and VAP increased over time in all concentrations tested. However, LIN and STR decreased over time in higher concentrations, being higher at 20 ng/ml.
Regarding the concentration used and comparing with the control, the concentration of 100 ng/ml showed the worst values after 2 h, since the parameters VSL, LIN, STR and WOB decreased.
CONCLUSIONS
The rrβ-NGF maintain the sperm viability and affects the sperm motility in a time and dose-dependent manner. Higher concentrations increase the velocity of sperm, and may enhance the energy consumption, which may produce a diminution of the sperm motility. This can be deleterious for the following assisted reproductive techniques.
Funds by AGL2015-65572-C2-2-R Grant and Predoctoral Contract UCM-Santander.

T18
SPERM FROM LOW AND HIGH FERTILITY BULLS DIFFER IN THEIR DNA METHYLATION PATTERNS

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BACKGROUND-AIM
Despite considerable international effort on the evaluation of bull semen quality, reliable prediction of bull fertility is still a major challenge. Recently, attention has turned to examining underlying molecular mechanisms which could uncover potential biomarkers of fertility. One such regulatory process is DNA methylation, which, together with other epigenetic mechanisms, is essential for the fertilising sperm to drive normal embryo development and establishment of a viable pregnancy. In this study, we investigated DNA methylation patterns in sperm from bulls used in artificial insemination with contrasting field fertility.
METHODS
The study was performed on frozen-thawed semen from 10 low and 10 high fertility Holstein Friesian bulls with average fertility scores of -6.6% and +6.5%, respectively (average of the population was zero). DNA was isolated from three pooled ejaculates per bull. The analysis of DNA methylation was performed by reduced representative bisulphite sequencing and differential analysis was performed by MethylKit software. A CpG10 was considered as a differentially methylated cytosine (DMC) when the adjusted p-value was less than 0.001, and the methylation difference between two groups was at least 35%. A differentially methylated region (DMR) was constituted by a minimum of three DMCs with a maximum inter-DMC distance of 100 bp.
RESULTS
Comparing low and high fertility bulls, we identified 661 DMCs, which were balanced between the groups in hypo- and hyper-methylation. They were preferentially located in intergenic regions, introns, repetitive elements, and open sea. We also identified 10 DMRs, covered by seven unique genes (SFRP1, STXBP4, BCR, PSMG4, ARSG, ATP11A, RXRA), which have been reported to be involved in spermatogenesis and early embryonic development.
CONCLUSIONS
In conclusion, using more stringent criteria than in previously published studies for analysis of sperm DNA methylation patterns, we found a relationship between sperm DNA methylation status and bull fertility within seven differentially methylated genes in sperm of subfertile bulls that may lead to altered gene expression during embryo development.

TOPIC Metabolism and reproduction

T19
FIRST POSPARTUM OVULATION AND PRESENCE OF OVARIAN CYSTS IN GRAZING DAIRY COWS MANAGED UNDER CONTRASTING CONDITIONS

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BACKGROUND-AIM
We aimed to study the effect of nutrition and cow management on ovarian physiology.
METHODS
In Uruguay, Holstein cows were blocked by expected calving date, parity, body weight and body condition score (BCS) and assigned to 3 treatments after calving until 75 days in milk (DIM): cows offered total mixed ration (TMR) ad libitum and confined (TMR group n=16), grazing cows confined at least 8 h when 30% of dry matter intake (DMI) of supplementation was offered (grazing/TMR group, n=16) or grazing cows staying outdoors offered equal supplementation than previous group (grazing group, n=15). Milk production was determined daily to 75 DIM and BCS every two weeks to 75 DIM when a blood sample was taken for IGF-1 determination (-15 to 60 DIM). An animal was considered to present first ovulation when a corpus luteum (CL) was detected by ultrasonography, and cysts when structures with a diameter > 17mm and a uniformly anechogenic antrum were present along with a flaccid uterus in the absence of CL. Milk, BCS and IGF-I concentrations were analyzed by repeated measurements and the proportion of cows ovulating and with cysts by Fisher exact tests.
RESULTS
Confined TMR group had higher milk yield (43.4±1.1 L a) than grazing/confined (34.6±1.3 L b) and grazing group (34.2±1.7 L b) (P=0.002). Confined TMR cows had also greater BCS (2.92±0.03a) than the other two groups (2.81±0.04b and 2.70±0.06b respectively, P=0.0007). Confined TMR cows had greater IGF-1 level than grazing cows (73.8±4.9a vs 50.3±4.9b ng/mL, P=0.01). In grazing/TMR cows IGF-I concentration was 65±6.1 ng/mL without difference with another groups. No differences in first ovulation or presence of cysts among the grazing groups were found, thus, data was pooled and presented as confined vs grazing groups. At 21 DIM a greater proportion of cows in the grazing groups had already ovulated when compared with confined TMR cows (17/31 and 3/16, P=0.007), but the proportion was not significant on day 60 DIM. Regarding ovarian cysts, confined TMR cows tended to present a greater proportion of cows with follicular cysts than grazing cows (5/11 vs 8/31, P=0.09), and no differences were observed on day 60 DIM.
CONCLUSIONS
Data suggest that the higher milk production in the confined group during the first 21 DIM may have determined worse energy balance, which may explain the low ovarian cyclicity and presence of cysts at 21 DIM.

T20
EVALUATION OF ACETYL-COA AVAILABILITY IN THE ACETYLATION OF HISTONES IN BOVINE OOCYTE

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BACKGROUND-AIM
Metabolic intermediates can be used by the oocyte both in energy production and for epigenetic events that control the transcriptional machinery. Among these epigenetic events is the acetylation of histone that is based on the principle of the insertion of acetyl groups in the lysines of the histone tails, causing changes in chromatin rearrangement and consequent transcriptional alteration. These acetyl groups may come from pyruvate metabolism and by converting mitochondrial citrate (produced in the TCA cycle) into cytoplasmic acetyl-CoA which is reused in the nucleus for the acetylation of histones. Therefore, the objective of this work is to evaluate how the availability of acetyl-CoA modulates histones' acetylation pattern and mitochondrial function in bovine oocytes along in vitro maturation (IVM).
METHODS
During IVM, oocytes were treated with drugs that promote (sodium dichloroacetate; DCA 1.5mM) or decrease (sodium iodoacetate; IA 5 µM) acetyl-CoA production. Then, oocytes were assessed individually for mitochondrial activity and accumulation of cytoplasmic lipids at 24 h; and at 0, 4, 8, 16, and 24h for lysine 9 histone 3 acetylation (H3K9ac immunofluorescence). Images were acquired using a fluorescence microscope and analyzed by Image J software. The results were compared by Student's t-test (treatment vs. control, n=3 replicates, ????=5%).
RESULTS
As expected, the stimulated group showed an increase in mitochondrial activity (p=0.007) and a decrease in lipid accumulation (p=0.003) compared to the Control, probably due to the activity of beta-oxidation of lipids. Surprisingly, IA group presented similar results (p<0.0001 and p<0.0044, respectively). In relation to H3K9ac, there was an increase in the DCA group at 24h (p<0.0001) and the other periods were marked by a significant decrease in acetylation (4h: p=0.0119; 8h: p=0.0020). Meanwhile, the IA group showed a decrease in H3K9ac at 16h (p<0.0001) and a surprising increase in acetylation at 24h (p=0.0009).
CONCLUSIONS
Changes in the availability of acetyl-CoA alter the dynamics of mitochondrial activity and histone acetylation in oocytes with possible impact to epigenetic maturation.
Acknowledgments: FAPESP (Process n° 2017/18384-0 and 2018/23142-9)

T21
INTERFERON TAU: INFLUENCES ON GROWTH AND DEVELOPMENT OF THE CONCEPTUS

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BACKGROUND-AIM
Interferon tau (IFNT), the pregnancy recognition signal secreted from trophectoderm cells of ruminant conceptuses abrogates the uterine luteolytic mechanism to ensure maintenance of functional corpora lutea for production of progesterone (P4). Importantly, IFNT, in concert with P4, also induces expression of genes in uterine luminal (LE) and superficial glandular (sGE) epithelia for transport and/or secretion of histotroph into the uterine lumen to support growth and development of the conceptus.
METHODS
Suffolk ewes (n=40) were assigned randomly to receive daily intramuscular injections of either 25 mg P4 (P4, n=20) in 1 ml corn oil (P4, n=20) or 1 ml corn alone (CO, n=20) from Day 1.5 through Day 8 of pregnancy. Ewes from each treatment group were hysterectomized on Day 9 (n=10) or Day 12 (n=10) of pregnancy. Uterine flushes, conceptuses and endometrium were analyzed to determine respective effects of P4 and IFNT.
RESULTS
IFNT and P4 induce transporters responsible for transport of glucose and arginine into the uterine lumen during the peri-implantation period of pregnancy. Arginine activates the mechanistic target of rapamycin (MTOR) nutrient sensing cell signaling pathway to stimulate proliferation, migration, differentiation and translation of mRNAs essential for growth and development of the conceptus. Glucose may be utilized directly by the conceptus or converted to fructose. These two hexose sugars are metabolized via aerobic glycolysis to produce metabolites used in the hexosamine biosynthesis pathway, pathways for one-carbon metabolism, and pentose phosphate pathway for synthesis of ribose sugars and NADPH. Arginine is metabolized to nitric oxide (NO) that stimulates angiogenesis in uterine and placental tissues, and to polyamines required for many cellular functions critical for growth and development of the conceptus.
CONCLUSIONS
IFNT and P4 regulate expression of genes for transport of select nutrients into the pregnant uterus during the peri-implantation period of pregnancy. Those nutrients are metabolized via multiple metabolic pathways to provide ATP and to serve as substrates for metabolism via four major metabolic pathways required for growth, development, and survival of conceptuses during the peri-implantation period of pregnancy.

T22
HEPATIC GENE EXPRESSION IS AFFECTED BY THE METABOLIC MEMORY AND IS ASSOCIATED WITH THE REPRODUCTIVE FUNCTION

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BACKGROUND-AIM
Our aim was to study the effect of undernutrition on liver gene expression and its association with the endocrine and uterine milieu in ewes with different body condition score at the beginning of the experiment (BCSi).
METHODS
In the University of Zaragoza, Spain during the breeding season, 36 multiparous-5 years old Rasa Aragonesa ewes were divided into 2 groups with different BCS: BCS>2.75 (high, H, 2.9±0.04) and BCS<2.25 (low, L, 2.1±0.04). Body weight (BW) of H and L group was 61.9±1.6 and 50.9±1.7 kg, respectively. Both groups received a diet to cover energy and protein maintenance requirements for 20 days, after which they were randomly assigned to 2 nutritional treatments: 1.5 (control, C) or 0.5 (undernourishment, U) times the daily maintenance requirements. The first day of the experimental diet, ewes were estrous synchronized with intravaginal sponges for 12 days, and mated with rams. Only ewes that had embryos on day 5 were included in this study setting up 4 groups: high-BCSi control (HC, n=6), high-BCSi undernourished (HU, n=6), low-BCSi control (LC, n=9) and low-BCSi undernourished (LU, n=7). Ewes were slaughtered at the end of the experiment. Variables were analyzed by ANOVA using a mixed procedure.
RESULTS
High-BCSi ewes presented higher hepatic gene expression of growth hormone receptor (GHR, P=0.03) and insulin like growth factor 1 (IGF-1, P=0.04) than low-BCSi animals, consistent with the highest plasma concentration of IGF-1 (P<0.01), ovulatory rate (P<0.05) and number of recovery embryos (P<0.05) previously reported for these ewes. Respect to nutritional treatment, only IGF binding protein-3 (BP3) hepatic gene expression was affected: ewes of U group presented lower gene expression than C animals (P=0.05), but this effect was not observed at uterine level. However, we previously reported that in uterus, IGF binding protein-2 (BP2) mRNA tended to be higher in U than in C group (P=0.08) without effect on BP3, suggesting a tissue dependent response of BP2/BP3 gene expression to a food restriction in ewes.
CONCLUSIONS
In conclusion, the negative effects of undernutrition in ewes with a better metabolic status are less marked, evidenced by a better endocrine milieu and a differential tissue gene expression and embryo quality.

T23
LIPID METABOLISM AND BASAL FOLLICULOGENESIS IN EWE.

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BACKGROUND-AIM
The key role of lipid metabolism was highlighted in terminal folliculogenesis in different species, but few data exist on its role during basal folliculogenesis. This work aimed to study the expression of different actors of lipid metabolism and the composition of lipid classes for ovine basal folliculogenesis.
METHODS
Peripubertal ovine follicles (80-850µm, 1389) were collected year-round from ovarian cortex strips (slaughterhouse) and dispatched in 5 follicular size classes (from preantral (PA) to small antral (SA)). The expression of 31 genes from lipid metabolism was analysed using the qPCR BioMark™ HD System (Fluidigm) and studied with a hierarchical clustering analysis. The protein expression and localisation of several actors were examined by immunohistochemistry on ovarian sections and/or by immunofluorescence on isolated oocytes and granulosa cells from follicular classes. Lipid classes were assessed by HPTLC in follicles. Data were analysed using Kruskal-Wallis tests with Dunn's multiple comparison post hoc tests or using Chi-square tests ($p < 0.05$).
RESULTS
The hierarchical clustering analysis showed the 3 classes of PA follicles were clustered separately from the 2 classes of follicles with antrum. Follicular development was accompanied with i) an increased expression of genes from lipolysis (LPL), FA transcription factor (PPARG), ii) with a decreased expression of genes from FA cytoplasmic transporter (FABP3, 5), FA oxidation (CPT1A), FA elongation (ELOVL2, 4, 5), triglyceride hydrolysis (PNPLA2) and synthesis (DGAT2). Percentage of stained oocytes for ELOVL2 and FABP3 proteins was increased during follicular development (+59% and + 19%, respectively, $p < 0.0001$). Percentages of phospholipids and neutral lipids were similar between the different classes of follicles.
CONCLUSIONS
Antrum appearance seems to be a critical point for expression changes of gene from lipid metabolism. These modifications are not accompanied by a change in the composition of several complex lipids. Follicles could prioritize the entry of outside lipids rather than in situ synthesis during basal folliculogenesis. Lastly, oocyte could be able to exert an active role in modifying the quality of stored FA.

T24
THE IMPACT OF SEVERE NEGATIVE ENERGY BALANCE EARLY POST-PARTUM ON THE PREOVULATORY GRANULOSA CELL TRANSCRIPTOME AT THE TIME OF BREEDING DEPENDS ON THE BLOOD ANTI-OXIDANT PROFILE

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BACKGROUND-AIM
Sever negative energy balance (NEB) in dairy cows results in elevated blood non-esterified fatty acid (NEFAs) concentrations during the early postpartum period (pp) with a negative impact on fertility. We hypothesized that high blood NEFAs at week 2 pp exert long-term effects on the growing follicles leading to alterations in the granulosa cell (GC) functions in the preovulatory follicle (POF) at the time of breeding (w8 pp). Furthermore we hypothesized that optimal blood antioxidant (AO) levels at w2 and/or w8 may protect against these detrimental effects.
METHODS
Blood was sampled at w2 and w8 pp from 47 multiparous cows in the same farm. GCs were collected by transvaginal aspiration from the POFs at w8 after estrous synchronization (pre-LH surge). Serum NEFAs and AOs (β-Carotene, βC; and Vitamin E, Vit.E) were measured. Representative GCs samples (n=16) with high or low concentrations of these parameters were selected for RNA sequencing, and their transcriptomic profiles were compared using DESeq2 (n≥3 per subgroup). Differentially Expressed Genes (DEGs: P-adj<0.05, 5% FDR) were determined and functionally annotated using Bioconductor packages in R.
RESULTS
Comparing cows in the highest vs. lowest quartiles of w2 NEFAs (0.86±0.16 vs. 0.30±0.08 mM) resulted in 64 DEGs in w8 GCs. These genes are involved in cellular response to lipids and ketones, immune responses, and response to oxidative stress. The same comparison for w8 NEFAs yielded no DEGs. Within the cows with high w2 NEFAs, a second comparison was made between cows with high (above median) vs. low βC & Vit.E at w2 (3±0.9 & 2.9±0.4 mg/L vs. 1.3±0.3 & 1.7±0.3 mg/L, respectively) and at w8 (5.5±1.9 & 6.5±1.4 vs. 2.3±6.5 & 3.0±1.0 mg/L, respectively). w2 AOs were linked with only 3 DEGs. w8 AOs were linked with 194 DEGs that are involved in MAPK, IGF and EGF receptor signaling, activation of meiosis, fertilization and acrosome reaction, which are indicative for a better oocyte supportive capacity.
CONCLUSIONS
These results show evidence that sever NEB in dairy cows can have a long-term impact on the GC functions in the preovulatory follicles at the time of breeding. Such effect might be mitigated by optimized AO concentrations before breeding. Further studies are needed to examine the consequences for oocyte quality and fertility.

T25
THE PIVOTAL ROLE OF PYRUVATE METABOLISM ON THE EPIGENETIC PROFILE AND TRANSCRIPTOMICS OF BOVINE EMBRYOS

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BACKGROUND-AIM
Embryos are known by their ability to adapt to nutrient availability maintaining the function of essential energy-related pathways, nevertheless, embryonic cells have other metabolic requirements beyond the ATP production. In embryonic stem cells the modulation of the glycolytic pathway and consequently the availability of Acetyl-CoA (the major donor for histone acetylation) is sufficient to alter the pattern of histone acetylation, releasing cells from pluripotency. In this study we explore the consequences of modulating the pyruvate metabolism during the pre-implantation development to the pattern of global epigenetic marks and its consequences to the molecular status of blastocysts.
METHODS
Modulation of pyruvate metabolism was promoted both by inhibition of the glycolytic pathway using sodium iodoacetate (IA), and also by stimulating the conversion of pyruvate into acetyl-CoA using sodium dichloroacetate (DCA), after major embryonic genome activation on day 5 (120 hpi). Blastocysts were collected at day 7 (168 hpi) and assessed for metabolic (RAMAN spectroscopy), molecular (H3K9 acetylation and RNASeq), and morphological parameters (cell number and allocation). Data were analyzed by ANOVA followed by LSD test and EdgeR analysis to verify differences in gene expression (DEGs) among groups.
RESULTS
After 48h incubation, interestingly, both DCA and IA treatment lead to diminished levels of acetyl-CoA, resulting in lower levels of H3K9ac. Despite this similarity, RNASeq revealed distinct adaptive mechanisms regarding aminoacids and lipids metabolism that could explain the maintenance of energy supply. The total cell number were higher in DCA blastocysts and lower in IA, corroborating the "cell cycle" as one of the affected biological processes identified by RNASeq. Both DCA and IA blastocysts presented a higher TE:ICM ratio, indicating that changes in epigenetic marks induced by changes in pyruvate metabolism were able to disturb the control of cell lineage differentiation.
CONCLUSIONS
Our results enlighten the pyruvate metabolism as essential for the establishment of epigenetic profile during the early stages of development. Funding: FAPESP 2019/22025-1 and 2019/25982-7.

T26
HERBAGE ALLOWANCE ON ENDOCRINE AND REPRODUCTIVE PARAMETERS OF PRIMIPAROUS BEEF COWS WITH TEMPORARY SUCKLING RESTRICTION AND FLUSHING

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BACKGROUND-AIM
To evaluate the effect of herbage allowance (HA) on body condition score (BCS), body weight (BW), probability of ovulation and pregnancy, milk yield, calf weight, estimated energy intake (EI), and insulin, IGF-1, and leptin concentrations in primiparous beef cows from the second trimester of gestation (STG)(-150 DPP) to weaning (195 DPP).
METHODS
Primiparous cows (n=31) on native pastures were allocated to two HA treatments that varied seasonally: annually 4 kg DM/kg BW, High HA (HHA) and 2.5 kg DM/kg BW Low HA (LHA). All cows were submitted to temporary suckling restriction with nose plates (12 days) and flushing (2 kg FB/cow/day whole-rice bran for 22 days), 6 days before bull introduction (86 DPP). Blood samples for hormone determination were also taken prior to suckling restriction, at suckling restriction, during flushing and 65 days after suckling restriction initiation (151 ± 12 days relative to calving).
RESULTS
HHA improved the concentrations of insulin and IGF-1, EI, and BCS, and BW at the end of the STG compared to LHA. During the last trimester of gestation EI, insulin, and leptin concentrations did not differ, while IGF-1 concentrations were greater in HHA than LHA. These results show that IGF-1 was associated with the STG HA. The probabilities of ovulation (0.94 vs. 0.75) and early pregnancy (0.73 vs. 0.48) did not reach significance, whereas final pregnancy was greater in HHA than LHA (0.9 vs. 0.61). The probability of ovulation was positively associated with IGF-1 concentrations during the LTG linking the effect of HA during STG with the reproductive response. Metabolic hormones did not differ postpartum and did not significantly influence reproductive response, milk yield, and calf weights. Management of HA during the STG improved the metabolic adaptation to the negative energy balance of the LTG which positively influence reproductive response. The HA during postpartum did not affect cows' nutritional and metabolic status and milk yield but HHA improves calf weaning weight.
CONCLUSIONS
The HA management during the second trimester of gestation improves the adaptation to the negative energy balance of the last trimester of gestation and the reproductive response of primiparous beef cows with temporary weaning and flushing at the start of the breeding season.

T27
DIFFERENTIAL MANAGEMENT OF DAIRY COWS IN THE FIRST 21 DAYS POSTPARTUM: IMPACT ON MILK PRODUCTION, BODY CONDITION SCORE AND OVARIAN CYCLICITY

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BACKGROUND-AIM
The aim of this work was to evaluate the immediate and residual effect of short-term differential management (first 21 days in milk, DIM) in primiparous (L1) and multiparous (L2) cows on milk production, body condition score (BCS) and the proportion of cows cycling.
METHODS
After calving, Holstein cows were randomly distributed in two treatments: T0, grazing + supplementation (TMR) (L1: n=8, L2: n=10), or T21, confinement in compost barn with TMR ad libitum during the first 21 DIM (L1: n=7, L2 n=13). At 22 DIM the T21 cows were managed as T0 cows. Milk production was measured daily and, BCS was evaluated weekly, from prepartum and up to 60 DIM. The proportion of cycling cows was determined by the presence of a corpus luteum at 21, 40, and 60 DIM by ovarian ultrasonography. Milk production was analyzed using Mixed procedure, and the other variables were analyzed using the Glimmix procedure SAS®.
RESULTS
Milk production was affected by the interaction treatment*parity (P=0.04) as milk production of L2 cows in the first 21 DIM was higher in T21 than in T0 (42.0±0.9 vs 37.7±1.1 L/day, P<0.0001), without differences thereafter. No differences were observed in L1 cows (T0-L1=29.7±0.8 vs T21-L1=29.6±0.9 L/day, P=0.9). Cows of T21 presented a higher BCS compared to T0 (3.1 vs 3.0±0.04, respectively; P=0.02), and a tendency treatment*parity was observed (P=0.07), where T21-L2 cows had higher BCS than T0-L2 cows (3.2 vs 3.0±0.05, respectively; P<0.01), while no differences were observed in L1 cows (3.0±0.06). The proportion of cows cycling at 21 DIM, was affected by the interaction between treatment*parity (P=0.03), being lower in T21-L2 than T0-L2 (23 vs 70%, P<0.05), while the proportion in L1 cows did not reach statistical significance (T21-L1=57% vs T0-L1=25%, P=0.2). No other differences were found.
CONCLUSIONS
The data suggest that the T21 management was depending on parity, as in multiparous cows favored the nutrient partitioning towards milk production in the first 21 DIM affecting negatively the reproductive axis (delaying the first postpartum ovulation), while this was not the case in primiparous cows probably explained by the lower decoupling of the somatotropic axis.

T28
USE OF ENDOGENOUS LIPIDS AND AMINO ACIDS AS UNIQUE OXIDATIVE SUBSTRATES FOR PORCINE OOCYTE NUCLEAR AND CYTOPLASMIC MATURATION IN VITRO

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BACKGROUND-AIM
Our aim was to study if amino acids (AA) and endogenous lipids may be used as unique oxidative substrates for nuclear and cytoplasmic maturation of porcine oocytes.
METHODS
Cumulus-oocyte complexes (COCs) were obtained by aspiration of antral follicles from ovaries of slaughtered gilts and matured in NCSU-37 (without oxidative substrates) and NCSU-37+Glucose as controls; NCSU-37+AA, NCSU-37+AA+Glucose, NCSU-37+AA+Salicylate (AA catabolism inhibitor); NCSU-37+L-carnitine (fatty acid oxidation inducer), NCSU-37+L-carnitine+Glucose and NCSU-37+Etomoxir (lipid catabolism inhibitor). Meiotic maturation (MII) rates were analyzed using Hoescht 33342. To analyze cytoplasmic maturation COCs were co-incubated 3h with 1x106 motile sperm/ml in modified Tris Buffer medium+0.4% serum bovine albumin+2.5mM caffeine. Zygotes were cultured in NCSU-23 and blastocyst rates were evaluated at day 7. The use of endogenous lipids was determined by oocyte lipid content using Nile Red stain, while the use of AA was evaluated by spectrophotometry, based on NADPH oxidation by glutamate dehydrogenase during the consumption of ammonia. Lipid levels and NADPH oxidation were analyzed by one way ANOVA, while MII and blastocyst rates were compared using a chi-square analysis. P<0.05 was considered significant.
RESULTS
Oocytes matured in NCSU-37+AA presented higher MII rates (52.4%) than those cultured in NCSU-37+Glucose (24%) or NCSU-37 (8%, p<0.05), but no difference was detected with those matured in NCSU-37+L-carnitine (35.3%). NCSU-37+AA+Glucose (67.6%) and NCSU-37+L-carnitine+Glucose (51.6%) presented higher MII rates compared with the use of each substrate alone (p<0.05). NCSU-37+AA+Salicylate and NCSU-37+Etomoxir showed no difference respect to NCSU-37. Blastocyst rates were higher in NCSU-37+Aa+Glucose than in the other media (54%, p<0.05). NCSU-37+L-carnitine and NCSU-37+L-carnitine+Glucose presented higher oocyte endogenous lipid consumption respect to the other treatments (p<0.05). As regards residual ammonia NCSU-37+AA resulted in a higher level, while NCSU-37+AA+Salicylate presented a reduction compared with the other groups (p<0.05).
CONCLUSIONS
These results suggest that both AA and endogenous lipids could effectively be used as unique substrates for porcine oocyte oxidative metabolism during maturation.

TOPIC Ovary and oocyte

T29
DIFFERENCIAL EXPRESSION OF ACETYLCHOLINE RECEPTORS IN THE BOVINE OVARIAN DYNAMICS

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BACKGROUND-AIM
Acetyilcholine is produced locally in the ovary by granulosa cells of growing follicles under the driving force of FSH and acts to regulate specific ovarian functions. Researches on this topic are restricted to rat and humans. Transcripts for nicotinic acetylcholine receptor (nAChR) were found in human and rat fetal ovaries, similarly muscarinic Ach receptors (mAChR) were also found. The aim of this study is to provide evidences on the presence of cholinergic receptors in the bovine ovary to explain our clinical observations that let us suppose a role for Ach in the bovine cystic ovarian disease (COD).
METHODS
Bovine ovaries were obtained from the local slaughterhouse. Slices of walls from luteal and follicular cysts, antral follicles, categorized according to their diameters, and corpora lutea at different age of development were recovered and used for protein extraction. The expression of nAChR and mAChR was validated by western blot using anti nAchR and m1AChR mouse monoclonal antibodies (Santa Cruz Biotechnology). The expression of each protein was related to GAPDH level. Gel bands were scanned and quantitate by density contrast (OD = contour x mm2) using Gel-Doc and Quantity One software. Data were analyzed for statistical significance by ANOVA test. Histological investigations were also carried out.
RESULTS
Western blot analysis showed that bovine ovary expresses both types of AChRs and that mAChR signal is always significantly higher compared to nAChR. For both receptors the signal intensity increases according to the stage of follicular development. In the CL, the expression level rises during development and decreases, remaining constant, during the regressed luteal stages. The positive signal of both receptors was found in the cystic structures.
CONCLUSIONS
The present results demonstrate for the first time: (1) the presence of nAChR and type 1 mAChR on bovine antral follicles and CL; (2) that they are differently expressed, (3) the expression of both type of AchRs is still present in the cysts. These findings let us to suppose an auto/paracrine action of the Ach in bovine ovary.

T30
MICRORNA EXPRESSION IN GRAAFIAN FOLLICLES BEFORE, DURING AND AFTER OVULATION IN CATTLE

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BACKGROUND-AIM
Despite the involvement of miRNAs in ovarian function, their participation in follicle ovulation and corpus luteum (CL) formation is unknown. Thus the aim of this study was to characterize miRNA expression in the bovine ovulatory follicle.
METHODS
The oestrous cycles of beef heifers were synchronized to manage the time of ovulation. Heifers (n=6) were slaughtered at a local abattoir at each of 4 timepoints (T): T1: 24h prior to ovulation; T2: 2h prior to ovulation; (T3) 24h post-ovulation and (T4) 72h post-ovulation. Theca and granulosa cells of bovine preovulatory (T1) and periovulatory (T2) follicles and early luteal tissue at 24h (T3) and 72h (T4) post-ovulation, were recovered by dissection and processed for NanoString nCounter Custom CodeSet miRNA expression analysis. The data were analyzed by linear mixed model (PROC MIXED) procedure of SAS and values with $P<0.05$ were deemed significantly different.
RESULTS
A total of 164 miRNAs were detected. Irrespective of time and tissue type, the most abundant miRNAs were bta-let-7a-5p, bta-let-7b, hsa-miR-4454, bta-miR-125b-2 and bta-miR-29b-1. The majority of detected miRNAs were more abundantly expressed in theca compared to granulosa and luteal -tissues. A total of 11 miRNAs in theca and 27 miRNAs in granulosa -tissues were found to be differentially expressed in T2 compared to T1 ovulatory follicles, of which bta-miR-155 and bta-miR-129-2 were commonly expressed and elevated in both T2 theca and granulosa -tissue. In contrast, only miR-1260a was differentially expressed in T3 compared to T4 -luteal tissue. Functional pathway analysis revealed the commonly abundant miRNAs to be involved in cell survival and steroidogenesis. The differentially expressed miRNAs in theca cells are known to be involved in mTOR signaling pathway, cell adhesion and cycle, and fatty acid metabolism, while differentially expressed miRNAs in granulosa cells were enriched in cell proliferation and growth, insulin signaling pathway, and fatty acid metabolism.
CONCLUSIONS
In conclusion, the present study demonstrates a high expression of miRNAs in the periovulatory follicle, particularly in theca cells, highlighting the complex orchestration of immune and somatic cells in follicle rupture, repair, and luteinization.

T31
PROSPECTS OF CULTURE AND VITRIFICATION OF CANINE AND MOUSE PREANTRAL FOLLICLES

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BACKGROUND-AIM
Ovary of the mammals contains high numbers of preantral follicles. These follicles are suitable for in vitro culture, can be cryopreserved and provides large repository of oocytes. Following in vitro maturation, these oocytes can be fertilized.The process makes the opportunity to preserve fertility in animals with unique genetics or in women before anti cancer treatment. However, in vitro culture protocols are not standardized, and the basal media and supplements, as well as preservation methods differs among laboratories and species as well.
The aim of our study was to analyse the characteristics of in vitro growth of mouse and canine follicles. Furthermore, we aimed to investigate the applicability of open pulled straw vitrification method.
METHODS
Preantral follicles of mice were isolated from ovaries of 8-12-week old females with mechanical separation. Canine follicles were isolated from ovaries (ovariohysterectomy) after collagenase treatment. Follicles of both species were cultured for 14 days in 20 µl droplets of modified medium (Advanced-MEM + 5% FBS + 100 mIU/ml eCG), covered by mineral oil. Half of the media were exchanged to fresh on every other day. Morphological and size assessment were carried out at the same days. OPS vitrification protocol was applied for cryopreservation. Thawing was carried out in HM with decreasing concentration of sucrose.
Follicle diameter, rate of live/dead cells and Cx43 content were assessed.
RESULTS
The supplemented medium provided continuous in vitro growth in both species.
Diameter of mice follicles showed 2.4-fold (1.8-3.1; CI 95%) growth from Day3 to Day5 ($p<0.0001$) and 3.2-fold (2.3-4.3; CI95%) from Day5 to Day11 ($p<0.0001$). The same tendency was detected in canine follicles from Day1 to Day5. Vitrification did not affect the live cell rate neither in mice, nor in canine samples. Cx43 level did not differ in fresh and vitrified follicles (105.8±17.3 and 94.15±36.4 arbitrary units, respectively).
CONCLUSIONS
Our data show that Advanced-MEM-based supplemented medium provides suitable environment for in vitro culture of preantral follicles both in mouse and dogs. The open pulled straw vitrification is a feasible method for cryopreserve these samples and can be applied without any sample quality loss.

T32
A TWO-STEP PROTOCOL FOR THE GENERATION OF AN OVARIAN 3D BIO-SCAFFOLD

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BACKGROUND-AIM
Over the last decades, great effort has been dedicated to identify the difference parameters that may successful support in vitro oocyte maturation and embryo development. Beside the soluble factors contained in the culture media, it has been recently demonstrated that gametes and embryos, as well as all somatic cells, respond to the bio-mechanical stimuli exerted by the surrounding microenvironment, which significantly affect cell response and behavior. Novel 3D platforms, able to mimic the ovarian in vivo milieu and its bio-mechanical cues, are therefore being generated.
METHODS
Here we describe a novel protocol consisting of two main steps: a) the generation of a 3D extracellular matrix (ECM)-based bio-scaffold that maintains the architecture and biological signals of the original ovarian tissue; b) the repopulation of the 3D bio-scaffolds with female germline stem cells (FGSCs).
RESULTS
In the first step, whole-ovaries were subjected to a decellularization protocol that involved a freeze-thaw cycle and sequential incubations in 0.5% SDS, 1% Triton X-100, and 2% deoxycholate. At the end of the process, histological staining and DNA quantification demonstrated successful removal of the cellular compartment. Histochemical analysis revealed preservation of the key ECM components, namely collagen, elastin, and glycosaminoglycan, with full retention of their microarchitecture. In the second step, bio-scaffolds were repopulated with FGSCs previously isolated through enzymatic digestion with 0.25% Tripsin-EDTA and 1 mg/ml Collagenase IV, and purified through magnetic activated cell sorting. FGSCs rapidly adhered and colonized the bio-scaffolds within 24h. During the subsequent 7 days of culture, they steadily expressed germline (DDX4, FRAGILIS, BLIMP1, DAZL) and pluripotency-related genes (OCT4, NANOG, REX1, SOX2).
CONCLUSIONS
Overall, the results obtained demonstrate that the two-step protocol here described is a powerful tool for the creation of an "artificial ovary" in vitro. This may represent a novel 3D platform for ovarian tissue in vitro modeling that may constitute a promising strategy to improve follicle culture and oocyte maturation.

T33
NUCLEAR PROGESTERONE RECEPTOR EXPRESSION IN GROWING AND FULLY GROWN BOVINE OOCYTES

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BACKGROUND-AIM
Progesterone (P4) plays a critical role in mammalian ovulatory cycle regulation and pregnancy establishment. The effects of P4 are mediated by its interaction with specific membrane and nuclear progesterone receptors (PR). Blocking of P4 signaling during bovine in vitro oocyte maturation leads to caspase 3 activation and altered mitochondrial distribution in oocytes and an increased incidence of abnormal metaphase phase spindles and chromosome alignment in resulting embryos and a decreased blastocyst development, indicating a role for P4 and PR in determining oocyte quality. In cattle, the final phase of oocyte growth occurs during high P4 concentrations. Therefore, the aim of the present study was to characterize the nuclear PR (nPR) in growing bovine oocytes from early antral follicles.
METHODS
Fully-grown and growing bovine cumulus-oocyte complexes (COCs) were recovered from ovaries collected at a local abattoir by the ovarian slicing technique. The COCs were denuded and the diameter of each oocyte was measured and oocytes were allocated to groups accordingly to their diameter (100-110µm and >120µm) and fixed to analyse the expression and localization of nPR by immunocytochemistry. Immunolabeled oocytes were visualized using a Carl Zeiss LSM 800 Airy confocal system. The resulting images were analysed in FIJI to determine the number of nPR foci. Data were analyzed by linear regression to evaluate the effect of the diameter on the foci and foci/µm 3.
RESULTS
The oocyte diameter did not affect the number of foci ($p=0.5$). However, there was a negative correlation between diameter and foci/µm3 ($p<0.001$). Thus, nPR expression appears to have reach maximum in growing oocytes >100 µm diameter.
CONCLUSIONS
The presence of nPR in growing oocytes in cattle suggests a role for P4 signaling during oocyte growth by promoting oocyte nuclear and cytoplasmic maturity and viability, as well as the acquisition of developmental competence.

T34
RELATIONSHIP BETWEEN COX-2 GENE EXPRESSION IN CANINE GRANULOSA CELLS AND GDF-9 AND BMP-15 LEVELS IN GROWING AND FULL -GROWN FOLLICLES

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BACKGROUND-AIM
Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) play crucial roles in ovarian functions, regulating follicular development, intrafollicular signals, and oocyte maturation. Both factors participate in the modulation of certain target genes in the ovary, stimulating cyclooxygenase2 (COX2) and prostaglandin E2 (PGE2) synthesis. The COX2/ PGE2 pathway produced within the periovulatory follicle is a critical mediator of oocyte maturation; however, this regulation has not been characterized in canines. The objective of this study was to examine in dogs the COX2 gene expression during the final stages of follicular development and the influence of BMP15 and GDF9 on COX2 gene expression in vitro.
METHODS
Granulosa cells from antral follicles and their corresponding cumulus-oocyte-complexes (COCs) and follicular fluid (FF) were obtained separately from 56 ovaries from adult bitches at estrus (n= 15) and proestrus (n=13) following ovariohysterectomy. Total RNA extraction was performed in granulosa cells and COX2 gene expression was achieved by relative quantification q-PCR analysis. The concentration of BMP15 and GDF9 was determined in the FF samples by ELISA assay. COCs were submitted to IVM with or without (control) recombinant GDF9 and BMP15 (200 ng/mL each). After 72 h culture, cumulus cells were removed from enclosed oocytes by pipetting and COX2 transcript analyses were performed by q-PCR. Data from at least three independent experiments were evaluated by the Student t-test.
RESULTS
There was an increased ($P<0.05$) of COX2 mRNA levels in granulosa cells obtained from follicles at proestrus with respect to those at estrus. COX2 gene expression in cumulus cells was four times greater ($P<0.01$) than control when both growth factors were added to IVM culture. However, the levels of BMP15 and GDF9 in FF decreased ($P<0.05$) from proestrus to the estrus phase.
CONCLUSIONS
In conclusion, COX2 gene expression and BMP15/GDF9 levels exhibited an inversed specific-stage variation in canine follicles in the late follicular development. But BMP15/GDF9 appears to upregulate the levels of COX2 mRNA transcript during IVM.
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T35
INDIVIDUALLY AND GROUP CULTURE OF SHEEP OOCYTES DERIVED FROM EARLY ANTRAL FOLLICLES

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BACKGROUND-AIM
To overcome the limited number of fully grown oocytes for in vitro embryo production (IVP) several techniques have been developed to exploit preantral follicles in humans and animals. The main challenge is to support follicular growth in vitro and reduce the degeneration rates. Recent works in cattle have demonstrated that long in vitro culture (LIVC) of cumulus-oocyte complexes (COCs) derived from early antral follicles support oocyte growth and the acquisition of meiotic competence (Barros, et al, 2020. Journal of Visualized Experiments, 161, p.e61625).
METHODS
To assess whether this protocol can be applied in the sheep, in this study, isolated COCs from sheep early antral follicles (350-400 µm) were retrieved by rupturing the follicle wall with a 21-gauge needle. Subsequently, they were washed and cultured as a single (control) or group (3 COCs) in a well of 96-well plate containing TCM199 supplemented with 0.15 µg/mL Zn sulfate, 0.0001 IU/mL FSH, 10 ng/mL estradiol, 50 ng/mL testosterone, 50 ng/mL progesterone and 1.7 µg/mL Cilostamide. After five days long in vitro culture (LIVC), the healthy COCs were selected and subjected to in vitro maturation.
RESULTS
Results indicated that COCs cultured in groups (n=81) had lower degeneration rates compared with those individually cultured (n=88; 48.1 vs. 65.9%, respectively; Chi-squared test $p<0.05$). Also, group culture improved the ability of COCs to resume meiosis and reach the metaphase II stage compared to the individual culture (20.6% vs. 13.3%, respectively), although this difference is not statistically significant. Therefore, there may be a signaling pathway that enhances the integrity and growth of COCs in the group culture method.
CONCLUSIONS
In conclusion, we demonstrated that the LIVC in the sheep could support a gradual transition of the oocyte from immature to mature stage, especially in the group culture method. This technology can increase the source of fertilizable gametes in the preservation programs and gives a prospective approach to livestock selection programs or conservation of endangered species.

T36
THE MEIOTIC COMPETENCE OF OVINE OOCYTES DERIVED FROM EARLY ANTRAL FOLLICLES IS INFLUENCED BY THE SEASON

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BACKGROUND-AIM
In seasonal breeders, the quantity and quality of immature oocytes obtained from the ovaries can vary greatly depending on the season. The present study aimed at evaluating the influence of the season (breeding and non-breeding) on the in vitro acquisition of meiotic competence of cumulus-oocyte complexes (COCs) derived from sheep early antral follicles (EAFs).
METHODS
For this purpose, Sarda sheep ovaries were collected from a local abattoir in the breeding (October – November) and non-breeding season (March-May). The COCs were retrieved from EAFs (350-400 µm) by rupturing the follicle wall with a 21-gauge needle. Subsequently, they were washed and cultured individually in a 96-well plate containing TCM199 supplemented with 0.15 µg/mL Zn sulfite, 10-4 IU/mL FSH, 10 ng/mL estradiol, 50 ng/mL testosterone, 50 ng/mL progesterone, and 5 µM Cilostamide. After five days long in vitro culture (LIVC), the healthy COCs were selected and subjected to in vitro maturation.
RESULTS
The results indicated that at the end of the culture period COCs had more intact granulosa cell layers with acceptable morphology (30/72, 41.6%) in the breeding season compared to the non-breeding season (18/67, 26.8%; Chi-squared test $P = 0.067$). The resumption of meiosis in the collected COCs during the breeding season was higher than in the non-breeding season (13.3 vs 11.3%, respectively: Chi-squared test $p<0.001$).
CONCLUSIONS
In conclusion, these results suggest that the LIVC of oocytes derived from sheep EAFs in the non-breeding season have a lower meiotic competence than those collected in the breeding season. Therefore, we recommend the breeding season as a suitable time for the LIVC of oocytes derived from sheep EAFs.

T37
DETERMINATION OF THE APPROPRIATE CONCENTRATION OF SODIUM ALGINATE USED FOR IN VITRO CULTURE OF CAT PREANTRAL FOLLICLES.

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BACKGROUND-AIM
In vitro culture of domestic cat ovarian follicles can be a suitable experimental model to develop oocyte conservation strategies in species of the Felidae family. The aim of this research was to determine the appropriate concentration of sodium alginate used for preantral follicle encapsulation before culture. Follicle development and steroid secretion were analyzed during culture of cat preantral follicles encapsulated in 0%, 0.5% and 1% of sodium alginate.
METHODS
Preantral follicles were isolated from cortical tissue obtained from cat ovaries after ovariectomy. Alginate was dissolved at 0.5 or 1% in PBS and autoclaved. Follicles in sodium alginate (0%, 0.5%, 1%) were released through a micropipette as droplets on 50Mm CaCl2 and 140Mm NaCl where those formed beads. Follicles, 4 per well, with 0% (G1), 0.5% (G2) or 1% sodium alginate (G3) were cultured with M199 supplemented with 100 ng/ml of FSH, EGF and IGF-I, for 7 days at 37°C, 5% CO2 and 99% humidity. Culture medium was replaced every 2 days, and samples were stored frozen until EIA of steroid hormones. Morphometric analysis of follicles was performed every 24 hours.
RESULTS
Follicles of G1 showed granulosa cell migration away from the oocyte, reaching larger apparent diameters (203.70 ± 5.82 µm; p< 0.05) than G2 and G3 follicles (157.89 ± 8.47 µm in G2; 95.23 ± 1.67 µm in G3) which maintained their tridimensional organization being larger in G2 than in G3 (p<0.05). On day 7 of culture, steroid concentrations were higher in G1 than in G3 (p<0.05): 60 ± 19 vs 0.88 ± 0.32 pg/mL estradiol (E2); 2.6 ± 0.84 vs 0.04 ± 0.02 ng/mL progesterone (P4); 1.3 ± 0.22 vs 0.61 ± 0.04 ng/mL testosterone (T); 1.6 ± 0.54 vs 0.22 ± 0.07ng/mL androstenedione (A4), respectively. Steroid concentrations in G2 were between those of G1 and G3 (23 ± 8.9 pg/mL E2; 1.1 ± 0.07 ng/mL P4; 0.85 ± 0.04 ng/mL T; and 0.62 ± 0.14 ng/mL A4).
CONCLUSIONS
In conclusion, 0.5% sodium alginate is apparently an adequate concentration to support cat preantral follicle development in culture because it maintains both three-dimensional arrangement and steroidogenic activity, in contrast with loss of tri-dimensional organization, and compromised steroidogenesis found in follicles directly seeded on growth surface or encapsulated in 1% sodium alginate, respectively.

T38
RNA-SEQ TRANSCRIPTOME PROFILING OF NUCLEOLUS-LIKE BODIES FROM FULLY-GROWN MOUSE OOCYTES

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BACKGROUND-AIM
Nucleolus-like bodies (NLBs) are atypical dense nucleoli present in fully grown oocytes and essential for oocyte/embryonic development. Determining the molecular composition of NLBs is important to understand their exact role which is still not fully understood. The objective of this study was to discover the RNA transcriptome profile of NLBs from fully-grown mouse oocytes.
METHODS
Mice females (CD–1 IGS, 6–12 weeks old) were injected intraperitoneally with 5-7.5 I.U. of PMSG and sacrificed by cervical dislocation after 44 hours of injection. Fully grown oocytes were aspirated from the antral follicles and enucleolated through a micromanipulation process. NLBs within pipette tips were washed several times in 1xPBS and then collected in lysis buffer for direct library preparation. RNA libraries were constructed from NLBs (triplicates, 25 NLBs each) using QIAseq UPX 3' Transcriptome Kit, in which each RNA molecule is tagged with a unique molecular index (UMI). Libraries were then sequenced on an Illumina NextSeq500. Raw data were de-multiplexed and FASTQ files for each sample were generated using the bcl2fastq software (Illumina). Data analysis was performed using the QIAGEN GeneGlobe bioinformatics tool. Trimmed reads were aligned to the mouse reference genome (GRCm38) and annotated to gene regions. Then, UMIs were counted for each gene and raw expression data were normalized using the trimmed mean of M-values (TMM) normalization method. Genes with values of UMI >5 and TMM >1 in each of the 3 replicates were considered to be expressed.
RESULTS
Transcriptome analysis revealed a total of 1385 genes in oocyte NLBs. Genes involved in the Skp-Cullin-F-box complex (SCF) including Fbxw14, Fbxw19, and Skp1a as well as, E3 Ubiquitin Protein Ligase gene (Rnf34) were among the top highly abundant genes in all 3 replicates. Ontological classification of expressed genes indicated the ubiquitin-mediated proteolysis as the top significant pathway. Moreover, protein phosphorylation and regulation of translation were the top of highly enriched biological processes.
CONCLUSIONS
In conclusion, this study discovers for the first time the mRNA composition of the oocyte NLBs. This will provide new insights into the significant role of NLBs in oocyte and early embryonic development.

T39
MICRORNA MAY REGULATE LUTEAL CELL SUSCEPTIBILITY TO CELL DEATH DURING ACQUISITION OF LUTEOLYTIC CAPACITY

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BACKGROUND-AIM
Prior to day 5 of the bovine estrous cycle, the corpus luteum (CL) fails to regress in response to prostaglandin (PG)F2A, whereas after day 5, CL regress when exposed to PGF2A, a phenomenon termed "acquisition of luteolytic capacity." MicroRNA (miRNA) are regulators of rapid changes in tissues, leading to the hypothesis that miRNA are regulators of functional changes in the CL during this time.
METHODS
Estrous cycles of 8 Holstein cows were synchronized. The day of estrus and ovulation induction was considered day 0. On day 4 or 6, CL were collected and frozen. miRNA and proteins were quantified and tested for differential abundance using Nanostring technology (Human v3 miRNA CodeSet) and Q-Exactive HF mass spectrometry (Data Independent Acquisition mode) respectively. TargetScan, Ingenuity Pathway Analysis (Qiagen), and Metascape were used for functional analyses.
RESULTS
Among the 138 miRNA detected on both days, 19 were more abundant on day 4, with 31 that were greater on day 6 (Padj < 0.05). An additional 19 miRNA were unique to day 4, and 2 to day 6. On day 4, miR-125b-5p was the most abundant miRNA in the CL (~20% of all reads), after which it decreased by more than 4-fold, making let-7a-5p the most abundant on day 6. Among 5179 proteins identified, 130 proteins were more abundant on day 4 and 736 were more abundant on day 6 (Padj < 0.15). Among differentially abundant proteins, 64% were predicted targets of, and inversely correlated with, at least one changed miRNA. miRNA that decreased on day 6 were predicted to target changed proteins associated with mitochondrial function and PGF2A-response pathways, including AMPK and MAPK signaling, and the 19 miRNA unique to day 4 and miR-125b-5p were predicted to regulate apoptosis signaling.
CONCLUSIONS
Changes in miRNA in the CL between days 4 and 6, including the switch in the most abundant miRNA in the CL, may make the CL more susceptible to PGF2A-induced cell death by releasing miRNA-mediated repression of PGF2A signaling pathways and apoptosis, and inducing changes in mitochondrial and metabolic activity. Overall, this study clearly implicates miRNA in acquisition of luteolytic capacity. This project was supported by USDA AFRI Competitive Grant no. 2012-67015-30212 to JLP and Predoctoral Fellowship no. 2017-67011-26062 to CHKH.

T40
SOMATIC SIGNALS REGULATE THE PI3K/AKT/MTOR PATHWAY IN BOVINE OOCYTES

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BACKGROUND-AIM
Communications between the oocyte and the surrounding follicular cells are critical for the oocyte ability to be fertilized and support embryo development, called developmental competence. Studies conducted in the mouse pointed out that the follicular/cultural environment affects the developmental competence by acting on the program of maternal mRNA translation, through the activation of the phosphatidyl-inositol-3-kinase-AKT-mechanistic target of rapamycin (PI3K-AKT-mTOR) pathway in the oocyte. Aim of the present study was to investigate whether similar mechanisms are also in place in not-murine oocytes and to dissect the signalling cascade that leads to the PI3K pathway activation in the oocyte.
METHODS
Cumulus oocytes complexes (COCs) were retrieved from abattoir-derived cow ovaries and in vitro matured with or without follicle stimulating hormone (FSH). Cumulus cells (CCs) were removed and phosphorylation of AKT at Ser-473 (p-AKT) was monitored by western blot. Denuded oocytes were also in vitro fertilized and the presumptive zygotes cultured for 8 days. In silico analyses were performed on deposited GEO datasets to identify putative factors, mainly ligands/cytokines, that upon FSH treatment might trigger the PI3K-AKT-mTOR cascade in the oocyte.
RESULTS
In a first set of experiments, we noticed that CCs removal by vortex triggered the AKT phosphorylation independent of the culture conditions. Therefore we developed a CCs removal system that, by incubation in Sodium Citrate and gentle pipetting, allowed oocyte survival, maturation and embryo development without interfering with the pathway of interest. Next, we observed that FSH induces a delayed and transient AKT phosphorylation in the oocyte, a key step in the activation of the PI3K pathway. Finally, candidate genes expressed by CCs in response to FSH that might be involved in the signaling cascade that ultimately leads to PI3K-AKT-mTOR activation in the oocyte were identified.
CONCLUSIONS
Our findings thus far seem to sustain that, as previously described in murine model, stimulation of CCs by FSH triggers the activation of the PI3K-AKT-mTOR pathway in the oocyte. Studies are ongoing to better define the signaling cascade and to verify an involvement in the regulation of maternal mRNA translation.

T42
EFFECT OF LIPOPOLYSACCHARIDE ON THE IN VITRO GROWTH, STEROIDOGENESIS, AND MATURATION OF OOCYTE-CUMULUS-GRANULOSA CELL COMPLEXES DERIVED FROM BOVINE EARLY ANTRAL FOLLICLES

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BACKGROUND-AIM
In postpartum dairy cows, lipopolysaccharide (LPS) derived from gram-negative bacteria causes uterine or mammary inflammation, resulting in low fertility. The present study aimed to investigate the effect of LPS on the in vitro growth (IVG), steroidogenesis, and maturation of oocyte-cumulus-granulosa cell complexes (OCGCs) derived from bovine early antral follicles.
METHODS
OCGCs were isolated from bovine early antral follicles (0.5-1 mm in diameter) and cultured in vitro for 12 days in the media supplemented with LPS (0, 0.01, or 1 μg/ml). The viability and antrum formation of OCGCs and the diameter of oocytes were determined at 4, 8, and 12 days of IVG culture. Half of the media was collected every 4 days, and estradiol (E2) and progesterone (P4) concentrations in the media were determined. After 12 days of IVG culture, oocytes surrounded by several layers of cumulus cells were collected from morphologically normal OCGCs and matured in vitro (IVM) for 22 h in the maturation media without LPS. The nuclear maturation of oocytes was determined by meiotic progression using acetic orcein. The mitochondrial membrane potential of oocytes was detected by a mitochondrial permeability transition detection kit.
RESULTS
The viability and antrum formation of OCGCs were lower when OCGCs were grown in IVG media supplemented with 0.01 and 1 μg/ml of LPS compared with OCGCs grown without LPS ($P<0.05$). No significant difference in oocyte diameter was observed among treatment groups throughout the culture period. E2 production from day 4-8 was suppressed by 0.01 and 1 μg/ml of LPS ($P<0.05$), and P4 production from day 0-4 increased by 1 μg/ml of LPS supplementation ($P<0.05$). The nuclear maturation rate after IVM was lower in oocytes exposed to 0.01 μg/ml LPS during IVG culture than in oocytes grown without LPS exposure ($P<0.01$). The mitochondrial membrane potential of post-IVM oocytes exposed to 1 μg/ml LPS during IVG culture was lower compared to oocyte grown in IVG media without LPS ($P<0.05$).
CONCLUSIONS
LPS inhibited the growth and steroidogenesis of OCGCs and impaired the nuclear and cytoplasmic maturation of oocytes derived from early antral follicles in vitro, suggesting that the detrimental effect of LPS on developing oocytes may contribute to the long-term attenuation of fertility in postpartum dairy cows.

T43
RESVERATROL MITIGATES CADMIUM-INDUCED CYTOSKELETON DISTURBANCE AND OXIDATIVE STRESS OF IN VITRO MATURED OVINE OOCYTES

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BACKGROUND-AIM
Cadmium (Cd) is a toxic heavy metal with adverse effect on female reproduction. Previous studies have shown that Cd-exposure altered the quality of oocytes and led to a defective nuclear and cytoplasmic maturation, through the induction of oxidative stress (OS). This study aimed to evaluate whether the addition of resveratrol (Res), a phytoalexin with antioxidant properties, to in vitro maturation (IVM) medium would protect against Cd-toxicity on maturation of ovine oocytes by evaluating the cytoskeleton morphology, intracellular reactive oxygen species (ROS) levels and the expression level of a panel of five OS-related genes.
METHODS
Cumulus-oocyte complexes (COCs) recovered from ovaries of slaughtered sheep were IVM in presence of 2μM $CdCl_2$ with (Cd-Res) and without (Cd) 1μM Res. COCs cultured in standard conditions were used as control (CTR). ROS levels, meiotic spindle and sub-cortical F-actin in MII oocytes were evaluated by confocal laser-scanning microscopy after incubation with 2',7'dichlorodihydrofluorescein-diacetate and immunostaining with anti-tubulin antibody/rhodamine-phalloidin, respectively. DNA was stained with Hoechst 33342. The expression of CAT, GPX1, GSR, SIRT1 genes was examined using qPCR. Data of nuclear maturation and cytoskeleton were analyzed by chi-square test, ROS levels and gene expression by ANOVA. Differences were significant when $p<0.05$.
RESULTS
Cd-Res oocytes had significantly higher rates of nuclear maturation (83.8%, n=88/105) and normal spindle structure (85.7%, n=42/49) compared to Cd-group (72.6%, n=106/146 and 66.7%, n=32/48 respectively). Res partially restored the normal F-actin configuration (81.6%, n=40/49) with a rate not statistically different from that of Cd-group (68.7%, n=33/48) but similar to CTR oocytes (88.2%, n= 45/51). ROS levels were significantly lower in Cd-Res oocytes compared to Cd ones. The expression of SOD1 and SIRT-1 was higher in Cd-Res group than in Cd-group; CAT, GPX and GSR expression did not differ among the two groups.
CONCLUSIONS
Res has a beneficial effect against Cd-induced disturbance of nuclear and cytoplasmic maturation of ovine oocytes. These results may provide a basis for developing defence strategies to avoid Cd-induced toxicity on the female gamete.

T44
INVESTIGATING MILLIFLUIDIC IN VITRO OOCYTE MATURATION

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BACKGROUND-AIM
In vitro oocyte maturation (IVM) could be improved by millifluidic cell culture systems [1]. In the present study, we analyzed the effects of millifluidic IVM (mIVM) by comparing three culture conditions, i.e. native COCs with no artificial coating, included in biocompatible box-shaped resin modular supports or embedded in alginate microbeads, on oocyte maturation rate and bioenergetics.
METHODS
IVM was performed in LiveBox1 bioreactor [2, 3], or in 4-well plates (CTRL) [4]. After IVM, nuclear maturation and mitochondria (mt) distribution pattern were assessed [4]. Data were analyzed by Chi-square test (statistical significance at P<0.05).
RESULTS
Culture of native COCs in mIVM significantly reduced the rate of Metaphase II (MII) oocytes (4/124, 3% vs 42/82, 51%; p<0.001) and increased that of Germinal Vesicle (GV) oocytes compared with CTRL (105/124, 85% vs 23/82, 28% p<0.001). Most of CTRL MII oocytes showed healthy perinuclear/subplasmalemmal (P/S) mt pattern (26/42, 62%) and the majority of oocytes found at the GV stage after mIVM showed irregular mt clusterization (46/61, 75%). When COCs were included in a box-shaped resin support, the MII rate was lower after mIVM than CTRL (5/71, 7% vs 57/95, 60% p<0.001) but most of GV oocytes (52/71, 73% vs 21/95, 22% p<0.001) showed P/S mt pattern (30/43, 70%). When COCs were embedded in alginate microbeads [3], MII rates (97/181, 54% vs 46/71, 65%) and P/S mt patterns (35/52, 67% vs 23/46, 50%) after mIVM were comparable to those of CTRL and higher than those of previously examined conditions.
CONCLUSIONS
In conclusion, mIVM in native condition expose COCs to flow-induced shear stress. This effect is partly slowed down by the box, which preserves COC viability while not ensuring meiosis resumption. Alginate microbeads protect the COC from mIVM-induced shear stress and ensure suitable oocyte nuclear and cytoplasmic maturation. The developmental competence of oocytes matured under mIVM will be the subject of future investigations.
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T45
HEAT STRESS MODULATES MITOCHONDRIAL ACTIVITY AND LIPID CONTENT IN BOVINE OOCYTES AND CUMULUS CELLS

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BACKGROUND-AIM
Cellular energy metabolism is of crucial importance for oocyte development and is influenced by exogenous factors. The objective of this study was to evaluate mitochondrial activity and lipid content of cumulus cells (CC) and oocytes in response to heat stress in dairy cows.
METHODS
Nine primiparous, non-pregnant Holstein cows were evenly allocated into either a heat stress (HS, n=3), control (C, n=3) or pair-feeding (PF, n=3) group. The HS cows were exposed to constant temperature of 28°C and temperature-humidity-index (THI) of 76 for 7 days with ad libitum feeding. The C group was exposed to 16°C and THI of 60 with ad libitum feeding for 7 days. The PF cows were exposed to the same environmental conditions as C cows but were offered only the amount of feed the HS cows ingested. After slaughter, the ovaries were taken, and the follicles were aspirated to obtain cumulus oocyte complexes (COCs). Viable COCs were slightly denuded, so that at least 1 layer of cumulus cells remain around the oocyte. COCs were stored in 4% paraformaldehyde at - 4° C until analysis. After washing COCs were stained with MitoTrackerTM Red CMXRos (300nM) and Bodipy® (3 µg /ml) and after that they were analyzed with a confocal microscope (HS = 17, C = 42, PF = 39). The fluorescence intensity was quantified using ImageJ software and statistical analysis was performed using MetaboAnalyst 5.0 program. After normalization, ANOVA was performed, followed by Tukey's test, considering $p < 0.05$.
RESULTS
Oocytes from HS cows showed higher mitochondrial activity than PF and C group ($p < 0.05$). However, the lowest lipid content was observed in HS group compared to PF and C groups ($p < 0.05$). A similar pattern was observed in CC. In HS cows, the mitochondrial activity of CC was higher than in PF and C cows ($p < 0.05$), whereas the lipid content was the lowest in HS compared to the others ($p < 0.05$).
CONCLUSIONS
It is concluded that oocytes and cumulus cells trigger lipolytic mechanisms through mitochondrial activity in response to heat stress. Such mechanisms seem to be specific to heat stress, since oocytes from PF cows showed a slight lipid accumulation.

T46
LIPID PROFILES IN BOVINE CUMULUS-OOCYTE COMPLEXES CHANGE IN RESPONSE TO HEAT STRESS

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BACKGROUND-AIM
Lipids are essential as an energy source and metabolic pathway in oocyte maturation and early embryonic development. The aim of this study was to investigate lipid profile of oocytes (Oo), cumulus cells (CC), follicular fluid (FF) and blood serum (BS) in response to heat stress in dairy cows.
METHODS
Primiparous, non-pregnant Holstein cows were kept in a climate chamber with ad libitum feeding and constant temperature of 28°C and temperature-humidity-index (THI) of 76 for 7 days (Heat stress - HS, n = 3) or under constant temperature of 16°C, THI = 60 and receiving the same amount of feed as heat-stressed cows, for the same period (Pair-feeding - PF, n = 3). After slaughter, the ovaries were taken, and the follicles were aspirated to obtain follicular fluid and cumulus-oocyte complexes (COCs). The viable COCs were denuded and Oo and CC were separately stored in groups corresponding to 5 COCs. Blood was collected from the jugular vein on the day of slaughter. Lipid profile was analyzed by LC-MS and statistical analysis was done by Volcano Plot using MetaboAnalyst 5.0.
RESULTS
The lipid profile of all samples evaluated varied significantly ($p < 0.05$) between groups, however the variation was more remarkable in Oo and CC than in BS and FF. In the oocytes, 22% (80/359) of the lipids were different and from these 19 were upregulated and 61 downregulated in HS cows. In CC, 24% (105/440) of the lipids were altered, among them 19 were upregulated and 86 downregulated in HS cows. Only 22 lipids were common between Oo and CC, and these were downregulated in HS cows. In FF, 4% (27/632) of the lipids were different, 10 were upregulated and 17 downregulated in HS cows. In BS all distinct lipids (5% - 17/345) were downregulated in HS cows. The lipid classes fluctuated between groups, the most abundant was phosphatidylcholines (PC) in BS (35%) and triacylglycerol (TG) in FF (26%), CC (69%) and Oo (56%) samples. Only TG 53:3 was observed in FF, CC and Oo, but it was upregulated in FF under HS conditions, whereas TG 53:3 was downregulated in Oo and CC in HS cows.
CONCLUSIONS
A clear difference in the lipid profile was observed between HS and PF cows, indicating that heat stress alters lipid metabolism, and could be one of the factors related to reduced oocyte competence of heat-stressed animals.

T47
EFFECT OF 150 KHZ ELECTROMAGNETIC RADIATION ON THE DEVELOPMENT OF POLYCYSTIC OVARIES INDUCED BY ESTRADIOL VALERATE IN SPRAGUE DAWLEY RATS.

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BACKGROUND-AIM
Polycystic ovary syndrome (PCOS) is the most common complex endocrine disorder affecting approximately 2-20% of reproductive-aged females. Tumour Treating Fields (100-300 kHz) is an innovative, non-invasive therapeutic approach to cancer therapy in recent times. This frequency as an alternative therapy for the management of polycystic ovaries has not yet explored. To explore the effect of full-body exposure of 150 kHz Electromagnetic Radiation (EMR), on the development of polycystic ovaries in estradiol valerate-induced PCOs rats' model.
METHODS
Twenty-one female adult rats were divided into three groups (n=7 each): control, Estradiol Valerate (EV), and EV + EMR groups. The EV + EMR group was exposed to full body exposure at 150 kHz EMR continuously for eight consecutive weeks. The Estradiol valerate was administered orally to induce polycystic ovaries in EV and EV+EMR groups. The body and ovarian weights were recorded and analyzed. The regularity of the estrous cycle was assessed in all three groups. The histological study of ovarian tissue carried out by hematoxylin and eosin staining. The serum concentration levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and testosterone were measured using the ELISA method.
RESULTS
The body and ovary weights did not differ significantly between the EV and EV + EMR groups. The estrous cycle was found to be irregular in both the EV and EV + EMR groups. The ovarian histology revealed near-normal morphology with little or no degenerative and morphological changes in developing follicles in the exposure group. The histometrical analysis showed an increased number of developing follicles, a significant reduction in the number and size of the follicular cysts ($p < 0.05$) in the EV+EMR group. The hormonal analysis depicted no significant difference in the testosterone and FSH levels between the EV+EMR and EV groups. However, the LH, LH/FSH ratio decreased significantly in the EV+EMR group than the EV group
CONCLUSIONS
The 150 kHz EMR appears to have positive effects like little or no degenerative and morphological changes in the developing follicles, increased number of typical developing follicles, a significant reduction in the number, and size of the follicular cysts ($p < 0.05$).

T50
IMPACT OF SHEEP FOLLICULAR FLUID MICROBIOTA METABOLITES ON MATURATION RATE AND EMBRYO CLEAVAGE OF PREPUBERTAL LAMB OOCYTES IN JIVET PROCEDURE

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BACKGROUND-AIM
The literature data for ovarian microbiome and its role in reproductive physiology/pathology are rare, and almost exclusively focused on human follicular fluid. Also, there is a lack of information about possible effects of microbiota metabolites in IVF technologies. Prepubertal livestock oocytes are used for juvenile in vitro embryo technologies (JIVET), but their competence remains reduced compared with adults. Efforts are done to increase the understanding of differences between prepubertal and adult oocytes with the ultimate goal to increase JIVET efficiency. In this study, beside evidences of microbiota presence in follicular fluids (FFs) of adult sheep ovaries (ASO), we also evaluated possible influence of their metabolites on in vitro prepubertal lamb oocyte (PLO) competence.
METHODS
The ASO FFs were collected from healthy slaughtered animals by aspiration from the dominant or 2-3 largest follicles. Nutrient-rich liquid medium tubes (nrLM) were inoculated by collected FFs, in order to propagate enrichment and metabolite production of eventually present microbiota. After 7 days of incubation, media with visible bacterial growth were centrifuged and supernatants (FF-S) collected. Three selected FFs (FF-S1, FF-S2 and FF-S3), as well as uninoculated nrLM supernatant (negative control, NEG-nrLM), were then used as IVM-medium supplementation in JIVET procedure with PLO [1].
RESULTS
PLO nuclear maturation rate, compared with NEG-nrLM (67%, 134/200), was significantly reduced by FF-S2 (55%, 93/168, P<0.05) and FF-S3 (51%, 49/97, P<0.01). Furthermore, although the total embryo cleavage at the JIVET day 2/48h post-IVF did not significantly differ in any of the 3 conditions (20-24% vs. 20% for NEG-nrLM), at the JIVET day 5/120h post-IVF this value was significantly higher with FF-S2 supplementation (31%, 46/149, P<0.05, vs. 19%, 32/169 for NEG-nrLM).
CONCLUSIONS
FF originated from healthy ASO can be colonized with microbiota, and follicular fluid microbiome metabolites may negatively and/or positively influence oocyte maturation and embryo cleavage in assisted reproductive technologies and, therefore, physiology of reproductive processes.
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T52
NICOTINIC ACID SUPPLEMENTATION ENHANCES THE DEVELOPMENTAL COMPETENCE OF PORCINE OOCYTES MATURED IN VITRO

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BACKGROUND-AIM
Sirtuins are a family of NAD+-dependent protein deacetylases that have been implicated in cellular aging and oocyte quality. Supplementing the diet of mice with the NAD+ precursor nicotinamide mononucleotide (NMN) has been shown to ameliorate the detrimental impacts of generational obesity on female fertility [1], suggesting involvement of NMN in promoting oocyte maturation. Using other NAD+ precursors, mouse oocytes matured with 10 mM nicotinamide (NAM) and nicotinic acid (NA) exhibited a delay in germinal vesicle breakdown (GVBD) and an increase in cumulus expansion respectively [2, 3]. Using small antral follicle-derived porcine oocytes, a well-established model of poor oocyte quality, the aim of this study was to examine the effect of NA supplementation during in vitro maturation (IVM) on oocyte developmental competence.
METHODS
Oocytes (n=50 oocytes per treatment, replicated 3 times) were matured for 44 h in defined IVM medium without (control) or with increasing doses of NMN (20, 50, 100 and 200 μM). Mature oocytes were artificially activated by sequential treatment with ionomycin and 6-dimethylaminopurine/cytochalasin B. Presumptive zygotes were cultured for 7 d in Porcine Zygote Medium-3. Cleavage and development to the blastocyst stages were assessed and total blastocyst cell numbers were determined.
RESULTS
Supplementing the IVM medium with 200 μM NA resulted in a significantly higher blastocyst formation rate (51.10 ± 7.88%) compared with the control group (21.51 ± 6.14%; p<0.01). Treatment with NA did not affect the rates of cleavage (range: 68.16 ± 13.16% to 87.54 ± 3.76%), degeneration (range: 9.29 ± 5.10% to 27.51 ± 11.86%) or blastocyst hatching (range: 9.72 ± 5.00% to 35.98 ± 11.82%; p>0.05). Total blastocyst cell number was significantly higher in the group supplemented with 50 μM NA (69.13 ± 6.11 cells) compared with the 20 μM supplemented group (52.38 ± 5.24; p<0.05).
CONCLUSIONS
The results show that supplementing the maturation media with NA improves the development of porcine embryos. However, further studies are needed to determine the processes by which NA influences the acquisition of developmental competence during oocyte IVM and its role in NAD+ production and modulation of sirtuin activity in the oocyte and developing embryo.

T53
INSIGHTS INTO THE UTILISATION OF NICOTINIC ACID FOR NUCLEAR MATURATION AND SPINDLE ASSEMBLY IN PORCINE OOCYTES

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BACKGROUND-AIM
Nicotinamide adenine dinucleotide (NAD+) is an essential cofactor in many cellular processes and its role in the production of energy is well characterised. Treatments that elevate NAD+ levels have been found to improve oocyte quality in mice (Bertoldo et al. 2019, Cell Rep, 30:1670-1681), suggesting that NAD+ production is vital during oocyte maturation. In addition, recent studies showed that supplementing in vitro maturation (IVM) medium with nicotinic acid (NA), a precursor of NAD+ biosynthesis via the Preiss-Handler pathway, enhanced the developmental potential of porcine oocytes. The aim of this study was to determine the effects of NA utilisation during IVM on nuclear maturation and spindle assembly in porcine oocytes.
METHODS
Porcine oocytes from small antral follicles were matured for 44 h in defined maturation medium (Pollard et al. 2021, J Reprod Dev, 67: 319–326) either without (control) or with the competitive inhibitor 2-hydroxinicotinic acid (2-HNA; 100 µM), NA (200 µM) or 2-HNA and NA combined. At 44 h, maturation rates were determined, and mature oocytes were fixed and stained to assess spindle assembly and chromosome alignment.
RESULTS
Inhibiting NA utilisation had no effect on oocyte maturation rate, but reduced spindle length (3.64 ± 0.28 µm) compared with the control (4.62 ± 2.34 µm) and NA groups (5.32 ± 0.28 µm; $P < 0.05$). Additionally, treatment with 2-HNA increased spindle width (12.26 ± 0.52 µm) and metaphase plate diameter (15.12 ± 0.60 µm) compared with the control (10.25 ± 0.41 µm and 12.73 ± 0.45 µm, respectively; $P < 0.05$). Inclusion of NA overcame some of the effects of 2-HNA, such that spindle width (9.71 ± 0.42 µm; $P < 0.0001$) and spindle length (6.31 ± 0.32 µm; $P < 0.01$) were rescued.
CONCLUSIONS
The results show that inhibiting NA utilisation by treating oocytes with 2-HNA impaired spindle assembly, while co-supplementation with NA rescued spindle assembly. Furthermore, the effects of 2-HNA treatment on the metaphase plate may increase the incidence of chromosome segregation errors. These findings suggest that NAD+ production via the Preiss-Handler pathway is vital for supporting meiotic spindle assembly and normal chromosome segregation in porcine oocytes.

T54
EFFECT OF RECOMBINANT-RABBIT NERVE GROWTH FACTOR (NGF) ON IN VITRO MATURATION OF RABBIT OOCYTES

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BACKGROUND-AIM
First identified during central nervous system development, NGF has been shown to have a role in the reproductive system. NGF expression and its receptors have been described in follicular fluid and ovaries of several species and may be involved in the control of oocyte maturation but no effect on rabbit oocyte competence has been previously described. The aim of the present study was to analyse the effect of the recombinant rabbit NGF (rrβ-NGF) on nuclear and cytoplasmic maturation of rabbit oocytes matured in vitro (IVM).
METHODS
Rabbit cumulus oocytes complexes (COCs) (n=837) were recovered from ovaries by follicular aspiration. COCs were morphologically selected and matured in IVM media for 16 h at 38.5ºC and 5% CO2 . Media was supplemented with 0 (n=269), 1 (n=150), 10 (n=220) and 100 (n=198) ng/ml of rrβ-NGF. Nuclear maturation was evaluated by Hoechst 33342 and propidium iodide staining while cytoplasmic maturation was assessed by mitochondrial (MitoTracker fluorescent dye) and cortical granule (CG, fluorescein-5-isothiocyanate) migration pattern. Apoptosis rate of granulosa cells was analysed by TUNEL labelling. Chi-square tests were used to analyse maturation parameters and One-way-ANOVA to analyse TUNEL data.
RESULTS
Metaphase II rate was similar for COCs matured with 0 and 1 ng/ml (79 and 70%), and significantly different compared with 10 and 100 ng/ml rrβ-NGF (53 and 42%, $p<0.05$). Only IVM with 1 ng/ml rrβ-NGF increased peripheral mitochondrial migration compared to control group (80 vs 65%, $p<0.001$). Supplementation with 1 ng/ml rrβ-NGF maintained CG peripheral migration similar to that on the control group (78 vs 75%) whereas addition of 10 and 100 ng/ml rrβ-NGF decreased CG migration when compared with the 0 ng/ml group (55 and 55 vs 75%, $p<0.05$). COCs matured with 10 ng/ml rβ-NGF showed less apoptosis than the 0 and 100 ng/ml groups (35±4 vs 51±4 and 52±3%, $p<0.05$) and similar rate than the 1 ng/ml group (51±4 vs 44±4%).
CONCLUSIONS
Addition of low doses of rrβ-NGF to IVM media can improve rabbit oocyte maturation and decrease granulosa cell death, whereas higher concentrations have a detrimental effect. rrβ-NGF supplementation during in vivo or in vitro maturation can be a tool to increase embryo production in rabbits. Funded by RTI-2018-094404-B-C22 and UCM Groups.

T57
VITRIFICATION OF BOVINE OVARIAN TISSUE SUPPLEMENTED WITH RESVERATROL DECREASES CELL DEGENERATION

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BACKGROUND-AIM

Vitrification is a widely used cryopreservation method in which the tissue is exposed to high concentrations of cryoprotective agents. Thus, it is essential to establish optimized protocols that do not cause further cellular damage. Supplementation of vitrification solutions with Resveratrol, due to its antioxidant and anti-inflammatory effects, is a possibility to establish protocols that enhance the preservation of cell integrity. The aim was to evaluate oxidative stress and cell degeneration levels and apoptosis in bovine ovarian tissue fragments vitrified in solution supplemented with Resveratrol.

METHODS

Bovine ovaries collected in slaughterhouse were fragmented (3x3x1mm) and divided in 3 groups: fresh control (FC), vitrified control (VC) and vitrified with resveratrol (VR). In VR, the same cryoprotectants base solutions were used with the addition of the Resveratrol (concentration of 20µM). After treatment, fragments from all groups were washed (PBS with 5% fetal bovine serum), and placed to stain in dark room in solution with 1µl/ml H2DCFDA (30`), then stained with 10µM/ml YO PRO 01 (30`), followed by a solution with 100µg/ml propidium iodide (15`) to identify reactive oxygen species (ROS) emission analysis, number of cells in apoptosis and degenerate cells, respectively. Fluorescence intensity at different depths was performed with LSM 710 ZEISS® microscope, and images were analyzed in the Zen 2008. Statistical analyses were performed on Sigma Plot 11.0 with F test. Results are presented as mean ± standard error.

RESULTS

VR fragments presented lower (P<0.05) levels of cellular degeneration (2.9±0.3) compared to those vitrified without supplementation (6.0±0.5), and similar values to FC (1.9±0.2). The levels of apoptosis and ROS, however, were similar in the vitrified groups (VC = 11.8±0.6 and 30.4±1.5; VR = 13.3±0.7 and 34.7±1.9, respectively), and greater in the FC (21.0±1.1 and 50.3±2.8, respectively).

CONCLUSIONS

Resveratrol addition in the vitrification solution of ovarian tissues decreases cellular degeneration and maintains the same levels of apoptosis and production of ROS detected in vitrified tissues without Resveratrol.

Acknowledgements: CAPES; FAPEMIG; CnPQ.

T58
MACROSCOPIC EVALUATION OF BOVINE OVARIAN TISSUE FRAGMENTS VITRIFIED IN SOLUTION SUPPLEMENTED WITH RESVERATROL AND SUBMITTED TO XENOTRANSPLANTATION

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BACKGROUND-AIM

After being subjected preservation methods such as vitrification and xenotransplantation, the ovarian tissue undergoes challenges such as the restoration of blood circulation and tissue oxygenation. The supplementation of vitrification solutions with Resveratrol before the xenotransplantation, due to its antioxidant and anti-inflammatory effects, could be an alternative to establish protocols that do not cause further tissue damage and preserve cell integrity. In this regard, the aim was to evaluate the scores of morphology, hemorrhage and adherence of bovine ovarian tissue fragments submitted to xenotransplantation after vitrification supplemented with Resveratrol.

METHODS

Bovine ovaries collected in slaughterhouse, were fragmented and divided in 3 groups: fresh xenotransplanted (FX), vitrified xenotransplanted (VX) and vitrified with Resveratrol xenotransplanted (VRX). In VRX, the same cryoprotectant base solutions were used with the addition of the Resveratrol (concentration of 20µM). For xenotransplantation, Balb/c line female mice were used (immunodeficient inbred mouse strain). After 7 days the fragments were recovered and evaluated in scores from 1 to 5 according to morphology, hemorrhage and adhesion characteristics, in which note 1 represented the worst and note 5 the best evaluation. Statistical analyses were performed on Sigma Plot 11.0 with F test and Spearman's correlation. Results are presented as mean ± standard error.

RESULTS

In the macroscopic evaluation of the xenotransplanted tissues, it was observed that the VRX presented lower (P<0.05) scores of morphology, adherence and hemorrhage (3.3±0.5b, 2.6±0.5b, 1.3±1.1b, respectively) than VX (4.3±0.5a, 3.9±0.4a, 2.4±0.5a, respectively) and FX (3.9±0.4a, 4.1±0.4a, 2.7±0.5a, respectively). Positive correlation (0.68) between adherence and hemorrhage in xenotransplanted tissues was also observed. Fragments with greater fixation in the transplanted area, presented a higher score of hemorrhage.

CONCLUSIONS

The supplementation of vitrification solutions with Resveratrol before xenotransplantation, according to the macroscopic evaluation performed, does not appear to be a satisfactory alternative to establish protocols that do not cause further damage to tissues.

Acknowledgements:CAPES; FAPEMIG; CnPQ.

T59
THE RECOMBINANT ZONA PELLUCIDA VACCINE INDUCES OVARIAN SHUTDOWN AND LEUKOCYTE INFILTRATION IN JENNIES – PRELIMINARY DATA AND DESCRIPTIVE ANALYSIS

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BACKGROUND-AIM
Zona pellucida (ZP) vaccine has been successfully used for contraception in approximately 80 species. The ZP is a complex glycoprotein matrix surrounding the mammalian oocyte. Despite the widespread application of ZP vaccines, relatively little is known of their mechanism of action. Primarily, it was suggested an antibody-mediated interference with sperm-zona pellucida binding and fertilization. Zona pellucida vaccines have however been associated with ovarian dysfunction. Therefore, the goal of this study was to evaluate the effect of a recombinant zona pellucida (reZP) vaccine on ovarian dynamics and histology of donkeys.
METHODS
Fifteen reproductively sound jennies were randomly assigned to treatment (n=12) and control (n=3) groups. Jennies received three treatments 35 days apart of reZP (Treatment; 250µg ZP3 and 250µg ZP4; Council for Scientific and Industrial Research) or a placebo (Control; Lactated ringer). Jennies were monitored by transrectal ultrasound weekly. A left flank ovariectomy was performed in the treated jennies when no follicles ≥10 mm were observed for ≥three continuous weeks (ovarian shutdown) and a concurrent ovariectomy was performed in the control jennies at this meantime. Immediately post-ovariectomy, the ovaries were fixed in 10% neutral buffered formalin and embedded in paraffin for histological evaluation. Tissues were sectioned at 5µm thickness and stained with hematoxylin and eosin. Sections from two different portions of the ovary of nine jennies (treated, n=7; control, n=2) were evaluated for the presence of follicles and inflammatory cells. Results are presented as mean±standard deviation.
RESULTS
Jennies treated with reZP stopped cycling 146.7±9.9 (n=12) days after the first vaccination. Leukocytic infiltration was observed in the treated group in secondary (4 jennies) and early tertiary (1 jenny) follicles. Only primordial follicles were observed in the sections from the other two treated jennies. Late tertiary follicles were observed in the control group but not the treated group. No inflammatory cells were seen in the ovaries from the control group.
CONCLUSIONS
These preliminary results suggested that the reZP vaccine induced ovarian shutdown in jennies through an immune response and consequent inhibition of follicle growth. Further analyses are required to confirm our hypothesis.

T60
IN VITRO EFFECTS OF ENNIATIN A ON CELL PROLIFERATION AND STEROID PRODUCTION BY BOVINE GRANULOSA CELLS FROM SMALL AND LARGE FOLLICLES

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BACKGROUND-AIM
Enniatins (ENNs) are hexadepsipeptidic mycotoxins produced by Fusarium species and are prevelant in grains. There are few data available on effects of enniatin A (ENNA) on ovarian function. The aim of this study was to determine if ENNA affects estradiol (E2) and progesterone (P4) production by granulosa cells (GC) from small (1-5 mm) and large (> 8 mm) bovine follicles.
METHODS
Ovaries were collected from cattle at a local slaughterhouse and follicular fluid was aspirated from small and large follicles to isolate GC. GC were cultured for 2 days in medium with 10% fetal bovine serum followed by 2 days in serum-free medium containing 500 ng/ml of testosterone (as an E2 precursor) and 30 ng/ml of follicle stimulating hormone (FSH) plus insulin-like growth factor 1 (IGF1; 30 ng/ml) in two experiments which were replicated with three independent pools of cells and treatments applied in triplicate for each pool. Medium was collected after 1 and 2 days of treatment for steroid radioimmunoassays and cell numbers assessed on day 2 via Coulter counting. In Experiment 1, GC harvested from small follicles were exposed to 0, 1, 3 and 5 µM ENNA, whereas in Experiment 2, GC collected from large follicles were treated with 0, 0.3, 1 and 3 µM ENNA. Data were analyzed as a 2 x 4 factorial ANOVA.
RESULTS
After 1 and 2 days of treatment, in small-follicle GC cultures, ENNA at 1, 3 and 5 µM inhibited ($P < 0.0001$) E2 production by over 99%. Similarly, P4 production was inhibited ($P < 0.0001$) by over 90% after exposure to ENNA at 1, 3 and 5 µM. In large-follicle GC, ENNA at 0.3, 1 and 3 µM inhibited ($P < 0.0001$) E2 production by over 80% and P4 production by over 70%. Large-follicle GC numbers decreased ($P < 0.001$) by at least 30% and 60% when ENNA was applied at 1 and 3 µM, respectively; 0.3 µM was without effect. Whereas ENNA significantly reduced ($P < 0.0001$) small-follicle GC numbers by 90, and 95% when applied at 3 and 5 µM, respectively; 1 µM was without effect.
CONCLUSIONS
In conclusion, ENNA has strong inhibitory effects on GC proliferation and steroid production. These results demonstrate that ENNA may affect bovine GC growth and steroidogenesis suggesting its potential to impair reproductive function in cattle.

T62
IMPACT OF SUPPLEMENTATION OF GRANULOSA CELL-DERIVED EXTRACELLULAR VESICLES DURING BOVINE OOCYTE MATURATION UNDER THERMAL STRESS CONDITIONS

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BACKGROUND-AIM
Climate change-induced thermal stress has been documented to compromise fertility by negatively affecting oocyte maturation and fertilization rates in both humans and animals. Recently, intrafollicular derived granulosa cells (GCs) have been noted to release extracellular vesicles (EVs), which shuttle protective messages to induce tolerance in recipient cells against recurrent thermal stress in vitro. Therefore, here we hypothesize that the supplementation of heat stress (HS) associated EVs during bovine in vitro maturation (IVM), will modulate the detrimental effects of HS through regulating associated genes and pathways.
METHODS
To achieve this, EVs were isolated from cultured granulosa cell-conditioned medium under normal (38.5°C) and thermally stressed (42°C) conditions using ultracentrifugation technique. At the time of maturation, cumulus-oocyte complexes (COCs) were arranged in a 2 x 3 factorial design for temperature (38.5°C or 41°C) versus EV supplementation (normal EVs [N-EVs], stressed EVs [S-EVs] and non-treated controls [NTC]) at 20% of the total maturation medium. In vitro fertilization of MII oocytes was performed using selected bull sperms to investigate the effect of EV supplementation on further developmental potential.
RESULTS
Significant upregulation of cumulus expansion marker genes PTX3, PTGS2, and EGFR was found in those cumulus cells supplemented with S-EVs when compared to non-supplemented ones under recurrent HS. Moreover, a significant reduction in the expression of stress marker genes ($p \leq 0.05$) including HSP70, HSP90 and NRF2 was observed in isolated cumulus cells from S-EVs supplemented group under recurrent HS, compared to those non-treated controls. Supplementation of both N-EVs and S-EVs during oocyte maturation resulted in increased cleavage rates (90.80±1.2%) and (88.98±0.85%), respectively compared to non-treated controls (85.11±1.7%). Blastocyst rates were higher ($p \leq 0.05$) when COCs were supplemented with N-EVs (20.8 ±2.1%) and S-EVs (21.9 ±1.2%) compared to the non-EV-supplemented groups (10.8 ±0.9%) under thermal stress conditions.
CONCLUSIONS
GC-derived EVs have a positive impact inducing thermal tolerance during oocyte maturation, thereby equipping with a carryover effect that positively affects the developmental competence to the blastocyst stage.

T63
PERIOVULATORY ANTICOAGULANT THERAPY IMPROVES EMBRYO RECOVERY IN SUPEROVULATED MARES

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BACKGROUND-AIM
The embryo recovery (ER) rate in superovulated mares is still low when compared to other domestic species. It is believed that the main factor influencing the embryo recovery in superovulated mares is a blood clots formation after the first ovulation(s), which avoids the subsequent oocytes to migrate through the ovulation fossa. The aim of this study was to evaluate the ER rates of superovulated mares treated with two different anticoagulants.
METHODS
Mares (n=11) were bred in four consecutive cycles in a crossover study, as follows: Group 1 (G1) control cycle; Group 2 (G2) superovulated mares + 40mL 0.9% saline solution (intravenous, IV); Group 3 (G3) superovulated mares + 450IU/kg/IV sodium heparin; Group 4 (G4) superovulated mares + 1mg/kg enoxaparin sodium (IV). Saline solution administration and anticoagulant therapy were performed 35h after ovulation induction. The superovulation protocol consisted of the administration of 25mg of equine pituitary extract (EPE) beginning on the 7th day after the previous ovulation associated with 5mg, i.m. of dinoprost-tromethamine during the first 2 days. In all groups, ovulation was induced with 250µg of histrelin acetate and 2500IU of hCG when a ≥35mm follicle was detected. Artificial inseminations (AI) were performed with 2 billion motile fresh sperm 24h after the induction of ovulation. Mares were monitored every 12h after AI and embryo flushing was performed nine days after the first ovulation. The mares in G3 and G4 had blood collected at 0 and 3 h after treatment for blood counting and 0, 1, 2, 3, 6, 9 and 12h for coagulogram. Statistical analysis was performed by Shapiro- Wilk test, ANOVA and Tukey test ($P<0.05$).
RESULTS
Mares in G2 (4.6±0.4), G3 (5.2±0.5) and G4 (4.9±0.6) had similar ovulation rates ($P>0.05$) but greater than G1 (1±0; $P<0.05$). The ER rate was superior in G3 (2.5±0.3) and G4 (2.6±0.6) than in G1 ($P<0.05$) and it did not differ ($P>0.05$) between G1 (0.6±0.2) and G2 (1.6±0.5). When the ER was evaluated regarding the number of ovulations, the G1 (64%), G3 (50%) and G4 (52%) groups had similar results, but G2 (36%) was lower compared with G1 ($P<0.05$). No hematological alterations were detected in mares after anticoagulant therapy.
CONCLUSIONS
In conclusion, periovulatory therapy using anticoagulants may be an alternative to improve embryo recovery rates in superovulated mares.

T64

CHARACTERIZATION OF LARGE AND SMALL EXTRACELLULAR VESICLES FROM FOLLICULAR FLUID IN COW

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BACKGROUND-AIM
Follicular fluid extracellular vesicles (ffEVs) participate in cell communications inside the follicle by exchange of different RNAs, proteins, and lipids between different follicular cells and enclosed oocytes, and thus affect cellular functions and signaling in target cells. We aimed to compare ffEVs of different sizes by comparative analysis of their morphology and protein cargo.
METHODS
Follicular fluid (FF) was aspirated from antral follicles of ovaries from slaughtered cows. Fractions of microvesicles (MV) were separated by centrifugation of cleared FF 30min at 12,000g. Fractions of small ffEV (exosomes, Exo) were obtained by ultracentrifugation of MV-depleted FF 90 min at 100,000g. Transmission electron microscopy (TEM) was performed on intact ffEVs and 1µM sections. The presence of EV markers was analyzed by Western blot. Peptide/protein profiles of ffEVs samples (n=24) were acquired by MALDI-TOF mass spectrometer (MS) RapifleX Tissuetyper (Bruker Daltonics) in positive linear ion mode in the 2,000 – 30,000 m/z range, in 9 technical replicates. Spectral processing and statistical analyses were performed using home R software based on MALDIquant & MALDIquantForeign packages (v1.19.3 & v0.12). Peaks annotation was obtained from Top-Down proteomics database.
RESULTS
CD81 and HSPA8 markers were detected in both MV and Exo fractions. By TEM, the mean diameter of MV and Exo was 170±72 nm and 60±22 nm, respectively. The ffEVs contain inclusions of different electron densities. MV fractions are more heterogeneous than Exo and contain the debris of different organelles like mitochondria, endoplasmic reticulum, and lipid droplets. Exo fractions contain protein agglomerates and ribosomes. By MS, 366 m/z peaks were detected from ffEVs mean spectra. Differential analysis of normalized peak height values between MV and Exo revealed an overabundance of 46 m/z in MV and 31m/z in Exo ($p<0.01$, fold change >2). Their annotation revealed both small proteins and proteolytic fragments.
CONCLUSIONS
Morphology, vesicular cargo, and peptide-protein fingerprints significantly differed between small ffEVs and MVs that revealed their different origin and potential roles in functional activity inside the follicle.
Funds: INRAE France; Russian Science Foundation (project 19-16-00115).

TOPIC Pregnancy, placental function and parturition

T66

GENETIC REGULATION OF EQUINE CHORIONIC GIRDLE DEVELOPMENT: LINKING INVASION WITH IMMUNOMODULATION.

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BACKGROUND-AIM
In early horse pregnancy, the invasion of the endometrium by the chorionic girdle trophoblast cells results from the explosive transformation of non-invasive trophoblast cells of the equine chorion to a rapidly dividing, highly motile phenotype over the course of only a few days. Transcriptional profiling has identified a large number of genes that are either up or down regulated during the development of equine invasive trophoblast, but relatively little is known about the molecular mechanisms governing these dramatic changes in gene expression.
METHODS
Recently, new methods have been developed for identifying regulatory elements in complex genomes. Chromatin run-on and sequencing (ChRO-seq) is a variant of global run-on and sequencing (GRO-seq), a technique that can precisely capture active transcription events in a cell population by focusing on engaged RNA polymerases at sites throughout the genome. A single ChRO-seq assay can reveal the location of thousands of non-coding functional elements, and in addition generate information on nascent transcripts equivalent to conventional RNA-seq.
RESULTS
We performed ChRO-seq assays on biological replicates of equine invasive and non-invasive trophoblast samples recovered at day 34 of gestation from normal horse mares. We also tested equine CD4+ T-cells and liver using the same assay. This large combined dataset allowed us to simultaneously compare gene expression across these four tissues and to identify putative transcription factor binding sites associated with genes that were highly expressed in placenta, and also genes that were differentially expressed between invasive and non-invasive trophoblast.
CONCLUSIONS
In our experience the ChRO-seq assay is robust, reproducible, and sensitive, producing data equivalent to that acquired using the traditional methods of the ENCODE Project, but with greater precision, at lower cost, and with reduced effort. ChRO-seq also has the potential to identify new regulatory elements not detected by earlier assays. Our data on expression of immune system genes in the invasive trophoblast may provide insights into tolerogenic mechanisms that are essential to pregnancy maintenance and survival.

T67
ELUCIDATION OF IMPRINTED GENES RESPONSIBLE FOR HYPERPLASIA IN SOMATIC CELL NUCLEAR TRANSFERRED PLACENTAS

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BACKGROUND-AIM
Somatic cell nuclear transfer (SCNT) is the sole reproductive technique to produce live animals from differentiated somatic cells. However, their birth rate is very low (usually less than 5% for transferred embryos) and many abnormalities are observed. We recently reported that one of the placenta-specific imprinted genes, Sfmbt2 microRNA cluster, was overexpressed in SCNT placentas by loss of imprint, resulting in placental hyperplasia in mice. We found that normalization of their expression level ameliorated their placental morphologies; however, their placental weights could not be corrected entirely to the level comparable to those of IVF placentas (0.10±0.004 g vs. 0.20±0.01 g). In this study, we investigated relationships between other placenta-specific imprinted genes and SCNT placental hyperplasia.
METHODS
We focused on three placenta-specific imprinted genes (Jade1/Phf17, Smoc1, Platr20) overexpressed in SCNT placentas. Their knockout (KO) mice lines were produced by the CRISPR/Cas9 system. Cumulus cells were collected from (C57BL x DBA/2)F1 female mice with a maternal KO allele and transferred into enucleated oocytes. Reconstructed oocytes were cultured in KSOM for 1 h and activated with Ca2+-free KSOM containing 2.5 mM SrCl2, 50 nM trichostatin A and 5 μM latrunculin A. 2-cell stage embryos were transferred into oviducts of pseudopregnant ICR female mice on the next day and SCNT fetuses were retrieved on day 19.5.
RESULTS
Two imprint genes, Jade1/Phf17 and Smoc1, revealed sublethal in homozygous KO fetuses. On the other hand, the homozygous KO of Platr20 did not show any lethality. When SCNT fetuses were produced with maternal KO cumulus cells from three KO lines, their birth rates were comparable to that of wild-type SCNT. In Smoc1 and Platr20 maternal KO, SCNT placental weights were not changed (wild-type: 0.32±0.02 g, Smoc1: 0.29±0.02 g, Platr20: 0.30±0.03 g), while those of Jade1/Phf17 maternal KO showed decreased placental weights (0.23±0.03 g).
CONCLUSIONS
Together with our previous study, we identified that loss of imprint of two imprinted genes, Sfmbt2 miRNA and Jade1/Phf17, could be the causes of placental hyperplasia in mouse SCNT. The details of placental morphologies are under-investigated at present.

T68
COMPARISON OF COMBINED THICKNESS OF THE UTERUS AND THE PLACENTA BETWEEN 2 BREEDS OF MARES AND THEIR POTENTIAL RELATIONSHIP WITH ESTROGENS DURING NORMAL PREGNANCY.

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BACKGROUND-AIM
To the best of our knowledge, combined thickness of the uterus and the placenta (CTUP) has never been compared between different breeds of horses, using the same settings. This study compares the CTUP in 4 to 11 months pregnant Spanish Pure-Breed (SPB) and showjumping Belgian Saddle-Breeds (SJ) mares. The potential relationships between CTUP and estradiol (E2), estrone (E1) or estrone sulfate (E1S) concentrations in maternal sera will also be investigated.
METHODS
Once a month, CTUP was measured and blood was collected in 15 SPB and 11 SJ mares. Mares presenting clinical signs of placentitis during the pregnancy or after foaling were excluded of this study. The CTUP was measured in 3 different places of the cervical area by transrectal ultrasonography using the previously described technique (Renaudin et al., 1997). The mean of the 3 measures was recorded and assigned to the month of pregnancy. Estrogens were assayed in serum using the previously validated Liquid Chromatography coupled to Mass Spectrometry technique (Dufour et al., 2021).
RESULTS
For the same month of pregnancy, no difference in CTUP was observed between breeds of mares. The CTUP gradually increased during the pregnancy and was significantly larger ($p<0.01$) at 11 months (mean: 8.39 ±2.02mm), showing a decreased and heterogenous echogenicity. No significant difference in CTUP was observed between 9th and 10th month (respective means: 6.29 ±1.23mm and 6.57 ±1.14mm), but they both tended to be higher ($p<0.1$) than those observed at 4, 5 and 6 months of pregnancy (respective means: 4.34 ±0.56mm, 5.45 ±0.65mm, 5.41 ±0.82mm). No correlation was observed between CTUP and estrogens concentrations.
CONCLUSIONS
Gradual increase of CTUP was observed from 4 to 10 months of pregnancy. During the last month, fœto-maternal unit thickened more quickly and it was associated with an heterogenous echogenicity. Some previous reports leaded to think that there were differences in CTUP between breeds of mares. However, with this design, such differences were not observed. The CTUP was not related to E2, E1, E1S concentrations, showing that morphologic and endocrinologic evolutions of placenta are not associated: maximal CTUP was observed at 11 months whereas estrogens peak is described between 5 and 6 months.

T69
DIFFERENCES IN BLOOD PARAMETERS AT CALVING AND PLACENTAL MORPHOLOGY BETWEEN HOLSTEIN AND JAPANESE BLACK DAMS WITH SIMILAR OR DIFFERENT FETUSES

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BACKGROUND-AIM
Our previous study demonstrated that nutrient supply from Holstein dams to fetuses of beef cattle (Japanese Black; JB and cross breed) was lower than that to Holstein fetuses (Kawashima et al., ASJ 2021). However, it remains unclear why the nutrient supply from dams to fetuses of different breeds is inhibited. Thus, in this study, we aimed to investigate the differences in blood parameters of dams, umbilical cords and calves, and morphology of the placenta in dams (Holstein; HOL or JB) between dams with similar or different fetuses.
METHODS
We examined HOL dams pregnant with similar (n = 23) or JB (n = 4) fetuses and JB dams pregnant with similar fetuses (n = 8). Blood samples were collected from dams immediately after calving, umbilical cords (veins and arteries) at calving, and calves immediately after birth. After expulsion of the placenta, the cotyledonary weight (CWT) and surface area (CSA) were measured. The distribution of each quartile for CWT and CSA were analyzed using the chi-square test. Other data were analyzed using ANOVA for comparison between the groups, and Holm–Sidak or Dunn's test was used for the post-hoc test.
RESULTS
Serum total protein levels in JB dams were higher than those in HOL dams with HOL fetuses ($p < 0.1$), whereas these levels in JB calves of HOL and JB dams were lower than those in HOL calves of HOL dams ($p = 0.03$; $p = 0.04$, respectively). Although all dams and umbilical veins showed similar serum glucose levels regardless of breed, JB calves of JB dams had lower serum glucose levels than HOL calves of HOL dams ($p < 0.001$), with JB calves of HOL dams being intermediate. In addition, the distribution of CWT and CSA differed among the three groups ($p < 0.001$); the most distributed quartiles in HOL dams with HOL fetuses, HOL dams with JB fetuses, and JB dams with JB fetuses were the 4th, 1st, and 2nd quartiles for both CWT and CSA, respectively.
CONCLUSIONS
Our study indicates that Holstein dams with JB fetuses might cause insufficient growth of the placenta and lower protein synthesis in the fetuses. Further studies are required to investigate the expression of transporters, blood flow, and function in the placenta, which is related to the nutrient supply capacity of the placenta of dams to fetuses in Holstein dams with JB fetuses.

T70
AMNIOTIC MICROBIOTA IN THE HEALTHY EQUINE PREGNANCY

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BACKGROUND-AIM
Microbial colonisation of the gastrointestinal tract (GIT) is believed to commence at birth, with the foetus developing in a sterile uterus. This colonisation is critical for the host's immune system and ongoing health. Recently, the in utero colonisation paradigm challenges this concept, suggesting that non-pathogenic microbiota exist in the amniotic fluid (AF), and are initial colonisers of the foetal GIT, and thus prepare the neonate for life outside the womb. This study investigated the AF of pregnant mares for the presence of microbes, with the aim of developing insights about the microorganisms potentially colonising the foetal GIT in utero.
METHODS
AF samples were obtained from 14 foaling mares during parturition, under conditions of maximum sterility, immediately snap-frozen and stored at -80C until analysis for microbiota. Genomic DNA was extracted and processed for 16S rRNA gene amplicon sequencing. Bioinformatics was carried out using QIIME2 and Calypso V8.84 software packages.
RESULTS
Microbes were detected in all AF samples in low diversity, as indicated by the Chao1, Evenness, Richness and Simpson's Index alpha diversity parameters. Despite some host variation, the most abundant Operational Taxonomic Units (OTU) detected in the majority of samples classified to the phyla Opisthokonta (100% of samples), Bacteroidetes (79%), Actinobacteria (57%) and Firmicutes (57%). At the family level, the most abundant OTU belonged to Staphylococcacea (79%) and Microbacteriaceae (71%). These microbes have previously been identified in equine oral, oesophageal, distal GIT and vaginal ecosystems, and suggest a maternal origin where microbiota translocate to the AF and foetus during pregnancy; a concept that is currently gaining traction in humans. Additionally, all samples were collected from healthy pregnancies, however, many OTUs identified belonged to bacteria that are opportunistic pathogens and have previously been confirmed in compromised equine pregnancies.
CONCLUSIONS
Overall, our results highlight the presence of low microbial diversity in AF from healthy equine pregnancies, predominantly comprised of environmental and gastrointestinal microbes likely of maternal origin. Their role in the developing foal is yet to be elucidated.

T72
MATERNAL ANTIOXIDANT SUPPLEMENTATION COUNTERACTS FETAL GROWTH RESTRICTION BEFORE THE LAST THIRD OF PREGNANCY IN SHEEP

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BACKGROUND-AIM
Undernourishment during sheep pregnancies results in restricted fetal growth, hypoxemia and oxidative stress, mainly in twins. Maternal oral supplementation with antioxidants (AOX), during almost the entire pregnancy prevent oxidative stress and fetal growth restriction. Our goal was to establish if the effects of maternal supplementation with herbal AOX (50 g/ewe/day of a commercial concentrate containing C-Power™ 10 g/Kg and Herbal-E™ 7 g/Kg; Nuproxa) are expressed before the fetal accelerated growth stage (last third of gestation).
METHODS
The study was carried out in single and twin-bearing ewes, selected by ultrasound at day 30 after mating, kept on natural pasture (CP: 3.3%, ME: 1.9 Mcal/kg, TDN: 45%) representative of Patagonian prairie at the INIA research farm (Chilean Magellan Patagonia, Lat 52° 36'; Lon 70° 56'). Animals were allocated to AOX treated (singles n=10; twins n=10) or control groups (n=10 each). The control groups received 50 g/ewe/day of the same concentrate but without AOX. At day 100 of pregnancy, umbilical blood sample were taken for gases and oxidative stress evaluation (malondialdehyde, MDA and total antioxidant capacity, TAC) and fetuses were weighed and measured after euthanized by pentobarbital overdose. Main fetal organs and semitendinosus muscle were also weighed. Results were compared by ANOVA; rank (single/twins) and AOX supplementation (with/without) were the variation factors.
RESULTS
Twins showed reduced umbilical blood PO2 and saturation of hemoglobin by O2 (P=0.04 for both), which was increased by AOX (P=0.06 and P=0.02, respectively), and lower TAC and higher MDA, which were reversed by AOX (P<0.05 for both). Lamb´s body weight, crown-rump length, and arms and leg length were lower in twins (P<0.05) and showed higher values in the AOX groups, but did not reach statistical significance. Semitendinosus muscle and brown adipose tissue showed a trend to be higher in singletons and AOX treated groups (P<0.1). Liver and heart weights were higher in singletons (P<0.05), without effect of AOX. Brain weigh was not affected by rank nor by AOX.
CONCLUSIONS
In conclusion, AOX supplementation counteracts fetal hypoxia, oxidative stress and growth restriction from as soon as at 100 days of gestation.
Ethical approval: Protocol # 11-2016
Support: FONDECYT 1160892, Chile; Nuproxa Switzerland Ltd.

T73
EFFECT OF GENDER ON FETAL-TO-NEONATAL TRANSITION PERIOD IN HOLSTEIN CALVES

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BACKGROUND-AIM
The success of immediate adaptation depends on the maturation of the lung parenchyma. Whenever a deficiency in full pulmonary maturation occurs, there is a higher incidence of Respiratory Distress Syndrome. In many species, fetal sex hormones play an important role in regulating lung development, with an inhibitory activity of androgens and stimulatory action of estrogen. Thus, this study aimed to compare the overall clinical and respiratory examination between male and female calves during the initial hours of the fetal-to-neonatal transition period.
METHODS
Holstein calves born from vaginal eutocia were assigned to two experimental groups, according to the fetal gender: Female Group (n=6) and Male Group (n=6). Calves were assessed through: neonatal vitality score (0-10) adapted for calves, which evaluates heart rate, respiratory rate, muscle tone, irritability reflex and mucous membrane color at birth, 5 and 60 minutes thereafter. Body temperature was measured at the same moments of evaluation. Oxygen saturation (SO2) were analyzed through hemogasometry of blood samples taken from the femoral artery at birth, after 2 and 4 hours of life. Body weight was also measured at birth. Interactions between groups and time were analyzed by ANOVA and the effects of groups were compared by Student's t-test (P≤0.05).
RESULTS
There was no difference between gender groups for the vitality score, respiratory rate, muscle tone, irritability reflex, mucous membrane color and body weight. For both groups, vitality score was satisfactory for neonate calves (9±0.2 for the Female Group and 8.6 ± 0.4 for the Male Group). Regardless of the moment of evaluation, heart rate of the Female Group was higher (165.2±7.2 bpm) compared to the Male Group (144±7.1 bpm). The Female Group (38.4±0.2°C) had higher body temperature at 120 minutes after birth compared to the Male Group (37.9±0.1°C). Oxygen saturation were within acceptable parameters for calves, indicating that satisfactory blood oxygenation occurred in both groups.
CONCLUSIONS
Eutocia-born calves born have adequate neonatal vitality, unrelated to neonatal gender. However, better adaptation to fetal-to-neonatal transition period was observed in females, with higher heart rate and normothermia compared to male calves.

T74
NOVEL PRIMER VALIDATION FOR FETAL SEX DETERMINATION FROM MATERNAL PLASMA IN MARES DURING PREGNANCY

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BACKGROUND-AIM
Preimplantation genetic sexing (PGS) is a fast-developing field not only in human but in veterinary assisted reproduction. It has been shown that a small proportion of fetal DNA passes through the equine multi-layer epitheliochorial placenta during pregnancy and can be detected with ploimerase-chain reaction (PCR) in maternal serum at various stages of gestation. This method requires extremely sensitive method not only for the PCR but for the DNA isolation as well.
The aim of this study was to create a novel, small primer which results in a short PCR product (105 bp) for more certain detection (multicopied presence in the samples).
METHODS
After collection of blood samples from different donors (1 non-pregnant, 14 pregnant mares from different gestation period, 5 mares at foaling and 1 stallion as a positive control), the plasma samples went through the DNA isolation (Macherey-Nagel Nucleospin Plasma XS kit). The new primer (TSPY=Testis Specific Protein Y) was designed with the aim of Primer Designer 4 programme and during the detection PCR the novel primer was used in comparison with 3 already published and extensively used original primers: Amelogenin X and Y (AMEL X, Y), and Sex-determining region Y (SRY). Samples have been amplified with Applied Biosystems, 2720 ThermalCycler (Life Sciences) PCR machine according to the suggested temperature and cycle for the different primers. The PCR products have been separated with agargel-electrophoresis (nanoPAC-300, Cleaver Sci. Ltd.). Detection of DNA content has been made under UV-light (Glite 900BW GelScaner System). Fetal sex have been detected from the animals retrospectively.
RESULTS
Sensitivity (84,6%) and specificity (100%) have been calculated and could be comparable with SRY primer values (76,9% vs. 77,7%) in this study. Fetal sex could be detected reliably at 210th gestational age in mares. Positive predictive value (100%) and negative predictive value (81,8%) and accuracy was 91% of the new TSPY primer.
CONCLUSIONS
No PCR method of fetal sex determination can reach 100% accuracy to date, and this novel TSPY primer could be used with high sensitivity and reliability as early as 210th day of gestation in mares. However, this method should be further refined to reach reliable results at early stages for gestation for routine use.

T75
MODULATORY ROLE OF FIBROBLAST GROWTH FACTOR (FGF) 2 ON OVINE TROPHOBLAST FUNCTIONALITY

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BACKGROUND-AIM
In sheep, during embryo implantation, trophoblast cells invade the endometrium to establish the foetal-maternal cross talk. Several endometrial-derived growth factors influence conceptus development, including fibroblast growth factor-2 (FGF2) implicated in multiple signaling pathways for cell survival. Uterine FGF2 production is related to the maintaining of placental growth and development by regulating trophoblast cells differentiation and function. Reduction of FGF2 release leads to impaired placentation associated with gestational complications, such as early pregnancy loss and intrauterine growth restriction. The aim of this study was to examine the FGF2 effects on ovine trophoblast cells (oTC) activity in the early stage of pregnancy.
METHODS
Primary oTCs were isolated from 21 days old sheep placenta and cultured in supplemented DMEM/F12 medium. oTCs were characterized by cell morphology, immunofluorescence and PCR for trophoblast markers (CK-7, e-CAD, INF-τ, oPL, PAG-11). oTCs were grown in media supplemented with FGF2 (50 ng/ml, rh-FGF) to study its effect on cell functionality vs controls. Cellular functionality was evaluated through cell proliferation (BrdU incorporation) and cell migration (Transwell) assays, gene expression profile and activation of key components of signaling pathway regulating cell growth and proliferation (qPCR and western blot analysis).
RESULTS
Our model showed mainly, mononuclear cells with epithelial cell-like growth and placental morphological properties, such as binucleate cells and multinucleated syncytium-plaques formation expressing peculiar trophoblast markers. FGF2 increased significantly proliferation rate starting from 12 to 48 hours (i.e. 24h, 33 vs 63%, $p < 0.0001$). Similarly, migration activity was two-fold higher in FGF-treated oTCs (12h, $p < 0.0001$). FGF2-treated oTCs showed modified expression of some trophoblast markers (e-CAD, INF-τ, PAG-11) and activation of Akt/mTOR signaling, mainly involved in regulation of cell proliferation and migration.
CONCLUSIONS
In summary, these findings support that FGF2 directly affects trophoblast cell functionality during the early stage of placentation in sheep. This work was supported by grant UNITO (TOSP_RILO_20_01).

T76
UTILISATION OF A CONCEPTUS-ENDOMETRIAL CO-CULTURE SYSTEM TO INVESTIGATE ENDOMETRIAL GENE EXPRESSION AND HISTOTROPH DEPENDENT ON CONCEPTUS ORIGIN

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BACKGROUND-AIM
During early pregnancy, luteal progesterone stimulates endometrial cells to secrete histotroph, supporting conceptus development. In cattle, the conceptus secretes interferon tau (IFNT), the maternal recognition of pregnancy signal and inducer of interferon stimulated genes (ISGs). Therefore, IFNT and other conceptus secretory factors modify endometrial gene expression and likely, uterine histotroph. Using a conceptus-endometrial co-culture system, a recent study identified endometrial transcripts that differed in response to Day 15 in vitro produced (IVP) compared to in vivo derived (IVD) bovine conceptuses. However, the exact day of the estrous cycle the endometrium was collected and extent by which co-culture effected the uterine histotroph was unknown.
METHODS
This study was designed to identify synchronized endometrial mRNA and histotroph components in response to Day 16 IVP compared to IVD bovine conceptuses. Angus-Holsten heifers underwent fixed-timed estrus synchronization and were either bred by AI (IVD conceptuses) or received age and sire matched IVP bovine blastocysts by embryo transfer (ET). A third group of synchronized heifers were not AI or received embryos and were used to generate Day 16 intercaruncular endometrium. On Day 16, uteri were harvested, and three treatments were established in 1 mL of RPMI medium: 1) endometrium alone (8 mm; Control; n=13), 2) endometrium co-cultured with IVD conceptus tissue (5 x 2 mm; IVD; n=15) and 3) endometrium co-cultured with IVP conceptus tissue (5 x 2 mm; IVP; n=13). After 12 h in standard cell culture conditions, medium and tissues were collected. Endometrial mRNA was isolated, and RT-qPCR relative expression calculated using the ΔΔCt method.
RESULTS
Data were statistically analyzed using a GLM. Compared to Control, endometrium treated with IVD and IVP had greater expression of ISGs ISG15, MX1, OAS1 and LGALS9 ($P < 0.001$) as well as other genes important for pregnancy establishment such as glycine transporter SLC6A9 ($P < 0.01$) and IL1B ($P < 0.05$). Further, expression of ISG15 was greater in endometrium treated with IVP compared to IVD conceptus tissue ($P < 0.05$).
CONCLUSIONS
In conclusion, synchronized endometrial gene expression, and possibly uterine histotroph, is altered by IVP compared to IVD bovine conceptuses.

T168
CAN WE USE TWIN SHEEP AS A MODEL FOR SINGLETON HUMAN PREGNANCIES? HEMODYNAMIC VALUES OF SINGLETON VS. TWIN PREGNANCIES IN A MEAT PRODUCING FLOCK

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BACKGROUND-AIM
Nutrient and oxygen supply to the fetus depends on proper uterine and placental blood flow which can be assessed by Doppler ultrasonography. In the ewe, Doppler ultrasonography during pregnancy has been used in singleton pregnancies as sheep models of human disease. However, most of our meat and wool producing sheep herds are selected to produce twin or triplets, and many singletons born from mature ewes are larger than the average newborn size for the herd. This trial was designed to obtain and compare the hemodynamic values of singleton vs. twin pregnancies in a meat producing flock.
METHODS
Mature Suffolk ewes from the Clemson Research Flock were examined by transabdominal ultrasound at 50 d post breeding to confirm pregnancy and estimate fetal number. Ewes carrying singleton (n = 9) and twin (n = 9) pregnancies were selected. Doppler ultrasonography was performed at 60, 90, and 120 (± 3) days post-breeding. Umbilical artery, fetal aorta, and maternal femoral artery blood-flow velocities and vessel diameter were measured. Blood flow velocities were used to calculate resistance index (RI). Vessel diameter and RI were analyzed as repeated measures over time, with an autoregressive covariance structure (Proc Mixed, SAS).
RESULTS
For umbilical artery RI, fetal aorta RI, and fetal aorta diameter, there was no significant ($p>0.05$) effect of fetal number; there was significant ($p<0.0001$) effect of time; and there was no interaction. Umbilical artery RI: 0.84 ± 0.01a; 0.64 ± 0.01b; 0.55 ± 0.01c, LSM ± SE for days 60, 90, and 120 respectively; fetal aorta RI: 0.79 ± 0.02a; 0.66 ± 0.02b; 0.61 ± 0.02c for days 60, 90, and 120 respectively; fetal aorta diameter: 0.22 ± 0.02a; 0.35 ± 0.02b; 0.62 ± 0.02c cm for days 60, 90, and 120 respectively. For maternal femoral artery RI, there was no significant ($p>0.05$) effect of fetal number, time, or interaction. Maternal femoral artery diameter had a significant ($p<0.005$) effect of time; and there was no interaction: 0.55 ± 0.01a; 0.51 ± 0.01b; 0.50 ± 0.01b cm, LSM ± SE for days 60, 90, and 120 respectively.
CONCLUSIONS
These preliminary results indicate that there are no major differences in the measured hemodynamic values between singleton and twin pregnancies in mature Suffolk ewes.

TOPIC Reproduction exotic anim and wild species

T77
POST-MORTEM RECOVERY OF EPIDIDYMAL SPERMATOZOA FROM HUEMUL (HIPPOCAMELUS BISULCUS, MOLINA 1782)

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BACKGROUND-AIM
The Huemul (Hippocamelus bisulcus, Molina 1782)) is an endangered species (Red List-UICN) of deer native to the mountains of Argentina (only 350-500 animals) and Chile. The huemul populations of Argentina are small and fragmented, and face continued decline. Due to the very low possibility of accessing individuals for the reproductive study in this species, it has been decided to communicate the study of the first observed case. The aim of this work is to describe some parameters of the huemul sperm cells obtained after death.
METHODS
A huemul male was found dead in the area of La Plata lake (Chubut, Argentina), during non-breeding season (October). The specimen was about 4 years old, with 73 kg of body weight. The scrotal contents were wrapped with paper, and put into a cell foam box. The storage temperature of the reproductive material ranged between 10 to 20°C, varying according to field and shipment conditions. Spermatozoa were recovered from both epididymes (caudal section), by retrograde flushing with needle inside the vas deferens. Time elapsed between the death and the samples recovery was about 72 h. Scrotal content weight, testicle measurements, volume recovered (spermatozoa + diluent), sperm cells concentration, total motility, plasma membrane functionality (HOST), live sperm cells and morphometry were assessed.
RESULTS
Testis + epididymis weights were 23 g for each one (right and left). The length of the testicles was 5.5 cm and the width was 3.0 cm (both sides). Volumes recovered were 150 µL and 200 µL (right and left epididymis, respectively), with a sperm cells concentration of 40 x 106 /mL, containing a total of 14 x 106 spermatozoa. Total motility of 25 ± 5 %, HOST of 39 ± 1% and live sperm cells of 36 ± 2 % were observed. Area of the sperm head of 34.25 ± 3.19 µm2, length and width of sperm head of 7.62 ± 0.56 µm and 5.26 ± 0.36 µm, respectively, length of middle piece of 10.60 ± 0.8µm and total length of sperm tail of 34.93 ± 1.40 µm were measured.
CONCLUSIONS
It can be concluded that it is possible to obtain live sperm, with acceptable functionality and morphometry 3 days after the death of the animal. This is encouraging when it comes to preserving germplasm in this endangered species.

T78
ISOLATION AND IN VITRO CULTURE OF FIBROBLAST CELLS FROM RED-RUMPED AGOUTI (DASYPROCTA LEPORINA LINNAEUS, 1758) AS A STEP TOWARDS FOR IN VITRO REPRODUCTION BY SOMATIC CELL NUCLEAR TRANSFER

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BACKGROUND-AIM
The isolation and in vitro culture of somatic cells is the initial step for their application in reproductive biotechniques such as somatic cell nuclear transfer. Thus, this knowledge has great relevance in wild species with ecological and scientific importance such as Dasyprcota leporina. The aim of this work was to isolate agouti fibroblasts and to characterize them in the second passage regarding morphology, viability, metabolic activity, population doubling time (PDT), levels of reactive oxygen species (ROS) and mitochondrial membrane potential (ΔΨm).
METHODS
Then, ear skin samples were obtained from six agoutis from to the Centre of Multiplication of Wild Animals at Federal Rural University of Semi-Arid. Samples were transported in minimal essential medium modified by Dulbecco (DMEM) supplemented with 10% fetal bovine serum and 2% penicillin and streptomycin solution at 37 °C. In the laboratory, fragments (9.0 mm³) were cultured under controlled atmosphere (38.5 °C, 5% CO2) and evaluated every 24 h. The cells were harvested when they reached 70% confluency and subcultured until the second passage for the analysis. Data were expressed as mean ± standard error, considering one animal/one repetition.
RESULTS
Thus, 48 fragments were cultured (eight fragments per animal), 98% presented adherence with an average of 3.7 ± 0.6 days, and the cell growth around the explants with 11.7 ± 0.5 days. Cells reached 70% confluence forming monolayer on day 15.0 ± 0.4. In general, from the morphological analysis, all cells had fusiform morphology with a centralized oval nucleus, showing to be like fibroblasts. For the trypan blue test, a viability of 92.3 ± 4.1% was obtained, with a percentage of metabolic activity of 100.0 ± 10.1%. Furthermore, the cells show a PDT of 16.5 ± 1.3 h with ROS levels and ΔΨm of 1.0 ± 0.1 and 1.0 ± 0.2 arbitrary fluorescence units, respectively.
CONCLUSIONS
In conclusion, it was possible to isolate somatic cells from agouti ear skin with high viability, metabolic activity, and proliferative activity, comprising initial and fundamental steps for the application of reproductive biotechnologies in the species.

T79
EVALUATION OF FSH CONCENTRATION ON IN VITRO MATURATION OF RED-RUMPED AGOUTI (DASYPROCTA LEPORINA LINNAEUS, 1758) OOCYTES

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BACKGROUND-AIM
The increase of oocyte in vitro maturation (IVM) rates is essential for the success of in vitro embryo production, especially in red-rumped agoutis. In this species, IVM rates are still considered low. In different mammals, the addition of follicle stimulating hormone (FSH) in IVM is beneficial in a dose-dependent manner. Therefore, the aim was to evaluate the FSH effect in two concentrations (10 e 50 mIU/mL) in the agouti IVM.
METHODS
Then, six agoutis were used for ovarian recovery. The cumulus-oocyte complexes (COCs) were recovered by slicing and classified under stereomicroscope. The viable COCs were transferred to the IVM medium composed by TCM199 with 0.23 mM sodium pyruvate, 10% FBS, 100 µM cysteamine and two different concentrations of FSH, 10 mIU/mL (FSH10) or 50 mIU/mL (FSH50). After 24 h of IVM, the structures were evaluated regarding the expansion and viability of cumulus cells, as well as the presence of first polar body (1PB). Subsequently, the structures were activated in the presence of strontium chloride and cytochalasin B for 6 h and then destined for embryonic culture in synthetic oviduct fluid (SOF) medium. Assessments of embryonic development were performed after 48, 96 and 168 h. Finally, data were expressed as mean ± standard error and means were compared using Fisher exact test with $P < 0.05$.
RESULTS
Thus, from the 12 collected ovaries was possible to recover 108 viable immature oocytes, distributed in three repetitions. No difference ($P > 0.05$) was observed between FSH10 and FSH50 in terms of qualitative parameters for cumulus cells expansion [95.2 ± 3.4% (40/42); 96.3 ± 1.4% (41/43)] and viability [81.9 ± 1.8% (286/356); 77.8 ± 0.6% (267/343)]. Moreover, no difference was observed for 1PB presence [51.4 ± 4.5% (19/37); 52.7 ± 4.1% (21/42)]. Regarding to embryonic development, oocytes matured in 10 mIU/mL [31.6% ± 17.5 (12/38)] or 50 mIU/mL [14.6% ± 8.9 (6/41)] FSH presented similar cleavage rates. Nevertheless, only in FSH10 was observed morula [25.0 ± 14.2% (3/12 morula/cleaved structures)].
CONCLUSIONS
In conclusion, oocytes matured with 10 mIU/mL FSH resulted in development of morula after artificial activation when compared to 50 mIU/mL FSH. This concentration may be suggested for further studies regarding the in vitro embryo production in D. leporina.

T80
CHARACTERISATION OF A STILLBORN SOUTHERN WHITE RHINOCEROS OVARY

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BACKGROUND-AIM
The northern white rhinoceros is functionally extinct with only two females left in the world. Advanced reproductive techniques are the only option for saving the species. The ovary contains a reserve of immature oocytes in primordial follicles. Establishing methods to grow and culture these oocytes in the lab would increase the chance of successful in vitro embryo production for endangered rhinoceros species. In order to establish culture methods, the basic physiology of the ovary needs to be revealed. In addition to investigating adult ovaries, stillborn or neonatal samples can provide insight into the development of a rhinoceros ovary. Therefore, this study investigated the structural and molecular characteristics of a stillborn rhinoceros ovary.
METHODS
The ovaries of a stillborn (died during birth) southern white rhinoceros calf were collected and one ovary was cut and fixed in 10% formalin. Haematoxylin-and-eosin stain (H&E), Masson Trichrome stain and hyaluronic acid staining were applied to investigate the viability and the general structure of the tissue. Detection of collagen I, Ki-67 (proliferation marker), SOX2 (pluripotency marker), DDX4 (also known as Vasa; germ cell marker) and anti-Müllerian hormone (AMH) was performed using immunohistochemistry with DAB peroxidase visualisation.
RESULTS
The stillborn ovary showed a unique and distinct structure. H&E and Masson Trichrome revealed that most of the ovary consisted of large cells organised in clusters separated by stroma. Follicles at all stages of development were present; from primordial to antral. Immunohistochemistry characterised the large undefined cells as mitotically active pluripotent cells with germ cell properties (DDX4 positive).
CONCLUSIONS
These results may indicate the ovary still contains ovigerous cords at the moment of birth, which would be a remarkable finding since in most mammals breakdown of the ovigerous cords happens in the second half of fetogenesis. However, more characterisation is required to confirm this hypothesis. Future studies will investigate steroidogenesis, other proliferation factors and apoptosis. This achievement is a precious step forward in revealing the development and physiology of the rhinoceros ovary.
This study is funded by Fondation Hoffmann to S. A. Williams.

T81
EVALUATION OF ENDOSCOPIC SALPINGECTOMY AND DEFERENTECTOMY AS A NEUTERING PROTOCOL IN COYPU (MYOCASTOR COYPUS)

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BACKGROUND-AIM
Neutering campaigns are key management options to avoid the impact of biological invasions of alien species. In Italy coypu population is widespread and well established and local permanent control campaigns in response to social pressures are common management policies, when public acceptance of eradication represents a possible side effect. The present study took place within the context of the control campaign held by the city of Turin started in 2018. The objective of the present study was to assess the feasibility and safety of laparoscopic salpingectomy and deferentectomy in coypu (Myocastor coypus) utilizing 3-mm laparoscopic instruments.
METHODS
Female (n=25) and male (n=15) coypus were captured, sedated and placed under general anesthesia. Pregnant female coypus (n=16) were excluded from the study and laparotomic ovariohysterectomy was performed. A 3-port technique was used with a 5-mm cannula for the endoscope and two 5-mm cannulas accommodating 3-mm endoscopic instruments including a plasma scalpel (OTECH Industry, airplasma® technology) and endoscopic grasping forceps. The abdomen was insufflated with CO2 to a pressure of 6-8 mmHg. The coypus were positioned in dorsal recumbency to endoscopically visualize, cauterize and excise the fallopian tubes in females and vas deferens in males. A cut was performed on left ear or right ear for female and male coypus respectively, in order to recognize treated animals and to monitor social behaviors at day 1, day 7 and day 14 after surgery.
RESULTS
The procedure was successfully performed in 22 animals (females n=7; males n=14). The average surgery times was 23±10 minutes for female coypus and 21±9 minutes for males; anesthesia times for females and males were 35±10 and 34±10 minutes, respectively. The coypus recovered smoothly from anesthesia and were set free four hours after appetite reappearance. Follow-up resulted in a total number of 28 observations of living neutered coypus.
CONCLUSIONS
Laparoscopic salpingectomy and deferentectomy with 5-mm laparoscopic instrumentation using a 3-port technique was feasible in coypus. Laparoscopic salpingectomy and deferentectomy can be considered as elective surgical techniques to prevent reproduction in coypus for its survival rate and the reduced postsurgical recovery time compared to traditional techniques.

T82
FIRST STEPS TO GENERATE ROE DEER INDUCED PLURIPOTENT STEM CELLS (IPSC) BY EPISOMAL VECTORS

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BACKGROUND-AIM
Induced pluripotent stem cells (iPSC) have been derived from humans and animals. From therapeutic to conservation strategies, this technology has been labeled as a breakthrough in the field of stem cell research. Embryonic diapause, a developmental arrest phase, is known to occur in several species. To date, its mechanisms are still not completely understood. The European roe deer (Capreolus capreolus) displays embryonic diapause over a period of 4 months. Embryo production for research purposes in this species is limited. Therefore, iPSC present an interesting source for studying cell potency and embryonic arrest in the roe deer. Here, we describe the initial attempts for generating roe deer iPSC.
METHODS
Primary fibroblasts were isolated from implanted embryos. Fibroblasts were transfected with episomal vectors carrying the human reprograming factors Oct3/4, SOX2, KLF4, L-MYC and LIN28 (Addgene, Okita et all 2011, Nat Methods, 8(5):409-12) using the Amaxa Basic Nucleofector kit. Cells were cultured for 7-9 days in DMEM/Glutamax, 20% FBS, non-essential amino acids, and 5 ng/ml bFGF (M20). The medium was after changed to ½ M20 and ½ reprograming medium (RPM, StemMACS iPS-Brew XF) and cells were seeded on Geltrex coated dishes. A day later, the medium was changed to either i) 100% RPM, ii) 100% RPM, 2.5 µM IWR-1, 0.5 µM CHIR99021, 7 µM Forskolin and 10 µM rhLIF (named IWR1), or iii) 100% RPM, 3 µM CHIR99021 and 10 µM SB431542 (named SB). After 1-2 days, cells from each treatment were moved to hypoxic conditions for colony appearance.
RESULTS
Seven days after transfection, areas of proliferation in island-like morphology were observed. After 17 days, the first intermediate colonies were picked. The 100% RPM and the SB media resulted in the development of epithelial-like morphology. The IWR1 was able to produce iPSC-like colonies after 8 passages.
CONCLUSIONS
So far, our results are promising that roe deer fetal fibroblasts can be reprogramed by human factors using episomal vectors in a serum- and feeder-free culture system. Further cell characterization will disclose their stem cell capacities. The small molecules cocktail supplementation efficacy might suggest a possible dependence on the Wnt/β-catenin signaling pathway for iPSC generation in the roe deer.

T83
BUSERELIN-ACETATE AS AN OVULATION INDUCTOR IN AFRICAN LIONS (PANTHERA LEO).

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BACKGROUND-AIM
Ovulation induction (OI) is required for the application of assisted reproduction techniques in felids. Most OI protocols include the use of repeated doses of exogenous gonadotropins, which often trigger side effects such as immunogenic responses, hyperestrogenism, superovulation, or luteal insufficiency. Minimally-invasive hormonal protocols that induce safe ovarian responses in non-domestic felids are thus still needed.
METHODS
This study examined the effect of a single dose of buserelin-acetate (BA; 20 µg, im, Receptal®, Intervet, South Africa) to induce ovulation on days 4 (n=3), 5 (n=5), and 6 (n=2) of estrus in African lions. For 12 months, daily monitoring of five captive lionesses enabled detection of females in natural estrus (high frequency of specific behavioral signs such as purring, flirting run, lordosis, allowing mount, and rolling; and high proportion of superficial cells, absence of neutrophils, large number of bacteria, and clean background in the vaginal smears). In parallel, blood sampling (n=188; 37.6 ± 4.07 samples per female; range: 23-47) took place 1-7 times per week, during positive reinforcement training. A competitive enzyme immunoassay utilizing antibodies against 5ß-pregnane-3ß-ol-20-one-3HS:BSA was used for serum progestagen (sP) quantification. Transrectal ultrasounds of the reproductive tract were performed on day 6 of estrus.
RESULTS
Induced ovulation occurred in all cases, on average 60.1 h (n=10, range: 24-96 h) after BA administration. Ovulation was confirmed by absence of estrous signs (although some lionesses showed estrous signs up to 72 h after ovulation), a predominant proportion of parabasal and intermediate epithelial cells associated, or not, with neutrophils and a dirty background in the vaginal smears, a rise in sP concentrations, and presence of ovarian corpora lutea (0.8 ± 0.84 CL; n=8 complete examinations; range: 0-2 CL). The CL observed about 48 h after BA administration, had a median diameter of 8.8 mm; the CL observed about 30 h after BA administration had a median of 12.5 mm. Induced pseudopregnancy was about 59 days in length (n=10; range: 56-65 days).
CONCLUSIONS
BA proved to be a valuable tool to induce ovulation in African lions. Its use may help to improve assisted reproduction techniques for this and other threatened large felids.

T84
"UNITED WE STAND": COMBINING REPRODUCTIVE TRACT ULTRASONOGRAPHY AND VAGINAL CYTOLOGY TO DETERMINE OVARIAN CYCLE STAGE IN WILD FELIDS.

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BACKGROUND-AIM
Despite current conservation efforts, most non-domestic felids are threatened with extinction. Reproduction is key to species survival. However, many wild and captive felids reproduce poorly. Thus, a deeper understanding of their reproductive physiology is needed to implementing assisted reproduction (ART) into these species conservation. This study aimed to compare vaginal cytology and female reproductive tract ultrasonography (US) in six species of captive non-domestic felids (Panthera leo, n=20; P. pardus kotiya, n=1; P. pardus tulliana, n=1; P. uncia, n=1; Catopuma temminckii, n=6; Neofelis nebulosa, n=1; and Acinonyx jubatus, n=1) for assessing the reproductive cycle.
METHODS
Transrectal US and vaginal smears were performed during routine anesthetic procedures over a period of three years. The ovarian cycle stage was determined separately by assessing the presence and size of ovarian follicles and corpora lutea (CL), and the percentage of cornified vaginal epithelial cells. Where possible, samples from the same animal were then compared and the ovarian cycle stage corrected when necessary.
RESULTS
Assessed US and cytologies revealed many commonalities between species. Overall, pro-estrus was characterized by small ovarian follicles and 60-90% cornification, while big follicles and >90% cornification were observed in estrus. Females in diestrus presented CL and 10-60% cornification. Quiescence ovaries and <10% cornification were detected in anestrus. Transrectal US proved to be a finer tool, enabling determination of the cycle stage in 71% of the animals assessed, compared to the 67.7% predicted with vaginal cytology alone. The combination of both techniques allowed a more accurate cycle stage prediction (87.1%). This was supported by a positive correlation between the size of the biggest follicle and percentage of epithelial cornification in P. leo (n=8; r=.76; p=0.030), and Catopuma temminckii (n=5; r=.89; p=0.042).
CONCLUSIONS
Besides being practical tools to routinely assess the reproductive cycle, these two techniques in combination may also be used for further research into the normal reproductive cycle of wild felids, which may be used to improve ART success rates, and in turn the conservation breeding programs of these endangered species.

T85
ESTABLISHING THE PERCOLL GRADIENT TECHNIQUE FOR SPERM SELECTION FROM COLLARED PECCARIES (PECARI TAJACU LINNAEUS, 1758)

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BACKGROUND-AIM
The collared peccary is a wild ungulate whose population is disappearing in some South American biomes. The development of assisted reproductive techniques as the in vitro fertilization (IVF) could contribute to peccary conservation; however, techniques should be stablished for the species peculiarities. The Percoll gradient has been used to select sperm from different mammals and shows variations in its concentrations that can influence the sperm quality. Thus, we evaluated the effect of different Percoll gradient concentrations on the quality and reactive oxygen species (ROS) levels of peccary sperm.
METHODS
The semen was collected from six males by electroejaculation and divided into three groups: fresh control, 45/90% and 35/70% Percoll gradients (centrifuged at 900g/15 min). The pellet obtained after selection was centrifuged to remove the Percoll. Sperm were evaluated for kinetic motility patterns, membrane integrity associated with mitochondrial activity and ROS levels. Data were expressed as mean ± SE and analyzed by ANOVA followed by Tukey test (P<0.05).
RESULTS
The total and progressive motilities were similar between the control (95.0 ± 1.8 and 70.8 ± 6.0%) and 45/90% (87.5 ± 3.6 and 75.3 ± 5.2%); however, the motilities were significantly lower in the 35/70% group (8.2 ± 2.1 and 3.3 ± 1.4%). The 35/70% gradient also negatively influenced other kinetic parameters. Moreover, the 45/90% gradient presented the highest values for velocity average pathway (96.8 ± 9.4 vs. 40.7 ± 6.1 vs. 69.7 ± 7.5 µm/sec), velocity straight line (88.4 ± 8.9 vs. 27.3 ± 7.0 vs. 56.0 ± 6.6 µm/sec) and linearity (66.0 ± 4.7 vs. 35.7 ± 6.7 vs. 46.0 ± 3.1%) compared to 35/70% and control, respectively. The membrane integrity associated with mitochondrial activity was similar between 45/90% gradient and control (71.5 ± 5.6 vs. 80.0 ± 3.1%), however, it was lower in the 35/70% (13.2 ± 2.7%). The ROS levels did not differ among treatments (control: 1.00 ± 0.04; 45/90%: 1.12 ± 0.05; 35/70%: 1.05 ± 0.06 fluorescence units).
CONCLUSIONS
In conclusion, the 45/90% Percoll gradient is efficient to select collared peccary sperm with better kinetic motility patterns, without causing membrane damage and oxidative stress. These results are fundamental for the development of embryo production by IVF in peccaries.

T86
INFLUENCE OF SEMINAL PLASMA ADDITION TO CRYOPRESERVED DONKEY SEMEN

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BACKGROUND-AIM
Artificial insemination using frozen donkey semen in jennies is still a challenge. This study was designed to determine the influence of seminal plasma (SP) addition to cryopreserved donkey semen using two extenders.
METHODS
Five ejaculates (n=15) from three Pega donkey jacks with proven fertility were collected and cryopreserved with extender I (BotuCrio, Botupharma, Brazil) and extender II (modified INRA-96, IMV Technologies, France). After thawing, samples were added or not to SP. Seminal samples were analyzed for kinetic motility parameters by computer assisted sperm analysis (CASA) and for plasma membrane and acrosome integrity (PMAI), lipid peroxidation (PER) and intracellular production of H2O2 (hydrogen peroxide; ROS) by flow cytometry. PER was assed using C11-BODYPY probe (D-3861; Molecular Probes, CA, USA) and the probe 5-(-6)-carboxy-2,7-dichlorodihydrofluoresceinyl acetate (carboxy-H2DCFDA; Molecular Probes, CA, USA) was used for ROS. The effect of the semen extender (I or II) was analyzed by Proc General Linear Models. The comparison of the mean was evaluated using the Tukey test.
RESULTS
The post-thaw progressive and total motility parameters (CASA) were similar for the two freezing extenders (P>0.05). However, the extender I provided a higher PMAI (20.7±7.4 vs. 14.0±4.8), decreased PER (20.6±15.8 vs. 88.8±15.0%) and ROS (12.3±10.7 vs. 63.4±40.4%) compared to extender II (P<0.05). Despite the addition of SP has shown a deleterious effect on motility parameters in both groups [Extender I: SP or Control (58.3±12.8% vs. 78.0±9.5); Extender II: SP or Control (44.1±20.2% vs. 68.5±18.45, P<0.05)], no differences were observed for PMAI (P>0.05). The increase in ROS for extender I (SP 81.9±32.5 vs. Control 12.3±10.7%) and for extender II (SP 95.2±7.5 vs. Control 63.4±40.4%) and a decrease in PER [Extender I: SP or Control (2.1±1.3 vs. 20.6±15.8%); Extender II: SP or Control (19.2±16.7 vs. 88.8±15.0%)] was also observed after addition of SP (P<0.05).
CONCLUSIONS
SP addition decreased sperm motility; however, it did not affect the sperm viability in both extenders. Moreover, the presence of SP increased ROS and decreased PER in post-thawed donkey semen.

T87
BRUCELLA CANIS DETECTION IN NON-DOMESTIC CANIDS IN CHILE: A POTENTIAL RISK TO REPRODUCTION AND CONSERVATION?

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BACKGROUND-AIM
Brucellosis caused by Brucella canis have been found worldwide in many environments, however, although it is normally found in domestic dogs, the role of non-domestic canids in the epizootiology of brucellosis is still unknown. In wild as in domestic canids, B. canis can produce reproductive failures, risking even more the survival of endangered species. Therefore, this study aimed to determine the infection with B. canis in non-domestic canids in Chile.
METHODS
Non-domestic canids, including Lycalopex culpeaus (N=25), L. griseus (N=8), Canis lupus (N=4), and Vulpes vulpes (N=4), from five rehabilitation centres and five zoos in the central area of Chile were evaluated by using serological and bacteriological methods. All animals were sampled by venipuncture over 8-month period. All blood samples were analysed for bacteraemia with trypticase soy broth enriched with sodium citrate (2% w/v) at 37 °C for 30 days. Every week, 100 µL were plated onto Brucella agar plates supplemented with 100 mg/L cycloheximide, 25,000 IU bacitracin, and 6,000 IU polymyxin B, for at least 72 h at 37°C. All suspicious colonies were identified by PCR with specific primers for B. canis using strain B. canis SCL as control strain (access number: NZ_LGAQ00000000.1). Serological evaluation was assessed by counterimmunoelectrophoresis (CIEF) with LPS-R of B. ovis as an antigen.
RESULTS
From all animals analysed, five (12.2%) were infected, being all of them detected by CIEF. In none of the samples, the pathogen could be isolated.
CONCLUSIONS
B. canis is present in Chilean populations of non-domestic canids, which could compromise the reproductive performance, which is fundamental to species survival. In addition to the potential zoonotic risk.
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T89
CHANGES IN THE PROSTAGLANDIN PROFILE IN THE UTERINE FLUID OF THE EUROPEAN ROE DEER DURING THE PREIMPLANTATION PERIOD ACQUIRED BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY (UPLC-MS)

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BACKGROUND-AIM
The European roe deer (Capreolus capreolus) embryo undergoes a period of decelerated growth at the blastocyst stage, delaying implantation by several months. This reproductive phenomenon is referred to as embryonic diapause. While molecular signals controlling diapause have been identified in other diapausing species, the molecular events guiding reactivation of embryonic growth have not been characterized in the European roe deer. Prostaglandins (PG) of maternal and embryonic origin are essential during the preimplantation period, as they are involved in maternal recognition of pregnancy and implantation. We have recently determined the PG secretion of the preimplantation bovine embryo in culture by ultra-performance liquid chromatography coupled with high resolution mass spectrometry (UPLC-MS) revealing that the hatched bovine blastocyst predominantly secretes PGE2 and Δ12-PGD2, whereas the elongated embryo produces mainly PGI2 (assessed by the determination of its stable metabolite 6keto-PGF1a) and PGF2a.
METHODS
We have revalidated the UPLC-MS method for the uterine fluid matrix and have subsequently quantified PGs in 75 uterine fluid samples covering the period from early diapause to embryo elongation.
RESULTS
Extraction recovery was ≥ 65%, and the method accuracy [bias %] and precision [CV%] were both ≤ 15% for most of the 31 PG under analysis. Similar to bovine, the abundance of all PG in the uterine fluid was 70-fold higher at elongation than at the diapausing hatched blastocyst stage. Interestingly, in the uterine fluid of diapausing embryos, 6keto-PGF1a was most abundant. During elongation, 13,14-dihydro-15keto-PGE2 displayed highest abundance, followed by 15keto-PGE2, and 13,14-dihydro-15keto-PGF2a.
CONCLUSIONS
In conclusion, we show an increase in PGE and PGF abundance at the time of embryo elongation in the European roe deer, most likely for preparation of implantation. The embryo and the maternal endometrium are potential sources. Future dissection of the relative embryonic and maternal contribution will shed further light on the intricate embryo-maternal communication around the period of embryo reactivation.

T90

LUTEAL CELL CULTURE OF ROE DEER AS A TOOL TO STUDY CORPUS LUTEUM FUNCTION DURING EMBRYONIC DIAPAUSE

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BACKGROUND-AIM

Corpus luteum (CL) is a transient endocrine gland on the mammalian ovary whose main function is the production of hormones ensuring pregnancy maintenance. The CL has been extensively studied in several species, but its maintenance and activity remain poorly understood during embryonic diapause. Embryonic diapause is defined as a temporary delay or arrest of development of the embryo. In Roe deer (Capreolus capreolus), diapause lasts for a period of five months, during which time the CL remains active and produces progesterone (P4). The concentration of P4 in CL tissue remains constant throughout the pregnancy. To better understand the function of the CL during diapause and identify its regulators, luteal cell cultures from Roe deer should be established and used for functional studies.

METHODS

Luteal cells were isolated from CL of Roe deer during diapause (n = 4). The CL tissue was digested with collagenase (types I and II, f.c. 0.1 %) and DNAse (f.c. 0.005 %). The isolated cells were plated on a 96 well plate and were treated with luteinizing hormone (0 and 100 ng/mL) over a two day period. The concentration of progesterone (P4) and estrogens were measured in the collected medium by ELISA. The steroidogenic activity of cells over the culture period was assessed by staining for activity of 3β-hydroxysteroid dehydrogenase (HSD3B).

RESULTS

The isolated cells had an average diameter of 17.8 ± 5 µm (n = 246). In the cell culture system, the steroidogenic cells produced P4 and estrogens. Treatment with 100 ng/mL LH caused a significant increase in P4 production during the first day of culture ($P<0.05$). Concentration of estrogens was not influenced by LH and its basal concentration was lower than the concentration of P4. Staining for activity of HSD3B indicates the presence of two types of luteal cells which had different steroidogenic activity over the culture period.

CONCLUSIONS

The described isolation and culture system for luteal cells of Roe deer provide a base for future functional studies. Here, we provide supporting evidence for a LH-influence in the in vitro system.

T91

NEGATIVE EFFECTS OF PROLONGED OVARIAN TRANSPORT TIMES ARE REVERSED USING THE ANTI-APOPTOTIC DRUG Z-DEVD-FMK IN IN VITRO MATURATION OF IBERIAN RED DEER OOCYTES

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BACKGROUND-AIM

In vitro embryo production (IVP) of Iberian red deer has some limitations compared to domestic species since their slaughtering usually takes place far from laboratories, resulting in long transport times. This may negatively influence oocyte quality and competence acquisition. In this context, we aimed to evaluate if the anti-apoptotic drug z-DEVD-fmk during in vitro maturation (IVM) could revert the negative effects on the oocyte due to the ischemic conditions maintained in the ovary.

METHODS

140 adult Iberian red deer ovaries were recovered and transported in saline solution for 13h at 25 °C. 350 Cumulus–oocyte complexes (COCs) were collected, of which 175 were matured in IVM control conditions and 175 matured in the presence of 200 nM z-DEVD-fmk for 22 h at 38.5 °C. Maturation rate, viability, mortality, and early apoptosis, as well as caspase-3 activity and DNA fragmentation were assessed. Moreover, we evaluated the relative abundance of apoptotic-related BAX, BCL2, CASP3, SHC1, TP53, and ITM2B transcripts by qPCR. The remaining COCs were fertilized and cultured in vitro to examine cleavage and blastocyst rates. Embryo quality was assessed by analyzing DNA fragmentation using TUNEL and determining total cell number. A General Lineal Model (GLM) and Bonferroni test were used to study the influence of z-DEVD-fmk on oocyte quality and embryo production.

RESULTS

Oocytes matured under z-DEVD-fmk conditions showed a significantly ($P<0.05$) higher maturation and viability rates (88.18% ± 1.99 vs. 74.01% ± 1.99, 44.44% ± 2.32 vs. 20% ± 1.8, respectively), and significantly lower ($P<0.05$) percentages of early apoptosis, DNA fragmentation and caspase-3 activity compared to matured control oocytes (41.48% ± 3.6 vs. 60% ± 2.79, 57.83% ± 1.91 vs. 74.62% ± 1.91 and 41.88% ± 3.42 vs. 67.1% ± 3.42, respectively). Moreover, though there were no significant differences in embryo quality and production rates ($P>0.05$), z-DEVD-fmk matured oocytes showed decreased ($P<0.05$) abundance of pro-apoptotic TP53 and ITM2B transcripts.

CONCLUSIONS

In conclusion, for wild species, like Iberian red deer, the negative effects of prolonged ovarian transport times on oocyte quality may be reversed using the z-DEVD-fmk inhibitor during IVM.

This work was supported by Spanish Ministry of Economy and Competitiveness (AGL2017-89017-R).

T93
ACCURACY OF THE USE OF VAGINAL HISTOLOGY AND CYTOLOGY TO PREDICT OVULATION IN GUINEA PIG (CAVIA PORCELLUS)

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BACKGROUND-AIM
Vaginal cytology and histology are commonly used in rodents to classify the stages of the estrous cycle. Initial studies reported a coincidence between ovulation and leukocyte infiltration in the vaginal smear, thus defining infiltration as an indicator of ovulation in guinea pigs. The objective of this study was to study the accuracy of the use of vaginal histology and cytology to predict ovulation in guinea pig which was objectively determined by the presence of a hemorrhagic body and oocyte with expanded cumulus cells in the preovulatory follicle.
METHODS
Female guinea pigs (n = 36) from 5 to 6 months of age were estrous synchronized with 0.22mg / Kg oral Altrenogest solution for 15 days. Synchronized females were randomly distributed into 6 groups of different times after the last dose of Altrenogest: 72h (G1), 84h (G2), 96h (G3), 108h (G4), 120h (G5) and 132h (G6) to evaluate opening, cytology, and vaginal histology. Total images for cytology and histological techniques were processed and observed at 100X. Epithelial cells were classified by cytology into basal-parabasal, intermediate, superficial and cornified(C). The presence of leukocytes was evaluated in relation to epithelial cells. The histology of the vaginal tissue evaluated and classified into stratum mucification, stratum corneum - stratum mucification, stratum corneum, stratum granulosum (SG), stratum germinativum and infiltration of leukocytes (IL). The variables were analyzed by ROC curve and binary logistic regression to determine the precision of the variable to predict ovulation. The variables were analyzed by ROC curve and binary logistic regression to determine the precision of the variable that would predict ovulation.
RESULTS
The results indicate that ovulation occurred after 108h (16.6%), 120h (83.3%) and 132h (83.3%) of the last dose of Altrenogest. Additionally, the variables C, SG and IL have an accuracy of 0.88, 0.88 and 0.83, achieved a ROC-AUC respectively to predict ovulation with p>0.05. Likewise, the mathematical model that allows predicting ovulation with 91.7% accuracy includes C, SG and IL.
CONCLUSIONS
Therefore, these variables determined by the cytology and histology studies can help to predict the time of ovulation in guinea pigs. FONDECYT (Peru) Research Grant N° 149-2019.

T94
FIRST LOW-TEMPERATURE CULTURE OF EARLY NAKED MOLE-RAT EMBRYOS

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BACKGROUND-AIM
Naked mole-rat (NMR) is subterranean eusocial rodent with origin in eastern Africa. Its peculiar features, such as unusual long-lifespan, cancer-resistance and tolerance to hypoxia, predetermine this species as an attractive model for cancer and aging research. A useful tool could be the establishment of NMR pluripotent stem cell line, but the reprogramming of induced pluripotent stem cells is problematic due to their stable epigenome. Another effective approach could be the establishment of embryonic stem cell line starting from NMR embryos. Within the colony, the reproduction is monopolized by a single dominant female (queen) and by 1-3 large males (pashas). The remaining members of colony (workers) show no sexual behavior. An easy way to obtain viable embryos is to sacrifice a pregnant queen and flushing the oviducts but her death leads to the colony collapse. To avoid this, the aim of this study was the in vitro production (IVP) of NMR embryos through piezo-ICSI oocytes collected from workers. As to date there is no knowledge regarding in vitro maturation (IVM) of NMR oocytes, preliminary experiments were conducted to assess the kinetic of meiotic resumption by the successive chromatin fluorescent staining. We concluded that after 24h at 32˚C, 5% CO2 and 5% O2 in humified atmosphere there is the highest proportion of matured oocytes.
METHODS
For IVP, oocytes with at least two compact cumulus cells layers underwent IVM. At the end of IVM, matured oocytes displaying a polar body were selected for piezo-ICSI and fertilized with fresh semen. Presumptive zygotes were cultured individually in KSOM or modified SOF medium at 32˚C, in 5% CO2 and 5% O2 for 7 days.
RESULTS
Appearance of pronuclei was detected 24h after injection by staining. First cleavage occurred after 48h of embryo culture. The maximum number of cells (9) was reached after 144h of culture. No signs of compaction were observed. The average cleavage rate (yield of cleaved embryos on day 2 over injected oocytes) in the group cultured was 43% (50/115) in KSOM and 45% (15/33) in mSOF.
CONCLUSIONS
Our results are the initial steps for creating the protocol for in vitro production of NMR embryos. Further efforts will be directed to the improvement of culture conditions in order to overpass the 4-8 cells development block.

T95
LAB-BASED AND ON-FIELD ANALYSIS OF PROGESTERONE METABOLITES IN FAECES SAMPLES: AGREEMENT OF DATA IN DIFFERENT SPECIES

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BACKGROUND-AIM
In the topic of reproductive cycle assessment and pregnancy diagnostics, there is an emerging need to develop immunoassays which are suitable for measuring fecal progesterone metabolite level. Faeces samples can be collected very easily and are alternative for blood sampling in zoo and wild animals. However, the extraction of hormonal content is a labor-intensive process, which requires relatively long time. This step can be shortened and centrifugation can be avoided, however, the agreement of the results from different extraction protocols is a key for the proper analysis.
The aim of this study was to analyse the agreement of data from different extraction protocols (a laboratory based and an on-fiel alternative) using faeces samples of different species.
METHODS
Fecal samples were collected from different species: cattle (n=2), elephant (n=2), giant anteater (n=3), and red panda (n=2). Ethanol-based extraction method were used in all the samples. Before the extraction, each sample was divided into two treatments: centrifugation and manual shaking. Furthermore, after extraction, all samples were refrozen and rethawed to analyse the possible degradation of hormonal content (5-8 repeats/sample). Progesterone metabolite analysis was carried out with ELISA (produced and validated by the laboratory). Intraclass coefficient of centrifuged and shaked samples were calculated to assess the agreement of data. Interassay coefficients of repeatedly frozen-thawed samples are calculated to assess the reproducibility of measurement.
RESULTS
Mean interassay cv% of hand-shaked samples of cattle, elephant, anteater and red panda were 7.76 ±5.02, 7.9±4.3, 20.8±12 and 7.18±2.64, respectively. These results did not differ from the interassay cv% of centrifuged samples. Intraclass coefficients of the results of the two treatments were 0.966, 0.91, 0.856 and 0.989 in cattle, elephant, anteater and red panda, respectively.
CONCLUSIONS
Our results show that extraction of progesterone metabolites from faeces sample can be carried out on the field. This method provides data which are in agreement with the results of laboratory-based extraction. Furthermore, even the repeated freeze-thaw cycle did not effect the intraclass coefficient and reproducibility.

T96
SPERM CONSERVATION TECHNOLOGIES FOR THE PROPAGATION OF ENDANGERED SPECIES: EVALUATION OF ARTIFICIAL INSEMINATION SUCCESS USING FRESH, REFRIGERATED, AND FROZEN SEMEN IN HOUBARA BUSTARD

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BACKGROUND-AIM
The Houbara conservation project is based on a strict genetic diversity management and the use of artificial insemination. Over the last 20 years, it has produced nearly half million birds (Chlamydotis undulata and macqueenii), to support both in and ex-situ conservation actions. Sperm conservation biotechnologies are keys to this success and the maintenance of genetic diversity, allowing flexibility in males' selection and the maintenance of a genome resource bank.
METHODS
Firstly, refrigeration of semen at 5°C (Chlamydotis undulata) was evaluated, Motility score (MS, 0-5), % Intact membrane (%IM) and % Normal sperm (%NS) were compared for fresh diluted (Lake 7.1) and after 3h, 24h, 48h and 72h of conservation. Secondly, cryopreservation (6% DMA, ultra-rapid pellet method) success was accessed. Finally, egg hatchability (H) and chick hatching weight (HW) were compared after AI with fresh, 5°C/24h and frozen semen. Different mixed models were built to compare results using an intercept nested random effect for variation between groups and within nested subgroups of multiple measures on the same sample. When significant effect was identified, developed models were followed with post-hoc analysis for pairwise comparison.
RESULTS
Significant decrease of MS was noticed after 3h of refrigeration and reduced gradually up to 72h (mean 2.6. % at 72h), IM were 2.32% (±0.5) lower after 3h and stabilized after 24h (89.6±3.5, 5% lower than fresh), %NS was not affected until 72h. The comparison of fresh and frozen samples, respectively, showed a significant difference for MS (3.2±1.0 vs 2.1±0.5), a significant decrease of both %IM (-32±2.6%, P<2*10-16) and %NS (-10±2,2%, P<0.001). The highest % of H was noticed for fresh semen inseminations, 65% and decreased to 56% with frozen and 51% with 5°C/24h. The mean average HW was 38.3 ±0.5g, a statistically significant difference of 1.2±0.4g was only noticed between frozen and fresh semen.
CONCLUSIONS
Despite differences found on sperm evaluations, 5°C/24h and frozen semen are suitable for AI. Hatching results were positive, proving the use of semen preservation techniques as key tools for houbara conservation. Cryopreservation of North African Houbara resulted in a semen bank of 20212 doses from 2217 males.

T97
MALE AND SPERM SENESCENCE IMPACT ON COLLECTION AND SPERM PARAMETERS IN NORTH AFRICAN HOUBARA BUSTARDS

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BACKGROUND-AIM
Senescing males can produce ejaculates with poor sperm quality, affecting reproductive success and offspring quality. Hence, male reproductive senescence can be due to pre-meiotic effects on the germline and post-meiotic effects on spermatozoa. Here, we tested whether pre and post-meiotic aging has additive or interactive effects in a bird with a promiscuous mating system, the North African houbara bustard.
METHODS
To assess the effect of pre and post-meiotic aging, we compared male collection (success and faecal contamination), and semen parameters (volume, concentration, motility and viability) between two age groups (154 young, 4 to7 years and 153 old, 13-17 years), at three collection intervals(1, 5 and 10). Thus, day 1 ejaculate are supposed to contain younger sperm than day 5 and day 10 collections. Generalized mixed models were used to investigate the effect of male's age and delay of collection, controlling the seasonal effect.
RESULTS
Highest collection success was achieved for old males at 1-day interval (Old: 99%±0.01: Young: 95%± 0.03; P<0.001). However, old males had 2.24±0.59 times more contaminated samples in all collection intervals (13.7% contaminated, P<0.005). Concentration and volume were overall higher (P<0.05) for young males (Young: 678±38.4*106sptz/mL, 84.7±3.30µL; Old: 494±33.1*106sptz/mL, 71.8±2.94µL). In both ages, concentration was higher (P<0.001) at 10-days (774±37.9*106sptz/mL) compared to 5-days (602±33.7*106sptz/mL) and to 1-day (400±25.5*106sptz/mL). Percentage of motile sperm was higher (P<0.05) for young birds (Young: 85.2±1.3%; Old: 81.6±1.3%). In both ages, Percentage of motile sperm was higher (P<0.05) at 5-days (86.06±1.1%) and 10-days (85.07±1.2%) in comparison to 1-day (78.5±1.60%). Viability was not affected by age and was higher (P<0.001) at 10-days (94.53±0.31%) compared to 5-days (93.3±0.38%) and 1-day (92.98±0.40%).
CONCLUSIONS
Results confirm the role of aging on male ejaculation and semen parameters. And suggest that North African houbara bustard sperm might require a longer epididymal or deferent duct transit to reach full maturation and optimal quality. This might reflect the pattern of mating frequency in a species with an exploded lek mating system and low densities.

T98
EFFECT OF SUMMER HEAT STRESS ON WATER AND FEED INTAKE OF WATUSSI CATTLE BORN IN HUNGARY

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BACKGROUND-AIM
Heat stress causes significant economic losses through various effects on reproduction-related and non-reproduction-related parameters in cattle. Selection and breeding of less temperature-susceptible species or breeds would be a productive way to adapt to more harsh environmental conditions. Watussi cattle (Bos taurus watusi) is indigenous in Africa and is adatped to extreme environmental heat stress. The aim of the study was to determine the water and feed intake, weight gain of watussi cattle during the summer heat stress period compared to Blonde D'aquitane cattle.
METHODS
Environmental temperature (minimum, maximum, average daily temperature), humidity, temperature-humidity index (THI), wind speed, water intake, feed intake and weight gain were recorded in watussi (n=5, group W) and Blonde d'Aquitane (n=5, group B) cattle from June 1st until September 1st in 2020 during a 3-months period at summer heat-stress. Animals were kept under the same conditions (same stall, and environment) in order to be able to compare results.
RESULTS
Starting body weight have been as follows: 325±48,9 kg (mean±SD) in group B and 309±29,9 (mean±SD) kg in group W. Significant difference was detected between the 2 groups at the end of the study; group B showed 77 kgs weight gain compared to group W with 45 kgs (P<0,05). Feed intake was not significantly different among the 2 groups. Environmental humidity was 71.56 ± 11.88 % (median 69.34) , temperature 25.35 ± 3.65 °C (median 25.5) and wind speed was 0.59 ± 0.33 (median 0.499) during the 3 months period. Water intake was influenced by environmental temperature, but only small proportion could be explained by temperature changes (R2 was 16,62, P<0,05). Humidity caused significant reduction in water intake in both breeds (both groups) but had less effect on watussi cattle (P<0,05). Temperature humidity index (THI =0.8*T + RH*(T-14.4) + 46.4) influenced water intake but not feed intake in either group.
CONCLUSIONS
Environmental summer heat stress affected water intake in beef and watussi cattle under summer months. Although differences have been shown in weight gain, feed intake did not show significant difference between groups. Temperature-humidity index increase caused a decrease in water intake but no effect was observed when only the temperature increased.

T99

FIXED-TIME ARTIFICIAL INSEMINATION OF PLAINS BISON WITH SEMEN COLLECTED AND FROZEN UNDER WILD CONDITIONS

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BACKGROUND-AIM
Plains bison in the wild are threatened by limited genetic diversity within isolated sub-populations and the presence of endemic disease. Assisted reproductive techniques such as semen cryopreservation, ovarian synchronization and fixed-time artificial insemination have been established using captive bison. However, bison populations range across geographically remote areas and these techniques remain to be tested under wild conditions. The present study was conducted to determine the fertility potential of semen collected under wild conditions for fixed-time artificial insemination in plains bison.
METHODS
Wild plains bison bulls (n = 2) were chemically immobilized, and semen was collected by electro-ejaculation at Elk Island National Park, Alberta, Canada (handling time from recumbency to recovery was 25 and 35 min). Immediately after collection, ejaculates were diluted with Tris-citric acid buffer (1:3) and transported to a field lab using a portable incubator at 32°C. Semen was further diluted with either egg yolk-based (TEYG, conventional) or cholesterol-based (CC-TG, animal product-free) extender to 50x106 sperm/mL, packaged into 0.5 mL straws, chilled for 4 h to 4°C, and cryopreserved by suspending 5 cm above liquid nitrogen, and transported to the University of Saskatchewan, Canada. Plains bison cows (n = 17) at the University of Saskatchewan Native Hoofstock Centre were synchronized and assigned randomly to be inseminated with either TEYG or CC-TG semen from one bull; cows were inseminated twice at a 24 h interval. Ovulation was ultrasonographically confirmed by the sudden disappearance of the dominant follicle within 48 h of insemination, and pregnancy rate was confirmed by ultrasonography 30 days post-insemination.
RESULTS
All but one bison ovulated by 48 h after insemination, and pregnancy rates per ovulation were similar between TEYG and CC-TG semen groups (3/7 [43%] and 4/9 [44%], respectively).
CONCLUSIONS
Results document the feasibility and utility of in situ collection of bison semen for use in a genome biobank to produce offspring with new genetic composition. This research was supported by NSERC Canada, the Government of Saskatchewan, and Agriculture and Agri-Food Canada.

TOPIC Reproduction in fish

T100

EFFECTS OF TEMPERATURE AND DIFFERENT ACTIVATING MEDIA ON THE MOTILITY OF EUROPEAN EEL (ANGUILLA ANGUILLA) SPERMATOZOA

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BACKGROUND-AIM
The European eel (Anguilla anguilla) is a critically endangered species, due to its overexploitation and habitat changes, that mainly lives in freshwater and migrates to the Sargasso Sea for spawning. The spermatozoa of marine teleost acquire motility upon hyperosmotic shock once released, and parameters such as temperature and osmolality are capable of influencing motility. Although limited literature data on European eel sperm motility are available, pH and ion concentration are known triggering factors of sperm activation. The aim of the work was to assess European eel sperm motility and kinematics upon activation with 3 different media: Artificial Sea Water (ASW), Tank Water (TW) and IMV Actifish® diluted 1:4 (Actifish), at 4 and 20 °C. Secondary aim was to test an Actifish® dilution mimicking the osmolality of sea water.
METHODS
Semen production was induced by weekly IM injection of hCG (1 IU/g); 24h after the last injection, milts from 26 specimens were collected by delicate abdominal pressure, diluted 1:10 with P1 medium, and maintained at 4 °C. Sperm concentrations were evaluated using a haemocytometer, viability by mean of Eosin-Nigrosin staining. Each sample was activated with ASW 3,7 % (pH 8.25; 999.67mOsm), TW from the breeding tanks (pH 8.14; 957.50mOsm), and Actifish diluted 1:4 with bidistillated water (pH 8.53; 592.75mOsm), at 4° and 20°C. For the second aim, Actifish was tested at the dilution of 1:2. Objective motility was evaluated by Computer Assisted Sperm Analysis (CASA) with custom settings for eel spermatozoa.
RESULTS
Samples showed similar sperm concentration and viability. At 4°C, the three media did not induce differences on sperm motility, while, at 20 °C, TW statistically reduced total and progressive motility. When diluted 1:2, Actifish reduced total and progressive sperm motility, increased slow and static spermatozoa and showed negative effect on many kinematics parameters.
CONCLUSIONS
To the best of the authors' knowledge, this is one of the first reports on the effects of different activating media and temperature on European eels sperm motility. The present data provide fundamental information for further studies on reproduction and breeding of this species.

T101
ASSESSMENT OF MOLECULES ASSOCIATED WITH THE PITUITARY STIMULATION FOR THE ENDOCRINE CONTROL OF REPRODUCTION IN THE YELLOWTAIL KINGFISH SERIOLA LALANDI

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BACKGROUND-AIM
Successful farming of yellowtail kingfish Seriola lalandi depends of understanding its reproductive physiology, and the study of the main molecules involved in the gonadotropic function may help improve reproduction control under captivity conditions. Literature data in model fish indicate that neuroendocrine factors as the gonadotropin releasing hormone (GnRH) and Kisspeptin (Kiss) stimulate the gonadotropic function. However, differences in both expression and function of these molecules have been observed among teleost species due to the wide differences in their reproductive strategies. In order to understand the molecular basis of the pituitary stimulation for the endocrine control of reproduction in yelowtail kingfish, this work aims to assess the mRNA expression levels of GnRH1, Kiss2, and their respective receptors (GnRH1r and KISS2r) throughout the reproductive cycle in females under captivity conditions.
METHODS
Every three months total RNA was extracted from brain and pituitary samples obtained of four females belonging to captive conditioned broodstock. Gene expression was assessed by RT-qPCR with an Illumina® Eco Real Time using the Maxima SYBR Green Master Mix (ThermoFisher). The relative expression of GnRH1, GnRH1r, KISS2 and KISS2r were estimated at each sampling season by ΔΔCt method, where beta actin (ACTB) and tubulin (TUB) were used as reference genes. Data were analyzed by ANOVA ($p<0,05$).
RESULTS
There were no differences in both GnRH1 and GnRH1r expression in samples collected between winter and summer. The level of GnRH1 mRNA during fall was lower compared to winter. GnRH1r expression decreased significantly in fall compared with the other seasons. Similar expression profiles were detected for KISS2 and KISS2r transcripts throughout the reproductive cycle in S. lalandi. These genes showed the higher expression levels during winter and spring and a significantly decrease during summer.
CONCLUSIONS
The spawn occurs in summer season in the captive broostock assesed for this study. Therefore, these results agree with the function of GnRH in both ovarian development and stimulation of the spawning process. Kisspeptin appear to stimulate the pituitary function during all gonadal development period in this species, before spawning period.
Supported by Fondecyt 1201343

TOPIC Reproduction in sheep and goats

T102
PRELIMINARY CHARACTERIZATION OF MORPHOMETRY AND DOPPLER PARAMETERS IN THE POST-SURGICAL MONITORING OF VASECTOMY IN RAM

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BACKGROUND-AIM
The use of vasectomized rams is an alternative to improve fertility in ovine artificial insemination. For this reason, studies are necessary to validate different vasectomy aspects such as surgery duration to reach a minimal invasion and incision. The aim of this trial was to characterize, for the first time, some morphometry, temperature and Doppler parameters in the postoperative evolution after vasectomy taking into account the time spent in surgery (more than 15 minutes -A- or less than 15 minutes -B-).
METHODS
Eleven adult Assaf rams were vasectomized and area of pampiniform plexus like morphometry parameter, testicular temperature and Doppler indexes (resistive index -RI- and pulsatility index -PI-) were measured at different times: before vasectomy -T0-, 24 postoperative hours -T1- and 7 postoperative days -T2-. Data were analyzed using ISAS 9.1 (Mixed Procedure).
RESULTS
Non-significant differences were observed among T0 and the rest studied times in area of pampiniform plexus ($p>0.05$). In contrast, there were significant differences ($p\leq0.05$) in testicular temperature at 24 hours postoperative according to the time spent in surgery (A: T0: 32.50 ± 0.45 °C; T1: 34.00 ± 0.26 °C; B: T0: 33.33 ± 0.18 °C; T1: 33.18 ± 1.02 °C). Furthermore, RI showed significant differences ($p\leq0.05$) at every studied times (A: T0:0.49 ± 0.02; T1: 0.43 ± 0.02; T2: 0.45 ± 0.02; B: T0: 0.45 ± 0.02; T1: 0.57 ± 0.03; T2: 0.55 ± 0.03) whereas PI did not differ ($p>0.05$).
CONCLUSIONS
These results could be explained considering the importance of RI pattern in the inflammatory processes. This index is the most sensitive parameter used in the detection of the testicular blood flow disturbances (Biagiotti et al., 2002). In conclusion, testicular temperature and resistive index are good indicators to conclude that surgery duration should be taking into account to decrease the inflammation process. Further investigations should be carried out to improve animal welfare and technique efficiency.
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T103
THE SEXUALLY ACTIVE BUCKS STIMULATE LH PULSATILITY IN BUCKS IN SEXUAL REST

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BACKGROUND-AIM
In seasonal anestrous goats, the introduction of a buck stimulates LH secretion and triggers ovulation, a phenomenon called "the male effect". The sexual and endocrine responses of females to the male effect varies with the sexual behavior displayed by bucks. Bucks rendered sexually active during the sexual rest by previous exposure to two months of artificial long days in Autumn and Winter, are more efficient to stimulate LH secretion and ovulation than control-sexually inactive bucks displaying a low sexual behavior. The aim of this study was to determine whether these sexually active bucks are able to stimulate LH pulsatility in bucks in sexual rest, as they do in female goats.
METHODS
Bucks from one group were kept under natural variations of photoperiod and formed the control-sexually inactive bucks (control-SI bucks; n=2). Bucks from the other group were rendered sexually active by exposure to two months of long days (16 h of light/day) followed by natural photoperiod (SA-bucks; n=2). On 1 April, two groups of SI bucks (n=6 each) were joined with control-SI or SA-bucks (n=2 in each group). Plasma LH pulsatility was determined every 15 min from 6 h before to 6 h after the introduction of bucks, and the next day during 6 h. LH pulse frequencies were detected using the DynPeak algorithm.
RESULTS
There was an interaction between time and groups in LH pulsatility ($P<0.01$). Before the introduction of bucks, LH pulsatility did not differ between those joined afterwards with the control-SI (0.2 ± 0.2 pulses) or SA bucks (0 pulses; $P>0.05$). By contrast, LH pulsatility was higher 6 h ($P<0.01$) and 24 h ($P<0.05$) after the introduction of the SA buck (3.2 ± 0.7 pulses in both periods) than in those joined with the control-SI buck (0.7 ± 0.2 and 0.8 ± 0.3 pulses, respectively).
CONCLUSIONS
Sexually active bucks stimulate LH pulsatility in males during the sexual rest by the new phenomenon called "buck-to-buck effect".

T104
THE EXPOSITION OF RAMS TO SEXUALLY ACTIVATED MALES IN SPRING INCREASES PLASMA LH CONCENTRATION OF EXPOSED RAMS

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BACKGROUND-AIM
The ram effect, which is the most well-studied socio-sexual factor for inducing ovulation in sheep during the seasonal anestrus, involves the reintroduction of males to a flock of ewes in anestrus that had been isolated from rams. The introduction of the rams induces an increase in the tonic secretion of LH of the females, and a proportion of them ovulates. A weak sexual behavior displayed by rams, which are also in their rest season, produces a weak ovulatory response in ewes, although this low response of the ewes is dramatically improved by the use of rams rendered sexually active during the anestrus, by previous exposure to artificial long days. The aim of this work was to determine whether a similar response to the introduction of activated males in spring is observed in rams.
METHODS
Eight adult Rasa Aragonesa rams, which acted as stimulating rams, and 12 rams which acted as stimulated rams, were used. The photoperiodic-treated stimulating rams (n=4) were induced into a sexually active state by exposure to 2 months of long days (16 h light/d) (15 Dec-15 Feb). At the end of the long-day period, rams were returned to the natural photoperiod. Control stimulating rams (n=4) were kept under the natural photoperiod. On April 20, stimulated rams were divided into 2 groups: exposed to activated (ACT; n=6) or control rams (C; n=6). The day of ram introduction, stimulated rams were blood sampled for 8 h at 20-min intervals, from 4 hours before to 4 h after ram introduction, and next day from 24 to 28 h after ram introduction, and analyzed for plasma LH concentrations.
RESULTS
Mean plasma LH concentrations (ng/ml) of stimulated rams were similar during the 4 h before stimulating-ram introduction (ACT: 0.59±0.03; C: 0.53±0.04). The introduction of the activated rams increased LH concentrations of stimulated rams during the 4 h after their introduction (1.14±0.37) compared with the C group (0.51±0.03) ($P=0.10$), especially during the first hour (ACT: 0.93±0.16; C: 0.49±0.03 ng/ml; $P<0.05$), and during the blood sampling period 24-28 h after ram introduction (0.75±0.07 vs. 0.58±0.04 ng/ml; $P<0.05$).
CONCLUSIONS
Rams sexually active in spring are able to stimulate LH secretion of another rams, as it has been observed in the traditional ram-to-ewe effect.

T105

GLYCOMIC PROFILING OF CERVICAL MUCINS IN EWE BREEDS WITH DIFFERENCES IN CERVICAL SPERM TRANSPORT FOLLOWING CERVICAL INSEMINATION

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BACKGROUND-AIM
Cervical mucus plays an important role in female fertility, as it regulates the entry of motile and morphological normal sperm while preventing the ascent of pathogens from the vagina. The dual function of cervical mucus is critically linked to its rheological properties that are in turn dictated by O-glycosylated proteins, called mucins. The overall aim of this study was to characterise the O-glycan composition in the cervical mucus of six European ewe breeds with known differences in pregnancy rates following cervical artificial insemination (AI) with frozen-thawed semen, which are due to reported differences in cervical sperm transport.
METHODS
These were Suffolk (low fertility) and Belclare (medium fertility) in Ireland, Ile de France and Romanov (both with medium fertility) in France and Norwegian White Sheep (NWS) and Fur (both with high fertility) in Norway (n = 28 to 30 ewes/breed). Cervicovaginal mucus was collected at the follicular phase of both a synchronised and natural cycle (3 replicates/ewe) and was pooled within breed (six pools of five ewes/breed) according to mucus viscosity. Following mucin purification, O-glycan composition was assessed by ultra-performance liquid chromatography (UPLC) and mass-spectrometry (ESI-MS) analysis.
RESULTS
We identified 124 O-glycans, from which 51 were the major glycans with core 2 and fucosylated glycans as the most common structures at the follicular phase of both types of oestrous cycle. The use of exogenous hormones for synchronisation did not affect the O-glycan composition in both high fertility ewe breeds (NWS and Fur) but did in the other four ewe breeds. Despite no differences in gross mucus properties previously reported by our group, we identified O-glycan changes and potential biomarkers that could be targeted for the improvement of cervical AI. For example, core 4 glycan and fucosylated glycan (GlcNAcβ1-3(Fuca1-2Galβ1-3)GalNAc) had higher abundance in the low fertility Suffolk breed compared with NWS (high fertility). In addition, the low fertility Suffolk breed had a higher proportion of sialylated O-glycans compared to the other ewe breeds.
CONCLUSIONS
In conclusion, our results indicate that biochemical changes in the O-glycans attached to the backbone mucin protein may be related to impaired cervical sperm transport.

T106
EFFECTS OF SEMINAL PLASMA OBTAINED DURING BREEDING AND NON-BREEDING SEASON ON RAM SEMEN CRYOPRESERVATION

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BACKGROUND-AIM
Seminal plasma (SP) has several proteins with different molecular weight which have been involved in the function, transit and survival of spermatozoa within the female reproductive tract (Druart et al, 2013). The addition of SP to sperm following cryopreservation increased post-thawed motility and fertility in ram, enhanced post-thawed sperm function and improved AI fertility (Maddison et al., 2014). The use of ram spermatozoa with SP before freezing protects them from freezing damage (Leahy et al., 2010). The aim of this work was to study the effects of different sources of SP (rams and seasons) added before the cryopreservation.
METHODS
Ejaculates from 6 Merino rams were obtained during the whole year (January to December). Individual samples of SP were obtained by centrifugation (7500 x g during 5 min at 4°C). For the frozen-thawed sperm trial, semen samples were obtained during the breeding season. Then, the ejaculates were pooled and centrifuged twice (600 x g, 10 min). The sperm pellet was resuspended in 12 sources of SP (6 rams and 2 seasons). These samples were diluted in a Tris-citric acid-glucose-glycerol-egg yolk extender, loaded into 0.25-mL straws, frozen and stored in liquid nitrogen. Post-thawing evaluation was based on index sperm motility (ISM), supravital stain (Eo-Ni), acrosome integrity (AI), HOST, incubation resistance (37°C, 4 h, TR-MI) and plasma membrane integrity (CFDA). The parameters were analyzed by ANOVA, with Fisher–LSD post hoc test.
RESULTS
Most of the post-thawing parameters showed no significant differences when breeding-season SPs were added before the cryopreservation. In this case, only CFDA showed significant differences ($p<0.05$) between two rams (18.25 ± 2.87% vs. 29.00 ± 2.58%, ram 6 and 3, respectively). When non-breeding-season SPs were added, ISM (1.15 ± 0.30 vs. 1.83 ± 0.28, ram 3 and 2, respectively), AI (38.50 ± 3.12% vs. 54.50 ± 3.97%, ram 3 and 5, respectively) and TR-MI (21.25 ± 6.57% vs. 42.50 ± 7.50%, rams 5 and 2, respectively) showed statistical differences ($p<0.05$).
CONCLUSIONS
We conclude that the breeding-season SPs allow a freezing process without major differences, regardless of the ram used, while the non-breeding season SPs show more differences between males, some SPs being more suitable for freezing semen than others.

T107
REPRODUCTIVE STATUS OF SHALL EWES DURING NON-BREEDING SEASON AND AFTER SUPPLEMENTARY FEEDING AND RAM EFFECT

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BACKGROUND-AIM
Ewes are seasonally polyestrous species. Different kind of variable such as geographic location, environmental temperature, breed, and nutrition can affect the duration of anestrus period. The objective of this study was to investigate the cyclicity status, the effect of supplementary feeding and ram effect on cyclicity of Shall ewes (native Iranian fat-tailed breed) during non-breeding season.
METHODS
Non-pregnant, weaned Shall ewes (n=77), 40.4 months of age (40.4±2.70) and BCS of 3 (3.0±0.08) were selected for 39 days study at the Veterinary Research Institute of University of Tehran, Tehran, Iran (latitude: 35°39' N; longitude: 51°26' E; altitude: 1029 m) during April and May. Ewes received ration according to NRC, 2007. in brief, they received corn silage (400gr), wheat straw (450gr), Alfalfa hay (700gr), and concentrate (400gr) as mixed ration. they also received 300gr extra concentrate as supplementary feeding. The cyclicity of ewes was investigated by measuring progesterone concentrations. The progesterone concentrations ≥ 1 ng/ml were considered as criteria to determine cyclicity in ewes. Two blood samplings, 9 days apart, were carried out at the beginning of this study. Concurrent with the second blood sampling, the supplementary feeding was initiated and lasted for 30 days. Ten days after the second blood sampling, the third sampling was conducted. Two weeks after the third sampling, the ram that was isolated for two months, was introduced to the herd. Six days after introducing ram, the fourth blood sampling was performed. The serum samples were isolated and frozen till progesterone assay. The concentration of progesterone was measured using ELIZA kit. Data were analyzed using Proc Freq and Genmod in SAS.
RESULTS
There was 19/77 (25%) cyclic Shall ewes in the first blood sampling ($P<0.0001$). Cyclic ewes increased to 47% in the second blood sampling ($P<0.01$). The frequency of cyclic ewes increased following supplementary feeding to 56% ($P>0.05$). Ram effect did not have great impact on enhancing the frequency of cyclic ewes in this study (58% cyclic ewes, $P>0.05$).
CONCLUSIONS
During April-May (non-breeding season), great number of Shall ewes displayed cyclicity (47%), without any nutritional and hormonal interventions. Supplementary feeding and ram effect did not have significant impact on cyclicity of Shall ewes under good nutrition and management during non-breeding season in Iran.

T109
LAPAROSCOPIC ARTIFICIAL INSEMINATION IN ILE DE FRANCE EWES UNDER TEMPERATE SEASONAL CLIMATE

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BACKGROUND-AIM
The ile de france breed has arrived to Hungary in the 1950s, where farmers usually apply natural breeding. This meat type breed plays an important role in hungarian sheep breeding, and hungarian flocks need genetic improvement from time to time by using import genetic material. The safest and most successful way of utilizing frozen sperm is the laparoscopic insemination. Our aim was to investigate the efficiency of artificial insemination in this breed with import frozen semen (Organisation de Sélection Ovine Nord, France) by laparoscopy in the temperate seasonal climate in Hungary.
METHODS
Donor ewes, aged from 2 to 5 years were choosen for the experiment from an east-hungarian flock where farmers keep the animals in a semi-intensive way. We inseminated 136 ile de france ewes between 2019 to 2021. Before the insemination we synchronized them with progestagen intravaginal sponges for 14 days and ewes were fasted for 24 hours before the anesthesia (detomidin intramuscular injection) and the operation. During the procedure ewes were placed dorsal decubitus in a cradle that was tilted 45 degrees. We inserted two trocars through the abdominal wall. An optic with light source was placed into the trocar to find the uterus and the ovaries and thereafter we injected the semen into the uterine horns with a special catheter. In autumn time, in season (October, November) 60 ewes, in spring time, out of season (March, April) 76 ewes were inseminated.
RESULTS
In the autumn season only 11 (18,3 %) ewes, in the spring months 54 (59,2 %) ewes became pregnant. The difference between the pregnancy rates of the two seasons is significant (p = 3,587*10-6) with Chi-squared test. The logistic regression model showed that an ewe 6,5 times higher become pregnant in Spring than in Autumn by using assisted reproductive techniques.
CONCLUSIONS
Based on our experience ile de france ewes gave a better response for the oestrus synchronization and the conception rates were better out of season. The laparoscopic artificial insemination with frozen sperm is more successful in spring in Hungary (out of season). Further investigation is in progress to find a connection between the weather conditions of the season and out of season time and the efficiency of artificial insemination.
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T110
A PRELIMINARY STUDY OF THE RELATIONSHIPS BETWEEN ECHOTEXTURAL CHARACTERISTICS OF THE MAMMARY GLAND AND CHEMICAL COMPOSITION OF MILK DURING EARLY LACTATION IN EWES

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BACKGROUND-AIM
A rapid method for determining milk composition would be an asset for agri-food industry, veterinary practice and biomedical research. The aim of this study was to examine ultrasonographic characteristics of the mammary gland in post-partum ewes of varying genotypes for correlations with the chemical composition of milk.
METHODS
Ultrasonographic images of the udder at 1, 2, 3 and 4 weeks after lambing were obtained using the 5.0- or 7.5-MHz transducers, in coronal (C) and sagittal (S) planes, from non-prolific Polish Mountain (PM) sheep (n=4) and prolific Olkuska (Olk) ewes (n=6). All ultrasonograms were subjected to image analyses to compute numerical pixel values (NPV) and heterogeneity (pixel standard deviation-PSD) of the mammary parenchyma.
RESULTS
In PM sheep examined with the 5.0-MHz probe, mean NPV-C correlated negatively with C17:0 (margaric acid) and C17:1 (heptadecenoic acid), whereas NPV-S correlated negatively with C17:0 content of milk. Mean NPV-S of the mammary gland correlated positively with the crude fat, dry weight and negatively with C18:3 n-3 (a-linoleic acid) content of milk samples in PM ewes examined with the 7.5-MHz transducer while PSD-S correlated positively with C16:0 (palmitic acid) and negatively with lactose. In the scans obtained with the 5.0-MHz in Olk sheep, mean NPV-C were directly related to C10:1 (decenoic acid), C14:1 (myristoleic acid), C16:1 iso (iso-hexadecanoic acid), C16:1 c9 (palmitoleic acid), and C18:0 (stearic acid). Mean PSD-C correlated positively with dry weight, C14:1, C15:0, C16:0, C16:1 iso, C16:1 c9, CLA (conjugated linoleic acids), and total saturated fatty acid content, and negatively with C18:1 n-7 (vaccenic acid), C18:2 n-6 (linoleic acid), and total polyunsaturated fatty acid content of milk. Mean PSD-S correlated directly with ash and total saturated fatty acid content of milk. Lastly, mean NPV-C of images recorded with the 7.5-MHz prober in Olk ewes related directly to ash and C12:0 (lauric acid), mean NPV-S related directly to ash and C16:1 iso, and mean PSD-C correlated positively with ash and total saturated fatty acid content.
CONCLUSIONS
Echotextural analyses of the mammary gland in ewes have the makings of the technique for determining milk content of certain chemical constituents in a breed-specific manner. Further studies are needed to verify the usefulness of ultrasonographic image analysis of the udder to predict milk quality and composition.

T112
USE OF DIPYRONE FOR PAIN CONTROL IN EWES AFTER SURGICAL EMBRYO COLLECTION

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BACKGROUND-AIM
Animal welfare is of utmost importance and the control of inflammation and pain must be considered in invasive procedures. Thus, this study assessed the benefits of administering dipyrone after surgical embryo collection in sheep.
METHODS
This study was approved by the Ethics Committee for the Use of Animals (#2717181220). Dorper ewes (n=22) were randomly allocated into two groups after surgical embryo collection: Control (CONT; n=11) and Dipyrone (DIP; n=11). All ewes received antibiotic (20 mg/kg I.M.) and flunixin meglumine (2.0 mg/kg I.M.) immediately after the laparotomy, 24 h and 48 h later. DIP-ewes also received dipyrone (50 mg/kg I.M.), at the same moments, while CONT-ewes saline solution. The anesthetic protocol used was 2% xylazine hydrochloride (0.1 mg/kg I.V.), and 2-4% isoflurane with 10-15 mL/kg/min of oxygen. After embryo collection, the following variables were recorded: interval from surgery to stand up and to eat; heart rate; respiratory frequency; rectal temperature; hemogram analysis, and inflammatory markers. These variables were recorded at 10 times: immediately before anesthetic induction (T1), during the surgery (T2), immediately after the surgery (T3), 1 h (T4), 3 h (T5), 12 h (T6), 24 h (T7), 36 h (T8), 48 h (T9), and 72 h after the surgical embryo collection (T10). Data were compared using a generalized linear model, including the treatment, time, and their interaction (treatment vs time). A $P<0.05$ was considered as significant, and data were presented as mean ± SEM.
RESULTS
No difference ($P>0.05$) was observed in the interval from surgery to stand up (~8.3 min), while DIP-ewes presented a shorter ($P<0.05$) interval from surgery to eat (12.4 ± 0.4 vs 9.5 ± 0.3 min). An interaction ($P<0.05$) between groups and time was observed for cortisol in T3, with beneficial effects in DIP-ewes (85.9 ± 13.7 vs 59.5 ± 6.8 ng/mL), glycaemia in T3 (124.9 ± 10.1 vs 147.8 ± 10.4 mg/dL) and T4 (153.1 ± 12.4 vs 116.9 ± 13.6 mg/dL), and in globulin in T9 (4.5 ± 0.5 vs 3.3 ± 0.3 g/dL), suggesting a positive role of DIP.
CONCLUSIONS
In conclusion, dipyrone administration to ewes after surgical embryo collection for three consecutive days brings beneficial effects to pain control, being positive to animal welfare.
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T113
EFFECT OF A PHYTOMELATONIN-RICH DIET ON RAM TESTICULAR PARENCHYMA EVALUATED BY MACROSCOPIC AND MICROSCOPIC ULTRASONOGRAPHY

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BACKGROUND-AIM
Melatonin is the hormone that regulates sheep seasonality and is usually used in subcutaneous implants in rams to counteract the effects of the non-reproductive season. Melatonin is also present in plants (phytomelatonin). Therefore, phytomelatonin-rich diets could be a green alternative for enhancing reproductive parameters in rams. Testicular ultrasonography is a non-invasive technique for the evaluation of macroscopic and microscopic characteristics of the testicular parenchyma. Thus, this study aimed to evaluate the effect of a phytomelatonin-rich diet on ultrasound testicular parameters in rams during the non-reproductive season.
METHODS
Eighteen Rasa Aragonesa rams were used in this study, nine fed with a commercial diet and nine with a phytomelatonin-rich diet (80% commercial diet and 20% of a mixture of pomegranate pulp, tomato pulp, and grape pomace) for five months during the non-reproductive season. Ultrasound videos were monthly recorded using a portable ultrasound scanner connected to a 7.5 MHz linear probe. The testicular echotexture analysis was performed using the ECOTEXT® software (Humeco, Spain), and the number of black (Ec1), white (Ec2), and grey pixels (Ec3), tubular density (TD), lumen area (LA), and lumen diameter (LD) of the seminiferous tubules were evaluated. Additionally, colour-doppler flow imaging was used in the testicular arteria to analyse the blood flow. Differences between groups were analysed using the mixed model ANOVA with SPSS (v.15.0) software.
RESULTS
Phytomelatonin-rich diet significantly increased ($p<0.05$) Ec2 in the fourth month of the experiment compared to the control group. An increase in Ec3 ($P=0.06$) was also detected at the same time. These parameters might indicate an increase in the number of cells within the seminiferous tubules. No significant differences were found in the other ultrasound parameters, nor testicular blood flow parameters.
CONCLUSIONS
Continuous feeding with a phytomelatonin-rich diet affects the echogenicity of testicular parenchyma in rams. However, it would be necessary to evaluate this effect on sperm quality and functionality.
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T114
INFLUENCE OF PLASMID DNA CONCENTRATIONS ON IVP OVINE EMBRYO DEVELOPMENT AND EGFP EXPRESSION

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BACKGROUND-AIM
Transgenesis in livestock allows improvement of desired traits besides being used as animal models for biomedical research. Intracytoplasmic microinjection (ICI) appears to be a favorable alternative for gene delivery to embryos. In this study, effects of different pEGFP-N1 concentrations on fluorescing embryo rate and embryonic development were investigated.
METHODS
Ovaries obtained from slaughterhouse were transported to the laboratory in PBS at 30-35°C and oocytes were isolated by slicing and matured for 24h. IVF was carried out using fresh semen which was prepared with Percoll density gradient technique. Sperm concentration was adjusted to 8x105 spz/mL and oocytes were co-incubated for 20-22 h. Following denudation putative zygotes were divided into four; control and 3 study groups (5, 10 or 20 ng/µL of pEGFN) for microinjections. Zygotes were subjected to ICI, cultured and observed for development. On 7th day postfertilization embryos were assessed by fluorescence microscopy to determine EGFP expression.
RESULTS
Relationship between microinjection groups and EGFP expression were evaluated using Chi-squared analysis. The cleavage rate in control, 5, 10 and 20 ng/µL groups were 82.1%, 79.4%, 75.4% and 35.0% respectively which revealed that 20 ng/µL group exhibited significantly lower cleavage rate than other groups ($p<0.01$). Development rates to the morula through hatched blastocyst stages in all injected zygotes were 46/67 (68.7%), 68/107 (63.5%), 73/114 (64.1%), 14/40 (35.0%) respectively. EGFP expression rates in all development stages of 5, 10 or 20 ng/µL groups were 35/107 (32.7%), 50/114(43.8%), 5/40 (12.5%) respectively. Results of EGFP expression in 10 ng/µL group was significantly higher than 20 ng/µL although there is no significancy between 5 and 10 ng/µL group ($p<0.01$).
CONCLUSIONS
Cytoplasmic microinjection of 10 ng/µL pEGFP-N1 into in vitro produced sheep zygotes reproducibly results in higher EGFP expressing embryos then other groups and does not impede embryonic development.
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T115
INDUCTION OF CYCLITY IN SHEEP USING A PROTOCOL BASED ON CIDR PLUS HCG

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BACKGROUND-AIM
The use of equine chorionic gonadotrophin (eCG) in protocols for estrus synchronization in sheep is currently challenged by future banning of the hormone so it is necessary to implement alternatives, mainly during the anestrous season. This research aimed to evaluate whether the administration of human chorionic gonadotropin, hCG, at the time of removal of a progesterone device may be effective at inducing estrus and ovulations in sheep during the natural seasonal anestrous
METHODS
Multiparous Dorper sheep were treated with one intravaginal CIDR device (CIDR® Ovis, Zoetis, Cd. de Mexico, Mexico) for seven days plus one intramuscular dose of 5 mg of prostaglandin F2a (Lutalyse, Zoetis, Cd. de Mexico, Mexico) at CIDR withdrawal. The group were divided into three experimental sub-groups based on the gonadotrophin treatment at CIDR removal. The first group (Group eCG, n = 11) received one intramuscular injection of 300 IU of equine chorionic gonadotrophin (GonActive® eCG, Virbac, Zapopan, Mexico), the second group (Group hCG, n = 13) received one intramuscular injection of 300 IU of human chorionic gonadotrophin (Chorulon®, MSD, Cd. de Mexico, Mexico), while the third group received one intramuscular injection of saline solution and acted as the control group (Group CON, n = 12).
RESULTS
The assessment of reproductive outcomes (estrus induction, ovulatory follicle dynamics, and pregnancy rate) demonstrated no response in the control non-treated group and the largest rate of narrowly synchronized estrus signs and ovulations in the eCG-group. The administration of hCG was effective for inducing estrus and promoting follicular growth but ovulation was delayed 74h.
CONCLUSIONS
The administration of hCG at the time of CIDR removal was effective for inducing estrus and ovulations and ovarian cyclicity, so it is useful for natural breeding, but the delay of ovulation may limit the implementation of artificial insemination protocols.

T116
REPRODUCTIVE PERFORMANCE OF EWES FED SECONDARY METABOLITES RICH-DROUGHT-TOLERANCE PLANTS

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BACKGROUND-AIM
The insertion of drought-tolerance plants could be an effective solution for the livestock sustainability after insuring their effects on animal performance. The effects of feeding different drought-tolerance plants with respect to their active components on oestrous behaviour, ovarian activity, steroid profile and reproductive performance of ewes were studied.
METHODS
Forty five Barki ewes were fed Trifolium alexandrinum (clover), Acacia saligna , Atriplex halimus and leucaena leucocephala and a basal concentrate diet. All diets were adjusted to meet nutritional requirments of animals during different reproductive stages and were iso-caloric and iso-nitrogenous. Feeding treatments started 2 oestrous cycles preceding oestrous synchronization and continued throughout the third synchronized oestrous cycle, mating and the first trimester of pregnancy. The active components of each plant were identified using hPLC. Overt signs of behavioural oestrus were monitored and oestrous rate, time of onset of oestrus and oestrous duration were estimated. Ovarian activity during the follicular phase and the luteal phase and pregnancy and numbers of emryos/fetuses were ultrasonographically monitored. Serum progesterone and estradiol concentrations were determined during pregnancy. Numbers and weights of lambs were recorded and used for estimating fertility data such as litter size and fecundity.
RESULTS
In atriplex and leucaena, pyrogallol was the dominant phenolic compounds. In acasia, rutin was the predominant compound, while quercetin was the predominant compound in clover. Leucaena significantly decreased number of total follicles compared to other roughages. Compared to clover, both acasia and leucaena significantly decreased number of corpora luteae, whereas atriplex showed an intermediate value. Acasia and leucaena tended to decreased serum progesterone concentrations compared to clover and atriplex, while clover and atriplex significantly increased serum oestradiol concentrations compared to the other roughages. Ewes fed acasia or atriplex expressed shorter significant oestrous duration than those fed clover, whereas ewes that fed leucaena expressed intermediate duration. Compared to clover, acasia and leucaena increased significantly total conceptus loss at day 50 of pregnancy.
CONCLUSIONS
Overall, atriplex seems to be the most suitable roughage for ewes during reproductive period under desertification conditions.

T117
DINOPROST TROMETHAMINE WAS MORE EFFECTIVE THAN D-CLOPROSTENOL TO ENHANCE SEXUAL BEHAVIOR, BUT CLOPROSTENOL IMPROVES SEMEN QUALITY IN YOUNG HAIR LAMBS.

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BACKGROUND-AIM
Searching to reduce the impact of the year season, hormonal strategies have been developed to stimulate male sexual performance, for example, prostaglandin analogs such as Dinoprost have been used in boars with positive results on sexual behavior. Also, D-Cloprostenol improved some of the characteristics of the ejaculate in hair rams. So, this study aimed to determine the effect of Dinoprost tromethamine (DT) or D-Cloprostenol (CD) over sexual behavior and semen quality of sexually inexperienced growing lambs.
METHODS
24 hair lambs (33.5 kg ± 6.0 kg) divided into three groups (n=8) were used in this research. 2 ml of saline was applied to the group I (control), 10 mg of DT to group II and 0.150 mg of CD to group III. All treatments were applied intramuscularly. Analogs were applied 5 minutes before the sexual behavior test and 20 before semen collection. Sexual behavior was evaluated twice a week, while seminal quality was assessed weekly. Weight and scrotal circumference controls were performed weekly. Experimentation lasted 10 weeks from May until July 2021. A mixed linear model was performed for the data analysis; the random effect was each lamb while the fixed effects were the PgF2a analogs, week test, and their interactions. Data were analyzed with SAS. Tuckey method was used when statistical differences were presented ($p < 0.05$).
RESULTS
Significant effects of Treatments were found in all variables of sexual behavior ($p < 0.05$) except for mount attempts and ejaculate mounts, DT was the treatment with best results. On the other hand, test number showed significant effects on the courtship start (<0.004), anogenital sniffing (<0.0001), Flehmen (<0.0009), and mount attempt (<0.03). Regarding body development and semen quality, CD showed consistent results, since it presented effects on live weight (<0.01) and scrotal circumference (<0.006); Likewise, CD positively affected volume ($P < 0.005$) and mass motility ($P < 0.04$). Week test, showed effects on all of the studied variables ($p<0.05$), except for progressive motility.
CONCLUSIONS
According to the results of this investigation, DT stimulates the libido of young lambs outside the reproductive season. Likewise, CD improves semen quality in young hair lambs.

T118
DEPENDENCE OF THE ACTIVITY OF NUCLEOLI ON THE NUMBER OF LAMBS IN THE LITTER

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BACKGROUND-AIM
NOR (nucleolar organizer region) can serve as a marker for characterizing the physiological state of the organism. The aim of our study was to study the relationship of NOR parameters in sheep depending on the number of lambs in the litter.
METHODS
The study protocol was approved by the Animal Care and Use Committee of the L. K. Ernst in accordance with the guidance of the Council for the control of animal experiments. The object of the study was three groups of ewes after lambing before weaning of lambs: group I with one lamb (n = 50); Group II with double (n = 70) and group III with triple (n = 38). Blood was taken from animals from a vein. Lymphocytes were isolated from blood using the ficoll-urographin density gradient sedimentation method. The preparations were fixed with methyl alcohol and stained with a 50% solution of silver nitrate according to the Havell-Blake technique. The resulting slides were examined using a Nikon Eclipse Ni microscope equipped with a DS-Qi2 digital video camera. The measurements and processing of the obtained images were carried out using the NIS-Elements BR4.30 software. Microsoft Excel-2010 software was used to process the obtained primary digital materials, and the SPSS v.23.0 software package was used for statistical processing.
RESULTS
The number of nucleoli in sheep with one lamb averaged 2.38 ± 0.18, the average area of nucleoli is 68.2 ± 2.73 pixel., In sheep with double lambs, 3.07 ± 0.23, the average area of nucleoli is 72.6 ± 6.54 in ewes with triplets during lactation, the content of nucleoli in intact lymphocytes averaged 4.76 ± 0.24; the average area of nucleoli in this group was 102.8 ± 7.81pixel. The difference between the groups in the number of nucleoli in intact lymphocytes is statistically significant, the largest number of nucleoli is observed in queens with triplets, the number of nucleoli in their lymphocytes is twice that in queens that brought and fed one lamb (p <0.001) and 55% more than from the queens who brought and fed two lambs.
CONCLUSIONS
The results of our research show that the number of nucleoli in intact lymphocytes of ewes during lactation is interrelated with the level of load on the body.

T119
EFFECT OF SPONGE TREATMENT DURATION (14 VS 15 DAYS) DURING AN ESTROUS SYNCHRONIZATION PROTOCOL USING LOW DOSE OF ECG (200 IU) IN SHEEP

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BACKGROUND-AIM
Estrous synchronization protocol for fix-timed artificial insemination (FTAI) in small ruminants usually requires the sponge to remain in the sheep for 12-14 days. Sometimes there is a need to split the AI tasks into two different days (i.e., large flocks, weather, etc.). Also, eCG effect may be affected by progestin length. The objective was to compare the effect of sponge-treatment duration (14 vs 15 days) on pregnancy, during a FTAI protocol combined with a low dose of eCG.
METHODS
The study was done in 2021, during the reproductive season, in INTA Bariloche, Argentina, were 223 Merino Sheep were enrolled, with an average BCS of 2,2 (scale 1-5). Sheep were kept in pasture. All the animals were randomly assigned to received (Day 0) an intravaginal sponge of 60 mg of Medroxyprogesterone (Progespon, Zoetis) for 14 (G14d; n=113) or 15 days (G15d; n=110) . At sponge removal, all animals received 200 IU of eCG (Ecegon, Biogenesis Bago) IM. FTAI was done 52-56 hours after the sponge removal by intracervical insemination using fresh semen. The semen was collected with artificial vagina and the progressive motility was evaluated to approve its use (> 80%). Pregnancy diagnosis was performed 35 days after AI by ultrasound. Data was analyzed with R Commander (R Core Team, 2016).
RESULTS
The global pregnancy rate of the flock was of 65.9%, where Group 14 days (G14d) obtained 63.7% (72/113) while G15d resulted in 68.2% (75/110) of pregnancy. The results did not show statistical differences between groups (P=0.48).
CONCLUSIONS
Results suggest that we have some flexibility in the implementation of synchronization protocols that combine sponge with 200IU of eCG, maintaining good reproductive achievements.

T120
OVINE CARUNCULAR ENDOMETRIUM TRANSCRIPTOME IS AFFECTED BY IMBALANCED NUTRITION AND FSH-INDUCED OVARIAN HYPERSTIMULATION

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BACKGROUND-AIM
Hormonal manipulation and/or imbalanced diet have modulating effects on the development of utero-placental feto-maternal contact, which is critical for the establishment of pregnancy. In sheep, depending on diet, FSH-induced superovulation alters caruncular expression of factors associated with uterine receptivity, like estrogen and progesterone receptors. In addition, FSH-treatment induces inclusions of lipid droplets in the endometrial surface and glandular epithelium. Based on these observations, our aim was to deepen our understanding of the biological mechanisms underlying the effects of FSH-induced superovulation and imbalanced nutrition on uterine function.
METHODS
Therefore, we used deep RNA sequencing (RNA-Seq) to investigate changes in the caruncular endometrial transcriptome. Ewes (n=4-5/group) were divided into feeding groups: normal fed (NF), overfed (OF) and underfed (UF), and were further treated with FSH (or saline, controls, C). Samples were collected on days 5 and 10 of diestrus. The SUSHI platform (FGCZ, ETH/UZH Zurich) was used for analysis. Gene ontologies, pathways and upstream regulators were evaluated in differently expressed genes (DEGs, P<0.01, FDR<0.05) between days 5 and 10 in NF_C (time-dependent effect), and in response to FSH under different feeding conditions on day 10 (the time of embryonal recognition in pregnant animals).
RESULTS
1484 DEGs were found on day 10 vs 5, predominantly associated with increased immune activity and cellular metabolic processes. A higher number of DEGs was identified in response to FSH in NF (1374) than in OF (168) or UF (18) samples. There were no common DEGs between OF and UF, indicating different transcriptomic effects in response to FSH, depending on diet. Genes found in NF after FSH treatment mapped mostly with terms associated with increased cell growth, proliferation, differentiation, suppression of cell-cell-, and cell-ECM adhesion and immune response. A decreased mRNA expression of several genes associated with these functional terms (e.g., FOXO1, PTGS2, CASP3, HIF1A) was determined in NF animals treated with FSH.
CONCLUSIONS
Our results show that FSH alters uterine gene expression depending on the maternal diet, which has implications for the implantation process and clinical outputs.

T122
EFFECT OF COASTING TIMES ON DEVELOPMENTAL COMPETENCE OF OVINE CUMULUS-OOCYTE COMPLEXES

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BACKGROUND-AIM
As the literature is still incipient regarding the ideal coasting time (time from the last FSH administration to oocyte collection) in sheep, the aim of this study was to assess the effect of coasting time of 12 (G12), 36 (G36) and 60 h (G60) on the number and quality of cumulus-oocyte complexes (COCs) recovered in Santa Inês ewes.
METHODS
Ewes (n=36) were subjected to the "day 0 protocol", with ovarian stimulation protocol consisting of 80 mg of FSH, varying only the coasting times. After collection, the COCs were morphologically classified in grades [good (GI/GII), viable (GI/GII/GIII), and degenerated (GIV)]. Analyses of brilliant cresyl blue (BCB) and configuration of germinal vesicle (GV) chromatin condensation were performed. Parametric and non-parametric data were analyzed by ANOVA and the Kruskall-Wallis test, respectively. Qualitative data were submitted to logistic regression analysis. The post hoc test of multiple comparisons used was Student-Newman-Keuls. Statistical significance was at P<0.05.
RESULTS
G12-ewes presented higher number (P<0.05) of good COCs (4.6±0.5) when compared to G36 (2.9±0.5) and G60 (3.0±0.9). However, G12, G36, and G60 were similar (P>0.05) in the following parameters, respectively: number of aspirated follicles (12.0±1.3, 10.4±1.0 and 10.0±1.3), structures recovery rate (54±5.7, 46±3.9 and 51±5.3%), number of viable oocytes (6.2±0.9, 4.3±0.5 and 4.8±0.9) and percentage of BCB+ (82.4, 71.2 and 89.7%). In relation to GV chromatin configuration, there was no difference (P>0.05) among G12 (2.5%), G36 (6.5%) and G60 (6.7%) in condensed chromatin around the nucleolus. However, the increase in the coasting time (60 h) seemed to be beneficial and let to an advanced development, since a lower (P<0.05) percentage of decondensed chromatin spread in the nuclear area and higher (P<0.05) percentage of condensed chromatin distributed near the nucleolus and nuclear envelope were observed on G60 (43.3 and 50.0%), compared to G12 (95.0 and 2.5%) and G36 (80.6 and 12.9%), respectively.
CONCLUSIONS
In conclusion, G12 presented more COCs of good quality in the morphological evaluation, however, the analysis of chromatin condensation suggests that COCs from G60 presented higher potential of development competence.
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T123
MALE SEXUAL BEHAVIORS INDUCED IN ANDROGENIZED FEMALE GOATS STIMULATE THE OVULATORY RESPONSE IN ANOVULTORY GOATS

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BACKGROUND-AIM
An intense sexual behavior by male goats is necessary to induce LH preovulatory surge and ovulation in seasonally anovulatory goats (Martínez-Alfaro et al., 2014, Vielma et al., 2009). Therefore, the aim of this study was to evaluate the importance of male sexual behaviors of androgenized female goats on the ovarian response of seasonally anovulatory goats.
METHODS
During seasonal anestrous, thirty-six creole multiparous female goats were used, divided into two groups homogenous by body condition score and physiological state. One group (Behavior group, BG; n=17) was exposed to full physical contact with two androgenized female goats (treated previously with two doses of propionate of testosterone, I.M. with interval of 14 days). A second group (Control group, CG; n=19), was directly exposed to 2 sexually active bucks, which allowed the auditory and olfactory communication, with sight and physical contact. The BG was located 100 m away from the CG, to avoid visual and auditory signals between the groups. Both, the sexually active males and androgenized goats remained in the respective groups of anovulatory female goats during 10 days. The ovulatory activity was assessed by the presence of corpora lutea through transrectal ultrasonography at 10 days after starting of the stimulation. The proportion of female goats that ovulated; and the frequencies of sexual behaviors were analyzed by chi-squared test.
RESULTS
The nudging behaviors in androgenized female goats was greater (563 vs. 226, respectively; P<0.0001) than in the sexually active bucks. These findings may to explain the fact that there was no difference (P>0.05), in the ovulatory response of female goats of BG (13/17; 76%) exposed to androgenized females respect to female goats of CG (18/19; 94%) exposed to sexually active bucks. When the female goats were exposed to androgenized females the response was not different to females exposed to bucks sexually actives.
CONCLUSIONS
In conclusion, male sexual behaviors seems to be the most important signal to induce the ovulation in anovulatory female goats

T124
OESTRUS BEHAVIOUR AND OVULATORY RESPONSE AFTER ADMINISTRATION OF PROSTAGLANDINS COMBINED WITH GNRH IN SHEEP

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BACKGROUND-AIM
Synchronization of estrus and ovulation in sheep are mostly based on intravaginal progestagens plus a single i.m. dose of eCG. However, the use of eCG is strongly compromised because the hormone is obtained from pregnant mares. Our group has developed a protocol based on administration of GnRH diluted in saline solution. The objective of the current study was to determine, by developing a preliminary study with a prostaglandin-based protocol, if the effect of the hormone may be improved by slowing its release by using a different vehicle (propylene-glycol).
METHODS
Occurrence of estrus and ovulation were synchronized in 18 ewes, during the breeding season (October), by two i.m. doses of 5 mg of prostaglandin F2a (Dinolytic®, Zoetis, Madrid, Spain), 7 days apart. At 32h after the second prostaglandin dose, a third of the animals received one i.m. injection of saline and remained as controls (Group C; n=6) whilst the remaining sheep were injected with a single i.m. dose of 50 µg of GnRH (Acegon®, Lab. Syva, Leon, Spain), in either saline solution (Group SS; n=6) or propylene-glycol (Group PP; n=6). The ovarian response, in terms of percentage and timing of onset of estrus behavior, was evaluated by individual detection of estrus signs with trained rams every 12h from 24h after the second prostaglandin dose. Afterwards, ovulatory efficiency was evaluated on Day 11 of the induced estrous cycle by determining presence and number of corpora lutea by transrectal ultrasonography
RESULTS
All the sheep in the Groups C and PP showed ovulation in response to the treatment; signs of estrus behavior were recorded in all controls (100%; timing: 48.0±0.0h) but only in five of six ewes in the Group PP (83.3%; 40.8±4.3h). Conversely, only four of six sheep in the Group SS showed estrus behavior (66.7%; 42.0±4.8h) and only three of six (50%) showed ovulation. Mean ovulation rates in responding animals were similar among groups (1.5±0.2 for Group C, 1.6±0.3 for Group PP and 1.3±0.2 for Group SS).
CONCLUSIONS
Efficiency of protocols based on GnRH administration may be improved by retarding the release of the hormone, and therefore the period of follicle maturation prior to ovulation, with propylene-glycol.

T125
REPRODUCTIVE SEASONS AFFECTS THE SEXUAL BEHAVIOUR AND THE SEMINAL QUALITY OF THE HAIR RAMS, REGARDLESS OF THE SOCIAL HIERARCHY IN NORTHEAST MEXICO

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BACKGROUND-AIM
In Mexico, there is an increase in the production of hair sheep, which, being native to the tropics, are considered non-seasonal. Neuroendocrine factors that regulate seasonal anoestrus in sheep are reported, but it is unknown if they are activated in hair sheep when they are moved to different latitudes. The objective was to evaluate sexual behavior and seminal quality in two reproductive seasons (winter and spring) according to the social hierarchy (SH) in Higueras Nuevo León, Mexico (latitude 25°88' and west longitude 100°02').
METHODS
Six rams with a live weight (LW) of 73.7±0.32 kg (mean±SD) were used, divided into 3 groups (2 rams/group: dominant-subordinate), previously identified by means of the feeding competition test described by (Synnott and Fulkerson, 1984), the dominants were marked with the letters D1, D2, and D3; and subordinates with S1, S2, and S3 respectively in each group, in addition, each pair was of the same breed and similar weight, which were assigned one to each group of 15 adult Saint Croix and Katahdin ewes in each breeding PV of 35, 4 ± 7.6 kg (mean ± SD). For each reproductive season (EB), the variables beginning of courtship, smell, lateral approaches, flehmen, mounting, mounting with ejaculation were recorded by means of eight video cameras distributed in the groups, recording for 24 hours/45 days. Weekly and for 8 weeks, the BW and scrotal circumference (CC) of each ram was recorded. The semen was collected with an electro-ejaculate and the ejaculate volume (V) (ml), mass and progressive motility, concentration (X10^6 sperm/ml), and % of sperm with progressive motility were evaluated. The data were analyzed with IBM® SPSS Statistics. Tukey's method was used when there were statistical differences ($p < 0.05$).
RESULTS
Breeding season affected ($p < 0.05$) on each of the sexual behavior variables, except lateral approaches and flehmen; Likewise, an interaction between the BS and the SH was presented regarding mounting attempts and the number of mounts. For seminal quality, except for ejaculate volume, there were differences ($p < 0.05$) in the breeding season, for social hierarchy, there was only one effect ($p < 0.05$) on progressive motility and concentration of sperm/ml. And for the BSxSH interaction, there was an effect on the total number of sperm and the number of sperm with progressive motility.
CONCLUSIONS
According to the results and conditions of this research, there was a greater sexual behavior and seminal quality in winter breeding in Northeastern Mexico.

T126
HIGH DILUTION RATES MODIFY THE MOVEMENT PATTERN OF RAM SPERM

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BACKGROUND-AIM
Conventionally, semen is diluted down prior to its conservation using a relatively low dilution rate (Ashworth et al.,1994). However, prolonged exposure of sperm to seminal plasma has been found to induce adverse effects on sperm function. These effects could be prevented by high dilution rates in order to minimize the effect of proteins and low molecular weight compounds of seminal plasma which reduce sperm quality (Martí et al., 2006; Palomo et al., 2017). The aim of this study was to assess the effect on physiological status of ram sperm induced by 3 dilution rates (1:2, 1:16 and 1:32).
METHODS
Semen samples were collected from 9 sexually mature Assaf rams in the breeding season using artificial vagina. Ejaculates from each 3 males were pooled and diluted down to 1:2, 1:16 and 1:32 (v/v) (D1, D2 and D3, respectively) in egg yolk-based extender (TTF 320-1-0). After 30 minutes motility and kinetic parameters were assessed using the CASA system (SCA Software) and viability (V) and mitochondrial functionality (MF) were assessed by flow cytometry (Zombie Violet® FVK and CellROX® Deep Red) at room temperature. The data were analyzed using a statistical MIXED procedure.
RESULTS
Non-significant differences ($p > 0.05$) were obtained among the 3 dilution rates with regard to V and MF. There were also non-significant differences ($p > 0.05$) on total motility (TM) and progressive motility (PM) among D1, D2 and D3. However, curvilinear velocity (VCL), velocity according to the smoothed path (VAP), velocity according to the straight path (VSL) and amplitude of the lateral displacement of the sperm head (ALH) were significantly higher ($p \leq 0.05$) at low dilution rate in comparison with the highest dilution rate (D1: 193.27 ± 38.82 µm/s VCL, 113.18 ± 18.48 µm/s VAP, 59.35 ± 9.37 µm/s VSL and 2.60 ± 0.48 µm ALH vs. D3: 148.78 ± 14.64 µm/s VCL, 92.38 ± 6.59 µm/s VAP, 46.09 ± 4.10 µm/s VSL and 2.05 ± 0.18 µm ALH).
CONCLUSIONS
In conclusion, high dilution rates seemed to induce an alteration in the movement pattern of ram sperm, characterized by a decrease in VCL, VAP, VSL and ALH. Further investigations could be necessary to implement additional methods to eliminate the adverse effects of seminal plasma under safety conditions. (Supported by MEC –fellowship FPU17/04142– and MINECO –AGL2017-83098-R–).

T128
THE CHARACTERIZATION OF ROS LOCALIZATION COULD BE A CRUCIAL FACTOR IN RAM SPERM QUALITY DETERMINATION

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BACKGROUND-AIM
It is widely known that low levels of reactive oxygen species (ROS) are necessary for the physiological function of sperm. However, high amounts of ROS are associated with oxidative stress and detrimental effects on fertility. As consequence, a deep characterization of ROS presence with different probes, the study of their intracellular localization and its relationship to other conventional parameters could improve semen preservation protocols in ram.
METHODS
To achieve this aim, semen samples were obtained from 7 Assaf rams (one ejaculate per male) using artificial vagina in each experimental group: breeding season (BS) with an expected higher sperm quality, non-breeding season (NBS) that usually provides low sperm quality and a positive control group (PC) treated with hydrogen peroxide (300 µM) as an indicator of damage. Motility and kinetics parameters were analyzed by the CASA system (SCA Software). Moreover, a multiparametric analysis including viability (Zombie Violet™), apoptosis (CellEvent™ Caspase 3/7), ROS content analyzed with two different probes (CellROX™ Green and Deep Red) was carried out by flow cytometry. Data were analyzed using Prism 8 (GraphPad Software).
RESULTS
As expected, total motility (TM), progressive motility (PM) and viability were significantly higher ($p<0.05$) in BS compared to NBS and PC. Contrary to this, apoptosis presented the highest value in PC compared with BS. Attending to ROS probes, CellROX™ Green presented a significantly ($p<0.05$) higher level in PC (99.04±0.58%) compared with BS (86.61±4.32%) in contrast to CellROX™ Deep Red that registered its highest level in BS (37.67±5.73%) sperm samples presenting significant ($p<0.05$) differences with PC (0.03±0.01%).
CONCLUSIONS
In conclusion, CellROX™ Green and Deep Red presented an opposite label pattern that was corroborated by fluorescence microscope obtaining a different probe localization. Attending to these results we can conclude that CellROX™ Deep Red was associated with high mitochondrial activity and it was located in sperm mitochondrial region, while that CellROX™ Green was associated with high ROS concentration and it was located in the cell nucleus producing sperm damage. (Supported by MINECO (PRE2018-086400, AGL2017-83098-R)).

T129
IDENTIFICATION OF MOLECULAR COMPONENTS FROM ESTROUS SHEEP SERUM WITH A KEY ROLE IN RAM SPERM CAPACITATION

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BACKGROUND-AIM
In small ruminants, estrous sheep serum (ESS) is often added to capacitating media to obtain optimal fertilization rates. However, little is known about the molecular components of ESS that are involved in sperm capacitation. The present study aimed to identify those proteins from ESS that interact or are transferred to ram spermatozoa during in vitro capacitation.
METHODS
ESS was extracted from five different ewes after estrus synchronization and detection with a vasectomized ram. All ESS were heated and then pooled. Four fresh ejaculates from four different rams were mixed and centrifuged through 45% Percoll. One fraction was incubated for 240 min in synthetic oviductal fluid (SOF) with 2% of ESS for capacitation and the other in SOF with 0.1% of polyvinyl alcohol. Reverse phase liquid chromatography coupled to mass spectrometry (RP-LC-MS/MS) was used to quantitatively characterize the proteome of ESS as well as the proteomic changes to ram spermatozoa following exposure to ESS.
RESULTS
ESS provided 14 proteins to ram spermatozoa during in vitro capacitation that were not found in non-capacitated spermatozoa, including albumin, dyneins, glycolytic enzymes, membrane-associated guanylate kinase, thioredoxin domain-containing protein-3, receptor protein-tyrosine kinase, vitellogenin domain-containing protein, fetuin-B and metallothionein-3. Such proteins are implicated in cholesterol efflux, lipoprotein remodeling, flagellar beat stimulation and regulation, zona pellucida's binding, cellular response to reactive oxygen species and energy production.
CONCLUSIONS
This is the first study that characterize proteins from estrous sheep serum that interact with ram spermatozoa. Our findings suggests that there are certain proteins in the ESS necessary to achieve capacitation in ram spermatozoa. A better knowledge of these proteins might allow their future use in cryopreserved spermatozoa to enhance their fertility.

T130
EFFECT OF BREED ON EMBRYO PRODUCTION IN SUPEROVULATED EWES

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BACKGROUND-AIM
The efficiency of an embryo transfer programme can be affected by many factors including the genotype of the ewe. The objective of the study was to evaluate the effect of ewe donor breed on ovulation rate, embryo recovery rate, and number of transferable embryos in superovulated ewes.
METHODS
Fifty four ewes from three breeds (18 Dorper (D), 18 Charollais (Ch) and 18 Pelibuey (P)) were superovulated. Ewes were synchronized with intravaginal sponges containing 20 mg of micronized fluorogestone acetate (FGA; Chronogest, Intervet) inserted for 12 days together with 75 µg of prostaglandins (Prosolvin, Intervet) per donor administered on day 10, considering as day 0 the day of sponge insertion. The FSH for superovulation was administered every 12 hours during 4 days through 8 intramuscular injections in a descending protocol (50, 46, 46, 30, 30, 26, 26, and 14 mg). The treatment started on day 10, 60 h before sponge removal and finished 24 h after. The ewes were inseminated 20 h after estrous onset through laparoscopy with 300 x 106 spermatozoa as fresh semen from a ram of the same breed of the donor with known fertility. Embryo recovery was attempted on day 7 after estrus and ovulation rate was determined through laparoscopy at the same time. The embryos were evaluated considering its morphological characteristics. The results were analyzed by analysis of variance or logistic regression as required and tested using a P≤0.05.
RESULTS
Ovulation rate was similar in P and Ch ewes (21.16 ± 1.80 vs 18.2 ± 0.99), however their responses were higher compared to the response of D ewes (10.7 ± 0.86). There were no significant differences in embryo recovery rate between Ch and P ewes (75% vs 87%) and between Ch and D ewes (75% vs 63%). However, the results were higher in P compared to D ewes (87% vs 63%). The number of transferable embryos was different among the three breeds, being higher in P compared to Ch, and Ch compared to D ewes (18.6 vs 12.8 vs 4.6, respectively).
CONCLUSIONS
The results showed differences between breeds in the response to the superovulatory treatment administered.

T131
EFFECTS OF TWIN PREGNANCY ON PLACENTAL CHARACTERISTICS OF EWES AND BIRTH WEIGHT OF MORADA NOVA LAMBS

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BACKGROUND-AIM
The placenta of mammals is the organ responsible for regulating the fetal growth and viability. As the pregnancy progresses, the cotyledons develop, which increases the maternal-fetal contact surface. Given the prolificacy of Morada Nova sheep, the aim of this study was to investigate whether placental features and lamb weight at birth could be altered by simple or twin pregnancies.
METHODS
The experiment was carried out at Embrapa-Brazilian Agricultural Research Corporation, São Carlos, Brazil. Forty-three Morada Nova sheep (44 months; 42.3±0.53kg) underwent an estrus synchronization protocol followed by controlled breeding. Sheep were kept on a single batch in semi-intensive production system throughout the gestational period. After uterine ultrasonography (Day30 of gestation), sheep were classified according to the number of fetuses: Simple Pregnancy (S, n=24) or Twin Pregnancy (TW, n=19). All births were supervised and no obstetric intervention was required, which resulted in 62 lambs at term. Immediately after parturition, the lamb birth weight (LW) and the total placental weight (PW) were individually measured on automatic scales. Subsequently, the cotyledons were dissected and evaluated for number (CN), diameter (CD), total cotyledon weight per placenta (TCW), and the mean cotyledon weight per placenta (MCW). Data were submitted to analysis of variance (Tukey test; p<0.01).
RESULTS
Twin-births showed significantly higher values (p<0.01) of PW, CN, TCW and MCW compared to single-births (PW: 455.5±24.3 vs 317.4±23.9 g; CN: 74.0±2.2 vs 62.1±3.3; TCW: 111.8±6.5 vs 72.1±3.9 g; MCW: 1.51±0.1 vs 1.19±0.1 g). No significant difference was observed for CD (21.26±0.6 vs 20.06±0.8 mm). LW was lower in Group TW (2.32±0.07 vs 2.94±0.08 kg; p<0.01). Although CD has not been different between groups, a more expressive growth of cotyledonary tissue in twin pregnancies indicates that the placenta of Morada Nova sheep has developed efficient mechanisms to support a multiple gestation. Also observed in other breeds, the lower lamb weight from twin calving did not compromise the viability of lambs.
CONCLUSIONS
The placental morphology of the Morada Nova sheep adapts according to the type of pregnancy, and no detrimental condition is observed in lambs born from twin births.

T132
EFFICIENCY OF CIDR-BASED PROTOCOLS INCLUDING GNRH IN PROPYLENE-GLYCOL FOR ESTRUS SYNCHRONIZATION IN SHEEP

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BACKGROUND-AIM
Traditional protocols for synchronization of estrus and ovulation in sheep, based on intravaginal progestagens plus eCG, are currently compromised because eCG is obtained from pregnant mares. Our group has developed a protocol using GnRH instead of eCG, with controversial results in breeding and non-breeding season. We aimed to evaluate if such protocol may be improved by slowing the release of GnRH, by using propylene-glycol instead of saline solution as vehicle, and the timing of injecting GnRH in propylene-glycol which may allow better results.
METHODS
The present study assessed, during breeding season (November), the response of sheep to the insertion of CIDR devices for five days plus a single 5 mg dose of prostaglandin F2a (Dinolytic®, Zoetis, Madrid, Spain) at CIDR removal. Four experimental groups were used, being treated with 400 IU of eCG (Foligon®, MSD Animal Health, Madrid, Spain; Group eCG; n= 8) or with a single i.m. dose of 50 µg of GnRH (Acegon®, Lab. Syva, Leon, Spain), in either saline solution at 56h after CIDR removal (Group SS; n=8) or in propylene-glycol at 24 or 36h after CIDR removal (Groups PP24 and PP36; n=11 and 10). The ovarian response, in terms of percentage and timing of onset of estrus behavior, was evaluated by individual detection of estrus signs with trained rams every 4h from 24h after CIDR removal. Afterwards, ovulatory efficiency and ovulation rate were evaluated by transrectal ultrasonography on Day 10 of the induced estrous cycle.
RESULTS
The Group PP24 showed a significantly lower occurrence of estrus behavior (18%; P<0.05) than the groups eCG, SS and PP36 (87.5%, 80% and 100% respectively). The timing of onset of estrus behavior in the Groups eCG and PP24 was significantly earlier (30.1±1.5 and 30±2.5h) than in the Groups PP36 and SS (40.0±2.4 and 47.0±3.2; P<0.05). All the responding ewes ovulated without significant differences in the ovulation rate (eCG: 1.5±0.2; SS: 1.6±0.1; PP24: 1.7±0.2; PP36: 1.8±0.2).
CONCLUSIONS
Efficiency of protocols based on GnRH administration either in saline solution at 56h or in propylene-glycol at 36h after CIDR removal, may offer similar results to traditional protocols based on eCG.

T136
ROLE OF FLUNIXIN MEGLUMINE IN AVOIDING PREMATURE REGRESSION OF CORPUS LUTEUM IN SUPEROVULATED DORPER EWES

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BACKGROUND-AIM
Premature regression of the corpus luteum (PRCL) occurs more frequently in animals submitted to the technique of multiple ovulations followed by embryo transfer. Thus, this study assessed the efficacy of flunixin meglumine to reduce the PRCL occurrence in superovulated ewes.
METHODS
Dorper multiparous ewes (n=23) received a conventional superovulatory protocol: 14 days (D14) of progesterone device (changed on D7), and 256 mg FSH (decreasing doses every 12 h, from D12 to D15), plus 200 IU eCG at device removal (D14), and 0.1 mg GnRH on D15. Ewes were randomly allocated into two groups: in FLU (n = 12), three doses of 2.2 mg/kg/day of flunixin meglumine were given on D18, D19 and D20, while in CONT (n = 11), saline was applied. Laparoscopic AI was performed twice on D16 using cooled semen. Embryo collection took place on D22, right after the laparoscopy, to check the CL number/viability. Ewes showing avascular CL (pink to whitish color) were considered to have PRCL. Qualitative data were analyzed by generalized linear models (GLM), with binomial distribution and logit link function. Quantitative data were analyzed by GLM with normal or Poisson distribution (log transformation).
RESULTS
One ewe (FLU) did not respond to superovulation and was excluded. The laparoscopy detected higher (P<0.05) PRCL in FLU (9%; 1/11) than in CONT-ewes (64%; 7/11). Although the total number of CLs in laparoscopy did not differ (P>0.05) between FLU (8.5 ± 1.3) and CONT (10.0 ± 1.2), the number of regressed CLs was lower (P<0.05) in FLU (0.3 ± 0.3 vs 4.8 ± 1.7), resulting in a higher (P<0.05) number of recovered structures (9.1 ± 2.1 vs 3.6 ± 1.4) and viable embryos (5.0 ± 1.1 vs 2.6 ± 1.2). The ova/embryo recovery rate was higher (P<0.05) in FLU (87.4 ± 16.0%) than in CONT (40.0 ± 14.6%). Blood progesterone concentrations were higher (P<0.05) in FLU than in CONT from D17 to D22 and those concentrations (on the day of embryo recovery) were correlated (P<0.05) with the number of CLs (r = 0.42) and number of structures recovered (r = 0.52).
CONCLUSIONS
In conclusion, flunixin meglumine was efficient to reduce the PRCL in Dorper ewes, leading to higher concentrations of progesterone, increasing embryo recovery rate, and number of viable embryos in treated Dorper ewes.
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T137
EFFECT OF SPIKENARD ESSENTIAL OIL AEROSOL ON THE ACUTE STRESS REDUCTION IN RAMS DURING SEMEN COLLECTION BY ELECTRO-EJACULATION

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BACKGROUND-AIM
Electro-ejaculation (EE) is a widely used technique to collect semen for breeding soundness examination in young rams.
The procedure is frequently stressful with a negative effect on animal welfare. In recent years, inhalation of essential oils (EO) as aromatherapy to reduce stress and anxiety in animals was studied. Here, we present the use of spikenard EO
aerosol as a non-invasive method with a potential on the acute stress reduction during semen collection in young rams.
METHODS
In this study, 30 young post pubertal rams of Jezersko-Solčava sheep were randomly divided into three groups: control
(C; no treatment), aerosol (A; 20 min exposure to 0.26% of EO aerosol before EE), and xylazine (X; sedation 10 min before EE with 0.1 mg/kg IM). Plasma cortisol concentration, respiratory (RR) and heart (HR) rate were determined to evaluate the effect of treatments on the stress response for each ram at three time points – before EE, as well as, 30 and 60 min after EE. Semen samples were collected to evaluate the effect of treatment on the semen quality.
RESULTS
Profile changes in cortisol and RR followed quadratic trend, and varied depending on a treatment group ($P = 0.029$, $P < 0.001$). There were no significant differences ($P > 0.05$) in treatments for cortisol levels before EE and 30 min after EE. On the other hand, there was a significant difference at 60 min after EE ($P = 0.029$) between X (5.79 ± 0.31 ng/mL) and C (12.82 ± 2.40 ng/mL), with no difference in A (8.74 ± 1.33 ng/mL). Significant differences in RR occurred with X at 30 min after EE (38.29 ± 2.29 bpm), which increased markedly ($P < 0.001$) as compared to before EE and 60 min after
EE, and as compared to C and A at 30 min after EE – all between 20.00 to 22.00 bpm. There were no differences in HR
between time points and treatments, although a decreasing trend was observed in A (85.20 ± 3.32 bpm) compared to
C (98.80 ± 5.27 bpm) at 30 min after EE. Treatment had no effect on semen quality.
CONCLUSIONS
According to these observations it could be concluded that spikenard essential oil has some tendency to reduce stress. However, these preliminary results suggested more researches in the future on this field to find an optimal aromatherapy approach to reduce stress when breeding soundness examination is performed in rams.

T138
IN VITRO REPRODUCTIVE POTENTIAL OF GENTILE DI PUGLIA SHEEP BREED

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BACKGROUND-AIM
Gentile di Puglia (GdP) is a sheep breed raised in southern Italy, with aptitude for meat, milk and wool production [1]. Despite the historical interest, its economic sustainability is often threatened and the risk of genetic erosion is high [2]. Therefore, conservation strategies are strictly necessary. In this study, the in vitro maturation rate (IVM) and bioenergetic/oxidative status of GdP prepubertal lamb oocytes were compared with those of lambs of other Italian (OI) sheep populations.
METHODS
Cumulus-oocyte complexes (COCs; n.389) underwent IVM and nuclear chromatin evaluation (x2 test). Oocytes at the MII stage were further assessed for mitochondrial distribution pattern (x2 test) and activity and intracellular reactive oxygen species (ROS) levels (Unpaired t-test) [3]. Data statistical significance at $p<0.05$.
RESULTS
The Metaphase II rate (MII %) was significantly higher in GdP oocytes in comparison to OI ones (67%, 115/172 vs 47%, 94/202; $p<0.0001$). The percentages of MII oocytes with heterogeneous perinuclear and subcortical mitochondrial distribution pattern, indicating cytoplasm maturity and competence, did not vary (47%, 53/113 vs 54%, 38/70; $p>0.05$) indicating that oocytes of any sheep population reached cytoplasmic maturity in our culture system. Mitochondrial membrane potential ($\Delta\Psi$: 579.09 ± 218.92 vs 794.21 ± 424.46; $p<0.0001$) and ROS levels (210.56 ± 72.60 vs 234.75 ± 120.71; $p<0.0001$) in MII oocytes were significantly lower in GdP than in OI ones.
CONCLUSIONS
Data indicate that GdP oocytes successfully reached nuclear and cytoplasmic maturation. Interestingly, oocyte $\Delta\Psi$ and ROS levels were lower in GdP, usually reared under pasture-based farming system, compared to OI sheep populations, mainly managed under semi-intensive practices with feeding based on industrial fodder and concentrates [2]. High-fat/high-sugar diet was related with oocyte and embryo $\Delta\Psi$ in a previous study [4]. Further studies will be carried out to investigate in vitro embryo development competence and cryotolerance of GdP oocytes.
Funded by PhD Program 2020 FSC - Piano Stralcio "R&I 2015-2017", University of Bari, Italy
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T139
EXOGENOUS PROGESTERONE SUPPORT DURING SUPEROVULATION INCREASES TGFB1 EXPRESSION IN IN VIVO-DERIVED SHEEP BLASTOCYST

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BACKGROUND-AIM
This study evaluated, in sheep, the effect of medroxyprogesterone acetate (MPA) or P4 (progesterone), during superovulation (SOV) on embryo gene expression.
METHODS
Thirty-six multiparous Santa Inês ewes were used. Prior to SOV ewes were submitted to a short oestrous synchronization protocol (Day 0 concept), consisting in a 60 mg of medroxyprogesterone sponge kept for six days, 24 h before removal 300 UI of eCG and 0.24 mg of cloprostenol were administered, and 36 h after sponge removal 0.025 mg of lecirelin was administered. SOV started 80 h after the sponge removal using 133 mg of FSH (Folltropin®; Vetoquinol) administered in six decreasing doses. At the first FSH dose ewes received a second progestin implant (that was removed at the fifth FSH dose) or non, and were randomly allocated into the groups: GMPA – receiving a 60 mg MPA implant (Progespon®; Zoetis); GP4 – receiving a 330 mg P4 implant (Eazi-Breed CIDR®, Zoetis); GC – without exogenous progestin/progesterone support. For all groups, at the sixth FSH dose 0.24 mg of cloprostenol was administered and, 12 hours later, 0.025 mg of lecilerin (Santos et al., 2020). Ewes were mated by fertile rams. Embryos were collected by laparotomy and classified (I = excellent to IV = poor). For gene expression analysis, total RNA from three pools of five blastocysts (grade I and II) were treated with DNAse prior to reverse transcription and transcripts quantification by RT-qPCR. The expression of each target gene was normalized using the average of GAPDH and H2AFZ and the groups comparison was performed by the comparative Ct method ($2^{-\Delta\Delta Ct}$).
RESULTS
Recovered structures and viable embryos, did not differ ($P > 0.05$) between groups. However, the viability rate ((viable embryos / total recovered structures) x 100) of the GP4 was higher when compared to the CG (72% vs 24%; $P < 0.05$). The mRNA abundance of genes related to pluripotency maintenance (Oct-4 and NANOG), apoptosis regulators (Bcl-2 and BAX), mitochondrial activity (NRF1) and trophectoderm differentiation (CDX2) were not affected ($P > 0.05$). TGFB transcript (cell differentiation and proliferation) was up-regulated ($P < 0.05$) in groups GP4 and GMPA.
CONCLUSIONS
In conclusion, P4 during SOV increases the yield of viable embryos and up-regulate TGFB gene expression.

TOPIC Testis

T141
A MATERNAL HIGH FAT DIET PROGRAMS SERTOLI CELL PRODUCTION OF GDNF, ETV5 AND IGF-I IN ADULT RAT OFFSPRING

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BACKGROUND-AIM
In rats, maternal high-fat diet increases the risks of obesity and premature aging of reproductive capacity in male progeny. We have previously shown that maternal undernutrition can affect transcription factors in the Sertoli cells of adult offspring, including those controlling insulin-like growing factor I (IGF-I), glial-cell line-derived neurotrophic factor (GDNF) and transcription variant 5 (ETV5). Given similarities in phenotypic outcomes for maternal undernutrition and high-fat diets, we hypothesized that maternal obesity would also affect expression of Sertoli cell transcription factors in the testis of adult progeny.
METHODS
We fed pregnant Wistar rats (5 per group) ad libitum with a standard diet (Control) or with a high-fat diet (HFat) throughout both pregnancy and lactation. After weaning, male offspring (n = 10 per group) were fed a standard diet until postnatal Day 160 when they were killed. Testes were collected postmortem, dissected and processed for histological morphometry and immunohistochemistry. Seminiferous tubule diameter and percentage of immunostaining area (%) of GDNF, ETV5 and IGF-I were measured. All data are reported as means ± standard error of mean (s.e.m.) with differences between groups analyzed by t-test with the level of significance at $P < 0.05$.
RESULTS
The diameter of the seminiferous tubules was greater in the HFat group (211.7± 5.2 µm) than in the Control group (195.5±5.2 µm; $P < 0.03$). Likewise, ETV5 percentage of immunostaining area was greater in the HFat group (6.2± 0.31 %) than in the Control group (3.5± 0.33 %; $P < 0.0001$). By contrast, there was less IGF-I immunostaining area in the seminiferous tubules in the HFat group (3.3 ± 0.62 %) than in the Control group (4.9 ± 0.3 %; $P < 0.01$). Similarly, GDNF immunostaining area in testis seminiferous tubules was reduced in the HFat group (7.6± 0.7 %) compared to the Control group (14.3± 0.6 %; $P < 0.0001$).
CONCLUSIONS
Maternal obesity during pregnancy and lactation leads to an increase in the diameter of the seminiferous tubules of adult offspring. Our observations suggest that this effect is mediated by an increase in ETV5 expression because that would increase the size of the spermatogonial cell pool. However, any increase in germ cell population would alter the balance between the numbers of Sertoli cells and germ cells, a major factor in the efficiency of spermatogenesis. Moreover, a maternal high-fat diet also leads to a decrease in the production of growing factors implying a negative consequence in the spermatogenic cycle in the testis of progeny.

TOPIC Uterus

T143
SINGLE-NUCLEI RNA-SEQ REVEALS HETEROGENEITY IN BOVINE UTERINE CELLS AND UNCOVERS CELL TYPE-SPECIFIC TRANSCRIPTOME CHANGES DURING THE ESTROUS CYCLE

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BACKGROUND-AIM
The mammalian uterus is a complex tissue containing three distinct layers- the endometrium, the myometrium, and the perimetrium. Each layer is composed of a variety of cell types. Interactions among those cells regulate uterine structure and function. Here, using single nuclei RNA sequencing, we investigated the cellular composition of the bovine uterus and explored changes in uterine biology that are affected by stage of the estrous cycle.
METHODS
To characterize the transcriptome changes occurring throughout the estrous cycle at the single cell level, endometrial-enriched uterine samples were collected from Holstein heifers in estrus (n=4), proestrus (n=4), and diestrus (n=4). Uterine samples were enzymatically digested, and the nuclei isolated for RNA sequencing. Single nuclei preparations were sequenced using the 10x Genomics Chromium platform, and the data processed using the Cellranger pipeline. Results were analyzed using the R toolkit Seurat and the Python-based toolkit Scanpy.
RESULTS
Unsupervised clustering of single cells identified 7 clusters of epithelial cells, 5 of stromal cells, 5 of endothelial cells, 3 of smooth muscle cells, 2 of neuron like cells, and 1 of immune cells. In one of the epithelial clusters, LGR5, a known epithelial stem cell marker in other tissues, was the third most significant marker gene detected across samples, indicating the presence of an epithelial stem cell population in the bovine uterus. Epithelial and stromal cells were the cell types with the greatest change in the transcriptome across the estrous cycle. A diffusion map for stromal and epithelial cells suggested that the process of cellular trans-differentiation occurred in our dataset. In murine models, mesenchymal-to-epithelial transition (MET) has been reported to contribute to endometrial regeneration during the postpartum period. MET and its reverse process, epithelial-to-mesenchymal transition (EMT), may also play a role in uterine regeneration and uterine remodeling during the estrous cycle in cattle.
CONCLUSIONS
Collectively, results from the present experiment demonstrate a large degree of cellular heterogeneity in the bovine uterus and provide insights into the mechanisms involved with cellular differentiation and renewal in the bovine uterus. Supported by NIH R01HD092254.

T144
CHARACTERIZATION OF BOVINE UTERINE FLUID EXTRACELLULAR VESICLES AT FOLLICULAR AND LUTEAL PHASES OF THE ESTROUS CYCLE

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BACKGROUND-AIM
Extracellular vesicles (EVs) are nanoparticles conveying biomolecules, which influence physiological and pathological conditions. EVs have been identified in uterine fluid (UF), however the bovine UF EV profile during different phases of estrous cycle has not yet been established. We compared UF EVs and their proteomic profile collected at follicular (FP) and luteal phases (LP) of the estrous cycle.
METHODS
UF samples of LP and FP were obtained from 3 live cows (LC) and 3 uteri acquired from slaughterhouse (SC). Isolation of EVs was done using tangential flow filtration in conjunction with size exclusion chromatography. EVs were characterized by nanoparticle tracking analysis (NTA), fluorescent NTA (FL-NTA), zeta potential (ZP), transmission electron microscopy (TEM) and mass-spectrometry (MS) was used to evaluate EV proteomic profile from live cows.
RESULTS
Particle concentrations (mean±SD) were higher ($p<0.05$) in FP than LP in both LC (FP 1.01x108±1.66x107 v LP 7.56x107±1.80x107) and SC (FP 1.17x108±2.34x107 v LP 9.12Ex107±9.77x106), respectively. The proportion of fluorescent membrane dye labelled EVs varied significantly ($p<0.05$) across live and slaughtered cows during different phases of the cycle (LC at FP 28.9±1.9% v LC at LP 19.3±2.8%, SC at FP 26.5±6.3% v SC at LP 27.3±2.7%), respectively. However, the ZP of fluorescent particles did not show a significant difference between live and slaughtered cows at different phases of the estrous cycle. In total, 41 proteins were differentially expressed in LP compared to FP, such as Lanosterol 14-alpha demethylase, Sodium/Potassium-transporting ATPase subunit beta-2, Methylsterol monooxygenase 1 and Aldehyde dehydrogenase family 3 member A2. Bioinformatic analysis showed involvement of altered proteins in cell adhesion and steroid biosynthesis pathways among others.
CONCLUSIONS
The results indicated variations in EV parameters when using different UF materials from live and slaughtered cows as well as different phases of the estrous cycle. Further research is needed to understand the effect of EV changes throughout the cycle and their function on endometrial receptivity.

T145
MACHINE-LEARNING IDENTIFICATION OF ENDOMETRIAL GENES INDUCED BY THE DAY-15 BOVINE CONCEPTUS INDEPENDENTLY OF INTERFERON TAU

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BACKGROUND-AIM
In cattle, conceptus elongation is initiated around day 13 of gestation. At this time, the conceptus starts to secrete increasing concentrations of interferon tau (IFNT), the maternal recognition of pregnancy signal, which significantly modifies the endometrial transcriptome. In addition to IFNT, other factors released by the conceptus may influence the microenvironment and play a role in maternal-conceptus communication. The objective was to employ machine learning (ML) algorithms to identify endometrial genes induced by factors, independent of IFNT, secreted by the Day-15 conceptus.
METHODS
Transcriptomic data were integrated from two independent experiments using endometrial explants collected on day 15 which were co-cultured for 6 h with: (i) nothing (controls, n=10), (ii) 100 ng/ml of IFNT (n=10), or (iii) a single Day-15 conceptus (n=33). Data analysis was done with the R software. After correcting for the study effect, data were pre-filtered, selecting the differentially expressed genes (DEG) between groups. Top genes were identified through Random Forest, comparing the importance of each gene with genes selected at random, repeating this process until all the top genes were confirmed. Finally, these genes were validated with two independent datasets (GSE56392 and GSE30694), using a support vector machine as a classifier.
RESULTS
From the DEG, 95 genes were chosen by the ML algorithm as the top genes, corresponding to 31 IFNT-independent genes upregulated by the conceptus, 54 IFNT-induced genes (but to some degree also upregulated by the conceptus) and 10 genes downregulated by both conceptus and IFNT. The same pattern was found for these genes in the external datasets. Furthermore, endometrial samples treated with IFNT or a conceptus in these datasets were correctly classified when using the expression of these genes as predictors. The 31 IFNT-independent genes were involved in TNF and chemokine signalling pathway (FDR<0.05).
CONCLUSIONS
The application of ML tools allowed the identification of conceptus-induced, IFNT-independent endometrial genes. Expression of these genes could have a biological role in the maternal-conceptus dialogue and thus in pregnancy establishment.
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T146
CHANGES IN ENDOMETRIAL STEROID RECEPTORS IN MARES WITH CHRONIC DEGENERATIVE ENDOMETRITIS TREATED WITH STEM CELLS

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BACKGROUND-AIM
Chronic degenerative endometritis (CDE) is characterized by degenerative changes of the endometrium, which are often associated with subfertility. This study aimed to determine the potential regenerative effects of stem cells therapy on the endometrium of mares with CDE.
METHODS
Fifteen mares with the endometrium classified as grade IIB or III were enrolled in this study. Ten mares were treated with an endometrial injection of autologous bone marrow mesenchymal stem cells (MSCs, 12 x 106 in 6mL of PBS), and five mares were sham-injected (6 mL of PBS). Uterine biopsies were taken during diestrus at 15 before (D0) and 60 (D60) days after endometrial injections. Endometrial score (H/E stained), intensity of fibrosis (Masson's trichrome stain), the extension of collagen type III, and progesterone and estrogen receptors (immunohistochemistry) were evaluated in endometrial samples before and after treatment. The intensity of fibrosis and extension of collagen type III were assessed in biopsy samples using the software AVSOFT BIOVIEW SPECTRA 4.0.1. Distribution of estrogen and progesterone receptors was evaluated using a semiquantitative score (0-4): 0, absence of staining; 4, more than 75% of positive cells.
RESULTS
An improvement in the endometrial score was observed in 6 mares (three mares improved to grade I and three to grade IIA) treated with MSCs, while no changes were observed in the remaining four mares. Morphometric evaluation of endometrial fibrosis showed a reduction (P=0.02) after treatment with MSCs (D0: 1541198±750341 pixel2; D60: 448714±241518 pixel2), as well as the collagen type III was reduced (P=0.007; D0: 726869±63200 pixel2; D60: 130685±7555 pixel2) in mares treated with MSCs. In addition to an increase in the distribution of progesterone receptors (P=0.03; D0: 3; D60: 4), immunolabelling for estrogen receptors tended to increase after MSCs therapy (P=0.06; D0: 1; D60: 3). There were no perceived changes in mares assigned to the control group.
CONCLUSIONS
In conclusion, endometrial MSCs transplantation surges as an alternative to decrease endometrial fibrosis and increases the expression of steroid receptors in the endometrium of mares suffering from CDE.

TOPIC Oviduct

T147
MULTILEVEL EFFECTS OF SUMMER HEAT STRESS ON OVIDUCTAL CHARACTERISTICS IN DAIRY COWS

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BACKGROUND-AIM
The summer heat stress (HS) numbers among the major threats for the sustainability of the dairy industry, due to the induced suppression of production and fertility of cows. It has been shown that HS causes endocrine aberrations and affects the developmental competence of oocytes and early embryos; however, little is known on the effects of HS on the oviductal environment.
METHODS
Here we examined the oviductal epithelial cells transcriptome and the oviductal fluid extracellular vesicles (EVs) characteristics under thermoneutral and HS conditions. Twenty cows were used during the spring (n=10) at Temperature Humidity Index THI =65.6±0.90 and summer at THI=78.36±2.73 (n=10). On day 4 (early diestrous) of a synchronized cycle, a blood sample was collected for progesterone (P4) determination, the animals were slaughtered, and their oviducts were collected. Oviductal fluid and epithelial cells from the ipsilateral and contralateral to the CL oviducts from 3 or 4 animals were separately pooled. To detect differential gene expression, a comparative transcriptomic approach, using RNASeq, in the ipsilateral and the contralateral oviducts was performed, and the results were analyzed using NoiSeq and EdgeR. EVs were isolated by Size Exclusion Chromatography (PURE-EV®), and their size and concentration were characterized by Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy, while specific proteins for EVs were detected by Western blotting.
RESULTS
P4 concentration was higher ($p<0.05$) during the spring. Among seasons, divergent expression of several genes related among others to immune system (haptoglobin), contractility (myosin light chain kinase), gametes' protection (antileukoproteinase) and having regulatory role (lncRNAs) were detected.
The size of the EVs did not differ between seasons. The EVs concentration in the ipsilateral oviduct (4.29 x 1010 EVs/mL) was numerically lower ($p=0.09$) from the contralateral (12.90 x 1010 EVs/mL) in the summer, but not in the spring.
CONCLUSIONS
Our results show for the first time that HS induces disarrangements of oviductal cells gene expression and affects the concentration of EVs in the oviductal lumen; these changes might be attributed to the suppressed P4 levels.

T148
NITRIC OXIDE: A NEW ASSIGNMENT IN MOLECULAR CONTROL OF OVIDUCT CELLS

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BACKGROUND-AIM
Reproductive events that occur in the oviduct depend on synchronized signaling processes, which involves communication between different cell types. Nitric oxide (NO), a short-lived free radical, has a variety of effects on cells, including metabolism and epigenetics processes. Recently, histone deacetylases were identified as intranuclear targets of NO. Thus, given its presence in the oviduct, we hypothesized that differences in NO availability to the bovine oviduct epithelial cells (BOEC) lead to alterations in histone acetylation profile.
METHODS
BOEC, collected from an abattoir, were selected from animals at luteal (L) and follicular (F) phases (six animal/phase), performed in three replicates (pools of two animals/replicate). BOEC were cultured (38°C, 5% CO2, high humid) until 70% confluency, moment of the treatments beginning: control follicular (CF) and control luteal (CL) – basic medium (DMEM+10%FBS); treated with S-Nitrosoglutathione (GSNO), a nitric oxide donor, in two concentrations, 100 and 500 µM, respectively, GSNO100F/GSNO100L and GSNO500F/GSNO500L. Cells were fixed after 4, 48, and 96h of culture and H3K9 acetylation levels were analyzed by immunofluorescence (H3K9ac) Microscopy images were processed by Fiji package and data analyzed by GraphPad Prism software (Kruskal-Wallis test, $p<0.05$).
RESULTS
The H3K9ac levels were dependent of the hormonal phase and treatments. In cells from follicular phase, it was higher in GSNO treatments than control, with higher levels in GSNO500 than GSNO100 in all time points. In cells from luteal phase, similar profile was observed, except for GSNO100 at 4 and 48h, when the highest levels of H3K9ac was observed. Interestingly, at 96h, H3K9ac was higher in cells from F than L, in all groups. Likewise,H3K9ac was higher in GSNO500F than GSNO500L in all time points. It is important to mention that, compared to control, both GSNO100 and GSNO500 treatments led to increase in H3K9ac, demonstrating the role of NO in the molecular control of BOEC.
CONCLUSIONS
NO has a profound effect on the modulation of histone acetylation in BOEC, leading to an overall increase in this epigenetic mark. Future research will reveal the mechanisms by which NO regulates the epigenome during reproductive processes. Supported by FAPESP (19/25982-7, 20/09051-0).

T149
REGULATION OF OVIDUCT EPITHELIAL FUNCTIONS VIA SEX STEROID HORMONES IS INFLUENCED BY CORTISOL

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BACKGROUND-AIM
Maternal stress and cortisol have been linked to the impairment of reproductive events within the oviduct. During estrous cycle, the oviduct epithelium undergoes cyclical transitions, which are mainly regulated by sex-steroids: estradiol (E2) and progesterone (P4). So far little is known about the mutual interaction of cortisol with sex-steroids in this organ.
METHODS
In this study, using the air-liquid interface culture system, porcine oviduct epithelial cells (N = 6) were stimulated with either single hormone (250 nM cortisol, 220 pM E2, 95 nM P4) or a mixture (cortisol & E2, cortisol & P4) from the basal side for 12h or 72h. Epithelial structure and cell populations were analyzed by histomorphometry, while epithelial barrier and bioelectric properties were monitored by measuring the volume of apical fluid, the trans-epithelial electrical resistance (TEER) and trans-epithelial voltage of cells. Statistical analysis was performed using paired t-test or paired Wilcoxon test ($P < 0.05$).
RESULTS
Results showed that in the 72h treatment group, E2 significantly increased the epithelial height and proportion of ciliated cells, while P4 had opposite effects. Likewise, the total cell numbers in the E2 group were higher than in the P4 group. Although cortisol alone exerted no effect on cell morphology, adding cortisol to P4 antagonized the decrease in epithelial height induced by P4. Treatment with E2 reduced the TEER while P4 elevated it. Cortisol alone failed to regulate the TEER, however addition of cortisol blocked the increase of TEER triggered by P4. The trans-epithelial voltage was increased by cortisol and P4, while the E2 induced drop of voltage was antagonized by cortisol. The volume of apical fluid was markedly elevated by E2, but not influenced by either cortisol or P4. In the 12h treatment group, the rise of TEER elicited by P4 was again blocked when cortisol is present, while other parameters remained unaffected by any of the treatment.
CONCLUSIONS
This study confirms that sex-steroid hormones are key regulators differentially modulating the function of the oviduct epithelium. In addition to its individual effect, cortisol could influence oviduct physiology through its interaction with sex-steroid signaling in vivo.
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T150
3D-MODELLING OF THE UTERO-TUBAL JUNCTION LUMEN ACROSS THE ESTROUS CYCLE IN CATTLE

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BACKGROUND-AIM
During the journey of sperm in the female genital tract, the utero-tubal junction (UTJ) has been reported as highly selective. Although 15-20 millions of spermatozoa are usually deposited in the uterus at the time of artificial insemination, only hundreds to thousands can reach the oviduct, where fertilization takes place. However, little is known about the anatomy of the UTJ. The aim of this study was to build a 3D-model of the UTJ lumen and evaluate if the lumen volume changed according to the stage of the estrous cycle in cattle.
METHODS
Bovine genital tracts were collected at a local slaughterhouse and classified into 3 stages according to the ovarian and corpus luteum morphologies (n=7 cows/stage): pre-ovulatory (Pre-ov), post-ovulatory (Post-ov) and luteal (Lut) phases of the estrous cycle. Only ipsilateral UTJ (0.5-cm of uterine horn and 0.5-cm of isthmus) were used. After dissection to remove fat and vessels, the UTJ lumen was filled with 4% low gelling temperature agarose mixed with Indian ink to create a contrast with the mucosa. The samples were embedded in Tissue-Tek and frozen in nitrogen vapors for 15 minutes then stored at -20°C until further processing. Serial transversal sections of 30-µm thickness were videotaped in a cryostat then sections were aligned using ImageJ software. The sections were converted into composite image stacks and the lumen volume was determined by using the Imaris software. Differences between stages were analyzed using the Kruskal Wallis test.
RESULTS
The 3D modelling of the UTJ lumen showed a high variability of its internal conformation between cows. The mean volume of the UTJ lumen was significantly higher during the luteal phase (mean ± SEM: 93.5 ± 12.4 mm3) and at Pre- ov (96.7 ± 17.3 mm3) compared to Post-ov (48.1 ± 6.3 mm3) ($P=0.04$).
CONCLUSIONS
This work provides the first 3D modelling of the internal UTJ in cattle. These preliminary data suggest that the UTJ lumen is a dynamic structure that changes in conformation across the estrous cycle in cattle. The possible role of the narrowing of the UTJ lumen in sperm selection requires further studies.

T151
CHANGES IN MORPHOLOGY AND HEAT SHOCK PROTEINS EXPRESSION IN THE BABOON PAPIO HAMADRYAS OVIDUCT DURING THE MENSTRUAL CYCLE

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BACKGROUND-AIM
The epithelial cells lining the oviduct create a unique environment that is essential in reproduction. Among the substances synthesized from this epithelium there are the heat shock proteins (HSPs), which are involved mainly in protein folding and unfolding and participate in a number of vital processes. We report for the first time the changes in morphology, localization and expression of HSP60, HSP70, and HSP90 in baboon Papio hamadryas oviduct during the menstrual cycle in association with ß-oestradiol (E) and progesterone (P) plasma levels.
METHODS
Oviducts were laparoscopically removed from 14 healthy adult female Papio hamadryas at the Safari Zoo (Fasano, Italy). E and P plasma levels were measured by ELISA in blood samples collected from cephalic vein. The expression of HSPs was evaluated through western blot (WB) using anti HSP60, HSP70, and HSP90 monoclonal antibodies (Santa Cruz Biotechnology). Histological investigations were carried out on fimbriae, ampulla and isthmus separately fixed in 4% (v/v) paraformaldehyde, embedded in paraffin wax, stained with hematoxylin-eosin for histological analysis or immunostained for the localization of HSP60, HSP70, HSP90 using the above-mentioned primary antibodies. Data were analyzed for statistical significance by ANOVA test.
RESULTS
Hormonal assay demonstrated that 4 subjects were in the follicular phase (FP) (E=96.5±18.7 pg/ml; P=0.2 ± 0.1 ng/ml), 5 baboons were in preovulatory phase (PP) (E=193.5±71.8 pg/ml; P=0.3 ± 0.1 ng/ml), 5 animals in the luteal phase (LP) (E=50.1 ±19.9 pg/ml; P=4. ± 0.5 ng/ml). WB revealed the presence of HSPs mainly in the PP oviducts. Histological analysis highlighted a high level of secretory activity in non-ciliated cells during the PP and showed a significant increase of the epithelium height in each oviductal segment from the FP to PP and it decrease in the LP ($P<0.05$). Immunohistochemistry revealed that 1) HSP60 decreased in the ampulla during FP and LP, 2) HSP70 decreased in the LP fimbriae and isthmus, and 3) LSP90 decreased in the LP ampulla.
CONCLUSIONS
The findings suggest that morphological changes and differential expression of HSPs occurring in the oviduct during the menstrual cycle participate in the reproduction of baboon Papio hamadryas.

T152
OVIDUCTAL EXTRACELLULAR VESICLES: MOLECULAR SNITCH OF EMBRYO QUALITY?

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BACKGROUND-AIM
Growing evidence points out the role of extracellular vesicles (EVs) in mediating the early embryo-maternal dialogue in the oviduct. We hypothesise that oviductal EVs and their molecular cargo differs depending on the embryo quality. Therefore, we aimed to characterise and decode the content of EVs derived from the coculture of bovine oviduct epithelial cells (BOEC) with early embryos of good (GE) or poor quality (PE).
METHODS
To do this, in vitro presumptive zygotes were cultured until 53 h after fertilisation and morphologicaly classified into GE (≥ 8 cell) or PE (< 8 cell). Then, embryos were cocultured (n= 50 embryos / group) with frozen-thawed BOEC (25 x 104 cell/ml), after reaching 80% of confluence (SOF without fetal calf serum, at 38.5°C, 5% O2, 5% CO2 was used). Additionally, embryos, BOEC, and media were cultured separately. After 24h, 500 ul of conditioned media (CM) was collected per group, to isolate the secreted EVs by a combination of serial centrifugation, size exclusion chromatography, and ultracentrifugation, followed by EV characterisation and RNA cargo analysis. BOEC viability (Propidium iodide/Hoechst 33342) and Day 7 and 8 blastocyst rates were assessed
RESULTS
BOEC viability was not affected by the co-culture with GE or PE. Blastocyst development (regardless of culture with or without BOEC) was higher for GE compared to PE (53 ± 0,02 % vs 10 ± 0,01% at Day 8, $P \leq 0,001$), confirming that the embryo selection was appropriately performed. Transmission electron microscopy, together with nanotracking analysis confirmed the presence of EVs in all experimental groups, except for the control media (size: 120 to 190 nm; concentration 6 x 108 to 109 particles/ml, with no significant difference among groups). Flow cytometry analysis showed that EVs were positive to CD9 and CD63 markers. The EV RNA profile was analyzed by Agilent Bioanalyzer, showing a typical EVs pattern with short RNA fragments and absence of rRNA peaks, and low RNA yield (2-20 ng/ul) but sufficient for low-input RNA-sequencing which is currently ongoing
CONCLUSIONS
The identification of the RNA cargo and data integration will elucidate if the oviduct can distinguish between good and poor-quality embryos by releasing different EV cargo, unveiling eventual biomarkers relevant for successful pregnancy.

T153

OVIDUCTAL MAGNETIC SPHEROID (OMS): A NEW 3D CULTURE SYSTEM FOR OVIDUCTAL CELLS

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BACKGROUND-AIM
It is widely known that the embryo-oviduct interaction can impact the embryo in ways such as epigenetic markers, metabolism, and improved quality. While studying the oviduct in situ faces access limitations, the in vitro models run into cellular dedifferentiation, low/no embryo production, limited cell lifespan, and/or very complex methodologies. Therefore, our aim was to develop a new oviductal cell culture system for the study of maternal-embryonic communication.
METHODS
For this, we use the magnetic 3D culture system (Greiner Bio-One CELLSTAR®), widely used in many cell types, and which, to our best knowledge, has not yet been used for the oviduct. Bovine oviduct epithelial and fibroblast cells, collected from a slaughterhouse, were separately cultured in a monolayer system. At 80% confluence, cells were trypsinized, counted, and magnetized by centrifugation with the nanoshuttle™-PL. Once magnetized, cells were placed in a 96-well plate cell-repellent surface atop of a magnetic plate, where the magnetic force centers the cells in the well.
RESULTS
In the first experiment, cells were seeded as 50,000, 25,000, 10,000, and 5,000 cells/well. Within three days of culture, both epithelial and fibroblast cells were able to aggregate forming 3D structures of attached cells denominated as Oviductal Magnetic Spheroid (OMS). Regarding the OMS size, the 50,000 and 25,000 cells were too big, resulting in a necrotic center of propidium iodide positive cells due to restriction of media supplements, whereas the 10,000 and 5,00 cells were satisfactory. Next, we performed OMS in a co-culture condition of epithelial and fibroblast cells (ratio of 7:3, 10,000 cells). Incredibly, the immunostaining of OMS revealed a capacity of self-organization, where fibroblast cells (anti-vimentin positive) were located in the inner of the OMS, while epithelial cells (anti-cytokeratin positive) were observed in one layer in the peripheral area, approximating to tissue architecture.
CONCLUSIONS
Altogether, these data show a strong possibility of using the magnetic system to perform a new in vitro culture system for oviductal cells. The future perspective is to be able to evaluate cell redifferentiation, response to hormone stimuli, and cell communication with embryo. Supported by FAPESP (19/25982-7, 20/02500-4).

TOPIC Embryogenesis in vitro

T154

EFFECTS OF EWE AGE ON OOCYTE VIABILITY AND THE TIMING OF EARLY EMBRYO CLEAVAGE

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BACKGROUND-AIM
The quantity and quality of oocytes decline with advancing age in mammalian females. Therefore, the advanced maternal/donor age may adversely affect the development of ovine embryos both in vivo and in vitro.
METHODS
Ovaries were obtained after slaughter from sixteen Longwool breed ewes aged 8-9 years (Group I-"old") and fourteen ewes aged 1.5-3 years (Group II-"young"). Cumulus-oocyte complexes were collected from scarified ovaries and in vitro maturation was performed in TCM 199 medium supplemented with Earle's Salt, 10% of FBS, and 5 µg/mL of LH/FSH at 38 degrees C for 24 h. After maturation, the oocytes were incubated with thawed, capacitated ram semen (IVF) for 19 h at 38 degrees C. Presumptive zygotes (n=48 in Group I and n=64 in Group II) were transferred to a 16-well dish containing Cult medium (Gynemed), and monitored with time-lapse (TL) video imaging for 8 days. The following variables were recorded: time from IVF to the attainment of two-cell (t2), three-cells (t3), or four-cell (t4) stage; morula stage (tM); blastulation (tSB); and blastocyst formation (tBL). The duration of the second cell cycle (cc2) and of the complete synchronous cell division (s2) were calculated, and developmental anomalies (e.g., fragmentation or direct/asymmetric cleavage) were noted.
RESULTS
In Group I, twenty-four zygotes (50.0%) underwent first cleavage division and five (10.4%) developed to the blastocyst stage. Sixteen embryos (33.3%) had morphological defects. In Group II, forty-four zygotes (68.75%) underwent first cleavage division, seventeen (26.6%) developed to the blastocyst stage, and seven embryos (10.9%) had different developmental disorders. Both the cleavage and blastocyst formation rates were higher ($P<0.05$) for Group II, but the percentage of embryos with various defects was greater ($P<0.01$) for Group I. The average time from IVF to t2 was 29 h 20 min and 25 h 42 min in Groups I and II, respectively ($P<0.05$). The duration of cc2 was 4h 14 min and 11 h 20 min, in Groups I and II, respectively ($P<0.05$).
CONCLUSIONS
Oocytes collected from "young" donor ewes had greater developmental potential and gave rise to the embryos with fewer aberrations compared with the oocytes obtained from "old" ewes. TL imaging provides a useful tool for studying the influence of donor age on embryogenesis.

T155
IN VITRO FERTILITY OF SEMEN FROZEN IN TWO COMMERCIAL EXTENDERS OF A URUGUAYAN CREOLE BOVINE SEMEN BANK

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BACKGROUND-AIM
The aim of the study was to evaluate the in vitro fertility (IVF) of semen of Uruguayan Creole (UC) bulls of the semen bank of the Veterinary Faculty. Conservation of local zoogenetic resources must include an ex-situ plan, for this reason our Faculty has got a semen bank of the only reserve of UC cattle and is planing to conserve embryos as well.
METHODS
We evaluated the in vitro fertility (IVF) of semen of 4 UC bulls, frozen in 2 extenders, one containing egg yolk, Triladyl (T) and the other one vegetal lecithin, Adromed (A). Individual motility and concentration of different straws were subjectively studied. 404 Oocyte-cumulus complexes (OCC), obtained from slaughterhouse ovaries, were matured in 100µl drops of TCM-199 + 5% FBS + hormones, 15 OCC/drop, covered with mineral oil, 22h at 38.5°C, 5%CO2 and 95% humidity. After maturation, 8 groups were formed and inseminated with the selected semen, frozen in both extenders: 1A; 1T; 2A; 2T; 3A; 3T; 4A and 4T. The capacitation was performed using Percoll gradients in Talp-Sperm and adjusted to 2x10 6 spz/ml with Talp-Fert. 100µl drops were formed, covered with mineral oil and the matured oocytes were placed in and co-cultured, 5h. The gametes were denuded by vortex and cultured in CR1aa + 5% FBS, in drops, in incubator. At 48h, division rate (DR) was evaluated, on day 7 embryonic development (ED) (IETS standards). The results were analyzed with x2 ($p<0.05$) using R software.
RESULTS
The straws of the different treatments had a concentration of 30-40 million/straw of 0.5ml, individual motility of 1A, was 30%; 1T, 50%; 2A, 20%; 2T, 30%; 3A, 30%; 3T, 30%; 4A, 70% and 4T, 50%. There were no significant differences in DR between 1 (73/109) and the 4 bull (68/101) ($p>0.05$), neither between them and bull 3 (77/102) and 2 (53/92). However, the 3 showed a greater DR with respect to the 2 ($p=0.008$). There were no significant differences in ED between the 4 bulls (35; 30; 37 and 35, respectively) ($p>0.05$). Comparing the behavior of the extenders, we found that did not have differences among them in DR (A: 143/207; B: 128/197), nor ED (69 and 68 respectively) ($p>0.05$).
CONCLUSIONS
The IVF rate observed in DR and ED had a good behavior in all samples. Both extenders seem to have a good performance. This work was the first one studying the UC semen in IVF, frozen in 2 extenders, providing a better knowledge of the IVF of the UC semen bank.

T156
THE EFFECT OF L-CARNITINE IN THE PRESENCE OF FATTY ACIDS DURING IN VITRO MATURATION ON BOVINE OOCYTE METABOLISM AND EMBRYO DEVELOPMENT

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BACKGROUND-AIM
Blastocyst development increases when β-oxidation is enhanced by inclusion of L-carnitine during mouse follicle culture. Additionally, L-carnitine enhances oocyte maturation within early antral follicles by increasing mitochondrial activity. L-carnitine can also regulate lipid metabolism during embryo culture. However, L-carnitine incorporation in semi-defined in vitro maturation (IVM) media has never tested in the presence of a specific mixture of fatty acids (FAs). The current study, therefore, sought to identify the effect of L-carnitine inclusion during IVM in the presence of this FA mixture on energy metabolism and embryo development.
METHODS
Six cycles of transvaginal follicular aspiration were performed in four sexually mature (12-15-month-old) Holstein-Friesian heifers. COCs were matured in TCM199 as described previously (Tutt et al., 2021; Theriogenology 161: 108-19), but without inclusion of serum, to which was added 25 µM palmitic, 15 µM stearic, 15 µM oleic, 5 µM linoleic, and 5 µM a-linolenic acid in either the presence or absence 2.5 mM L-carnitine. Embryo development and spent media composition were established from the first four cycles. In the final two cycles, following IVM, COCs were denuded for ATP and mtDNA copy number analyses. Data analyses employed mixed-linear models in REML which assumed binominal errors for proportions.
RESULTS
Proportion of Day 8 embryos increased ($P=0.008$) in the presence than absence of L-carnitine (0.298 ± 0.0303 vs 0.157 ± 0.0253); as did ATP production (0.406 ± 0.0413 vs 0.347 ± 0.0401; $P=0.064$) and mtDNA copy number (1330 ± 136.2 vs 692 ± 133.6; $P<0.001$) in matured oocytes. Analyses of spent IVM media indicated that both glucose uptake (0.101 ± 0.0289 vs 0.148 ± 0.0289 µmol/mL) and lactate production (0.196 ± 0.0517 vs 0.300 ± 0.0517 µmol/mL) FAs only and with L-carnitine, respectively, were not altered significantly ($P=0.109$).
CONCLUSIONS
L-carnitine increases mtDNA copy number and ATP production in oocytes; the latter possibly from β-oxidation of FAs, as glucose uptake and lactate production appeared to be reduced. Importantly, L-carnitine incorporated into IVM media improves embryo development in the presence of our cocktail of FAs.
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T157
METABOLOEPIGENETIC MODULATION DURING EARLY DEVELOPMENT PROMOTES ALTERATIONS IN ENERGY HOMEOSTASIS THAT LAST UP TO BLASTOCYST STAGE

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BACKGROUND-AIM
Embryos are interesting models to study metaboloepigenetics, since they undergo broad changes in metabolism and widespread epigenetic remodeling. In a previous work, our group demonstrated that the modulation of a-Ketoglutarate (AKG) and Succinate (SUC) ratio, two intermediates of the tricarboxylic acid cycle (TCA), was capable to alter the levels of 5-methylcytosine (5mC) in embryos (Ispada, 2020). Nevertheless, TCA cycle also generate the reducing equivalents NADH and FADH2, required to transfer electrons to the mitochondrial respiratory chain, which may alter energy homeostasis and consequently, embryo development. Thus, in the present work we assessed the consequences of AKG:SUC ratio modulation on NADH and FAD+ and mitochondrial function of bovine blastocysts.
METHODS
Bovine embryos were in vitro produced using standard protocols and cultured as control (CO) or treated from day 0 (of cleavage) until day 4 with analogs for AKG or SUC. Embryos were collected at day 4 and 7 and the levels of mitochondrial membrane potential (MMP - MitoTracker Red CMXRos dye) and NADH and FAD+ (autofluorescence) were evaluated. Data was quantified in embryos from day 4 and day 7 (inner cell mass-ICM) with ImageJ and analyzed using GraphPad Prism.
RESULTS
Embryos from SUC treatment presented higher MMP at day 4 (CO= 38.2±8.1AU; SUC= 47±10.3AU; p<0,05) and lower MMP at day 7 (CO= 23.7±7.8AU; SUC= 18±6.2AU; p=0,05) when compared to the control. This group, while not presenting difference in NADH or FAD+ amounts on day 4, had respectively lower (CO= 40.4±11.8AU; SUC= 30.9±8.5AU; p=0.05) and higher (CO= 43.8±13.8AU; SUC= 51.3±20.2AU; p<0.004) quantity of those on day 7. The treatment with AKG reduced NADH (CO= 40.4±11.8AU; AKG= 28.6±9.4AU; p<0.05) in embryos at day 7, without any alteration in embryos at day 4 or FAD+ and mitochondrial activity both at day 4 and 7.
CONCLUSIONS
Considering the changes promoted by AKG:SUC supplementation, it is possible to assume that beyond epigenetic alterations, changes in the availability if these intermediates during in vitro embryo development affect mitochondrial function, with consequences to energy homeostasis.

T158
MELATONIN SLIGHTLY IMPROVES EMBRYO QUALITY DURING IN VITRO CULTURE OF BOVINE EMBRYOS IN SERUM-FREE MEDIUM

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BACKGROUND-AIM
In vitro culture is known to induce oxidative stress and melatonin (MEL) is a potent antioxidant. Effects of MEL supplementation to in vitro culture medium of bovine embryos in a serum free culture system were assessed.
METHODS
Bovine oocytes were in vitro matured for 24h (TCM-199 with 0.5 µg/ml FSH and 3mg/ml BSA), followed by in vitro fertilization (1.0x106 sperm cells/ml in TALP medium) for 18-20h; both cultures under 5% CO2 in air at 38.5oC. Zygotes were washed and placed in culture medium (CR2aa with 3mg/ml BSA) supplemented with MEL (0, 10-11, 10-9 and 10-7M) for seven days under 5% O2, 5% CO2 and 90% N2 and 38.5oC. Cultures were in four well plates (500µl/well) without oil. Cleavage and blastocyst rates were determined on days 2 and 7 (186-191 oocytes/group). Day 7 blastocysts were fixed and stained for TUNEL assay (31 to 37/group), and total cell numbers and apoptosis rates determined. Next, day 7 blastocysts cultured in vitro without (n=38) or with 10-9M MEL (n=37) were vitrified. After vitrification-warming, reexpansion and hatching rates were determined after 72h culture. Experiments were replicated four times and data analysed by one-way ANOVA followed by Tukey test at 5% significance.
RESULTS
Cleavage (87.1±8.9 to 90.1±8.4%) and blastocyst rates (34.6±4.5 to 41.3±9.0%) were not affected by MEL (P<0.05). Total cell numbers were increased (P<0.05) in 10-9M MEL (165.3±38.5 cells) relative to control and 10-7 M (~129 cells in both groups), while 10-11M did not differ from any group (144.7±49.4 cells, P>0.05). The proportion of apoptotic cells was similar among groups (5.6±4.2 to 7.2±4.8%, P>0.05). Most embryos reexpanded and survived vitrification at 72h in both groups (97.5±2.5 and 88.4±1.9 % for control and melatonin groups, respectively, P>0.05). Hatching rate also did not differ (81.9±14.3 and 65.7±4.2%, respectively, P>0.05).
CONCLUSIONS
In conclusion, melatonin addition to a semi-defined serum free medium during in vitro culture did not improve development rates of bovine embryos, but did slightly improve embryo quality increasing total cell numbers; MEL showed limited benefit under the culture conditions studied. Funding: SSC FAPESP-Brazil 2019/2596-4-9; CLVL FAPESP-Brazil 2019/18019-6; CNPq-Brazil 304276/2018-9.

T159
EFFECT OF MELATONIN DURING IN VITRO CULTURE ON BOVINE EMBRYO PRODUCTION, NUMBER OF CELL COUNT AND MTDNA COPY

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BACKGROUND-AIM
Melatonin (MLT), synthesized in pineal gland and several other tissues, shows varied actions including effects on reproductive competence, influence on different cell signaling pathways and antioxidant and antiapoptotic activities. Mitochondria are essential for energy production, but also site of generation of reactive oxygen species (ROS), which can lead to mtDNA damage. The aim of this study was to assess the effect of MLT during in vitro culture on embryo development, total and differential cell number count and mtDNA copy number in bovine blastocysts.
METHODS
Cumulus-oocyte complexes were matured in vitro for 24h and fertilized in vitro for 20h in BO-IVM and BO-IVF (IVF Bioscience), respectively. Presumptive zygotes (n=50/treatment; 3-4 replicates) were cultured in vitro in bovine optimized embryo culture medium with 10% fatty acid free bovine serum albumin (SANTIQUET et al., 2017) supplemented with MLT (0, 10-11, 10-9 and 10-7M) for 7 days at 38.5°C, 6% CO2 and 5% O2. Data were analyzed by one-way ANOVA and Tukey test (p<0.05; GraphPad Prism software).
RESULTS
Cleavage rates (day 3) did not differ between control (0M MLT) and different MLT concentrations (range: 65.11± 0.53 to 70.80±0.21 %, p>0.05). Blastocyst rates were also similar among groups (range: 19.46 ± 0.47 to 23.17±0.38 %, p>0.05). Day 7 blastocysts showed no difference for cell number in inner cell mass (range: 36.4 ± 4.3 to 52.8 ± 3.8 cells, p>0.05). Both trophectoderm and total cell numbers were similar among groups (trophectoderm range: 72.3 ± 9.4 to 105.4 ± 8.4 cells and total range: 108.7 ± 13.4 to 158.3 ± 11.3 cells, p>0.05). For mtDNA copy number, treatments were also not different (range: 2379.3 ± 19.3 to 2425.1 ± 13.6, p>0.05).
CONCLUSIONS
In conclusion, under the culture conditions assessed, MLT was unable to influence embryo production or cell numbers and mtDNA copy number in day 7 blastocysts.
Funding: FAPESP-Brazil (HF-2016/24884-3, 2018/19852-0, CLVL-2015/20379-0; 2019/18019-6).

T160
CO-CULTURING LOW-QUALITY SHEEP OOCYTES WITH DENUDED OOCYTES DURING IN VITRO MATURATION

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BACKGROUND-AIM
The low-quality cumulus-oocyte complexes (COCs) are less likely to generate embryos, due to their low number of granulosa cell layers (GC). In addition, denuded oocytes secrete factors that regulate a broad range of granulosa cell functions, therefore, they could support the competence of low-quality COCs and, therefore, embryo development. The study aimed to evaluate co-culturing low-quality sheep COCs with denuded oocytes during in vitro maturation, and its effect on embryo development.
METHODS
COCs were obtained of ovaries from a slaughterhouse and classified based on the number of GC layers. Only low-quality COCs (<3 GC layers and homogeneous cytoplasm) and denuded oocytes were considered. The in vitro maturation was carried out in TCM medium (In vitro, Mexico City, Mexico) supplemented with 10% SFB (Mayimex, Mexico City, Mexico) and hormones for 24 h at 5% CO2, 38.5 °C, and humidity at saturation. The fertilization was carried out with fresh semen (1 × 106 mL-1) in a fertilization medium (In vitro, Mexico City, Mexico). The presumed zygotes were cultured in Cleavage and Blastocyst medium (Cook IVF, Brisbane, Australia) for 72 and 96 h, respectively, until the blastocyst stage. The experiment was a completely randomized design, where the COCs were assigned to one of two treatments, T1 (control)= low-quality COCs maturation (n= 160) or T2 = co-culturing low-quality COCs + denuded oocytes (n= 160 + 80, respectively). The morula, blastocyst, and grade one blastocyst rate were analyzed with GENMOD, and the blastocyst diameter with GLM from SAS 9.3.
RESULTS
No difference (p> 0.05) was found between T1 and T2 in morula rate (68.5 ± 3.65 vs. 75.6 ± 3.39%). However, the blastocyst rate was higher (p< 0.05) in T2 than in T1 (26.8 ± 3.50 vs. 17.9 ± 3.01%). The blastocyst diameter was similar in T1 and T2 (222.62 ± 9.92 vs. 235.20 ± 7.97 µm, p> 0.05). Nevertheless, the grade one blastocyst rate was higher (p< 0.05) in T2 than in T1 (58.7 ± 7.26 vs. 36.6 ± 8.79%).
CONCLUSIONS
In conclusion, co-culturing low-quality sheep COCs with denuded oocytes during in vitro maturation improved the blastocyst and grade one blastocyst rate, under the conditions of this study.

T163
EFFECT OF FOLLICULAR FLUID EXOSOME-LIKE EXTRACELLULAR VESICLES ON THE QUALITY OF BOVINE OOCYTES MATURED IN VITRO.

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BACKGROUND-AIM
Follicular fluid extracellular vesicles (ffEVs) affect different functions of follicular cells and can improve quality of enclosed oocytes due to ffEV cargo of different proteins, RNAs and lipid. The aim of the study was to determine optimal concentration of ffEVs preparations in IVM medium that is beneficial to blastocyst development and quality.
METHODS
Differential centrifugation of follicular fluid (FF) from 3-8 mm follicles was used for extraction of ffEVs. IVM medium (TCM199 with 100 ng mL-1-1 EGF and 3 mg mL-1-1 BSA, used as a control) was supplemented with ffEVs in concentration either equivalent to FF (ffEV extracted from 1 mL of FF was added to 1 mL of IVM medium, 1:1), or twice concentrated (2:1), or twice diluted (1:2). Cumulus-oocyte complexes from the same size follicles underwent IVM during 24h, followed by 16h IVF in BO-IVF medium, and embryo development in BO-IVC medium (IVF Bioscience, England), until Day 5, and then in the same medium supplemented with 5% FCS up to Day 7, at 38.5°C and 5% CO2. Blastocyst total cell number and apoptosis rate were determined using DAPI and TUNEL staining. The data from 3-4 independent experiments were analyzed by ANOVA and Tuckey test.
RESULTS
Maturation and cleavage rates, determined during post-IVF oocyte stripping and at Day 2 of IVD, respectively, did not differ between conditions, whereas blastocyst rate was higher in the groups supplemented with FF-equivalent (33.1±3.2%, $p<0.05$) or concentrated ffEVs (36.0±2.1%, $p<0.01$) than in control group (22.4±2.3%). Moreover, corresponding blastocysts showed lower apoptotic rate (3.8±0.4 and 3.3±0.4%, respectively, $p<0.05$), compared to control group (6.0±0.9%). The most significant effect in term of embryo cell number was observed with two-fold concentrated ffEV preparations (82.7±2.3 vs 69.0±1.6 in control, $p<0.01$) suggesting more efficient ffEV uptake by the oocytes at this concentration.
CONCLUSIONS
In conclusion, simple preparation of ffEVs by differential centrifugation is enriched in exosome-like vesicles, and may be used during IVM, at concentrations at least equivalent, or superior to FF, to obtain higher quality blastocysts. The study was supported by the Russian Science Foundation (project No. 19-16-00115).

T165
IMPACT OF EXPOSURE TO HIGH NON-ESTERIFIED FATTY ACIDS DURING IN VITRO FERTILIZATION ON TRIMETHYLATION OF HISTONE H3 AT LYSINE 27 IN BOVINE EMBRYOS

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BACKGROUND-AIM
Exposure to elevated levels of non-esterified fatty acids (NEFA) such as steric acid (SA), palmitic acid (PA]) and oleic acid (OA) during in vitro oocyte maturation (IVM) and embryo culture can impair preimplantation embryo development in cattle. However, the effect of high NEFA during the fertilisation process per se have been less studied. In the present study histone H3 at lysine 27 trimethylation (H3K27me3) was analysed in bovine embryos exposed to high NEFA during in vitro fertilisation (IVF). H3K27me3 is a repressive epigenetic mark that undergoes gradual erasure from fertilisation to the time of embryonic genome activation (EGA) to allow a correct EGA and progression to the blastocyst stage (Epigenetics 2012;7:976-81).
METHODS
After IVM, IVF took place under different NEFA levels representing physiological (Control-1[C1], 28µM SA, 23µM PA, 21µM OA) and pathophysiological (High-NEFA, 280µM SA, 230µM PA, 210µM OA, levels found in cows experiencing negative energy balance) concentrations. A second control (C2) group contained solvent. Presumptive zygotes were then culture, and resultant 2- and 4-cell embryos were collected on day 2 (IVF=day 0) to examine H3K27me3 protein expression by immunofluorescence and confocal microscopy. Fluorescence levels were quantified with the Imaris software. Data were analysed (SPSS statistical software) by ANOVA and T-test (mean±SEM), with percentage data arcsine transformed before analysis.
RESULTS
Cleavage rate was decreased in the high-NEFA group compared to controls (C1=57.1±7.4%, C2=59.1±7.1%, High-NEFA=29.8±2.6%, P=0.012). H3K27me3 levels (Log10 transformed data) in 2- (C1; n=31, C2; n=29, High-NEFA; n=29) and 4-cell (C1; n=23, C2; n=32, High-NEFA; n=13) embryos was not different between the groups. There was no difference in H3K27me3 expression between 2-cell and 4-cell embryos in the control groups. However, in the high-NEFA group, 2-cell embryos displayed a higher H3K27me3 expression than 4-cell embryos (P=0.038).
CONCLUSIONS
Our data suggest that activation of key developmental genes may be delayed in embryos experiencing a high NEFA microenvironment during fertilization, which partially explain the decreased blastocyst formation previously observed in this model of high NEFA exposure (ESHRE meeting 2021, P-176).

T166
GENE EXPRESSION ANALYSIS OF TROPHECTODERM MARKERS IN DOMESTIC CAT BLASTOCYSTS CULTURED WITH AND WITHOUT ZONA PELLUCIDA

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BACKGROUND-AIM
Domestic cat embryos cultured without zona pellucida have a reduced implantation capacity after embryo transfer at the blastocyst stage. The objective of this study was to evaluate the expression of trophectoderm markers in domestic cat blastocysts cultured without zona pellucida.
METHODS
Two experimental groups were done: 1) Domestic cat embryos cultured until the blastocyst stage (Zona intact group, ZI). 2) Domestic cat embryos cultured until the blastocyst stage without zona pellucida (Zona free group, ZF). Ovaries were collected from domestic cats subjected to ovariohysterectomy. Cumulus-oocyte complexes (COCs) were matured in vitro in supplemented Medium-199, in 5% CO2, at 38.5°C for 26 hours. For IVF, 20-30 COCs were co-cultured with 1.5-2.5 x 106 spermatozoa/mL in supplemented TALP medium, in 5% CO2, at 38.5°C for 24 hours. After IVF, the zona pellucida was removed by incubation in 2 mg/mL pronase for 4 minutes. ZF-zygotes were cultured in microwells using the well of the well system (WOW). In both groups, IVC was done in supplemented SOF, in 5% CO2, 5% O2 and 90% N2, at 38.5°C, for seven days. The cleavage, morula and blastocyst rates were estimated. The relative expression of the trophectoderm markers CDX2, YAP1, TEAD4 and E-cadherin and the apoptosis marker CASP3 was evaluated in the blastocysts by RT-qPCR using the ΔΔ-Ct method. SDHA was used as internal control. The Wilcoxon test was used to evaluate differences ($P < 0.05$).
RESULTS
No differences were observed in the in vitro development between the ZI and ZF groups. Cleavage rate: ZI = 310/641 (48.4%); ZF = 216/375 (57.6%). Morula rate: ZI = 180/310 (58.1%); ZF = 124/216 (57.4%). Blastocyst rate: ZI = 81/310 (26.1%); ZF = 55/216 (25.5%). No statistical differences were found in the relative expression of CDX2, YAP1, TEAD4 and CASP3 between blastocysts from the ZI and ZF groups. The relative expression of E-cadherin was higher in ZF-blastocysts than in ZI-blastocysts. This higher expression might be needed to enhance cellular adhesion and compaction of blastomeres in the ZF-embryos.
CONCLUSIONS
In conclusion, the culture without zona pellucida did not affect the in vitro development of domestic cat embryos and their expression of trophectoderm markers.
ANID FONDECYT 3200352

T167
CO CULTURE OF EMBRYOS WITH CUMULUS CELLS PROVIDE METABOLIC SUPPORT FOR OPTIMAL BLASTOCYST DEVELOPMENT IN VITRO

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BACKGROUND-AIM
In vitro embryo development rates are suboptimal when compared to in vivo. Despite of significant advances in embryo culture, an optimal approach is still lacking. We developed a co-culture protocol of pre-implantation embryos with cumulus cells (CCs), further analyzing the impact on embryo metabolism and morphology.
METHODS
Cumulus-oocyte complex collection 23-day-old female mice were superovulated with equine chorionic gonadotropin. 48h later, ovulation was induced with human chorionic gonadotrophin. Animals were sacrificed 14h later and expanded COCs were retrieved from the ampulla. IVF and embryo culture COCs were placed in IVF medium and motile sperm were added. After incubation, oocytes were denuded and randomly assigned to embryo culture dishes corresponding the 2 culture conditions: Simple Culture group (SC) and Co-Culture group (CC). The embryos were cultured for 5 days, in presence of fresh, age matched CCs from donor mice for the CC group, or without CCs for the SC group. Embryo development assessment Two cells embryo rate was assessed 24 after insemination. On day 5, blastocyst rate and hatching were evaluated. Metabolism pattern analysis Levels of lactate, pyruvate, citrate, alpha-ketoglutarate, malate, NADPH and NADP+ were detected in embryos. Also, activity levels of lactate dehydrogenase, glucose-6-phosphate, phosphofructokinase and aconitase were identified. Detections were assessed colorimetrically or fluorimetrically using specific kits and a spectrophotometer.
RESULTS
No significant difference were observed in the 2-cells embryos rate for the SC (82%, n=103) and CC group (80%, n=112). Blastocysts formation rates were comparable as well, 80% for SC group (n=82) and 81% for CC group (n=91). However, Embryos from CC group presented higher levels of lactate, pyruvate, NADP+ and alpha-ketoglutarate, and higher activity level of aconitase enzyme ($P<0.05$). None of the analyzed parameters was shown to be higher in SC group.
CONCLUSIONS
The presence of CCs allowed the embryos to fully activate their metabolic pathways, producing higher rates of intermediates necessary for proper glucose metabolization, biosynthesis and energy production. We present co-culture with CCs as a simple, free and effective additive to improve in vivo embryo culture.

TOPIC Camelid reproduction

W01
COMPARISON OF TWO COMMERCIAL EXTENDERS FOR LLAMA SEMEN COOLING

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BACKGROUND-AIM
The use of commercial extenders for semen cryopreservation instead of the ones being handmade allows more repeatable results due to their less variable composition. There are no reports of the use of commercial extenders for cooling llama sperm. The objective of this study was to compare the effect of Andromed® (AM) and Androstar Plus® (AS) extenders on cooled llama sperm.
METHODS
The study was carried out in the Faculty of Veterinary Sciences of the University of Buenos Aires, Argentina. Fourteen ejaculates were collected from 4 adult llama males using electroejaculation. Each ejaculate was evaluated, divided into two aliquots and diluted with AM and with AS (0 h), achieving in both a final concentration of 50x106 sperm ml-1. Samples were cooled at 5 °C in Equitainer® and the following seminal characteristics were evaluated after 24 and 48 h of storage: motility, membrane function, viability, acrosome integrity, morphology and chromatin condensation. Data were analyzed using a factorial design (raw semen, AM0, AS0, AM24, AS24, AM48 and AM48).
RESULTS
A significant decrease in total sperm motility was observed in AS 24 and 48 h when compared to 0 h (AS48: 16.4±15.3%, AS24: 23.4±12.9% and AS0: 52.6±13.8%, mean ± SD). While, samples cooled with AM showed a significant decreased in total motility only after 48 h of storage (AM48: 21.0±21.4%, AM24: 29.5±25.4% and AM0: 51.1±17.4%). A significant decrease in the percentage of live sperm with intact acrosome was observed in AM 48 h when compared to 0 h (AM48: 27.6±22.6% and AM0: 60.0±19.6%). However, a significant increase in the percentage of live sperm with reacted acrosome was observed in AM 48 h when compared to AS 24 and 48 h (AM48: 7.5±7.5%, AM24: 3.8±4.3%, AS48: 0.3±0.4% and AS24: 0.3±0.5%). Regarding membrane function, a significant decrease was observed in AS 48 h comparing to raw semen (AS48: 28.7±12.6% and raw: 50.9±18.4%). Although no significant differences were observed in sperm with normal morphology and with condensed chromatin, a greater percentage of abnormal tails was observed in AS samples comparing to raw and AM samples (AS48: 21.3±12.6%, AS24: 19.2±6.0%, AM48: 9.5±4.7%, AM24: 10.9±5.2% and raw: 11.8±7.9%).
CONCLUSIONS
The Andromed® extender showed better results and could be considered as an option for cooling llama sperm.

W02
COMPARING THE EFFICIENCY OF TWO SIMPLIFIED SUPEROVULATION PROTOCOLS IN DROMEDARY CAMELS

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BACKGROUND-AIM
Traditionally, superovulation (SO) was induced by twice daily administrations of FSH for 4-7 days in dromedary camels. We have developed a simple SO protocol for camels, in which two doses (48 h apart) of slow-release preparation of FSH (FSH dissolved in hyaluronan solution) induces successful SO (Manjunatha et al., Theriogenology 2019;126:214-221). Recent studies in our centre showed that a single dose of eCG produces a comparable embryo yield to the traditional SO protocol and this protocol would be more simple, economical and also practical. This study was aimed to compare the superovulatory response of camels treated with either two doses of slow-release Folltropin-V or a single dose of eCG.
METHODS
Follicular development of camels (aged 7-18 years) was synchronized by GnRH on Days -22 and -12 and PGF2a on Days -15 and -5. On Day 0, animals were treated with a further injection of GnRH to synchronize wave emergence and were randomly divided into two groups. Camels in group I (n = 22) received 200 mg of slow-release Folltropin-V (Folltropin-V diluted in 5mg/mL hyaluronan solution) in two IM injections on Day 4 (120 mg) and Day 6 (80 mg). Camels in group II (n = 28) received a single IM dose of 3000 IU eCG on Day 4. PGF2a was administered on Day 7 and camels were mated on Day 11. The ovaries were examined by ultrasonography on Days 11 and 13 to record the number of ovulatory follicles (≥ 9 mm in diameter) and ovulations. Animals were flushed 8 days after mating (Day 19) and all embryos counted and graded (Scale: Grade I-IV) based on gross morphological appearance, stage of development. Statistical analysis was performed using linear MIXED model in GENSTAT (version 17, VSN Int.).
RESULTS
The mean (± SEM) number of ovulatory follicles (14.6 ± 1.1 versus 13.2 ± 1.1), ovulations, (13.9 ± 1.0 versus 12.3 ± 1.0), total embryos (5.8 ± 0.9 versus 5.1 ± 0.8) and transferrable embryos [Grade I-III (includes only hatched blastocyst stage): 5.4 ± 0.8 versus 4.9 ± 0.6] did not differ between groups (P > 0.05). All camels responded with ≥ 5 ovulations in both groups.
CONCLUSIONS
Camels treated with Folltropin-V and eCG resulted in similar superovulatory response. This indicates that eCG can be used as an alternative to FSH, however, repeated use of eCG to induce SO needs to be determined in this species.

W03
METHODS FOR ELIMINATION OF SEMINAL PLASMA VISCOSITY AND ITS RELATIONSHIP WITH OXIDATIVE STRESS IN ALPACA SPERMATOZOA

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BACKGROUND-AIM
The high viscosity of alpaca semen obtained through artificial vagina makes laboratory handling difficult, for this reason, are used different methods allow viscosity elimination of seminal plasma in alpaca. Has been proved that these methods can affect the structure and functionality of spermatozoa, but not if exist an increase of reactive oxygen species production and hence oxidative stress. This study aimed to evaluate the effect of three seminal plasma viscosity elimination methods on oxidative stress in alpaca spermatozoa.
METHODS
Ten ejaculates of alpaca obtained by artificial vagina coupled on a dummy were employed in this study. Each sample was divided into three aliquots for the assessment of Mechanic Method (MM), Centrifugation (C), and Enzymatic Digestion (ED) with Papain and E-64. After treatments, flow cytometry was used to evaluate sperm viability (SYBR14/PI), mitochondrial membrane potential (MitoTracker Deep Red FM), lipid peroxidation (BODIPY® 581/591), and mitochondrial superoxide production (MitoSOX™ Red). Sperm motility was also assessed. The effect of the methods on the percentages of motility, sperm viability, mitochondrial membrane potential, lipid peroxidation, and mitochondrial superoxide production were evaluated using a one-way ANOVA.
RESULTS
Percentages of sperm motility, sperm viability, and mitochondrial membrane potential, were higher on samples treated with the ED method ($p<0.05$). In addition, percentages of mitochondrial superoxide production and lipid peroxidation and were lower on samples treated with the ED method compared to the other two techniques.
CONCLUSIONS
We conclude that treatment with Papain and E-64 (Enzymatic Digestion) for the elimination of seminal plasma viscosity in alpacas is better than mechanic method and centrifugation because produces between 10 to 20% less oxidative stress, respectively.

W04
EFFECT OF 1000 IU OF SYNTHETIC ECG LIKE GLYCOPROTEIN ON FOLLICULAR DEVELOPMENT AND EMBRYO RECOVERY IN LLAMAS

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BACKGROUND-AIM
The aim of this study was to evaluate the effect of a synthetic eCG like glycoprotein on follicular development and embryo production, as an alternative to native eCG in llamas.
METHODS
Twenty non-gestating and non-lactating llamas were examined daily by transrectal ultrasonography (tUS) to asses ovarian status (MyLab One Vet, ESAOTE) until a growing follicle with a diameter ≥7 mm, considered ovulatory in this species, was observed. At that moment, females received 8 µg of a GnRH analog (IV) (buserelin acetate, Gonaxal® Biogénesis Bagó, Argentina) (Day 0). On Day 3, tUS was performed to confirm the absence of follicles > 5 mm and then animals were divided in two groups: eCG-N (n= 10) received 1000 IU of native eCG (IM) (Novormon®, Syntex, Argentina), and eCG-R (n= 10) received 1000 IU of a synthetic eCG like glycoprotein (IM) (Syntex, Argentina). On Day 7, all llamas were injected with 112.5 µg of cloprostenol (IM) (Enzaprost®, Biogénesis Bagó). On Day 10, the number of ovulatory follicles were determined by tUS and then llamas were mated with a male with proven fertility. Afterwards, females were injected with 8 µg of buserelin acetate (IV) and 24 h later, natural mating was repeated with another male, in order to minimize the male effect. On Day 18, the number of corpus luteum (CL) that developed after mating were assessed by tUS and then embryo recovery was performed by uterine flushing. The number of follicles that developed after treatment and the number of collected embryos between groups were compared by Mann-Whitney test. The number of CL that developed after mating between groups were compared by unpaired t-test. Values are expressed as mean ± SEM.
RESULTS
No significant differences were observed between groups in the number of ovulatory follicles observed on Day 10 (10.5±2.9 vs. 8.4±1.3 in the eCG-N and eCG-R, respectively), nor in the number of CL that developed after mating (9.2±3 vs. 8.2±1.2 in the eCG-N and eCG-R, respectively). The number of collected embryos did not show significant differences between groups (2.6±1.1 and 3.3±1.1 in the eCG-N and eCG-R, respectively).
CONCLUSIONS
The synthetic eCG like glycoprotein shows a similar effect with regards to follicular development and embryo collection than the native eCG.

W05
OVULATION INDUCTION METHOD MODIFIES THE CONCENTRATION OF INFLAMMATORY MEDIATORS AND METABOLOMIC PROFILE OF UTERINE FLUID OF LLAMAS (LAMA GLAMA).

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BACKGROUND-AIM
Llama is an induced ovulatory species with waves of follicular growth, where >95% of gestations take place in the left uterine horn and present high embryonic mortality. The purpose of this study was to know the role of inflammatory mediators and metabolomic profile under different reproductive physiological conditions in llamas.
METHODS
Llamas (lama glama) around 6 to 8 years of age, 130 to 150 kg of weight were used for this study. Ovaries were examined daily by transrectal ultrasonography using a 7.5 MHz lineal array transducer (Esaote, Mediclinic, Chile). When a growing follicle ≥8 mm in diameter was detected, llamas were randomly assigned to the following groups: Follicular phase (n=5) or Luteal phase induced by: an i.m. dose of 50 µg gonadorelin acetate (GnRH, Ovalyse, Pfizer Chile SA, Santiago, n=5), or an intrauterine infusion of 5 ml of seminal plasma (n=5), or mating with a proved fertile male (n=5). Females were submitted to a non-surgical uterine flushing using a 16 F Foley catheter with 5 ml of PBS and the fluid of both uterine horns was recovered separately. The uterine fluid was centrifuged at 1000 x g for 15 min. at 4 ° C to separated debris and the fluid phase was stored at -80 ° C. To evaluate the concentration of inflammatory mediators, the analysis of IL-1β, IL-6, IL-8, IFN γ, TNF-a and PGE2 was carried out through ELISA. The metabolomic analysis was performed by gas chromatography and mass spectrometry (GC-MS).
RESULTS
The luteal phase induced by seminal plasma showed the highest concentration of IL-8, IL-1β and PGE2. The luteal phase induced by mating, produced a greater activation of metabolic pathways within the 77 metabolites detected in the uterine fluid of llamas.
CONCLUSIONS
The method of induction of ovulation in llamas modified the concentration of inflammatory mediators and metabolomic profile of uterine fluid of llamas, because the luteal phase induced by seminal plasma increased the concentration of IL-8, IL-1β and PGE2; and the luteal phase induced by mating increased the activation of metabolic pathways, which can influence the process of implantation and the fertility of this species.
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W06
COMPARISON BETWEEN TRANS-VAGINAL AND RECTO-VAGINAL TECHNIQUES FOR TRANSFERRING EMBRYOS IN THE DROMEDARY CAMELS

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BACKGROUND-AIM
The outcomes of embryo transfer (ET) in dromedary camels in terms of pregnancy rate and pregnancy losses are variable. Factors related to donors, recipients, embryo quality, and environment are the main factors affecting the outcomes of ET in dromedary camels. The aim of the present study was to investigate the effect of the technique of transferring camel embryos (recto-vaginal vs. transvaginal) into recipients on the pregnancy rate and rate of pregnancy losses.
METHODS
A total of 494 embryos (hatched blastocyst) were recovered by uterine flushing of the donor females at Day 9 after mating and transferred non-surgically into 442 recipients. Two hundred and fifteen (215) embryos were transferred into 186 recipients by trans-vaginal technique (Group 1), while 279 embryos were transferred into 256 recipients by recto-vaginal technique (Group 2). Pregnancy diagnosis was carried out on Day 10 after embryo transfer (Days 19) after mating of the donors by using the progesterone ELISA test. At Day 60, pregnancy was confirmed by trans-rectal ultrasonography of the recipients. The pregnancy rates and rate of LEM/EFM were compared between the two groups using Fisher`s exact test.
RESULTS
The overall pregnancy rates at Days 19 and 60 and the rate of late embryonic (LEM) and early fetal mortalities (EFM) were 53.2 %, 29.4% and 44.7%, respectively. Significant higher pregnancy rates at Days 19 (61.3% vs. 47.3%, respectively; P< 0.005) and 60 (40.8% vs. 21.1%, respectively; P< 0.001) were obtained in recipients receiving embryos by recto-vaginal technique when compared with those of recipients receiving embryos by trans-vaginal technique. Furthermore, a significantly higher LEM/EFM between Days 19 and 60 of gestation (55.4% vs. 33.3%, respectively; P< 0.005) was recorded in recipients receiving embryos by trans-vaginal technique when compared with that of recipients receiving embryos by recto-vaginal technique.
CONCLUSIONS
In conclusion, the technique of transferring camel embryos into recipients greatly affects the outcome of ET in terms of pregnancy rate and rate of pregnancy losses between Days 19 and 60 in dromedary camel. The trans-vaginal technique is not recommended for embryo transfer in dromedary camels due to increased pregnancy losses.

W07
DOES MONOZYGOTIC TWINNING OCCUR AFTER TRANSFER OF A HATCHED BLASTOCYST IN THE DROMEDARY CAMEL?

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BACKGROUND-AIM
Twinning in the dromedary camels is rare and eventually terminates in abortion. Monozygotic twinning after transfer of a single unhatched blastocyst has been reported in humans (Schachter et al., 2001, Human Reproduction, 16:1264-1269).
METHODS
During three breeding seasons, donor females (n=116) with or without superovulation were naturally mated after a mature follicle(s) was detected on the ovaries by using transrectal ultrasonography. Flushing of the embryos was done at Day 8 or 9 post-mating and the recovered embryos were washed several times in a commercial holding medium (IMV Technologies) and loaded in 0.25 ml plastic straws. A single hatched blastocyst was transferred into a synchronized recipient (n=924) within 20 minutes of flushing. All the procedures were carried out at room temperature and under aseptic conditions.
RESULTS
Transfer of embryos resulted in 167, 225, and 255 pregnancies (> 3 months after ET) in seasons 2017-2018 and 2018-2019, 2019-2020, respectively. The overall pregnancy rates at Days 18 to 19 and 60 were 56 and 43%, respectively. In season 2017-2018, a monozygotic twin (MZT, males) was aborted at 8 months of gestation. In season 2018-2019, 2 MZT (females) were aborted at 10 and 12 months of gestation, respectively. In season 2019-2020, so far, a MZT (males) was aborted at 7 months of gestation. The overall incidence of MZT after ET in the three breeding seasons (2017-2020) was 0.6% (4/647). The MZT was recorded after transferring embryos recovered from donor females with and without superovulation. No pregnant recipients gave birth to twins after a normal gestation period.
CONCLUSIONS
This case report demonstrates for the first time the occurrence of MZT after transfer of a single hatched blastocyst in the dromedary camels. The possible explanations of MZT after single ET in the dromedary camel will be discussed.

W08
IN VITRO ACTIVATION OF ALPACA PRIMORDIAL FOLLICLES WITH THE SUPPLEMENTATION OF KIT LIGAND AND/OR BPV(HOPIC)

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BACKGROUND-AIM
In mammalian, the activation of primordial follicles to a primary follicle is a progressive and highly regulated process. There is evidence in mice that the silencing of phosphatase and tension homolog (PTEN) is a major negative regulator of phosphatidylinositol 3-kinase (PI3K), initiating the activation of dormant follicles. This study evaluated the effects of Kit Kigand (KL) and/or PTEN inhibitor, bpV (HOpic) on the morphology and development of alpaca preantral follicles (fresh control) after 24 hours of in vitro culture in a-Minimal Essential Medium (a-MEM; control medium) containing KL (100 ng / ml), bpV (HOpic) (10 µM) or a combination of the two products.
METHODS
Alpaca (vicugna pacos) ovaries (n = 14) were dissected in slices and randomly divided into 5 groups according to culture media supplemented with KL and / or bpV-HOpic. The fragments were cultured for 24 h at 39 °C with 5% O2, 5% CO2 and 90% N2. After in vitro culture, all samples (fragments) were individually fixed overnight at room temperature in 4% paraformaldehyde (PBS, pH 7.4) for routine histology. The histological sections were mounted and stained with hematoxylin. Follicles were classified according to their developmental stage (primordial, primary, and secondary) previously defined by Silva et al. (2004). Follicles that showed pyknotic oocyte nuclei, cytoplasmic retraction and disorganized granulosa cells were classified as abnormal follicles.
RESULTS
The difference between groups was analyzed by analysis of variance (ANOVA), followed by Duncan test for samples with normally distributed data. The results show a significant increase ($p<0.05$) in the percentage of primary follicles in culture media supplemented with KL and/or bpV-HOpic (61.2 to 63.6%), compared with the fresh control (37.17%), control medium 50.27% (a-MEM). The percentage of abnormal follicles was not affected by culture of ovarian tissue.
CONCLUSIONS
In conclusion, the supplementation of KL (100 ng/ml) and / or bpV-HOpic (10 µM) promoted the activation from primordial to primary follicles after in vitro culture of alpaca ovarian tissue. Peru Grant FONDECYT 385-2019.

W09
COMPARISON OF CRYOPROTECTANT AGENTS ON THE POST THAW QUALITY OF ALPACA (VICUGNA PACOS) SPERMATOZOA

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BACKGROUND-AIM
Cryoprotectant agents (CPA) prevent sperm membrane damage caused during cryopreservation. In alpacas, glycerol, ethylene glycol and dimethyl sulfoxide (DMSO) have traditionally been used, but evidence in llamas and stallions indicate that amides (dimethylacetamide (DMA), dimethylformamide (DMF) and/or methylformamide) could have better effects. However, all of these polyols, sulfoxides and amides cryoprotectants have not been properly compared in alpacas. The objective of this study was to compare the effect of different concentrations of glycerol, ethylene glycol, DMSO, DMA, DMF and methylformamide, as CPA during the cryopreservation of epididymal alpaca spermatozoa.
METHODS
As obtaining alpaca semen is some difficult, seventy-two alpaca sperm samples were recovered from cauda epididymides after mincing them with a scalpel and diluted with 2.5 mL of an extender based on skim milk, fructose and egg yolk. A 6x3 factorial design formed by six CPA at three different concentrations (1.0, 3.5 and 7.0%) were used. Samples were processed in 3 main groups (n=24). In this way, 24 samples were used for assessment of glycerol and methylformamide; 24 samples for ethylene glycol and DMSO; and last 24 samples for DMA and DMF. All sperm samples were loaded into 0.5 mL plastic straws at room temperature; frozen using an automated system (Straws were cooled from 18 to 5°C during 90 minutes, holded at 5°C for 30 minutes; and then a temperature drop followed until freezing and stored in liquid nitrogen). After thawing (1 minute at 37°C), sperm viability (SYBR14/PI) and mitochondrial membrane potential (MMP) (MitoTracker Deep Red) were evaluated by imaging flow cytometry. Sperm motility was also evaluated subjectively. The effect of the treatments on the percentages of motility, sperm viability and MMP were evaluated using a two-way (CPA and concentration) ANOVA and the Tukey test.
RESULTS
In general, better results were found when CPA were used at concentrations of 1 or 3.5%. DMSO, glycerol and methylformamide showed higher ($p<0.05$) percentages of sperm motility, however all CPA had similar percentages of viability and MMP. In addition, there were found interactions between CPA and concentrations in sperm motility, viability and MMP, where groups glycerol-3.5% and methylformamide 3-5% had the higher percentages in the three parameters assessed.
CONCLUSIONS
In conclusion, the use of glycerol or methylformamide at 3.5% could be a good option for cryopreservation of alpaca epididymal spermatozoa.

W10
APOPTOSIS-RELATED MARKERS IN EPIDYDIMAL ALPACA (VICUGNA PACOS) SPERMATOZOA

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BACKGROUND-AIM
Apoptosis is a mode of programmed cell death that can occur in spermatozoa during spermatogenesis or epididymal transit, as well as in ejaculated spermatozoa. A high percentage of apoptotic sperm would be a poor indicator of the freezability of a raw seminal sample. In alpaca spermatozoa, this phenomenon has not been studied; however, in spermatozoa from other species, different markers for apoptosis such as Annexin V and caspases have been found. Therefore, the objective of this study was to describe the levels of apoptosis in raw alpaca spermatozoa.
METHODS
Ten (n=10) alpaca sperm samples were recovered from cauda epididymides and diluted using skim milk, fructose and egg yolk. Each sample was divided in 2 aliquots for incubation with apoptosis markers: Annexin V (FITC Annexin V/Dead Cell Apoptosis Kit) and caspase (CellEvent™ Caspase-3/7 Green Detection Reagent) for detection of externalization of phosphatidyl serine and activation of caspase, respectively. Camptothecin (CPT) at 10 µM was used as an inducer of apoptosis (positive control). All aliquots were also incubated with MitoTracker® Deep Red FM and propidium iodide to differentiate viable and necrotic cells, respectively. Samples were assessed by imaging flow cytometry assessment using 488 and 642 nm lasers.
RESULTS
Mean percentage of raw alpaca spermatozoa with translocation of phosphatidyl serine (Annexin V positive) was 8.07 ± 4.56, while raw alpaca spermatozoa with activated caspases 3/7 (Caspase green positive) was 10.86 ± 4.51. Incubation of alpaca spermatozoa with CPT (10 µM) triggered apoptosis to 52.6% when Annexin V was used, and to 36.6% when caspase was used.
CONCLUSIONS
In conclusion, levels of apoptosis in raw alpaca spermatozoa obtained from epididymides is around 10% when Annexin V or caspases are used as apoptotic markers. Later, it could be interesting to know if cryopreservation process triggers apoptosis in alpaca spermatozoa. This project was funded by 135-2020-FONDECYT.

TOPIC Embryo development and differentiation

W11
PROGESTERONE RECEPTOR (PGR) IS NOT REQUIRED FOR BOVINE HYPOBLAST MIGRATION AND EMBRYONIC DISC FORMATION IN VITRO

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BACKGROUND-AIM
Progesterone has been positively associated with conceptus length and embryo survival in cattle. However, it is unclear whether this positive effect is exerted through a direct effect upon the embryo or indirectly, by promoting changes in uterine fluid composition. While progesterone supplementation to conventional embryo culture up to Day 8 (D8) is not required for embryo development, post-hatching embryo culture (D8 to D12) requires the addition of multi-compound supplements containing progesterone. The objective of this study was to determine the developmental effects of progesterone receptor (PGR) ablation.
METHODS
In vitro matured bovine oocytes were injected with mRNA encoding for Cas9 alone (control group, C) or combined with sgRNA against PGR (C+G group). Following fertilization and culture, blastocysts were cultured in a post-hatching system up to D12. At D12, embryos were fixed and epiblast and hypoblast development were analyzed by immunocytochemistry for SOX2 and SOX17, respectively. Following individualized image analysis, samples were recovered and genotyped by miSeq to determine which embryos in C+G group were KO.
RESULTS
PGR ablation did not impair blastocyst formation, as similar developmental rates were obtained in C or C+G groups (33.4±2.6 vs. 26.7±4.3 %, mean±s.e.m. for C and C+G, respectively, t-test p>0.05). In vitro development from D7 to D12 was also similar between groups (85.4±5.7 vs. 81.3±6.3, mean±s.e.m. for C and C+G, respectively, t-test p>0.05). 22 out of 45 D12 embryos analyzed in C+G group were KO. D12 embryo diameter was not affected by embryo genotype (772±74 vs. 648±64 vs. 731±44 µm, mean±s.e.m. for WT, edited non-KO and KO, respectively, ANOVA p>0.05). The proportion of embryos showing complete hypoblast migration was similar in WT (23/32, 72 %), edited non-KO (12/20, 60 %) and KO (19/22, 86 %) embryos (Chi-square p<0.05). Epiblast survival (SOX2+ cells) rates were also similar in WT (26/32, 81 %), edited non-KO (9/20, 45 %, 60 %) and KO (12/20, 60 %) and no differences were noted on embryonic disc (ED) formation rate (20/32 63 % vs. 6/20 30 % vs. 10/22 45 % for WT, edited non-KO and KO, respectively Chi-square p>0.05).
CONCLUSIONS
Direct progesterone signaling to the embryo through PGR is not required for hypoblast migration and ED formation.

W12
TEAD4 IS DISPENSABLE FOR BOVINE CONCEPTUS ELONGATION

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BACKGROUND-AIM
TEAD4 plays an essential role on trophectoderm (TE) differentiation in mice, where its ablation impedes blastocyst formation. However, in a previous study we found that bovine embryos lacking TEAD4 (TEAD4 KO) develop to blastocyst. The aim of this study was to determine if subsequent preimplantation development was affected by the ablation.
METHODS
In vitro matured bovine oocytes were injected with mRNA encoding for Cas9 and sgRNA against TEAD4 (C+G group). Following fertilization and embryo culture to D7, blastocysts were transferred to a Day 6.5 recipient ewe. Nine days after ET, conceptuses were recovered at a stage developmentally equivalent to bovine E14. Recovered conceptuses were measured, fixed and subjected to immunocytochemistry (ICC) to determine lineage development using antibodies for CDX2 (TE), SOX17 (hypoblast) and SOX2 (epiblast). Following ICC embryos from C+G group were genotyped by clonal sequencing to determine which harbored only frame-disrupting alleles on TEAD4 sequence (i.e. KO alleles).
RESULTS
32 Day 7 bovine blastocyst developed in vitro following microinjection were transferred to both sides of the uterus (16+16) of a recipient ewe. Nine days after ET 14 conceptuses (7+7) were recovered by uterine flushing. All conceptuses were edited, 3 harbored at least one in-frame (non-KO) allele and 11/14 were KO. The development of extraembryonic membranes (TE+hypoblast) was not affected by the ablation, as conceptus length was comparable between KO and non-KO conceptuses (10.9±3 vs. 9.8±2.3 mm, mean±s.e.m. for non-KO vs. KO, respectively, t-test p>0.05) and all conceptuses but one KO (the smallest embryo) showed complete hypoblast migration along the inner surface of the TE. A SOX2+ embryonic disc (ED) was detected in all conceptuses but one KO (the same smallest embryo), and ED diameter was similar in both groups (260±82 vs. 229±33 µm, mean±s.e.m. for non-KO and KO, respectively, t-test p>0.05).
CONCLUSIONS
In contrast with the crucial role observed in murine embryos, bovine embryos do not require TEAD4 for trophectoderm specification and maintenance up to tubular stages.

W13
GENERATION OF TROPHOBLAST-LIKE CELLS FROM EPIGENETICALLY ERASED ADULT DERMAL FIBROBLASTS

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BACKGROUND-AIM
The first differentiation event in mammalian embryos is the formation of the trophectoderm (TR), which supports the fetus during intrauterine life. Limited amount of information is available in large mammals and humans, where TR cells can be isolated with poor efficiency. A better characterization of TR cells would be useful for different aspect of reproductive medicine. In this regard, the creation of in vitro models that mimic the mechanisms and local controls at work during the initial steps of placenta development could help to better elucidate decline in fertility in general and, more in particular, age-related senescence, due to motherhood postponing. To this purpose, we describe the epigenetic conversion of adult dermal fibroblasts into TR-like cells.
METHODS
We used fibroblasts obtained from female human patients, 34 and 96 years old (Coriell Institute from Medical Research). Cells were first treated with 1μM 5-aza-CR for 18h and then differentiated towards the TR lineage, using Mouse Embryonic Fibroblasts conditioned medium, supplemented with 10 ng/ml bone morphogenetic protein 4 and 1μM inhibitors of the Activin/Nodal and 0.1 μM FGF2 signaling pathways in 5% O2.
RESULTS
At day 2 of TR induction, both young and aged donor-derived fibroblasts acquired the tight adherent epithelial morphology typical of TR stem cells and expressed the early TR marker CDX2. However, efficiency was significantly lower in cells from the aged donor. By day 5, cells showed a mature TR morphology, exhibited round or ellipsoid shape, round nuclei, and well-defined border. Both groups expressed KRT7, PAPPA, PSG6 and GCM1, although the levels of transcription for these genes were significantly higher in cells obtained from young patients.
CONCLUSIONS
Our results demonstrate the possibility to obtain TR-like cells from fibroblasts, using chemical reprogramming and avoiding the use of viral vectors. The protocol was successful to convert both young and aged donor-derived fibroblasts, however, higher efficiency was achieved when using cells from the younger person, suggesting that a drift in cell response to differentiation cues is likely to take place during ageing. This may find useful applications in order to clarify age-related TR defects and embryo implantation mechanisms in older patients.

W15
IMPACT OF IN VITRO CULTURE (IVC) ON MITOCHONDRIAL ACTIVITY AND DISTRIBUTION IN MOUSE PREIMPLANTATION EMBRYOS

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BACKGROUND-AIM
Studies in the field of mitochondrial dynamics have identified an intriguing link between energy demand/supply balance and mitochondrial architecture. Those suggests that inappropriate culture conditions may inhibit mitochondrial functions and negatively affect embryo development.
Therefore, this study was conducted to evaluate whether In Vitro Culture (IVC) may affect mitochondrial function in mouse embryos, and whether mitochondrial activity and distribution have an impact on embryo developmental competence.
METHODS
IVC and in vivo produced (IN VIVO) mouse embryos at the 2-cell and blastocyst stage were subjected to mitochondrial analysis (distributions, organisation, membrane potential and dynamics), expression of mRNA and localization of proteins involved in regulation of mitochondria functions, as well as number of mitochondrial DNA (mtDNA) copies.
RESULTS
Results showed that mitochondria in IN VIVO 2-cell embryos were numerous and homogeneously distributed in both blastomers, while IVC ones were less numerous and localised mainly in the pericortical region of the cytoplasm. Mitochondrial in IN VIVO blastocyst created elongated mitochondrial networks along the cells, while IVC ones were fragmented, rounded, and aggregated mainly at the perinuclear area. Time-lapse analysis showed that the mitochondria of IN VIVO embryos moved back and forth along their long axis on radial tracks, while IVC blastocysts displayed mobility alterations. Moreover, results indicate that the IVC group had reduced mRNA expression of mitofusin 1, mitofusin 2, and optic atrophy 1 responsible for mitochondrial fusion. Additionally, mtDNA copy number for IVC blastocysts (92 337±5860) was significantly lower than that of IN VIVO blastocysts (169104±16322; $P < 0.02$). Percentage of the blastocysts [78% (106/136) vs 98% (100/102); $P < 0.02$] and total cell number of the IVC embryos was decreased compared to IN VIVO embryos (31±1.1 vs 52±2.3, respectively).
CONCLUSIONS
Taken together, those results indicate that embryos cultured in in vitro conditions are more susceptible to perturbations in mitochondrial number and function, which is associated with decrease developmental competence of mouse embryos.

W16
ELONGATING BOVINE EMBRYOS DISPLAY GENES INVOLVED IN METABOLISM AND GROWTH FACTOR SIGNALING, SUGGESTING A DIETARY EFFECT ON ELONGATION

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BACKGROUND-AIM
Differential gene expression of embryos of the same age but different developmental stages may indicate indispensable pathways for successful embryo elongation. We have previously reported a promotion of embryo elongation by maternal dietary n-3 fatty acid (FA) compared to n-6 FA supplementation in heifers (Giller et al., 2018). The current analysis assessed the transcriptome of these elongating embryos.
METHODS
In brief, heifers were supplemented with 450 g of rumen-protected fish oil (rich in n-3 FA; n=16) or sunflower oil (rich in n-6 FA; n=11) for 8 weeks. Following cycle synchronization and artificial insemination, animals were slaughtered at day 15 of gestation. By flushing the uterus, embryos ranging in length from 0.4-20.3 cm (6.1 ± 0.9 cm (mean ± SEM)) were recovered and classified into short (S) and long (L) embryos (S: 0.4-9.5 cm; L: 10.5-20.3 cm (Richard et al., 2015)). The n-6 FA supplementation led to the recovery of only S (n=11) embryos, while n-3 FA revealed S (n=10) and L (n=6) embryos. Total RNA was isolated from individual embryos and subjected to RNA sequencing on an Illumina HiSeq4000. Data was processed and analyzed using the Sushi Platform (Functional Genomics Center Zurich). Genes showing altered expression with a false discovery rate (FDR) below 1% were considered differentially expressed genes (DEGs). A gene ontology (GO) analysis was performed using PANTHER 14.1.
RESULTS
No DEGs were found comparing n-3 with n-6 embryos. A total of 357 DEGs in L vs. S embryos was identified (247 up- and 110 downregulated genes). The GO analysis revealed 94 biological processes (BP), 6 cellular components (CC) and 10 molecular functions (MF) significantly regulated for the upregulated DEGs in L vs. S embryos whereas 4 BP, 7 MF and no CC were identified for the 110 downregulated genes. The overrepresented BP for upregulated genes encompassed "insulin receptor signaling pathway", "glucose homeostasis", "cellular response to insulin and growth factor stimulus", "positive regulation of phosphorylation", and "membrane lipid biosynthetic process" (all p-values ≤ 0.001), among others.
CONCLUSIONS
These data indicate that the promoted embryo elongation on day 15 of gestation during maternal n-3 FA supplementation is likely based on an increase of metabolic and growth factor driven processes.

W17
TARGETED INSERTION OF LARGE DNA FRAGMENT INTO BOVINE EMBRYOS USING CRISPR/CAS9

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BACKGROUND-AIM
Gene editing of CRISPR/Cas9 technology allowed efficient and relatively easy targeting of specific genomic regions. Studies of gene function by random indels after CRISPR became feasible in embryos of species in which gene knockouts were not available. Even the knock-in of short DNA fragments is possible. However, targeted knock-in of longer fragments is still a challenge. We hypothesized that microinjection after a shorter period after insemination of Cas9 enzyme fused to streptavidin (mSA) and biotinylated donor DNA into bovine zygotes would enhance the chances of a knock-in.
METHODS
To test this hypothesis, we designed gRNA targeting the stop codon region of the SOX2 gene and Cas9-mSA was cloned under a SP6 promoter. A donor plasmid vector containing mCherry fluorescent protein was engineered by cloning 5' and 3' homology arms of the targeted SOX2 gene region. Both gRNA and Cas9-mSA mRNA were in vitro transcribed and the donor DNA was obtained after PCR using biotinylated primers. We microinjected in vitro produced bovine zygotes using different concentrations of gRNA, Cas9-mSA and donor DNA: respectively 50-75-50; 50-75-100; 100-100-100, all expressed in ng/µl, at 8 hours post insemination (8hpi) or 16hpi, with at least 2 replicates each. Blastocyst formation was evaluated at 186hpi, embryos were imaged for fluorescence detection and then submitted to genotyping by PCR with one primer upstream the 5' homology arm and the other within the 3' arm.
RESULTS
No difference in blastocyst rates was observed in all conditions, including non-injected controls. Surprisingly, not a single embryo displayed mCherry fluorescence, but genotyping revealed that knock-in embryos were present in all groups, except when injected with 50-75-100. Injection of 100-100-100 provided more edited blastocysts, 9/14 at 8hpi and 5/6 at 16hpi, but no statistical difference was observed within these groups. Interestingly, the only homozygous edited embryos were present in embryos injected at 16hpi.
CONCLUSIONS
In conclusion, the use of Cas9-mSA and biotinylated donor DNA allowed the knock-in of large DNA fragments in bovine embryos at 8hpi or 16hpi.

W18
INHIBITION OF FGFR IMPAIRS EXPRESSION OF PRIMITIVE ENDODERM MARKER SOX17 IN BOVINE EMBRYOS

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BACKGROUND-AIM
The first cell differentiation event of the embryo results in the trophectoderm (TE) and the inner cell mass (ICM) lineages. Next, the ICM differentiates into epiblast (EPI) and primitive endoderm (PE). In the mouse, NANOG promotes the expression of FGF4, which activates the FGFR/ERK pathway in neighboring cells. This results in suppression of NANOG and expression of SOX17, triggering PE segregation. However, in bovine embryos, this differentiation event is not fully elucidated. In this study, we aimed to assess the role of FGF signaling in the specification of PE in bovine embryos through inhibition or activation of the FGF pathway. For this purpose, two experiments were performed using in vitro produced embryos. We wanted to test the hypothesis that FGF4 signaling through FGFR is determinant for PE establishment in bovine embryos.
METHODS
In the first experiment, embryos were treated with 1µg/mL FGF4 (FGF) or 0,1% (w/v) BSA in PBS as a vehicle (PBS group). In the second experiment, embryos were treated with 1mM AZD4547, a FGFR inhibitor (Cayman Chemical - AZD), 0,01% DMSO as vehicle (DMSO group) and an untreated control group (CTRL). In both experiments, embryos were treated in three different windows of time: days 5 to 7 (D5-D7) of in vitro culture, 7 to 9 (D7-D9), or 5 to 9 (D5-D9) in five replicates. PBS and DMSO were added from D5-D9 in respective experiments. Blastocyst rates were evaluated at D9 and embryos were fixed for SOX17 immunofluorescence and cell count. Five to seven embryos had their cells counted in each group. Data were analyzed by ANOVA followed by Tukey comparison of means, using a 5% level of significance.
RESULTS
In the first experiment, blastocyst rates, SOX17-positive cell number and total cell number of the embryos were not affected by FGF treatment when compared to the PBS group. AZD treatment reduced blastocyst rates significantly only in D5-D9 (5.72 ± 3.58%) versus D7-D9 (21.47 ± 3.58%). SOX17-positive cells were absent in both D7-D9 and D5-D9 AZD treatments.
CONCLUSIONS
In conclusion, exogenous FGF4 did not increase the number of cells positive for SOX17. However, AZD treatment until D9 ablated SOX17 expression, indicating that the formation of PE cells in bovine embryos depends on the FGF receptor activity.

W19
BLASTULATION TIME AFFECTS TO POST-HATCHING IN VITRO DEVELOPMENT OF BOVINE EMBRYOS

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BACKGROUND-AIM
Traditional embryo selection of bovine embryos is fundamentally based on morphological characteristics. However, this method is subjective and results in unreliable embryo selection. Therefore, it is necessary to include non-invasive criteria such as blastulation time. The aim of this study was to evaluate the effect of blastulation time on post-hatching in vitro development of bovine embryos.
METHODS
Cumulus oocyte complexes (COCs) were matured for 23 h and then fertilized by incubating the COCs with the sperm (10000 sperm per oocyte) for 18 h. Presumptive zygotes were placed in microwells, in droplets of commercial culture medium. Digital images of developing embryos were captured every 15 min by Primo Vision TL system (EVO+; Vitrolife, Göteburg, Sweden). The following time intervals were recorded: from IVF to the attainment of blastulation (tSB) and blastocyst formation (tB). Viable embryos were determined by a system of extended in vitro culture of bovine embryos until day 9.5 (post-hatching development). The criteria to define a viable embryo at day 9.5 post IVF was blastocyst diameter greater than 200 µm and total cell count greater than 190. To verify the predictive power of early (value 1) or late (value 0) blastulation the receiver-operating characteristic with determination of area under the curve (AUC) was analyzed. The embryo viability was compared in both groups using a Chi-square test with a significance level of 0.05.
RESULTS
Ninety-six of 208 (46.2%) embryos selected for time-lapse observations, started blastulation at an average time of 149.8 h, being this the cut-off point to discriminate as early or late blastulation. Out of these 96 embryos, 89 (92.7%) progressed to the blastocyst stage (day 7 post IVF). Blastulation time (tSB) was significantly associated with the blastocyst formation (tB) (Spearman's rho 0.522). After extended in vitro culture, embryo viability was higher in the group of early blastulation (early: 26/49, 53.1%; late: 7/47, 14.9%; p = 0.0038). Additionally, early blastulation achieved a ROC-AUC of 0.717 (95% CI, 0.61.6–0.824; P <0.001) to predict viability at post-hatching development.
CONCLUSIONS
Therefore, blastulation timing may be helpful for selection of embryos with high capacity of post-hatching development. Peru Grant FONDECYT 143-2020.

W20
THE EMERGING ROLE OF PROGESTERONE RECEPTOR MEMBRANE COMPONENT 1 IN BOVINE EARLY EMBRYOGENESIS: A PRELIMINARY STUDY

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BACKGROUND-AIM
Progesterone Receptor Membrane Component 1 (PGRMC1) is a multifunctional protein that was initially identified in the liver as a progesterone binding protein, which accounts for its name. In the maturing bovine oocytes PGRMC1 localizes in the cytoplasm and the nucleus of immature oocytes, while it associates with the chromosomes and the spindle during chromosomes segregation. Strikingly, PGRMC1 localization in the maturing oocytes mirrors its localization in dividing bovine granulosa cells (bGC), suggesting a possible common role in mitotic and meiotic cell division. This hypothesis was confirmed by experiments showing that disturbance of PGRMC1 function with an inhibitor (AG205) or its downregulation with RNA interference (RNAi) impairs the oocyte ability to emit the polar body (PB) and properly arrange the chromosomes in a MII plate. Further time lapse imaging analysis of PGRMC1-RNAi treated bGC confirmed PGRMC1's role in mediating the final stages of cell division (1). Starting from these observations, our ongoing studies aim at investigating PGRMC1 function during the early stages of bovine embryonic development, when blastomeres are actively dividing.
METHODS
PGRMC1 localization was assessed by immunofluorescence (IF) analysis in developing embryos. Bovine zygotes were treated with AG205 to assess the effect of perturbing PGRMC1 function on segmentation. The possible interaction between PGRMC1 and the cytoskeletal component Myosin was assessed by in situ proximity ligation assay (PLA) in bGC.
RESULTS
As expected, PGRMC1 localization in bovine 6-8 cell stage embryos parallels the expression in somatic cells, localizing in both the nuclear and the cytoplasmic compartments of the blastomeres. Our data indicates that treatment with AG205 impairs the formation of mononucleated blastomeres, significantly increasing the percentage of multinucleated and non-nucleate blastomeres (28.2% vs 69.4% in the CTRL and AG205 groups, respectively, $P<0.05$ Chi square test). IF and PLA studies reveal that PGRMC1 colocalizes and interacts with Myosin II.
CONCLUSIONS
Altogether our data suggest a possible role in mediating cytoplasmic rearrangements that drives blastomeres division.
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1 Terzaghi et al 2016 Cell Cycle 15(15): 2019-2032

W21
TRANSCRIPTOMIC AND EPIGENOMIC ANALYSIS OF BOVINE EMBRYONIC STEM CELLS DERIVED FROM IN VITRO FERTILIZED AND SOMATIC CELL NUCLEAR TRANSFER EMBRYOS.

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BACKGROUND-AIM
Bovine embryonic stem cells ESC (bESCs) can be derived from in vitro fertilized (IVF) and somatic cell nuclear transfer embryos (SCNT). Given that SCNT embryos often present epigenetic dysregulation that can lead to abnormal development, we set out to assess the transcriptomic and epigenomic status of SCNT-derived ESCs.
METHODS
bESCs were derived from day-7 SCNT or IVF blastocysts in N2B27 base media containing BSA, FGF2 and IWR1. For each ESC group, genome-wide transcriptome and histone profiles (H3K4me3, H3K9me3, H3K27me3) were generated for three independent cell lines using 3'-Tag-Seq and Cut&Tag techniques, respectively, and compared to bovine fetal fibroblasts (FIB).
RESULTS
After 4 weeks in culture, well-defined colonies appeared in both groups with an equivalent efficiency (30%). All ESC exhibited pluripotency features: alkaline phosphatase activity, pluripotency marker expression and euploidy. Principal component analysis of gene expression as well as histone profiles of H3K4me3, H3K9me3 and H3K27me3 showed that IVF-ESC and SCNT-ESC clustered together but separate from FIB, suggesting that transcriptomes and epigenomes of both ESC sources were similar, but distinct from FIB. Specifically, 2,378 differently expressed genes (DEG, $p<0.01$; $FC>2$) were found in bESC compared to FIB and only 46 DEG were found between SCNT-bESC and IVF-bESC. Additionally, we identified 439 H3K27me3 peaks, 214 H3K4me3 peaks, and 1,041 H3K9me3 peaks that differed between both bESC sources. However, those modifications were spread out over all chromosomes without a predominance in regions surrounding DEG, promoters or body genes. Finally, categorization of chromatin states genome wide showed that both ESCs shared the same epigenetic pattern in promoters. Minor differences found between both ESC could be stochastic in nature, possibly related to genotype variability or the techniques themselves.
CONCLUSIONS
These results suggest that SCNT-ESC resemble IVF-ESC transcriptionally and epigenetically, enabling the generation of pluripotent stem cells from adult individuals, thus, allowing the expansion of agricultural and economically important production traits.

W22
BONE MORPHOGENETIC PROTEIN 4 PROMOTES IN VITRO MESODERM DEVELOPMENT IN SHEEP EMBRYONIC DISCS

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BACKGROUND-AIM
Most pregnancy losses in farm ungulates and humans occur during the second and third weeks of pregnancy. At this time, the epiblast develops into an embryonic disc (ED) that initiates gastrulation by the expression of the mesoderm marker BRACHYURY (T) at the posterior pole. However, these processes remain poorly understood because until now they could only be studied in vivo, with a high cost and effort. Bone morphogenetic protein 4 (BMP4) is involved in mesoderm formation in mouse, rabbit, primate and ungulate embryos and its supplementation to mouse and human embryonic stem cells induces mesoderm differentiation. The objective of this study was to analyse the effect of BMP4 supplementation on mesoderm development in a recently established in vitro post-hatching culture system that sustains sheep embryo development up to gastrulating stages.
METHODS
Day (D) 6/7 in vitro-produced sheep blastocysts were cultured in N2B27 supplemented with activin A and ROCK inhibitor (post-hatching culture system; NAR). At D12, embryos were cultured in 1) NAR (n=50); 2) NAR + 1 ng/ml BMP4 (NAR1; n=63); 3) NAR + 10 ng/ml BMP4 (NAR10; n=68); and 4) NAR + 100 ng/ml BMP4 (NAR100; n=51) until D14. Surviving embryos were imaged, fixed and the development of specific lineages was assessed by immunostaining for SOX2 (epiblast), FOXA2 (hypoblast) and T (mesoderm).
RESULTS
BMP4 addition at 1-100 ng/ml did not affect embryo survival, embryo size, the percentage of the embryo covered by hypoblast cells, epiblast cell number and the percentage of embryos developing an ED (Chi-square / One-way ANOVA; $p>0.05$). T+ mesoderm cells were detected in 3/8 (~37%) NAR; 4/13 (~31%) NAR1; 9/19 (~47%) NAR10; and 4/12 (~33%) NAR100 embryos developing EDs. The number of T+ mesoderm cells was significantly higher in NAR10 (169.4 ± 36.5) and in NAR100 (240.5 ± 61.72) than in NAR (47.67 ± 28.18; One-way ANOVA; $p<0.05$).
CONCLUSIONS
BMP4 supplementation at 10 or 100 ng/ml from D12 to D14 promoted mesoderm development by enhancing the number of T+ cells in sheep embryonic discs developed in vitro.

W23
SLOWED, BUT NOT STOPPED: CONTINUOUS DEVELOPMENT THROUGHOUT EMBRYONIC DIAPAUSE IN THE ROE DEER

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BACKGROUND-AIM
The European roe deer (Capreolus capreolus) was the first species in which embryonic diapause, a temporary reduction of developmental pace, was described. In contrast to other species, the roe deer blastocyst continuously proliferates during diapause. Moreover, the embryoblast changes morphologically, suggesting differentiation and developmental progression.
METHODS
We recently acquired RNA-Seq data from diapausing blastocysts and elongating embryos collected by uterine flushing between September and January in 2015 to 2017. These embryos varied in diameter between 400 µm and 4 cm and their cell number ranged from around 200 to > 300'000 cells. We employed an alternative strategy to re-assemble and annotate genes relevant for embryo development and to analyse differential mRNA transcription to enhance the underlying first version of the roe deer transcriptome (van der Weijden et al., 2021). To corroborate the previously observed morphological changes, we additionally stained individual roe deer blastocysts using phalloidin.
RESULTS
We found dynamic changes in transcript abundance of developmentally relevant genes over the course of diapause. Our results point towards the formation of the primitive endoderm prior to the earliest stages of diapausing blastocysts analysed. After an initial increase at the beginning of the diapause period, the transcript abundance of pluripotency factors declined in embryos with > 3`000 cells. The morphological changes occurring in the embryoblast during diapause coincided with changes in cell polarity and were associated with changes in WNT5A, SOX17, GRHL2, and PAX2 expression. These genes are possibly linked to Wnt-signalling as well as ongoing polarization and epithelialisation events. Gastrulation likely occurs during embryo elongation and thus after diapause.
CONCLUSIONS
Taken together, our results indicate that next to proliferation, embryonic development is - whilst slow - ongoing during diapause in the roe deer. Diapause in this species therefore offers a unique opportunity to study the regulation of developmental pace, the coordination of differentiation and proliferation, as well as the determinants of embryo survival and embryo-maternal communication.

W24
PROTEOMIC ANALYSIS OF EARLY BOVINE EMBRYOS DEVELOPED IN VIVO

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BACKGROUND-AIM
Early mammalian embryo development is a well-orchestrated process taking place in the oviduct and uterus and involving important molecular and structural changes leading to implantation. So far, very few proteomic studies were conducted on mammalian embryos and most of them were done with in vitro-produced embryos. In this study, we used a mass spectrometry (MS) approach to elucidate proteomic changes of bovine embryos collected in vivo.
METHODS
Eleven Holstein females (9 cows, 2 heifers) were synchronized for estrus, treated for ovarian superovulation and inseminated twice at 12 and 24 h after standing estrus with frozen-thawed semen. Between days 1.7 and 7.5 after the first AI, embryos were recovered by oviduct and uterine flushing less than 20 min after slaughter and stored at −80°C. Proteins from pools of grade-1 embryos at the 4-6 cells, 8-12 cells, morula, compact morula and blastocyst stages (4 embryos/pool; 3-4 pools/stage; total of 18 pools) were analyzed by nanoliquid chromatography coupled with tandem MS using a TimsTOFPro instrument (Bruker). Proteins were identified using the Bos taurus UniProtKB database and quantified by a label-free spectral counting method. Data were analyzed using principal component analysis (PCA), ANOVA and hierarchical clustering. Functional analysis of differentially abundant proteins (DAPs) was carried out using Metascape.
RESULTS
A total of 2757 proteins were identified and quantified, of which 1627 were shared between embryonic stages. PCA of all data showed a clear separation of embryo pools according to their stage of development. Two main clusters of DAPs ($p<0.05$) between stages were evidenced: proteins that increased (n=626) and those that decreased (n=400) in abundance during development. Major changes in protein abundance were seen between the 8-16 cell and morula stages (corresponding to the embryonic genome activation) and between the compact morula and blastocyst stages (corresponding to the first lineage specification). The main pathways and processes overrepresented among upregulated proteins were protein translation, RNA metabolism and carbon metabolism whereas protein processing in endoplasmic reticulum, vesicle transport and neutrophil degranulation were overrepresented among downregulated proteins.
CONCLUSIONS
These data provide new insights into the molecular pathways governing embryo development in vivo.

W25
WHAT DOES THE MOTHER BRING TO THE EARLY EMBRYO? PROTEOMIC COMPARISON OF BOVINE EMBRYOS PRODUCED IN VIVO OR IN VITRO

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BACKGROUND-AIM
Despite many improvements with in vitro systems and culture media for mammalian embryos, we still question the normality and viability of cultured embryos compared to their in vivo counterparts. To bring knowledge to answer this question, we used a mass spectrometry (MS) approach to compare the protein content of bovine embryos from the 4-6 cell to the blastocyst stages that were conceived in vivo or produced in vitro.
METHODS
Eleven Holstein females were synchronized for estrus, treated for ovarian superovulation and inseminated twice with frozen-thawed semen. Between days 1.7 and 7.5 after the first artificial insemination, in vivo-conceived embryos were recovered after slaughter by flushing of the oviducts and uterus and stored at −80°C. Additional embryos were produced in vitro using slaughterhouse bovine ovaries and the same male semen. Proteins from pools of grade-1 embryos at the 4-6 cells, 8-12 cells, morula, compact morula and blastocyst stages (3-5 embryos/pool; 3-4 pools/stage, total of 38 pools) were analyzed by nanoliquid chromatography coupled to tandem MS. Proteins were identified using the UniProt Bos taurus database and quantified by label-free spectral counting method. Data were analyzed using principal component analysis (PCA), ANOVA and hierarchical clustering. Functional analysis of differentially abundant proteins (DAPs) was carried out using the Metascape on-line tool.
RESULTS
A total of 3103 proteins were identified and quantified. The PCA of all data showed a clear separation of embryo pools according to their origin (in vivo vs. in vitro) and stage of development. Two main clusters of DAPs ($p \leq 0.05$) according to the origin were evidenced: proteins upregulated (n=297) and those downregulated (n=228) in vivo compared to in vitro. Pathways and biological processes overrepresented among upregulated proteins included carbohydrate metabolism, immunity and cell adhesion whereas vesicle-mediated transport, metabolism of amino acids and cell response to stress were overrepresented among downregulated proteins.
CONCLUSIONS
These data provide new insights into the molecular contribution of the oviduct and uterine environment to the preimplantation embryo and may help improving in vitro media.

W26
IMPACT OF ORAL E2 EXPOSURE ON MATERNAL ENDOMETRIUM, SYSTEMIC HORMONE CONCENTRATIONS AND CONCEPTUS PREIMPLANTATION DEVELOPMENT IN PIGS.

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BACKGROUND-AIM
Endocrine disrupting chemicals (EDC) are exogenic compounds that interfere with the endocrine system by binding to hormone receptors and evoke an erroneous response. The exposure to EDC at very low doses is linked to adverse direct effects on reproductive organs as well as epigenetic perturbances of the developing conceptus.
METHODS
As many EDCs have an estrogenic effect, we orally exposed gilts to estradiol-17β (E2) during the preimplantation development to assess the physiological and molecular consequences of low-dose E2. Post insemination, animals were randomly distributed in 4 groups receiving different doses of E2 twice daily (0.05, 10 and 1000 µg/kg body weight/day, or carrier control) from day 1 of gestation onwards. Samples were taken at day 10 of gestation at slaughter 1h after the last E2 exposure. Epithelial cell types of the endometrium were isolated by laser microdissection and embryos were manually separated into trophoblast and embryoblast. The distribution and metabolization of E2 in the maternal organism were analyzed by enzyme immunoassay and liquid chromatography– tandem mass spectrometry (LC-MS/MS). Transcriptome analyses were applied on samples of the endometrium, the trophoblast, and the embryoblast. The methylome of the embryoblast was assessed by whole genome bisulfite sequencing (WGBS).
RESULTS
The oral E2 exposure neither impacted on the pregnancy rates, on the morphology of the embryos, nor on the number of embryos per mother. In maternal plasma, E2 and E1-glucoronide conjugates were significantly higher in animals exposed to the highest dose. Interestingly, E1-glucuronide was significantly elevated in animals exposed to the no-effect level (10 µg/kg body weight/day) of E2, pointing to an accumulation of inactive metabolites with a potential of a local metabolization to their active form. Luminal and glandular epithelial cells of the endometrium displayed DEGs (FDR < 0.05) in a dose dependent manner.
CONCLUSIONS
Taken together, oral uptake of E2 lead to an accumulation of E2 metabolites in the maternal organism and evoked transcriptome changes in endometrial epithelial cells. The molecular characterization of the embryos will deepen the understanding of whether and how exogenic E2 affects preimplantation development via a maternal exposure.

W28
IMPROVEMENT IN THE PRODUCTION OF BOVINE TETRAPLOID EMBRYOS THROUGH THE FUSION OF TWO-CELL ZONE-FREE EMBRYO

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BACKGROUND-AIM
Tetraploid embryos differentiate into trophectoderm when they are complemented with diploid embryos. The objective of this study was to optimize the electrofusion of two-cell embryos to generate tetraploid (4n) blastomeres in the presence or absence of zona pellucida (ZP) at two different voltages.
METHODS
After 29 up to 30 hours of initiating the IVF, all embryos with two blastomeres were randomly assigned to the following groups a) ZP free fused with 0.8 Kv/cm (ZP-Free-0.8), b) ZP free fused with 1 Kv/cm (ZP-Free-1), b) intact ZP with 0.8 Kv/cm (iZP-0.8), c) intact ZP with 1 Kv / cm (iZP-1). For ZP-free embryos, ZP was removed by a treatment with a protease. Then embryos were treated with 0.8 Kv/cm or 1 Kv/cm for the fusion of the two cells of each embryo. Each treatment involved 2 pulses of 30 µs separated by 0.1 s of rest. Two hours after fusion, presumptive 4n zygotes were cultured independently in microwells with synthetic oviductal fluid (SOF) medium in 6.5% CO2 in air at 38.5°C for 7 days.
RESULTS
The best fusion rate was observed in the ZP-Free-0.8 group, with 74.83% of fused embryos, compared to 55.75%; 60.37%, and 38.78% for the groups ZP-Free-1; iZP-0.8 and iZP-1 respectively ($p<0.05$). Furthermore, better blastocyst rates were obtained in the ZP-Free-0.8 and iZP-0.8 groups (32.74% and 22.22% respectively) compared to the ZP-Free-1 and iZP-1 groups which showed significantly lower blastocyst rates (4.06%; 9.37% respectively) $p<0.05$.
CONCLUSIONS
Our study demonstrated that removal of the zona pellucida and an intensity of 0.8 Kv/cm improve fusion and blastocyst rates in tetraploid embryos.

TOPIC Female reproductive physiology

W29
GENE EXPRESSION PATTERNS IN UTERUS AND OVIDUCT DURING THE PREOVULATORY PERIOD IN EWES

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BACKGROUND-AIM
The uterine and oviduct environment soon after ovulation plays a major role on fertilization and embryo development. However, the knowledge about the uterine/oviductal environment during the preovulatory period is not fully understood. This study investigated the gene expression oviduct (ampulla and isthmus) and uterus (horns) during the preovulatory period in multiparous ewes.
METHODS
Multiparous Corriedale ewes (n=10) were synchronized during breeding seasonwith intravaginal sponges and equine chorionic gonadotrophin (Menchaca and Rubianes 2004). Estrus was detected after sponge removal by using an androgenized ram. The ewes were euthanized (thiopental sodium Tiobarbital and T-61) from 36 to 48 h after sponge removal (i.e., before ovulation), the reproductive tract was dissected and the oviducts (ampulla and isthmus) and a portion of the upper third of the uterine horns were sampled to be stored at -80°C. Gene expression of PGR (nuclear progesterone receptor), ESR1 (estradiol receptor alpha), IGF2 (Insulin-like Growth Factor 2), PTGS2 (Prostaglandin-Endoperoxide Synthase 2), Serpina14 (Uterine milk protein), SOD1 and SOD2 (Superoxide Dismutases 1 and 2) as housekeeping genes BACT (Beta-Actin), and HPRT (Hypoxanthine-Guanine Phosphoribosyltransferase) were determined by real time PCR. All variables were analyzed using a mixed model procedure in SAS.
RESULTS
The expression of most of the genes (PGR, ESR1, IGF2, PTGS2, SOD1, SOD2) was greater (P<0.001) in the oviduct compared to the uterine tissue. Only the Serpina14 gene had greater (P<0.001) RNA expression in the uterus (62.3±9.0) compared to both ampulla (1.9±9.7) and isthmus (18.1±9.0). Regarding the oviduct tissue, the genes IGF2, PTGS2, SOD1 and SOD2 had greater (P<0.001) RNA expression in the isthmus (2.1±0.2; 8.6±1.4; 2.0±0.2; 2.1±0.1) compared to the ampulla (0.1±0.2; 1.3±1.2; 0.9±0.2; 0.7±0.1), respectively.
CONCLUSIONS
During the preovulatory period, the isthmus segment seems to have a greater gene expression activity of certain genes compared to the other regions, likely improving the environment for the cumulus oocyte complex transport, sperm migration for fertilization, and early embryo passage to the uterus.

W30
BISPHENOL S ADMINISTERED TO PREGNANT EWES WITH CONTRASTED METABOLIC STATUS IMPAIRED THE OVARIAN FOLLICULAR DEVELOPMENT OF FETUSES AND LAMBS

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BACKGROUND-AIM
The size of the ovarian reserve, which is established by the number of primordial follicles, is defined before birth in ovine. The prenatal period is a critical window of sensitivity to environmental factors. Maternal metabolic status during gestation can influence fetal programming, and affect the ovarian reserve. Bisphenol A (BPA), a plasticizer used in food packaging, has deleterious effects on fetal folliculogenesis. It has been banned from the food industry and mainly replaced by bisphenol S (BPS). The objective of this study was to determine the effects of BPS 50 µg/kg/d in utero exposure on female fetuses and lambs from ewes with contrasted metabolic status on follicular population by ovarian histology.
METHODS
This study was divided into two experiments performed on pregnant ewes exhibiting a contrasted metabolic status (lean versus well-fed). First, pregnant ewes were exposed to BPS by daily subcutaneous injection for 3 months, to analyze the ovarian development of the fetuses at 130 days of gestation. Second, pregnant ewes were exposed daily to BPS through food for 3 months and the lambs were then monitored up to 4 month-old (pre-puberty). Follicular population was characterized through follicle classification and counting on ovarian sections.
RESULTS
In utero BPS exposure led to an increase in the number of pre-antral ($p = 0.005$) and antral follicles ($p < 0.001$) in fetuses and conversely to a decrease in 4 month-old lambs ($p = 0.007$). Besides, a significant interaction between maternal metabolic status and BPS exposure during gestation was reported for the number of primordial ($p = 0.019$), pre-antral ($p = 0.003$) and antral ($p < 0.001$) follicles of fetuses. In offspring of fat mothers, the plasma anti-Mullerian hormone decreased in 1 and 2 month-old lambs ($p < 0.001$). In offspring of well-fed mothers, the body weight of the female fetuses increased ($p < 0.001$). Moreover, the body weight of female lambs that were in utero exposed to BPS increased compared to control ($p = 0.040$).
CONCLUSIONS
In conclusion, bisphenol exposure during gestation had deleterious consequences on the body and folliculogenesis of the offspring. BPS effects also vary according to the maternal metabolic status. Further research will be needed to determine if these alterations will have deleterious effects on the ovarian reserve and on the reproductive function in adulthood.

W31
BISPHENOL S IMPAIRED HUMAN AND OVINE GRANULOSA CELL STEROIDOGENESIS

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BACKGROUND-AIM
Human being is exposed to bisphenol A (BPA), a plasticizer used in food containers, through diet. Granulosa cell (GC) play a fundamental role for oocyte growth and maturation. BPA impaired GC steroidogenesis and has been classified as an endocrine disruptor (ED). BPA was banned from the food industry and replaced by structural analogues, particularly bisphenol S (BPS). Given that the ewe has similar follicle kinetic compared to women, the evaluation of the effects of BPS on the ovine would allow to investigate whether the ewe is a suitable model to study ED effects in human. Our objective was therefore to assess BPS effects, and its mechanisms of action, on both human and ovine GC.
METHODS
After puncture of follicles from women undergoing in vitro fertilization or ewe ovaries (slaughterhouses), GC were collected, purified and treated in complemented serum-free Mc Coy Medium, with BPS (1 µM, 10 µM or 50 µM) for 48-h. We analysed GC viability (adenylate kinase activity assay) and proliferation (incorporation of BrDU), secretion of oestradiol and progesterone (ELISA essay), expression of steroidogenic enzymes (Western Blot), hormonal receptor gene expression (quantitative RT-PCR) and MAPK3/1 signalling pathway, as it is involved in both survival and proliferation cellular process (Western Blot). Results were analysed using non parametric permutational ANOVA and Tuckey post-hoc test.
RESULTS
BPS 10 µM significantly decreased progesterone secretion of both human (16 %; $p = 0.0059$) and ovine (22 %; $p = 0.0402$) GC compared to control. BPS 50 µM significantly decreased oestradiol secretion in human GC (46%; $p < 0.0001$), while it was significantly increased with BPS 10 µM in ovine GC (198%; $p = 0.0082$). In both ovine and human GC, BPS did not affect cell viability, proliferation, protein expression of steroidogenic enzymes, PR gene expression, or MAPK3/1 phosphorylation.
CONCLUSIONS
Thus, the effects of BPS on GC appear harmful and similar between human and ovine; except for oestradiol secretion, likely due to the ovulatory stage of GC collection for women. Our data confirmed that the ewe is a relevant model, in term of sensitivity, for the human female reproduction. Ewe GC will allow us to further study the detailed mechanisms of action of BPS on female reproduction.

W33
INTERRELATIOSHIP BETWEEN INTRAFOLLICULAR DOPAMINE AND ESTRADIOL CONCENTRATIONS IN CYCLING MARES

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BACKGROUND-AIM
Follicular hormonal changes associated with oocyte maturation can be modulated locally by monoamines (Bódis et al., 1993). Animal models, ovariectomy decreases the release of dopamine (DA), that can be reversed with subsequent treatment of estradiol (E2) (Thompson and Moss, 1994). This is consistent with the elevation of DA presumably in relation to high levels of E2 during the estrous cycle. This response suggests that DA in FF could play an important role in the mechanism of ovulation in humans (Bódis et al., 1992). In mares, the effects of DA on follicular growth may be mediated by FSH secretion (King et al., 2008). However, the relationship between DA and E2 concentrations has not yet been investigated. The objective of this study was to investigate the interrelationship between DA and E2 in follicular fluid (FF) in different categories of follicular sizes in cycling mares.
METHODS
A total of 60 samples of FF by aspiration of slaughterhouse ovarian follicles of 30 clinically healthy mares aged 6.6 ± 1.3 years are evaluated. FF samples were classified according to diameter as: small (20-30 mm), medium (31-40 mm) and large (≥ 41 mm) size. Intrafollicular DA (pg/mL) concentrations were measured with a radioimmunoassay technique (Labor Diagnostika Nord GmbH, Nordhorn, Germany). The concentrations of E2 (ng/mL) in FF are determined by a competitive enzyme-linked immunosorbent assay (Estradiol sensitive ELISA Demeditec DE4399) validated specifically for FF in the equine species.
RESULTS
The intrafollicular concentrations of E2 in small follicle were significantly lower (652.9 ± 241.3 pg/mL) than in medium (1.498.9±205.8 pg/mL) and in large (1.692.7±146.8 pg/mL) follicle sizes ($P < 0.05$). In FF, the concentrations of DA in medium size follicles were significantly higher (707.9±360.7 ng/mL) than in small (306.2±113.8 ng/mL) and large (351.2±132.5 ng/mL) follicle sizes ($P < 0.05$). DA and E2 concentrations are positively correlated ($r=0.66$; $P < 0.05$).
CONCLUSIONS
These results suggest that, as is the case at the circulating level, ovarian estrogens are involved in dopaminergic activity in follicular development, or that catecholamines modulate the stimulating effect of estradiol in follicular fluid.

W34
ANTIOXIDANT CAPACITY OF FOLLICULAR FLUID IN RELATION TO FOLLICULAR GROWTH AND HORMONAL STATUS IN CYCLING MARES

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BACKGROUND-AIM
Follicular fluid (FF) contains diverse components as steroid hormones, reactive oxygen species (ROS) and antioxidants (Ambekar et al., 2013) among other. The most common antioxidants detected in the FF are superoxide dismutase (SOD), selenium-dependent glutathione peroxidase (SeGPx), catalase (CAT), thioredoxin and glutathione peroxidase (GPx), vitamins (A and C), selenium (Nashiri et al., 2017) and ferric reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) (El-Shahatam and Kandil, 2012). Variations of ROS/antioxidants in FF have been studied in humans (Nashiri et al., 2017) and buffaloes (El-Shahat and Kandil, 2012). Indeed, the normal balance between ROS/antioxidants is related to the metabolic activity of follicular cells, reflects the physiological status of the follicle, is essential for oocyte maturation and fertilization, granulosa cell proliferation, differentiation, ovulation and subsequent pregnancy (Hennet and Combelles, 2012). Until now, these mechanisms remain unknown in the mare. The objective of this study was to determine the total concentrations of antioxidant capacity during follicle development, and his relationship with steroid hormones in cycling mares.
METHODS
A total of 27 follicles of 18 mares categorized as small (20-30 mm; n=10), medium (31-40 mm; n=10) and large size (≥ 41 mm; n=7) were analyzed. FRAP and CUPRAC were determined by means spectrophotometric assays specifically validated for equine species. E2 and P4 were determined by a competitive enzyme-linked immunosorbent assay and RIA, respectively.
RESULTS
FRAP and CUPRAC concentrations decreased significantly with follicular growth ($P < 0.05$). Both antioxidants were significantly correlated ($r=0.80$; $P < 0.05$). FRAP and CUPRAC were negatively correlated with the follicular diameter ($r=-0.48$ and $r=-0.59$; < 0.05), E2 ($r=-0.31$ and $r=-0.45$; < 0.05) and P4 ($r=-0.40$ and $r=-0.42$; < 0.05) concentrations, respectively.
CONCLUSIONS
The reduction of intrafollicular antioxidants inverse to the increase in follicular dimensions could represent a physiological mechanism of follicular protection against oxidative damage for the maintenance of normal cellular function. Future studies are needed to determine ROS and their relation with antioxidants in follicular fluid in normal mares.

W171
NUCLEAR PROGESTERONE (P4) AND ESTROGENS RECEPTORS IN OVINE PLACENTA ON DAY 22 OF PREGNANCY: EFFECTS OF ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)

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BACKGROUND-AIM
Because steroids mediate uteroplacental development, we hypothesized that protein expression of progesterone (P4) and estrogens receptors will be altered in placenta after application of ART techniques.
METHODS
Uteroplacental tissues were collected from natural (NAT) pregnancies or after embryotransfer (ET) from natural mating of FSH-treated ewes (NAT-ET), in-vitro fertilization (IVF) or in vitro activation (IVA; parthenotes) on day 22 of gestation (n = 4-7/group). Immunofluorescent staining of formalin-fixed tissue sections was used to detect P4 receptors (PGR) AB, and estrogens receptors (ESR) 1 and 2, followed by generation of images of the entire tissue cross sections using the Zeiss MosaiX tiling and image stitching protocol. Intensity of staining was quantified via image analysis to determine expression levels in selected placental compartments including fetal membranes (FM), luminal epithelium (LE), endometrial glands (EG), endometrial stroma (ES) and myometrium (Myo). Data were analyzed using SAS application package.
RESULTS
PGRAB, and ESR1 and 2 were detected in utero-placenta, and intensity of fluorescence of PGRAB and ESR1, but not ESR2, was affected by application of ART in selected compartments. PGRAB expression in ES was less ($P<0.05$) in NAT-ET than NAT and IVA, and in Myo was less ($P<0.03$) in NAT-ET and IVF than IVA. ESR1 expression in FM, LE, EG, ES and all compartments combined was less ($P<0.0001$-0.05) in NAT-ET, IVF and IVA than NAT.
CONCLUSIONS
Application of ART affected expression of PGRAB and ESR1, but not ESR2, in placental compartments indicating that PGR and ESR1 may play different functions for selected placental compartments during early pregnancy in sheep. Changes in PGRAB and ESR1 expression in selected compartments in NAT-ET, IVF or IVA groups may explain altered placental development and function after application of ART. Supported by NIH grant R03HD076073 to LPR and ATGB.

TOPIC Horse reproduction

W35
EXPRESSION OF HEAT SHOCK PROTEINS IN THE TESTIS OF CRYPTORCHID HORSE

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BACKGROUND-AIM
The reproductive activity of cryptorchid animals is potentially compromised. A rise in testicular temperature leads to reduced sperm output, decreased sperm motility, increased proportion of morphologically abnormal spermatozoa and predisposes to the onset of testicular tumors. In the horse the incidence of this developmental defect is ~ 9% and the testis migration from the abdomen to the scrotum occurs between 30 days before birth to 10 days after birth and it is necessary to lower temperature allowing spermatogenesis. To acquire thermotolerance and avoid damages because of the increased temperature, cells synthesize heat shock proteins (HSPs), which are categorized in families according to their molecular weight. Since HSP60, HSP70, and HSP90 are involved in spermatogenesis, steroidogenesis and tumorigenesis, in this study we evaluated their expression in the horse cryptorchid testis.
METHODS
Proteins were extracted from ten cryptorchid stallions: five with complete abdominal and five with inguinal testis retention; contralateral descended testes were used as a control. The HSPs expression was evaluated by western blot using anti HSP60, HSP70, and HSP90 monoclonal antibodies (Santa Cruz Biotechnology). The expression of each protein was related to beta-actin level. Gel bands were scanned and quantitate by density contrast (OD = contour x mm2) using Gel-Doc and Quantity One software. Data were analyzed for statistical significance by ANOVA test. Histological investigations were also carried out.
RESULTS
The expression of HSP60, HSP70, and HSP90 was increased in the undescended testes when retention was inguinal ($P<0.05$); on the contrary it remained unchanged in respect to the normal testis when retention was in abdomen.
CONCLUSIONS
The findings suggest that temperature rise occurring when testis is retained represents a positive stimulus for the significant increase of HSP60, HSP70, and HSP90 expression (retained testis vs normal testis). This increase could have a role in inducing functional modifications that can lead to infertility and to a high risk to develop testicular tumors.

W36
ARE THE UTERINE EXTRACELLULAR VESICLES BIG PLAYERS IN THE MATERNAL RECOGNITION OF PREGNANCY IN MARES?

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BACKGROUND-AIM
Uterine extracellular vesicles (EVs) have emerged as key signaling mediators of the embryo-maternal dialogue leading to a successful pregnancy. However, limited is known about uterine EVs and their molecular cargo in equine pregnancy. Moreover, the embryonic signals or mechanisms of maternal recognition of pregnancy (MRP) remain elusive in horses compared to other mammals. We hypothesize that uterine EVs carry signals for the MRP in horses. Therefore, our objective was to determine the RNA and protein content of uterine EVs collected from pregnant and non-pregnant cyclic control mares.
METHODS
Low volume uterine lavage (LVL) (100 mL PBS) was performed in pregnant mares on days 10, 11, 12, and 13 (P10-P13, n=4/day), and on days 10 and 13 (C13 and C13, n=4/day) post-ovulation in non-pregnant. Pregnancy was confirmed by ultrasonography and embryo recovery after LVL. The isolation of EVs from LVL was conducted by combining Centricon ultrafiltration, size-exclusion chromatography, and ultracentrifugation. Characterization of EVs was performed by electron microscopy, immunoblotting, and nanoparticle tracking analysis.
RESULTS
These analyses confirmed the presence of a population of exosomes at 40-100 nm range, positive for known exosomal markers (CD9, CD81, Alix, and TSG101) and a smaller population of microvesicles in the 100-250 nm range. The RNA-sequencing of the EV RNA cargo showed a dynamic transcriptome profile across all samples. The highest number of differential RNAs was observed between C10 and C13 (FDR<5%: 1359 RNAs), followed by C13 vs. P13 (202 RNAs) and with almost no differences between C10 and P10 (FDR<5%: 4 RNAs). Comparison between pregnant samples revealed the highest differences between P10 and P13 (FDR<5%: 162 RNAs). In addition, 13 miRNAs were differentially abundant among groups. Mass spectrometry identified 2458 proteins, with 53 proteins (FDR <10%) differentially abundant among all groups. Marked differences in EV protein cargo were found mainly in C10 vs. C13 and P10 vs. P13.
CONCLUSIONS
In conclusion, this is the first extensive RNA and protein profiling of uterine EVs around the time of MRP in horses. Our ongoing integrative analysis of EV components with embryo and endometrium datasets will further illustrate the potential roles of EVs in signaling MRP in horses.

W37
THE IMPACT OF DAYS OF GESTATION ON THE LIPID PROFILE AND THE AMOUNT OF ADIPOSE DEPOSITION IN LACTATING AND NON-LACTATING MARES

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BACKGROUND-AIM
Studies report an incidence of obesity of more than 50% in the equine population and it is associated with altered metabolic and reproductive activities, and decreased insulin sensitivity. In pregnant mares, no study has evaluated subcutaneous fat throughout pregnancy and there is little data on lipid profile.
METHODS
Seven non-lactating (NL) and 5 lactating (L) reproductively normal mares, 5 to 15 years old, BCS between 4-8 were used. They were evaluated on the day of ovulation (D0), during gestation (55, 110, 165, 220, 275, 330 days), and day 21 postpartum (D21pp). Subcutaneous fat ultrasound of the croup (CR),12 intercostal space (ICS) and retroperitoneal area (RE), was performed. Triglyceride (TG) and NEFA concentrations were evaluated. Data (mean ± SEM) were analyzed for significant effects of lactational status (L vs NL), and days before, during and after pregnancy, and interaction between them.
RESULTS
There were no differences over days on CR; but it was higher in the NL group (p<.0001); similar to ICS (p=0.003), which was also affected by day (D0:0.58±0.09abc;D55:0.63±0.05 ab;D110:0.76±0.07a;D165: 0.72±0.07ab;D220:0.59±0.07abc;D275:0.61±0.05ab;D330:0.60±0.05bc;D21pp:0.44±0.04c, p=0,01); RE decreased over days (D0:0.62±0.06ab;D55:0 .65±0.06ab; D110:0.63±0.05ab;D165:0.5 ±0.02abc; D220: 0.5±0.03abc;D275:0.49±0.01bc;D330:0.49±0.02bc;D21pp:0.44±0.04c, p=0,02), no differences in RE were present between groups. There was a significant interaction of lactational status and days on NEFA and TG: for L, TG increased during gestation, but decreased on D21pp (D0:19.61±1.58bc; D55:16.12±1.16c;D110:17.58 ±1.81c;D 165:28.32±2.96bc;D220:34.38±3.9ab;D275:34.61±4.95ab; D330:46.58±5.56a;D21pp:16.8±1.81c, p<0.0001), for NL did not change along gestation and decreased on D21pp. For NEFA, in L mares there was no day effect while in NL mares, day effect was significant (D0:0.22±0.03b;D55:0.24±0.04b; D110:0.26±0.04b; D175: 0.39±0.05ab; D220:0.38±0.06ab ;D275:0.62±0.07;D330:0.41±0.05ab; D21pp:0.35±0.08ab, p=0.0001).
CONCLUSIONS
All variables, except RE, were higher in NL showing an association of lactational status on the lipid profile and the body fat composition. Moreover, only in NL there was an effect of gestation time on NEFA concentrations, demonstrating greater fat mobilization in this group at D275. Supported by CAPES and FAPESP.

W38
OXIDATIVE PROFILE IN MULE NEONATES

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BACKGROUND-AIM
The birth of a foal causes sudden changes from the intrauterine to the extrauterine environment, stimulating physiological adaptation events and oxidative conditions, with the production of reactive oxygen species (ROS). When an unbalance occurs, it can result in oxidative stress, cell damage and neonatal affections. The oxidative profile has been studied in different species, including horses, although reports were not found in mules. Thus, the aim of the present study was to evaluate the oxidative profile in mule neonates, compared to equine neonates during the first month of life.
METHODS
Eleven mule foals and eleven equine foals were evaluated at birth, with 1, 6, 12 and 24 hours, 7 and 30 days of life (committee on ethics in animal use n° 6001260715). Lipid peroxidation, through the test of thiobarbituric acid reactive substances (TBARS) was measured. The antioxidant system was evaluated by the enzymatic activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD). The SAS System for Windows 9.3 (SAS, 2000) program was used for data analysis, and p<0.05 was considered significant.
RESULTS
There was no interaction between time and group (TBARS p = 0.48; SOD p = 0.50 and GPx p = 0.36); however, they were all affected by time (TBARS, p <0.0001; SOD p = 0.005; GPx, p <0.0001). TBARS showed a progressive decrease over time, while GPx remained similar until 7 days of life, with an increase at 30 days. SOD was statistically similar at all times, with the exception time 1h, which had lower activity. Regarding groups, mule neonates had lower TBARS values (p = 0.01) and higher GPx activity (p = 0.01).
CONCLUSIONS
As expected, the lipid peroxidation was higher after birth but it decreased with time and with the action of antioxidants enzymes. It suggests a different oxidative profile in hybrids at this time of adaptation to the extrauterine environment, but effective considering that all foals studied were healthy.

W39
ENDOMETRIAL OXYTOCIN RECEPTORS IN REPRODUCTIVELY NORMAL AND MARES WITH DELAYED UTERINE CLEARANCE

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BACKGROUND-AIM
Mares with delayed uterine clearance (DUC) released greater amounts of 13,14-dihydro-15-keto-Prostaglandin F2a (PGFM) than reproductively normal mares after oxytocin administration on day 3 of Estrus. Hypothesis is that endometrial inflammation increases uterine oxytocin receptors (OTR). OTR concentrations during estrus were determined in normal and DUC mares.
METHODS
Mixed breed mares, age from 4-14 years, were categorized as reproductively normal (Group 1, n=6) or with DUC (Group 2, n=5). Group 1 mares had no history of endometritis, cleared an intrauterine inoculum of 106 Streptococcus zooepidemicus within 96 h and during the following estrus cleared more than 50% of radio-labelled colloid in a 2-hour period. Group 1 mares had a grade I or II endometrial biopsy scores based on inflammation, fibrosis, and lymphatic lacunae. Group 2 mares had infertility due to endometritis and were unable to clear an intrauterine inoculum of 106 Streptococcus zooepidemicus within 96 hours and, during a second estrus cleared less than 50% of a radio-labelled colloid in a 2-hour period. Group 2 mares had a grade II endometrial biopsy score. During a 3rd cycle on day 3 of estrus, or when the dominant follicle was >35 mm, 2 endometrial biopsies were taken from each mare and frozen immediately in liquid nitrogen. OTR Assay for was performed, and data analyzed using General Linear Model Procedure of SAS.
RESULTS
Of the variables measured (Protein, DNA, OTR/protein, OTR/DNA and Kd), only DNA concentrations differed between groups, being lower in DUC mares (1.58±0.2 mg/g vs 2.2±0.2 mg/g, p<0.07). Oxytocin receptors per cell (OTR/DNA) did not differ (1784±531 fmol/mg vs 1206±442 fmol/mg, p>0.1). Lower concentration of DNA in mares with DUC may indicate lower rate of cell proliferation and greater amount of uterine stretching and fibrosis, which may result in less cells per unit area of tissue. Variances for OTR/protein and OTR/DNA were greater in DUC mares (Group 2) compared to Normal Mares (Group 1). Estimates of the Kd and variances did not differ between groups 1 (1.120±0.11 nmol/L) and 2 (1.014±0.14 nmol/L).
CONCLUSIONS
Abundance of OTR is not responsible for the difference in PGFM responses to oxytocin administration between the two groups.

W41
THE ACTIVITY OF PARAOXONASE TYPE 1 IN DONKEY SEMINAL PLASMA IS RELATED TO SPERM CRYOTOLERANCE.

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BACKGROUND-AIM
Paraoxonase type 1 (PON1) is an extracellular enzyme associated with high-density lipoproteins (HDL) that possesses antioxidant and anti-inflammatory properties, preventing the oxidation of low-density lipoproteins and HDL and thus protecting cells against oxidative stress. This enzyme has been recently identified in the seminal plasma (SP) of some species, where high levels of PON1 activity have been related to an improvement in motility, fertility and freezing capacity of spermatozoa, together with a decrease in the generation of intracellular ROS. Therefore, the objective of this study was to describe for the first time the presence of PON1 in donkey semen and to determine if its activity levels are related to sperm cryotolerance in this species.
METHODS
Ejaculates from 15 Catalonian donkeys were collected and separated into two aliquots of equal volume. The first one was used to isolate the SP by centrifugation and to determine the levels of PON1 activity, whereas the other aliquot was cryopreserved. After thawing, the ejaculates were classified hierarchically as of good (GFE) and poor freezability (PFE) according to the percentages of total motile sperm (MT) and of sperm with an intact plasma membrane (SYBR14+/PI-). The levels of PON1 activity found in GFE (n=8) and PFE (n=7) were compared by means of a t-test for independent samples ($P < 0.05$), using the R software (V4.0.3, R Core Team, Austria).
RESULTS
The levels of PON1 activity in the SP of donkey ejaculates classified as GFE were significantly higher ($P < 0.01$; ranging between 0.2 IU/L to 0.7 IU/L) than those found in ejaculates classified as PFE (ranging between 0.1 IU/L to 0.2 IU/L).
CONCLUSIONS
In conclusion, the levels of PON1 activity are related to sperm cryotolerance in donkey semen and could be used as a freezability marker.

W42
HETEROLOGOUS IN VITRO FERTILITY EVALUATION OF STALLION SPERM AFTER TWO DIFFERENT CRYOPRESERVATION METHODS: PRELIMINARY RESULTS

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BACKGROUND-AIM
Recently, vitrification of stallion sperm has been successfully developed in straws as an alternative method of cryopreservation. In vitro fertilization (IVF) is one of the most accurate indicators to evaluate the fertilizing capacity of sperm. The use of equine oocytes for research is limited. Therefore, heterologous IVF using zona-intact bovine oocytes has been performed in previous studies to evaluate the in vitro fertilizing capacity of frozen stallion sperm. The aim of this work was to assess the fertilizing ability of cryopreserved stallion sperm after conventional freezing or vitrification.
METHODS
Samples (n=2) were collected from two stallions. Conventional freezing was performed in 0.5 ml straws frozen in LN2 vapours. Vitrification was performed in covered 0.25 ml straws plunged directly into LN2. Frozen sperm from a single bull was used as control. IVF ability was evaluated by pronuclear formation at 20 h post-insemination (hpi) and embryo cleavage rate after 48 hpi; for this purpose, oocytes were fixed and stained with Hoechst 33342. IVF parameters were compared between treatments by ANOVA. Results were expressed as mean ± standard error.
RESULTS
Homologous group showed a significant higher ($P < 0.05$) pronuclear formation (71.43 ± 8.7 %) and cleavage rate (73.33 ± 8.2 %) than frozen or vitrified stallion sperm. No differences were found between frozen or vitrified stallion sperm in pronuclear formation (frozen= 11.54 ± 4.5 %; vitrified= 18.37 ± 5.6 %) or cleavage rate (frozen= 42.86 ± 6.0 %; vitrified= 47.89 ± 6.0 %).
CONCLUSIONS
Therefore, we can conclude that heterologous IVF using zona-intact bovine oocytes can be performed in order to assess the fertilizing ability of either vitrified or frozen stallion sperm. Further studies are needed, including a large number of animals and ejaculates, as well as the evaluation of pronuclear formation at different times post-insemination.

W43
SPERM DNA DAMAGE ASSAYS FOR STALLIONS CAN PREDICT REPRODUCTIVE OUTCOME

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BACKGROUND-AIM
Despite the male being such a large contributor to fertility loss during assisted reproduction, the causative factors accounting for ~30% of issues have no currently defined origin and therefore, no rational solutions. Frequently, sufficient numbers of sperm are produced and under inspection appear normal, however, it is the poor quality of gametes produced, commonly containing compromised DNA and other forms of cellular damage, that drive fertility loss. This makes the standard microscopic assessments that are still routinely used for fertility monitoring in many animal species, a poor predictor of male fertility and reproductive outcome. A key barrier to advancing the field is our limited understanding of the mechanistic basis of sperm dysfunction, however sperm DNA damage assays are facilitating the progress.
METHODS
Dismount samples from 15 commercial thoroughbred stallions were analysed using Comet and Halo assays. Sperm DNA damage parameters from these samples were correlated to conception and pregnancy data. Statistical significance was determined using t-tests between stallion groups, with differences of $p < 0.05$ considered statistically different.
RESULTS
Sperm DNA damage, via the Comet assay, was not related to conception rates based on the 14 day pregnancy scan. However, from the dismount samples of stallions associated with late term pregnancy loss, a significant elevation of DNA damage was observed, compared to stallions that completed a successful breeding season resulting in multiple healthy offspring. Oxidative stress markers, in the dismount samples, align with DNA damage levels.
CONCLUSIONS
The Comet assay can provide valuable predictive fertility data from stallion dismount samples. The potential role of sperm oxidative stress in driving the DNA damage that contributes to late stage pregnancy loss, provides an opportunity for the rational management poor reproductive efficiency.

W44
REPRODUCTIVE EFFICIENCY (RE) CAN BE USED AS AN INDIRECT ESTIMATOR OF MARE´S FERTILITY IN ARGENTINEAN CREOLE HORSES

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BACKGROUND-AIM
Fertility is one of the most difficult traits to estimate in mares due to the lack of large and reliable phenotypic datasets. Even more, in a species in which fertility management varies from no ART´s allowed (in Thoroughbreds) to clones (in polo ponies). However, new methodologies based on the analysis of reproductive traits estimated from pedigree records are being developed. In this study, we analyzed the use of Reproductive efficiency (Re) as a fertility estimation in a large population of Argentinean Creole horses bred under extensive conditions.
METHODS
First, we determined two well-validated reproductive traits (the age at first (AFF) and last foaling (ALF) in a large database of 8025 mares (55,579 foaling records) produced during the last 40 years. Then we determined the Reproductive efficiency (Re), as the relationship (in percentage) between the actual and optimal parity number of a mare during its entire reproductive life, as a method of indirect fertility evaluation in horses. Finally, we performed a genetic evaluation of the traits using a REML multivariate model including herd size (3 levels), breed (2 levels), and the mare´s foaling number (14 levels) as fixed effects, inbreeding of the mare as a lineal covariate and the residual, animal, and HYS as random effects
RESULTS
AFF and ALF averaged 62.18 and 165.09 months respectively, demonstrating the fertility of the breed even at old ages. Average Re was 81.16%, showing a heritability of 0.168, supporting the existence of a genetic effect controlling the trait. Results were also in agreement with previous estimations in Pura Raza Española horses, in which the trait was developed. In addition, the genetic values for Re ranged from -16.10 to 8.11, validating the possibility to increase the fertility of the individuals by genetic selection. Finally, Re showed moderate (-0.31) and high (0.71) genetic correlations AFF and ALF respectively, suggesting that Re is a better global estimator for fertility.
CONCLUSIONS
We demonstrated, by analyzing pedigree records, a quantitative genetic effect on fertility Re in a large population of horses bred under extensive conditions. Our results were in agreement with previous reports in European breeds. Heritabilities and correlations with two additional reproductive traits suggested the validity of the Re as a fertility trait in mares.

W45
MARE AGING AS YOUNG AS 10 YEARS OLD ALTERS GENE EXPRESSION AND STRUCTURE OF TERM PLACENTA WITHOUT AFFECTING FOAL GROWTH

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BACKGROUND-AIM
Maternal aging, excluding extreme ages, is associated with increased foal weight/size and heavier/more voluminous placenta. Maternal parity is a confounding factor as it is often strongly correlated with maternal age. The aim of this study was to analyze the effect of mare age, while excluding a parity effect, on placental structure and gene expression and foal growth and health.
METHODS
Placentas from 10 young (6-year-old, YM) and 12 older (>10-year-old, OM) multiparous mares of similar parity were recovered, measured and weighted after foaling. Placental structure was analyzed by stereology on HES stained histological slides. Paired-end RNA-sequencing was performed on 6 placentas/group (Illumina, NextSeq500). Differential expression was analyzed (DESeq2) using a false discovery rate <0.05 cutoff. Gene Set Enrichment Analysis was performed using KEGG and GO BP databases. Foal growth was monitored from birth to 18 months of age (m). At 6 and 12 m, carbohydrate metabolism was assessed using frequently sampled intravenous glucose tolerance tests and joint X-rays were performed to detect osteoarticular lesions. Stereology, growth and FSIVGTT parameters were analyzed using a linear model with permutations. Fisher tests were used to analyze the prevalence of osteochondrosis (OCD).
RESULTS
Placental weight, volume and surface did not vary according to maternal age. The area of allantoic vessels was reduced (p=0.02) while volume of microcotyledon connective tissue (p=0.05) and total microcotyledon (p=0.08) tended to be increased in OM. Of the 14,716 expressed genes identified in placentas, only 9 were differentially expressed between groups. Pathways enriched in OM were related to immunity whereas those enriched in YM were related to tissue genesis. Neither foal growth nor OCD were affected by maternal age. A decreased insulin pancreas response (p=0.08) with increased basal glycemia (p=0.05) tended to be present in female OM foals at 6m. Basal glycemia (p=0.02) and insulinemia (p=0.03) were increased in YM foal at 12m.
CONCLUSIONS
Mares' age did not alter gross morphology but slightly perturbed term placenta histology and function, suggesting long term adaptation. Growth and prevalence of OCD did not differ but carbohydrate metabolism of foals was affected by maternal age.

W46
THE HORSE A SPECIES WHERE KISSPEPTIN RECEPTOR ACTIVATION INCREASES CIRCULATING GONADOTROPINS BUT DOES NOT ELICIT OVULATION.

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BACKGROUND-AIM
The role of the kisspeptin (Kp) system in mammals' reproduction is well established. Nevertheless, species-specific differences exist. Previous studies in the horse have shown that administration of the shorter endogenous Kp isoform, Kp10, is unable to trigger ovulation even though it could increase gonadotropins plasma concentrations. However, Kp10 has a half-life of a few seconds that could reduce its efficacy. To overcome this issues Kp analogs with a greater half-life than Kp10 were developed. One of these analogs, C6, is able to trigger ovulation in sheep and goat. To further explore the possibility to trigger ovulation in the mare two Kp analogs, one based on the equine Kp10 sequence, eC6, and one on the ovine Kp10 sequence, oC6, were tested.
METHODS
Three experiments were performed to evaluate the efficacy of eC6 and oC6. In a first experiment, eleven anoestrus mares were used to compare the effect of the two analogs. After, the best analog, oC6, was selected to compare, the effect of an intravenous administration and an intramuscular administration (N=7 per group). Finally, the effect of oC6 was tested on sixteen mare in pre-ovulatory state to elicit gonadotropin increase and induce ovulation
RESULTS
During the non-breeding season both molecules at the dose of 150 nmol/mare were mildly active but oC6 provide a better stimulation of FSH than eC6. Furthermore, oC6 was more effective when injected iv compared to im. During the breeding season oC6 (150 nmol/mare) was probed for ovulation induction. The molecule was injected during the preovulatory phase when the follicle diameter was comprised between 34 and 37 mm and a uterine edema was observed. oC6 consistently increased the total amount of gonadotropins released, (FSH P=0.01 and LH P=0.02). However, as shown by transrectal echography and plasma progesterone levels, it was unable to anticipate ovulation compared to control mares.
CONCLUSIONS
Our results further support the observation that Kp10 or its analogs are unable to anticipate ovulation in the mare. This study and previous one corroborate the hypotheses that in the horse the Kp system works differently compared to small ruminants. The question whether longer form of Kp, such as Kp54, would trigger ovulation remains open.

W47
CHARACTERIZATION OF PROSTAGLANDIN E2 AND OXYTOCIN RECEPTORS IN THE STALLION ACCESSORY SEX GLANDS

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BACKGROUND-AIM
While prostaglandins are known to be important in many aspects of reproductive processes in the mare, little is known about the role of prostaglandins in the reproductive tract of the stallion or gelding. Prostaglandins and oxytocin are occasionally administered to augment semen collection; however, their effects on the individual accessory glands are unknown. Additionally, differential expression of oxytocin receptor has been documented in the diseased human and rat prostate, but importance and distribution of oxytocin receptor in the stallion accessory sex glands is unclear. The objectives of this study were to characterize gene expression of EP2, EP4, and OXTR in the equine accessory sex glands using immunohistochemistry (IHC), and to determine if gene expression varied based on age or reproductive status.
METHODS
Ampulla, prostate, vesicular, and bulbourethral gland tissue were collected from mature stallions (n=3), mature geldings (n=3), and 280-day gestation male fetuses (n=3) at time of euthanasia. Fresh tissues were fixed in 10% neutral buffered formalin, then embedded in paraffin. Tissues were sectioned in 5uM slides, and stained with rabbit anti-human polyclonal antibody for EP2 or EP4, or mouse anti-human monoclonal antibody for OXTR. Slides were processed using an IHC Select HRP/DAB kit according to manufacturer instructions. Protein localization of EP2, EP4, and OXTR was evaluated by IHC, and staining was characterized as absent, mild, moderate, or strong.
RESULTS
Prostaglandin E2 receptors EP2 and EP4 and OXTR were expressed in all glands. EP4 was strongly expressed in the luminal epithelium of all glands, moderately expressed in the smooth muscle of the ampulla and prostate, and mildly expressed in the submucosa of the vesicular gland. EP2 was mild to moderately expressed in the luminal epithelium of all glands. OXTR was moderately expressed in the epithelium and mildly expressed in the glandular stroma.
CONCLUSIONS
The presence of EP2, EP4, and OXTR, were confirmed in all male equine accessory sex glands. The relative roles of these receptors in during ejaculation or disease in the stallion are still to be determined.

W48
PREGNANCY RATES IN JENNIES IMPROVED AFTER DEEP-HORN ARTIFICIAL INSEMINATION WITH FROZEN SEMEN RE-EXTENDED IN AUTOLOGOUS SEMINAL PLASMA

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BACKGROUND-AIM
A variety of donkey breeds are under the threat of extinction, and storage of frozen donkey semen is one way of helping to preserve the genetic diversity for the future. In the literature, the very low pregnancy rates after artificial insemination (AI) with frozen semen in donkeys were improved in one study after re-extension in autologous seminal plasma (SP) [1]. The aims of our study were i) to describe in vitro post-thaw parameters of donkey jackass semen after re-extension in seminal plasma or in INRA96 and ii) to compare pregnancy rates in jennies bred with frozen-thawed semen using two different AI protocols.
METHODS
Semen collected from two Amiata donkey stallions was frozen in INRA96 supplemented with 2% egg yolk and 2.2% glycerol, SP was filtered and stored at -20°C. Ovarian activity and sexual behavior of ten cyclic, non-lactating Amiata jennies, were monitored daily by transrectal palpation and ultrasound and by teasing with an adult donkey stallion. When the dominant follicle reached a diameter of ≥ 32 mm and estrous behavior was shown, ovulation was induced with 0.4 ml of GnRH agonist subcutaneously (hour 0). For both deep-horn AI protocols, with (DHAI+SP) or without re-extension in SP (DHAI), an ultrasound was performed at 14, 38 and 42 hours. Jennies were inseminated once, at 38 hours after the induction of the ovulation. Jennies that ovulated after 42 hours were not included in the study.
RESULTS
No positive effects on donkey semen kinematics were observed in in vitro conditions when semen was re-extended in SP. When comparing the different protocols used, DHAI yield to a numerically lower (although not statistically different) pregnancy rates than DHAI+SP (2/9; 22.22% vs 6/10; 60%, respectively, P=0.17).
CONCLUSIONS
Although no statistical difference was found between groups, DHAI+SP was able to obtain pregnancy rates (60%) in the highest range reported in literature for this species. The results obtained in the present study require confirmation with a larger number of animals, and more studies on the characterization of donkey seminal plasma content and their interactions are required to fully understand the potential of seminal plasma in this species.
[1] Rota et al., (2012). Therio, 78(8), 1846-1854.

W49
INFLUENCE OF SUPPLEMENTATION OF FERTILIZATION MEDIUM WITH EQUINE OVIDUCTAL FLUID ON THE FERTILIZING CAPACITY OF FROZEN/THAWED EQUINE SPERMATOZOA

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BACKGROUND-AIM
A repeatable protocol for conventional in vitro fertilization (IVF) has remained elusive in the horse. Recently this group has demonstrated that addition of equine postovulatory oviductal fluid (OF) at 0.125 % (v/v) induces stallion-dependent protein tyrosine phosphorylation (PY) in fresh equine spermatozoa when incubated in a capacitating medium. Hence, the aim of the study was to test the effect of OF on PY induction in frozen equine spermatozoa and determine its usefulness in conventional IVF.
METHODS
Thawed equine spermatozoa (37 ˚C for 60 s; n = 3, one ejaculate per stallion) were incubated for 2 h at 37 ˚C in Modified Whitten's medium (MW; pH = 7.25) in presence or absence of OF at 0.125 % (v/v). After incubation, spermatozoa were fixed and PY was evaluated by immunofluorescence using an anti-phosphotyrosine monoclonal antibody (clone 4G10). In parallel, equine cumulus–oocyte complexes (COCs) retrieved in vivo or post-mortem were matured in Tissue Culture Medium 199 (TCM-199) added with 5 mU/mL of follicle-stimulating hormone and 10 % of fetal bovine serum for 28-30 hours. Mature COCs were then co-incubated in MW medium added with OF at 0.125 % (v/v) for 20-24 hours in a 5 % CO2/95 % air atmosphere at 38.5 °C with 1 x 106 spermatozoa/mL (5 ejaculates from 5 different stallions). After co-incubation, oocytes (n = 78, 6 replicates) were denuded, fixed and stained with 2.5 µg/mL of Hoechst 33342. PY and oocyte fertilization were evaluated by fluorescence microscopy.
RESULTS
The individual percentage of PY positive sperm (stained tails) in control for stallion 1 was 31 %, for stallion 2 was 28 % and for stallion 3 was 26 %, and OF addition yielded 48 %, 22 % and 30 % respectively. The data of the study showed an increase in the percentage of PY positive sperm in stallions 1 and 3 when OF was added, however no effective fertilization was observed.
CONCLUSIONS
Hence, even when PY can be induced in frozen-thawed equine spermatozoa using OF at 0.125 %, this PY induction does not correlate with the sperm´s ability to fertilize an oocyte in the horse. IVF in the horse still remains a matter of study and more research is needed to develop repeatable protocols.

W50
EFFECT OF SEMINAL PLASMA FROM GOOD AND BAD COOLER STALLIONS ON EPIDIDYMAL SPERM-OVIDUCT BINDING

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BACKGROUND-AIM
In horses, sperm binding to the oviduct sperm reservoir appears to be more complex than in other species. Seminal plasma constituents are related to capacitation, binding and release of sperm in the oviduct epithelium. Sperm recovered from epididymis tail do not receive seminal plasma. In this sense, the aim of the study was to evaluate the effect of seminal plasma addition on kinetics and the rate of binding to fallopian tube explants of sperm retrieved from the tail of epididymis.
METHODS
Mini Horse stallions (n=8) were castrated and the sperm from the tail of epididymis was recovered by the retrograde flow technique. Each sample was split into 4 groups: skim milk extender (SM), egg yolk extender (EY), seminal plasma from a good cooler stallion with high fertility rate (GSP) and seminal plasma from a bad cooler stallion with low fertility rate (BSP). Total motility (TM), progressive motility (PM) and rapid sperm (RAP) were measured by CASA method and plasma membrane integrity (PMI) was performed by the epifluorescence microscopy technique. Sperm-oviduct binding test was perfomed using explants from cow's oviducts. Sperm suspensions (1x106/mL) were added to the explants-dropplets for each group and incubated at 38.5°C in 5% CO2 for 30min. Two images were captured (400x) per sperm-oviduct explant. The number of bound spermatozoa was counted per square millimeter (mm2) of explants. Statistical analyses were performed using Shapiro-Wilk and Tuk ey Tests, followed by SAS software.
RESULTS
The SM, EY, GSP groups presented increased values, respectively, of TM (38.8±6.1, 57.8±4.9, 74.4±2.6), PM (10.2±2.5, 21.6±2.5, 34.8±2.2) and RAP (27.0±5.7, 46.0±5.1, 64.1±3.2), whereas BSP (TM: 65.5±3.7, PM: 28.5±2.8, RAP: 48.0±5.5) differed from SM but had similar values to EY and GSP. Plasma membrane integrity did not differ between groups. The number of bound epididymal sperm was similar between the groups (SM: 470.6±106.1, EY: 511.1±70.5, GSP: 455.9±175.3, BSM: 497.8±89.6). However, a high number of sperm-linked per area was observed when compared to other studies, probably due to the selection of the oviducts from cows without corpus luteum.
CONCLUSIONS
The addition of seminal plasma did not increase the epididymal sperm binding rate by explant area to bovine oviducts.

W51
ALTERNATIVE THERAPY FOR SEMINAL VESICULITIS IN STALLIONS

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BACKGROUND-AIM
Seminal vesiculitis is an important reproductive disorder in stallions, due to its persistent nature and high recovery rate. It is caused by bacterial infection inside the seminal vesicles, which P. aeruginosa is one of the most common bacteria isolated. The objectives were: induce seminal vesiculitis and monitor its development; evaluate local infusion of 30% metacresolsulfonic acid and formaldehyde solution (Lotagen®) as treatment; and characterize seminal vesicles and semen quality before and after treatment.
METHODS
Endoscopy (300cmX9.8mm) was used to induce seminal vesiculitis in 3 stallions by delivering P. aeruginosa and 8 wk later 60mL of Lotagen was delivered inside the vesicles. Semen evaluations were done weekly, since 1 wk before infection to 7 wk after treatment. After semen collection by artificial vagina, total motility (TM), progressive motility (PM) and rapid sperm (RAP) were evaluated by CASA; plasma and acrosomal membrane integrity (PAMI) by flow cytometry; percentage of neutrophils (%N) by semen cytology; and bacterial culture.
RESULTS
There were changes in semen color in 3/3 stallions along the study. For stallions 1, 2 e 3, respectively, before infection parameters were: TM% (76, 84, 84), PM% (44, 47, 44) and RAP% (66, 81, 73). During infection: TM% (68, 86, 84), PM% (39, 49, 42) and RAP% (61, 80, 79,). After treatment: TM% (40, 77, 70) and PM% (25, 39, 37) and RAP% (32, 67, 57). Higher values for PAMI% (48, 78, 72) were observed after treatment in comparison to infection period (34, 69, 62), and were similar to before infection (58, 81, 76). Higher %N were observed during infection (24, 17, 18) when compared to after treatment (4, 4, 1). Bacterial culture of semen was negative (2/3) 7 wk after treatment. Stallion 1 developed bilateral epididymitis by S. equi zooepidemicus. During infection, seminal vesicles had purulent material. After treatment, partial occlusion of glands (2/3) and mucosal fibrosis (1/3) were observed by endoscopy. No sings of pain were observed subsequently the treatment.
CONCLUSIONS
Local infusion of 30% Lotagen® solution was effective, halting growth of P. aeruginosa, reducing percentage of neutrophils in semen and maintaining seminal quality (2/3). This approach has promise for an alternative treatment of equine seminal vesiculitis.

W52
THE RELATIONSHIP BETWEEN MITOCHONDRIAL PERMEABILITY TRANSITION PORE FORMATION AND MITOCHONDRIAL MEMBRANE POTENTIAL LOSS IN STALLION SPERM

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BACKGROUND-AIM
Cyclosporin A (CsA) does not prevent mPTP formation in human sperm according to the Calcein-AM/CoCl2 (C-AM) assay, casting doubt on sperm mPTP formation. The JC-1 assay is also used to measure mPTP formation, and the aim of this study was to compare the assays to measure mPTP formation in response to oxidative stress (arachidonic acid; AA) or high intracellular calcium (ionomycin).
METHODS
Exp 1: stallion sperm were pre-loaded with 0, 5, 10 and 20μM CsA, followed by ± 500nM ionomycin. mPTP formation was measured flow cytometrically using the C-AM assay and mitochondrial membrane potential (MMP) was measured using the JC-1 assay. Exp 2: sperm were exposed to ionomycin (0, 0.25, 0.5, 1 and 4μM) or AA (0, 10, 20 and 40μM) for 15 min at 37°C. Live (Far Red LIVE/DEAD negative), high MMP populations (JC-1) were gated and assessed for mPTP formation (C-AM). Exp 3: the temporal effect of 1μM ionomycin and 20μM AA on MMP loss was investigated following incubation at 37°C at 15 min, 1, 2, 3 and 4h using the triple staining technique described in Exp 2.
RESULTS
Exp 1: ionomycin-induced mPTP formation (C-AM: 878±397 to 127±12 AU; P≤0.01) wasn't inhibited by CsA at any concentration. While ionomycin also decreased MMP (37.8±8.3% JC-1 positive) compared to the control (69.3±11.1%; P≤0.05), CsA at 10μM and 20μM inhibited MMP loss (57.1±12.7 and 64.2±13.6%). Exp 2: MMP did not decrease until 4μM ionomycin and 40μM AA (99.5±1.0 vs 87.6±7.4 and 80.9±5.6%; P≤0.05 and 0.001 respectively). Conversely, the C-AM signal (from high MMP cells) was abolished at 0.25μM ionomycin (90.3±7.5 to 0.3±1.4%; P≤0.0001) and in a dose-dependent manner between the control, 20 and 40μM AA (90.3±7.5 vs 45.6±14.8 and 3.9±0.5%; P≤0.001 and 0.0001 respectively). Exp 3: 1μM ionomycin decreased MMP between 15 min and 1h (92.4±3.1 to 45.1±6.6; P≤0.01), and abolished MMP by 3h (5.4±3.9%; P≤0.0001). 20μM AA reduced MMP over time, but this was not significant until 4h (15 min: 95.7±2.3 vs 4h: 79.7±6.9% high MMP).
CONCLUSIONS
While CsA prevents the loss of mPTP-initiated MMP in stallion sperm, it does not directly inhibit mPTP, and prolonged mPTP formation results in a loss of MMP over time. This study proved that it is possible to have mPTP formation without a loss of MMP, and therefore the JC-1 and C-AM assays are not interchangeable.

W53
ANTI-MULLERIAN HORMONE CONCENTRATIONS AND ITS ASSOCIATION WITH AGE AND PREGNANCY STATUS IN MARES

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BACKGROUND-AIM
A previous study (Almeida et al. 2011) reported no effect of the day of the estrous cycle or month of gestation on AMH concentrations. The present study investigated the association between anti-mullerian hormone (AMH) concentrations, age and pregnancy status.
METHODS
A total of 67 mares (n=55 pregnant, n=12 non pregnant) that ranged from 4 to 25 years located in the Reproduction Center Los Callejones del Duende, Aranjuez, Spain were included in the study. Blood samples were taken for AMH and insulin determination, centrifuged and serum was stored at -20 C until hormone determination. Insulin was determined by radioimmunoassays and AMH by ELISA. Animals were categorized according to their age in Group 1 (4 to 6 years, n=14), Group 2 (7 to 14 years, n=32) and 3 (> 14 years, n=21). Data was analysed by ANOVA using a mixed procedure including status (pregnant vs non pregnant), age category and their interaction.
RESULTS
Age category did not affect AMH concentrations (2.67±0.2, 2.75±0.1 and 2.35±0.2 ng/mL for Groups 1, 2 and 3 respectively, P=0.1988). When the analysis was performed in mares < 14 years compared to mares >14 years, a tendency was found for lower concentrations in the elderly group (2.71±0.13 vs 2.36±0.19 ng/mL, P=0.096). The reproductive status affected AMH concentrations, as pregnant mares had lower AMH concentrations than empty mares (2.29±0.10 vs 2.80±0.23 ng/mL, P=0.018). Insulin concentrations were not affected by age (20.0±4.3, 24.3±2.6 and 19.0±3.5 uUI/mL for Groups 1, 2 and 3 respectively, P=0.3863), or reproductive status (20.6±4.2 vs 21.7±1.9 uUI/mL, P=0.8154).
CONCLUSIONS
As far as we know this is the first report showing that pregnancy is associated with lower anti-mullerian hormone concentrations in mares.

W54
PROTEOMIC PROFILING OF FERTILE, SUB-FERTILE, AND INFERTILE STALLIONS REVEALS NOVEL FERTILITY BIOMARKERS

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BACKGROUND-AIM
Stallions are retired from racing with no prior knowledge of their fertility status. Where a severely sub-fertile or infertile stallion commences an ill-fated breeding career, a host of unnecessary economic and welfare cost – stemming from unsuccessful repeated breedings – are incurred. To date there are no effectual processes in place to tackle this problem. As such, this study aimed to compare the sperm proteomic profiles of fertile, infertile, and severely sub-fertile stallions, to identify a library of proteins that could be used to detect unsuitable sires in the future.
METHODS
Semen samples were collected from two infertile, four sub-fertile (conception rates 0–33%) and 15 commercially 'fertile' stallions. Samples were fractionated using an Equipure gradient to isolate high-quality cell populations, and subsequently assessed using LC-MS/MS. Proteins and peptides were identified using the SequestHT algorithm searching against the SwissProt Mammalian and UniProt Equus caballus databases.
RESULTS
A total of 2203 proteins were identified, of which, 10 were significantly more abundant in fertile samples, and 19 more abundant in non-fertile samples (grouped infertile and sub-fertile; $P \leq 0.05$, $FC \geq \pm 1.3$). These included α-aminoadipic semialdehyde dehydrogenase (ALDH7A1; $P \leq 0.001$, FC = -2.1) and acrosin ($P \leq 0.05$, FC = 1.7). When comparing the proteomic conservation, a notable 236 proteins were exclusively conserved to fertile samples. Of note, stress-70 protein, mitochondrial (HSPA9); arylsulfatase A (ARSA); and phospholipase C zeta 1 (PLCZ1) are associated with asthenospermia, fertilisation and oocyte activation, and were completely absent from infertile samples. On the other hand, testisin (PRSS21); α-enolase (ENO1); alipoprotein E (APOA1); and tektin-3 (TEKT3), were absent from sub-fertile samples, and are associated with zona-pellucida binding, fertility, and varicocele.
CONCLUSIONS
Taken together, this preliminary analysis offers a library of putative, protein biomarkers that may have substantial potential for assessing stallion reproductive fitness. These findings will serve to inform the development of novel diagnostics to improve pregnancy success rate, thereby dramatically reducing the economic wastage and welfare implications associated with unsuccessful breedings.

W56
CHARACTERISATION OF PAF AND PAFR IN THE MARE REPRODUCTIVE TRACT

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BACKGROUND-AIM
Platelet-activating factor (PAF) is a potent phospholipid mediator produced and released by embryos of all mammalian species studied to date, but not previously investigated in the horse. PAF acts through a single G protein-coupled receptor (PAFR) on the cell surface. PAF is associated with platelet coagulation, but has been detected in human follicular fluid and has been purported to play important roles in ovulation, sperm-egg interactions and embryo development. This study seeks to determine whether PAF and its receptors are present in the mare's reproductive tract.
METHODS
For detection of PAF by liquid chromatography-mass spectrometry (LC-MS), follicular fluid samples were collected transvaginally from cycling mares (n = 6) via ultrasound-guided aspiration, filtered and immediately frozen in liquid nitrogen. After extraction, analysis was performed on a SCIEX QTRAP 6500. Transition fragments unique to each of the homologs were detected by LC-MRM (negative mode) with 250pg of isotopically labelled C16 PAF -d4 spiked (100 pg/µl).
Intact mare oviducts were obtained from slaughterhouse specimens and fixed in 4% paraformaldehyde (n = 12) and sectioned. Presence of PAF receptor proteins was detected by Western blot and their localisation assessed by immunohistochemistry and confocal microscopy.
RESULTS
PAF was quantitatively detected and identified in equine follicular fluid samples by LC-MS. PAFR protein expression was membranous and was identified predominantly in the apical, but also the basolateral epithelial cells of the epithelium. Immunofluorescence showed receptors were evenly expressed over the luminal epithelial membrane, notably at the level of the ampulla-isthmus junction.
CONCLUSIONS
This is the first time that PAF and PAFR have been identified in mare reproductive tissue. These observations show that equine follicular fluid is enriched with PAF and equine oviduct expresses PAF receptors at the anatomical point of fertilisation. PAF may play a role in ovulation, sperm-oocyte interaction and/or early equine embryo-maternal dialogue. In order to further develop our understanding of early equine pregnancy, future studies should examine the functional contribution of PAF in the processes of conception, particularly within the oviductal environment.

W57
PRELIMINARY RESULTS ON EQUINE FOLLICULAR FLUID PROTEOMICS

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BACKGROUND-AIM
Media used for equine oocyte maturation can be further improved but, until now, the proteomics of equine follicular fluid remains unknown. There is no optimized protocol for equine albumin depletion, which decreases the efficiency of the analysis. We aimed to test the efficiency of albumin depletion using specifically developed columns for the equine species.
METHODS
Follicular fluid was recovered from 1 preovulatory follicle (>35 mm) postmortem, centrifuged and kept at -80°C. The fluid was processed using the ProteoExtract Albumin/IgG Removal Kit (CalBiochem, San Diego, CA, USA), and the samples were analyzed by nanoliquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). A crude sample was also run in parallel. Follicular fluid purification with the above-mentioned columns allowed to reduce the content of serum albumin (isoforms A0A3Q2H333 and P35747), improving proteomic profiling by 62%. The purified follicular fluid revealed the presence of 114 proteins (Mascot scores ≥ 30, significant protein matches ≥ 2). 60 out of 114 proteins were mapped using Equus Caballus as reference gene list and were sifted with the last versions of Gene Ontology, DAVID, and Panther databases. Only categories with p<0.05 and FDR<0.05 (Fisher's exact test) were taken in consideration.
RESULTS
Thirty-five proteins were associated to the categories: extracellular region (GO:0005576), extracellular exosome (GO:0070062), membrane invagination (GO:0010324), vesicle budding from membrane (GO:0006900), and endocytosis (GO:0006897). Moreover, different proteins, as Angiotensinogen and Adiponectin A1, A4, C1Q, and D, were involved in the regulation of protein, steroid and lipid metabolic processes (GO:0051246, GO:0019216, and GO:0019218) and in the regulation of cholesterol transport (GO:0032374). Interestingly, twelve proteins were associated to the Serpin domain (IPR023796).
CONCLUSIONS
Our preliminary results reveal that the columns used increase the efficiency of the analysis. Interesting data were obtained showing that the proteins present in equine follicular fluid are involved in lipid metabolism and extracellular transport which may be essential for the acquisition of developmental competence. Funding: AGL2017-84681 & RYC2017-21545 (AEI/FEDER/UE); GR18094 & TA18008 (Junta de Extremadura-FEDER).

W58
SECRETOME RETRIEVED FROM EQUINE PREOVULATORY FOLLICULAR FLUID IMPROVES IN VITRO MATURATION RATES OF EQUINE OOCYTES

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BACKGROUND-AIM
In the horse, oocytes are retrieved immature and subjected to in vitro maturation (IVM) prior fertilization, but the composition of commercial media does not match the physiological environment. Hence, our aim was to test if the addition of secretome retrieved from equine preovulatory follicular fluid enhances maturation rates of equine oocytes.
METHODS
Follicular fluid (FF) was retrieved from 2 healthy mares. An ultrasound was performed and when a preovulatory follicle (> 35 mm) and uterine oedema grade 3 (scale 1 to 3) was detected, 3000 IU of hCG were administered intravenously. Thirty-two hours later, FF was retrieved by flank aspiration. Three ml of the fluid from both mares was pooled, diluted 1:1 in sterile phosphate buffer saline and centrifuged (4000 g, 1 hour at 4 °C) using a 10K Amicon® Ultra-15 Centrifugal Filter Unit. Protein concentration of the solution collected (secretome) was measured and frozen at - 80 °C until use. Equine cumulus-oocytes complexes (41 in total) were retrieved by ovum pick up from 6 mares in 3 different sessions. The oocytes were subjected to IVM in DMEM/F12 medium added with 10 % of FBS and 5 mU/ml of FSH (control medium; 19 oocytes) or in control medium added with 32 µg/ml of secretome (22 oocytes) for 26 hours in 5 % CO2/95 % air atmosphere at 38.2 °C. Then, the oocytes were denuded, fixed in 4% formaldehyde in PBS and stained with 2.5 µg/ml of Hoechst 33342 for 10 minutes at 37 °C. The oocytes were mounted on slides using glycerol and the chromatin was visualized by fluorescence microscopy. The Student's t test was used to compare groups; significance was set at $p < 0.05$ and data are presented as mean ± standard deviation (SD).
RESULTS
In the control group, 39 ± 1.7 % of the oocytes were at metaphase II, in the secretome group, the maturation rate reached 68.7 ± 5.7 %; $p < 0.001$). In the control group, 34 ± 10.4 % of the oocytes were in germinal vesicle stage while in the secretome group this percentage dropped to 11.7 ± 10.1% ($p > 0.05$).
CONCLUSIONS
Our data demonstrate that the addition of secretome retrieved from equine preovulatory FF improves maturation rates in equine oocytes undergoing IVM. More studies are required to fully establish if cytoplasmic maturation is also improved resulting in enhanced blastocyst rate.

W59
LUTEAL BLOOD FLOW AND SIDE EFFECTS IN SMALL BREED JENNIES AFTER INDUCTION OF LUTEOLYSIS WITH DINOPROST TROMETHAMINE AND CLOPROSTENOL SODIUM

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BACKGROUND-AIM
Administration of luteolytic agents in donkeys has been reported to use the doses recommended for horse. However, there are no studies evaluating the side effects of PGF2a and its analogue after treatment. The aim of the study was to evaluate the blood flow and side effects in jennies after treatment with two luteolytic drugs
METHODS
Five days after ovulation, eight jennies (144 ± 22.5 kg and height 95.5 ± 113 cm) were randomized in a crossover design to receive either cloprostenol (250 mcg/animal), or dinoprost tromethamine (5 mg/animal) intramuscularly. B-Mode and Doppler ultrasonography examinations were performed 15 min before (-15) administration of either drug and at times 0, 15, 30, 45, and 60 min and 2, 3, 4, 5, 6, 7, 8, 12, and 24 h after application. Animals were observed from a distance for side effects at-15minutes-intervals for 1 h after administration of the luteolytic agents. Data were assessed for normality Shapiro Wilk's test. Comparisons of the CL area and luteal blood flow were performed using PROC MIXED of SAS 9.4. Jennies were accounted as a random effect, whereas time and luteolytic agents were fixed effects. Interactions of fixed effects were also assessed. Statistical significance was considered as $p \leq 0.05$. Data are presented as mean ± SEM
RESULTS
A significant increase in CL blood flow was observed 60 min and 45 min after drug administration for dinoprost and cloprostenol, respectively. There was a significant increase in CL blood flow at 4 h after dinoprost administration in comparison to group 2. However, at 5, 6, 7 h jennies that received cloprostenol had greater vascularity than group 1 animals. . Both drugs had a progressive reduction at later times. Blood flow and corpus luteum area decreased gradually during the first 24 h in both groups. Regarding the observation of side effects, group 1 presented a major score of sweating ($p<0.05$), while in group 2, greater abdominal discomfort and diarrhea were evidenced as a major side effect ($p<0.05$).
CONCLUSIONS
While both drugs seem equivalent to induce luteolysis in donkeys, dinoprost resulted in more profound side effects. Further studies are needed to determine whether lower doses may effectively induce luteolysis with minimal side effects in small breed donkeys.

W60
THE EFFECT OF HEAT STRESS ON EARLY EMBRYONIC LOSS IN EMBRYO TRANSFER MARES

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BACKGROUND-AIM
Heat stress has long been identified as detrimental to reproductive success in several livestock species, but little is known about its impact in horses. As global temperatures rise and extreme weather events become more frequent, their effect on equine reproduction needs to be explored. Thus, the aim of this study was to investigate the relationship between ambient climatic conditions, as measured by temperature-humidity index (THI) between day 7 and day 14 of gestation, and early embryonic loss (EEL) in embryo transfer mares.
METHODS
Data from 834 embryo transfers was used from an equine breeding facility in Victoria, Australia. Pregnancy loss was indicated by a positive embryo transfer on day 7 and negative transrectal ultrasound on day 14 of gestation. Maximum THI for the day of transfer (day 7) and mean THI between day 7 and day 14 was established. Other recorded variables included quality and age of the embryo, quality of the recipient mare and quality of the embryo transfer. Results were based on odds ratios generated by multivariable logistical regression after the confounding variables were adjusted for.
RESULTS
EEL was observed in 21% of embryo transfers. A five-unit increase in maximum THI on the day of transfer was associated with a 1.18-times increase in early embryonic loss ($P = 0.01$), after accounting for changes due to the impact of embryo and recipient grade. Similarly, the likelihood of EEL increased by a factor of 1.25 for each five-unit increase in mean THI between day 7 and 14 ($P = 0.003$).
CONCLUSIONS
In conclusion, these results suggest that both single and cumulative heat stress are correlated with an increased likelihood of EEL in embryo transfer mares. This has significant implications for breeding management of mares, particularly during the summer months.

W61
REPEATABILITY OF ANTRAL FOLLICLE COUNT AND REPRODUCTIVE CHARACTERISTICS IN MARES OF DIFFERENT AGES

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BACKGROUND-AIM
Advancing age in mares reduces the fertility, but the relationship with antral follicle count (AFC; follicles ≥2 mm diameter) is still limited. Therefore, the aim of this study was to determine repeatability of AFC, preovulatory follicle diameter, and degree of uterine edema in young and old mares.
METHODS
To evaluate the AFC and reproductive characteristics, Quarter Horse mares (n=15), ranging from 3 to 17 years of age, healthy and in good nutritional status were divided into mares considered young (3 to 9 years) or old (10 to 17 years).
Maintained in the same management system during ovulatory season, a minimum of 3 and maximum of 10 cycles were monitored by ultrasonography (a single practitioner), every 24 to 48h during the follicular phase (estrus). In each evaluation, AFC (both ovaries), dominant follicle diameter (mm) and degree of uterine edema (0 to 4 scale) were determined. For statistical analysis, AFC, maximum diameter of ovulatory follicle (mm) and uterine edema were analyzed by general linear model (GLM). Repeatability was calculated from #2 animal / (#2 animal + #2 error) according to Boni et al. (1997).
RESULTS
Data are presented as the mean ± standard error (P ≤0.05). AFC ranged (P < 0.001) from 4 to 14 follicles among mares (8.3 ± 0.1). The average number of antral follicles was higher (P = 0.04) in young (8.9 ± 0.5) than in old mares (7.9 ± 0.5). However, throughout the evaluations and among cycles, AFC proved to be highly repeatable, 0.97 for younger and 0.98 for old mares. The maximum diameter of ovulatory follicle was similar (P = 0.17) between young and old mares (38.7 ± 0.3 and 37.7 ± 0.9 mm, respectively). However, young mares had a higher degree of uterine edema (P = 0.04) than old ones (3.3 ± 0.1 vs. 2.9 ± 0.2).
CONCLUSIONS
In conclusion, AFC is a variable reproductive parameter among mares but highly repeatable in same female regardless of estrous cycles. Also, younger mares have higher AFC throughout cycles and develop greater uterine edema during the follicular phase.

W62
TRANSCRIPTOMIC ANALYSIS OF EQUINE PREMATURE PLACENTAL SEPARATION

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BACKGROUND-AIM
Premature placental separation (PPS; red bag) accounts for 5–10% of equine abortion, stillbirth, and perinatal death. However, the molecular mechanisms underlying PPS are poorly understood. Therefore, the current study characterized the transcriptome of equine chorioallantois (CA) retrieved from mares with PPS compared to normal postpartum CA.
METHODS
A total of 29 CA, including 4 control and 25 PPS, were collected from Thoroughbred mare. Mare's age were 10.5 ± 4.7 in control and 11.5 ± 3.9 in PPS. RNA-Seq was performed using Illumina NovaSeq6000 and mapped to EquCab3.0 using STAR 2.7.2a. Mapped reads were quantified using Cufflinks 2.2.1 with the NCBI annotation. PPS samples were classified into three clusters using principle component analysis. Differentially expressed genes (DEGs) between control and each cluster were determined using Cuffdiff 2.2.1 based upon an FDR<0.05. In order to gain insight about the biological function of DEGs, DAVID Gene Ontology analysis was carried out. In order to determine the key regulators triggering the transcriptomic changes in each cluster, IPA® upstream regulators analysis was used.
RESULTS
Overall, 1,204 DEGs were overlapped between the three clusters. Collagen fibril organization and cell adhesion were significantly overrepresented in biological processes. In cellular components, proteinaceous extracellular matrix, extracellular space, extracellular matrix, extracellular exosome, focal adhesion, and basement membrane were significantly overrepresented. Collagen (COL1A2, COL5A1, COL5A2, COL6A1, COL6A2, COL6A3, COL11A1, COL14A1, COL15A1, COL16A1, COL18A1, COL21A1, COL27A1) and proteoglycan (VCAN, BGN, DCN, FMOD, LUM) transcripts were up-regulated in the three clusters. Although collagenases (MMP1, 8, 13) were not changed, the inhibitor gene (TIMP1) was up-regulated. Eleven upstream regulator genes (CPXM1, FGFR1, HIF1A, IGF1, JUNB, SAA1, SMAD3, TGFB2, TGFB3, TNFRSF1A, and TYROBP) were overlapped between the three clusters.
CONCLUSIONS
In conclusion, the upregulation of multiple transcripts related to the extracellular matrix in the CA from PPS cases might explain the failure of rupture and premature separation of the CA. Moreover, the current study identified 11 potential regulators which could be involved in the pathogenesis of the PPS.
Supported by the Clay Endowment and the Clay Visiting Fellowship of the University of Kentucky.

W63
SUCCESSFUL CRYOPRESERVATION OF LARGE EQUINE EMBRYOS USING A COMBINATION OF LASER ASSISTED TECHNOLOGY AND PIEZO MANIPULATOR RESULTING IN PREGNANCY

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BACKGROUND-AIM
In comparison to other species, equine embryo cryopreservation presents some challenges. Cryopreservation of equine embryos is affected by their size, blastocoel volume, and capsule. Cryoprotective chemicals cannot penetrate the thick embryonic capsule of equine large embryos, and making standard cryopreservation techniques impossible. Large blastocoel volume increases the likelihood of ice crystal formation during the vitrification process. Shrinkage of the blastocoel appears to be the best solution for this problem. The aim of this study was to improve cryopreservation of equine large embryos via combination of laser assisted technology and piezo manipulator.
METHODS
Artificial inseminations were conducted by using frozen semen from an Irish Connemara pony stallion approximately 32 hr following hCG injections. Embryo collections were done via the non-surgical transcervical procedure from Hokkaido native pony and its cross breeds. Embryos were punctured 20 µm with the both of a laser system and piezo micromanipulator. Punctured embryos were transferred in an equilibration medium for a maximum of 15 min at room temperature. After that, embryos were transferred through the vitrification solutions, loaded onto the Cryotop® and plunged into liquid nitrogen within 1 min of exposure to the final vitrification solution.
Embryos were thawed and cultured for 3 or 24 hr. After culture, embryos and capsule size were measured, and the quality of the embryos were assessed. Embryo transfer were performed non-surgically, and one week after transfer, pregnancy was determined by the presence of a spherical embryonic in the uterus, then pregnancy was confirmed 35 days after transfer by detecting fetal heartbeat by ultrasound examination.
RESULTS
In this study, six embryos were cryopreserved, thawed and transferred. They were in the hatched blastocyst stage, with a collection size ranging from 713 to 1323 µm. On day 35, two out of six transferred embryos (33.33 %) resulted in a positive pregnancy that resulted in two healthy foals born.
CONCLUSIONS
This study focused on cryopreservation of equine large embryos (≥700 µm) and to the best of our knowledge is the first positive cryopreservation and pregnancy and live birth in large equine embryo using a combination of laser and piezo manipulation.

W66
INFLUENCE OF PROCESSING TIME AND LUTEIN ADDITION ON EPIDIDYMAL EQUINE SPERM VITRIFICATION

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BACKGROUND-AIM
Epididymal sperm preservation in the equine specie is a feasible option for those stallions that have to be sacrificed or have died from a pathology. Knowing how many time we have to process the testicles after been obtained is important to have the best results with sperm preservation. As well, the use of lutein in vitrification media could improve this sperm quality, because it´s carotenoid that protects sperm against lipid peroxidation, so will improve semen parameters after devitrification. The objective of this work has been to assess if it is possible to keep the testicles for 24 hours in refrigeration before processing, and what is the optimal lutein concentration in vitrification media.
METHODS
Fourteen epididymis of testicles from healthy stallions were processed after their slaughter in the Municipal Slaughterhouse of Zaragoza (Mercazaragoza). Seven were processed immediately and the other seven after 24 hours at 4 ° C. The control extender (C) was INRA 96® supplemented with 1% BSA and 0.15M trehalose. With this base the different vitrification media were prepared, supplemented with lutein: 5 µM (LT5), 10 µM (LT10) or 15 µM (LT15). The sperm were obtained by retrograde flushing using INRA 96®. The final concentration for the different media was 50x106 sperm/ ml. For vitrification, 50 µl of each suspension were dropped on a cryotube containing 300 µl of N2L. After samples were warmed, sperm motility (ISAS® Proiser), viability and integrity of the acrosome (PNA-FITC staining) and DNA fragmentation (Acridine orange stain) were assessed. The statistical analysis of the data obtained was performed with the SPSS statistical package, version 22.0 for Windows, using a general univariate linear model (GLM).
RESULTS
Progressive motility were significantly better when sperm was vitrified at 0h compared to those at 24h in the case of media C, LT 5 and LT 10. For viability and DNA fragmentation values, there are no significant differences between different processing times. Nevertheless, the percentage of intact acrosomes was higher at 0h compared to 24h.
CONCLUSIONS
From the results of this study it is evident that the processing of the testicles and the vitrification of their spermatozoa is better immediately after slaughter and that there is no significant protective effect of the addition of lutein in the vitrification media in the equine specie

W67
GNRH IMMUNIZATION REDUCE BLOOD FLOW TO THE TESTIS BEFORE SPERM QUALITY AND SPERM PRODUCTION DROP.

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BACKGROUND-AIM
Immunocastration is an alternative to surgical castration that allows reversible suppression of sexual behavior and reproductive function. The aim of this study was to evaluate the short-term effect of GnRH-immunization on the vascular perfusion of the testis, sperm production and sperm quality in equines. We hypothesized that GnRH-vaccination causes a disturbance to testicular vascular perfusion prior to sperm dysfunction.
METHODS
Seven stallions (4-12 years old) were used in this study. Five stallions were vaccinated in January and February (at a 35-day interval) using 150 µg of a GnRH-protein conjugate (Improvac® Zoetis, Spain). Two control stallions received an equivalent volume of saline solution. Reproductive examinations were performed in January (T0), February (T1) and March (T2). Semen evaluation included total motility(TM), progressive motility(PM) and curvilinear velocity(VCL) (CASA system), morphoanomalies and total number of sperm (TNS). Testicular volume (TV) was assessed using B-mode ultrasound. Total Arterial Blood Flow (TABF: TAMV x A) and TABF rate: TABF/TTV x 100 were evaluated using pulse-Doppler mode.
RESULTS
The TM (80.4±2.9 vs 26.0±11.4%), PM (55.5±4.7vs9.7±4.1%) and VCL (54.8±6.64vs 129.9±4.7µm/sec) were significantly reduced at T2 in stallions treated with Improvac® (p≤0.05). The percentage of abnormal sperm increased from 36.7±3.4%(T0) to 73.2±4.7%(T2)(p≤0.05). There was also a decrease at T2 in TNS (4776±1381x106 vs 2538±1094x106) and TV (230.7±57.6 vs 137±45.6 cm3)(p≤0.05) in vaccinated stallions. In the control group, an increase in both parameters was observed as the reproductive season progressed. Regarding vascular perfusion, treated stallions experienced a lower blood supply to the testis compared to controls as early as T1 (TABF:26.8±3.3vs 85.6±25.8mL/min and TABFrate:29.3±8.2vs52.9±9.9)(p≤0.05) and at T2 these differences increased (TABF:17.9±3.2vs98.3±22.1 and TABFrate:27.4±10.9vs61.0±18.4(p≤0.05).
CONCLUSIONS
Improvac caused a decrease in sperm production and testicular functionality two months after vaccination. The anti-GnRH vaccine reduced testicular blood flow before a drop of sperm quality and sperm production (T1). Evaluation of testicular vascular perfusion is a good early marker of testicular dysfunction. PID2019-107797RA-100/AEI/10.13039/501100011033, IB20163.

W68
CYSTINE INCORPORATION THROUGH SLC7A11 ANTIPORTER INFLUENCES THE ABILITY OF CRYOPRESERVED STALLION SPERMATOZOA TO BIND TO HETEROLOGOUS ZONAE PELLUCIDAE

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BACKGROUND-AIM
Spermatozoa are redox-regulated cells, and deregulation of their redox status is considered to affect male fertility and to reduce their fertilizing ability following biotechnological procedures, such as cryopreservation. As Cystine (CysS), after incorporation in sperm via SLC7A11 antiporter, has been demonstrated to increase intracellular GSH content (doi: 10.1093/biolre/ioz069), this study was aimed at determining the effect of CysS supplementation during post-thaw incubation on equine sperm after in vitro induction of capacitation
METHODS
Thawed sperm from 7 stallions (2 ejaculates/stallion) was washed and resuspended in Tyrode's medium; each sample was divided in 4 groups: Control (Ctr), 0.5 mM Cystine (CysS), 500 µM Sulfasalazine (SS), a specific inhibitor of SLC7A11 antiporter, and 0.5 mM CysS + 500 µM SS (CysS+SS). After 1 h of incubation at 37°C, samples were washed twice, resuspended in capacitating BWW medium supplemented with 5 mM db-c AMP, 0.5 mM Methyl-β-cyclodextrin and 3 mM Caffeine and incubated at 38 °C under 5% CO2. After 30 and 60 min, sperm motility, viability and tyrosine phosphorylated protein immunolocalization, used as capacitation status index, were evaluated. After 30 min of capacitation, 4x105 spermatozoa were coincubated with 20 denuded pig oocytes in 400 µl of capacitation medium for the heterologous binding assay for 30 min
RESULTS
No differences of the sperm parameters studied (motility, viability and tyrosine phosphorylation) were recorded. However, the number of spermatozoa bound per oocyte (mean ± SEM), tended to increase in CysS group (44.0 ± 12.3) respect Ctr (40.8 ± 10.8) while decreased in SS group (32.4 ± 7.8) ($p<0.01$). Moreover, CysS+SS group showed a lower binding rate (32.1 ± 10.0) compared to CysS ($p<0.001$)
CONCLUSIONS
Our results suggest that CysS supplementation of thawed stallion spermatozoa may influence their ability to bind to heterologous zona pellucidae as the inhibition of CysS incorporation by SLC7A11 reduced the number of sperm bound per oocyte. This effect does not seem to be ascribed to a modification of sperm motility, membrane integrity and tyrosine phosphorylation.
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W69
EFFECT OF MESENCHYMAL STEM CELLS THERAPY ON TESTICULAR HISTOLOGY FROM STALLIONS SUBMITTED TO SCROTAL HEAT STRESS

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BACKGROUND-AIM
Testicular degeneration is the most frequent cause of reproductive disorder in stallions, causing subfertility. Mesenchymal stem cell therapy (MSC) have shown an increased regeneration of damaged tissues. The aim of this study was to evaluate the effect of MSC treatment on the testicular histology of stallions submitted to scrotal heat stress.
METHODS
Ten healthy ponies were divided into 2 groups: control (CG) and treatment with MSCs (TG). Degeneration was induced by thermal blanket (42-45°C) for 3 hours during 3 days. One week after degeneration, the TG received testicular therapy with MSCs, while CG only PBS. MSCs were collected from a healthy stallion by puncturing the bone marrow in the sternum. MSCs (10x106) were suspended in 5mL of PBS, and, by prior antisepsis, injected in 5 points in the medial regions of the testicular curvature between the head and tail of the epididymis, using 26G hypodermic needl with the aid of ultrasound, forming a diamond-like figure with a center point.. Testicular biopsies were collected at 4 different times: 7 days before the heat stress (B1); 7 days after the heat stress (B2); 7 days (B3) and 14 days (B4) after PBS or MSC application. The biopsies were performed using a TRU-CUT 16G needle. The fragments were fixed in formalin 10% and stained with H&E. Histopathological evaluations were performed quantitatively (400x) and scores were established for: Tubular architecture lesion (0-absent, 1-mild, 2-moderate, 3-severe); Sperm count inside the seminiferous tubule (0- absence, 1- 1-20, 2- 21-50, 3- 51-100 , 4- >101); Seminiferous tubular cells (0-absence of cell layers, 1-sertoli cells only; 2-up to 2 cell layers, 3-up to 4 cell layers, 4- > 4 cell layers). Statistics was performed using SAS 9.1.3 software (P<0.05).
RESULTS
No signs of inflammation were observed after treatment with MSCs. In the TG, all scores decreased at B2 and B3 compared to B1, and at B4 scores increased compared to B2 and B3 and were similar to B1 moment. In the CG, all scores showed no differences between B2, B3 e B4.
CONCLUSIONS
Treatment with MSCs showed a faster recovery of the semiferous tubules epithelium in comparison to control group, returning to the initial histological patterns. This technique proved to be safe as a treatment for testicular degeneration in stallions.

W70
COMPARISON BETWEEN A TRADITIONAL AND A NEW PROTOCOL FOR EX COPULA EJACULATION IN STALLIONS INCAPABLE OF BREEDING

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BACKGROUND-AIM
Physical, neurological, and behavioral disorders in stallions, including lameness, ejaculatory disorders, erectile dysfunctions, and penile tumors, can prevent semen collection using traditional methods. Thus, some protocols have been used to induce ex copula ejaculation in stallions, however, results are variable. Recently, a new protocol using imipramine, detomidine and oxytocin was developed and seems to be a promising alternative. Therefore, no studies were performed in stallions incapable of copulate. The aim of this study was to evaluate the efficiency of 2 protocols for ex copula ejaculation in stallions incapable of breeding.
METHODS
Stallions (n=10) received the protocols to induce ejaculation, with 2 trials of each one, with a minimum interval period of 48 hs. The traditional and the new protocol were, respectively: IX-imipramine (I; 3mg/kg, orally) plus xylazine (X, 0.66mg/kg, iv) and IDO-imipramine (3 mg/kg, orally), detomidine (0.01mg/kg, iv) and oxytocin (20 IU, iv). The stallions were affected with: penile schamous cells carcinoma (2/10), penile paralisis (2/10), idiopatic ejaculatory dysfunction (3/10), tendinitis (2/10) and broken leg (1/10). The ejaculates were collected in a plastic bag inside a cup collector held by a plastic ring positioned over the prepuce and suspended by a light rope.
RESULTS
Overall, 8 of 10 disable stallions ejaculated. IX protocol induced ejaculation in 4 stallions in all attempts (100%). Equally, IDO induced ejaculation in 4 stallions in 13 of 20 attempts (65%). Interestingly, none of the stallions that responded to xylazine responded to detomidine. The interval from xylazine treatment to ejaculation ranged from 2 to 5 min and interval from detomidine treatment to ejaculation ranged from 3 to 6 min.
CONCLUSIONS
In conclusion, both protocols had similar success rates and IDO protocol was effective in triggering ejaculation in most stallions in which IX was not effective. Therefore, attempts using both protocols are encouraged and show promising results in disable stallions.

W71
OBESITY IN PREGNANT CRIOLLO MARES: ENDOCRINE AND METABOLIC PROFILES

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BACKGROUND-AIM
We investigated the effect of body condition score (BCS) on endocrine and metabolic profiles in Criollo pregnant mares.
METHODS
Criollo pregnant mares (n=56) were categorized according to their BCS (scale 1-9, Henneke et al. 1983) in obese (7 to 9 BCS, n=32) and non-obese BCS (<7, n=24). Animals were bled at two moments during gestation: second (OBS 1) and third trimester (OBS 2). Insulin was determined by Radioimmunoassays and biochemistry by spectrophotometer. HOMA index was estimated as Glucose (mg/dL) x 0.0555 x (Insulin)/22.5. Variables were analyzed by ANOVA using a mixed procedure including BCS group, observation (time of gestation) and their interaction.
RESULTS
Obese mares had greater concentrations of insulin than non-obese mares (15.1±1.1 vs 10.7±1.2 µIU/mL, P=0.0078), insulin concentrations increased with gestation (9.0±1.1 vs 16.8±1.1 µIU/mL, P<0.0001) and no interaction was found among these factors. Glucose concentrations were affected by BCS (78.6 vs 70.5 mg/dL for obese and non-obese mares, P=0.0015), the observation P<0.0001 and their interaction (P=0.012) as while differences according to BCS were found in OBS 1, no differences were found in OBS 2. HOMA index was greater in obese mares than non-obese (2.98±0.24 vs 2.21±0.27, P=0.04) and in OBS 2 compared to OBS 1 (3.65±0.28 vs 1.53±0.24, P<0.0001). Cholesterol and triglycerides concentrations were affected by observation only, being greater in OBS 2 (P<0.01). Both ALP and GOT were affected by BCS, being 722±27 vs 639±31 IU/L for ALP (P=0.04) and 381±13 vs 327±16 IU/L for GOT (P=0.01) in obese and non-obese mares respectively. Levels of ALP were lower in OBS 2 when compared to OBS 1 (P<0.0001) and the contrary was found for GOT concentrations (P=0.05). The interaction among group and observation was significant for ALP concentrations as the decrease found in OBS 2 was greater in obese mares than non-obese mares.
CONCLUSIONS
This study shows that obesity in pregnant mares is associated with insulin dysregulation (increased insulin and glucose concentrations and HOMA index) and altered liver functionality (enzyme data).

W72
HEAT SHOCK TRANSCRIPTION FACTOR HSF1 IMMUNOEXPRESSION IN MARE'S ENDOMETRIUM DURING OESTROUS CYCLE AND ANESTRUS PERIOD.

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BACKGROUND-AIM
We have recently showed that heat shock protein HSP90 is detected in mare's endometrium in normal oestrus cycle and anestrus period. HSP90 function is known to be regulated by the heat shock transcription factor HSF1 in human and mouse models. However, the expression of HSF1 factor has not been determined yet in the mare´s endometrium during the oestrous cycle and anestrus period.
METHODS
Endometrial biopsies of 10 healthy nulliparous quarter horse mares were analysed to detect the localization and immunoexpression of heat shock factor HSF1 during winter anoestrus, oestrus and diestrus phase. The biopsies were processed and immunohistochemistry was performed against HSF1 using mouse polyclonal antiHSF1 antibody (ab52813, abcam, USA). Immunostained area of endometrial lining epithelium and superficial and deep glands were expressed as mean ± SEM and compared by one-way ANOVA. Differences between groups were assessed with post hoc analyses Tukey's test and considered significant when p< 0.05.
RESULTS
Heat shock factor HSF1 was localized at cytoplasm and nucleus of superficial and basal cells of lining epithelium, supranuclear region and nucleus of superficial and deep endometrial glands cells during anestrus, oestrus and diestrus. The immunoexpression in endometrial lining epithelium and superficial endometrial glands was higher in diestrus than in oestrus and anestrus (p<0.0001). However, the immunoexpression in deep endometrial glands was lower in anestrus (p<0.0001) and showed no differences between diestrus and oestrus.
CONCLUSIONS
In conclusion, HSF1 factor was immunoexpressed in mare's endometrium, being higher its immunoexpression during diestrus. The rise of HSF1 immunoexpression during diestrus suggests a role in secretory cells activity mediated by progesterone. Given the protective role and regulation of inflammatory cytokines of HSF1 in human endometrium we suggest a similar protective mechanism in mare's endometrium during diestrus. Future comparative studies of HSF1 factor expression should be done under pathological conditions such as endometritis.

W73
MULTIOMICS APPROACH TO DIFFERENTIATE PREGNANT AND NON-PREGNANT MARES: IDENTIFICATION OF EARLY PREGNANCY PLASMA BIOMARKERS

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BACKGROUND-AIM
Diagnosis of early pregnancy in mares is an important component of equine breeding practice, as early embryo loss is relatively common and incurs substantial economic loss. This is compounded by a short breeding season, placing pressure to achieve pregnancies early. Furthermore, a precise signal or mechanism for maternal recognition of pregnancy has not yet been elucidated in horses with current detection only being possible at day 14. As a prelude to developing an on-farm robust early pregnancy test, we have undertaken multiomics analyses to compare the blood plasma profiles of pregnant (7P) and non-pregnant (7NP) mares at day 7 post-ovulation to identify pregnancy-induced biomarkers.
METHODS
We conducted a proteomics study (total samples=264) in parallel with pilot lipidomics study (total samples=12). Using a batch mode approach, we compared 7P, 7NP, 14P and 14NP (66 samples/group for proteomics; 3 samples /group for lipidomics), coupled to our established bioinformatical pipelines.
RESULTS
This study revealed a plasma protein profile of 234 proteins and a lipidome composed of ~700 lipid ions. Amongst these profiles we identified 14 proteins and 24 lipids that were significantly up- or down-regulated between 7P and 7NP. Proteomics revealed serpin A6, a member of the serine proteinase inhibitor (serpin) plasma proteins, to be significantly increased in 7P plasma. Serpins are known to be synthesised by the uterus in many species, and serpin A6 is the principal transport protein for cortisol and progesterone. Other differentially abundant proteins detected in this study may be important in the immunological recognition of pregnancy such as immunoglobulin lambda light chain variable region, alpha 2 macroglobulin and complement C8 gamma chain. Lipidomic analysis revealed a group of ceramides to be significantly increased in 7P plasma, suggesting a role for lipid-mediated signalling in early pregnancy. Moreover, pathway analysis implicated ceramides in many reproductive hormone signalling pathways, notably progesterone synthesis.
CONCLUSIONS
These novel findings support the utility of mass spectrometry driven omics platforms for pregnancy biomarker discovery and indicate that systemic physiological changes occur as early as day 7 following fertilisation in the pregnant mare. Overall, this study represents significant progress toward establishing a potential panel of biomarkers with ongoing research work to validate these findings for detection of early pregnancy in the mare.

W74
EFFECT OF OZONE ON EQUINE SEMEN REFRIGERATING: PARTIAL DATA

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BACKGROUND-AIM
Reactive oxygen species (ROS) are critical for sperm cell physiology, participating in important fertilization events, as well as acrosome reaction, capacitation and hyperactivation. However, when there is an imbalance between ROS production and antioxidant capacity, an oxidative stress process is established. Sperm motility is a sensitive indicator of oxidative stress, and may be one of the first parameters affected when the oxidation process begins. Ozone is a radical that when used in small doses causes a precondition to oxidation, activating the transcription factor Nrf2 and its inhibitor Keap1, thus stimulating a strong antioxidant response. NrF2 is a regulator of genes that protect cells from endogenous and exogenous effects of oxidative stress. The expression of NRF2 mRNA showed significantly lower semen levels in patients with oligozoospermia and asthenozoospermia. Thus, the objective of this unpublished work was to evaluate a possible protective effect of ozone on sperm cells of horses subjected to refrigeration.
METHODS
Five stallions were used, four ejaculated from each (n=20). After collected, the ejaculates were divided into 5 groups, control (without addition of Ozone) and 4 other groups where different concentrations of Ozone were added to the refrigeration medium: 5µg/m³, 15µg/m³, 30µg/m³ and 60µg/m³ (O3-5, O3-15, O3-30, O3-60 respectively), and then evaluated at 24h of cooling. The sperm kinetic evaluations were performed by computerized semen analysis (CSA).
RESULTS
There was a significant increase (P <0.01) in total and progressive motility in the O3-15 group (60.3 ± 3 and 40.7 ± 3.4 respectively) when compared to the control groups (54.9 ± 4 and 35.0 ± 4.4 respectively), O3- 30 (53.8 ± 2.3 and 34.1 ± 4, respectively) and O3-60 (52.8 ± 4 and 34.0 ± 3.8, respectively).
CONCLUSIONS
Therefore, this study demonstrated that the dose of 15µg/m³ of ozone in the cooled equine semen had a beneficial effect on the total and progressive motility and doses greater than 15µg/m³ of ozone had no positive effects on the sperm quality of the cooled equine semen.

W75
DOES AGE INFLUENCE ON OXIDATIVE BALANCE IN PREGNANT AND POSTPARTUM MARES?

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BACKGROUND-AIM
During pregnancy and postpartum, physiological changes occur to maintain homeostasis, with oxidative adaptation being one of the most notorious. During these periods, there is an increase in energy demand and, consequently, in the availability of oxygen, which can culminate in oxidative damage if there is an imbalance between reactive oxygen species (ROS) production and antioxidants defenses. In order to cope with this challenge, an increase in protective effect of antioxidants during the pregnancy, labor and postpartum is necessary. Thus, the present study aimed to compare the antioxidant activity and oxidative stress from the 8th month of pregnancy until the 2 second postpartum ovulation according to the age of the mare.
METHODS
Seventeen mares were allocated into three groups: 3-7 years (n=7); 8-11 (n=4) and ≥ 12 years (n=6) to evaluate the influence of the age. Mares were evaluated at 6 time-points: 8th, 9th, 10th and 11th months of pregnancy, 12 hours postpartum and second postpartum ovulation. A venous blood sample was collected for glutathione peroxidase (GPx), superoxide dismutase (SOD), and TBARS (thiobarbituric acid reactive substances), protein oxidation, magnesium and iron analysis. Variables were estimated by repeated measure analysis of variance, if no significant interactions occurred, variables were analyzed by Student's T and LSD tests (P≤0.05).
RESULTS
Mares with 3-7 years showed an increase in Magnesium concentration postpartum when compared to the other time-points. Mares with 8-11 years had decreased Magnesium at the 9th month in comparison to the other months. Mares ≥ 12 years had higher GPx concentration compared to 8-11 years mares, regardless of the time evaluated. In addition, throughout gestation a progressive decrease of GPx between the 8th and 10th month was observed, with a progressive increase at the 11th month, regardless of group. Moreover, protein oxidation, TBARS, SOD and iron analysis did not show differences between the evaluated groups and times.
CONCLUSIONS
In conclusion, age is a factor that directly affects the concentration of antioxidant to maintain the oxidative homeostasis in pregnant and postpartum mares . Ethical approval CEUA-FMVZ-USP number 5888210814. Authors thank CAPES and FAPESP (2020/10260-3) for their financial support.

W76
VIABILITY AND ULTRASTRUCTURAL ANALYSIS OF FROZEN-THAWED STALLION SPERMATOZOA FROZEN WITH DIFFERENT CONCENTRATIONS OF SDS IN A BASE EXTENDER

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BACKGROUND-AIM
Sodium Dodecyl Sulfate (SDS) has been included in extenders used for frozen semen in some domestic species, obtaining good results. The aim was to study the protective effect of different concentrations of SDS in a base extender (Lactose-EDTA-egg yolk-Dimethylformamide) on equine sperm during the freeze-thaw process.
METHODS
Ten stallions Criolla Argentina breed, aged between 5 and 8 years, clinically healthy and fertile, were used. Two ejaculates of each animal were collected. After filtering, semen evaluation (Motility [MOT, AndroVision®, Minitüb GmbH], percent alive (PAL, % of live, Eosin-nigrosin stain); HOS test (HOS, % rolled tails) and percent intact acrosomes (PIA, Pisum sativum agglutinin-fluorescein isothiocyanate) was performed. After that, one aliquot was used for ultramicroscopic analysis in a JEM 1200 EX II 60-80 Kv TEM. The other semen aliquot was diluted 1:1 in a Kenney extender, aliquoted, centrifuged and resuspended at 200x106 sperm/mL in a Kenney extender with different concentrations of SDS: SDS0 (0%); SDS0.6 (0.062%); SDS12 (0.125%); SDS20 (0.25%); SDS50 (0.50%). The frozen-thawed semen was subjected to the same tests as fresh semen. Data were analyzed by ANOVA using the PROC Glimmix, SAS® 9.4, and the results are presented as LSM±SEM. Significance was defined as P<0.05.
RESULTS
All parameters studied were higher in fresh semen compared with frozen-thawed semen 70.0±37.4 vs 24.3±6.9 [MOT]; 86.9±8.8 vs 60.2±15.7 [PAL]; 57.1±14.1 vs 25.7±13.8 [HOS]; 82.6±8.1 vs 57.9±18.6 [PIA]; P <0.05). No differences in post-thawed parameters were observed between SDS0, SDS0.6, SDS12, and SDS20. However, SDS50 showed lowers post-thawed parameters compared with SDS0, SDS 0.6, SDS12 and SDS20 (30.0±1.0 vs 3.4±4.7 [MOT]; 64.0±14.0 vs 33.7±14.2 [PAL]; 28.0±8.0 vs 8.0±7.2 [HOS]; 63.0±11.0 vs 14.8±15.8 [PIA]; P <0.05). The ultramicroscopic study showed the deleterious effect of cryopreservation on the acrosomal and plasmatic membrane.
CONCLUSIONS
Although no protective effect of SDS was observed at low concentrations, our results showed the deleterious effect of high concentrations of SDS on the sperm cell. It is possible that concentrations lower than 0.062% of SDS added on the extender could show a protective effect on stallion sperm during cryopreservation.

W77
GENERATION OF EQUINE CHORIONIC GIRDLE ORGANOIDS

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BACKGROUND-AIM
Equine chorionic girdle (CG) is comprised of specialized invasive trophoblast cells that begin formation ~25 days after ovulation. Mature CG cells differentiate into binucleate endometrial cup cells that secrete equine chorionic gonadotropin (eCG), which has variable LH and FSH-like activity in non-equine species and has been utilized for these properties in vivo and in vitro. To collect eCG, large volumes of blood are collected from pregnant mares, which impairs equine welfare. Production of eCG by culture of CG explants has not been successful beyond 180 days, with peak eCG production at 30 days of culture. Organoids are 3D cell clusters that self-organize and remain genetically and phenotypically stable throughout long-term culture. Human trophoblast organoids have been reported to produce human chorionic gonadotropin long-term. The objective of this study was to optimize the use of organoids to produce eCG from equine CG.
METHODS
CG was collected from Day 33-34 equine conceptuses (n=3). CG was isolated manually using a dissection microscope. The first CG was enzymatically digested fresh and cultured as organoids using media reported to support equine endometrial organoids (Trial 1). The second CG was cryopreserved in 10% DMSO and later cultured as organoids using media reported for human trophoblast organoids (Trial 2). The third CG was digested fresh and cultured as organoids using human trophoblast organoid media (Trial 3). CG organoids were assessed using immunohistochemistry (IHC) and by quantifying equine LH (as a proxy for eCG) via radioimmunoassay (RIA) of conditioned media.
RESULTS
Trial 1 resulted in organoid growth and proliferation over 2 weeks, including 1 passage. These produced high levels of eCG (242.3 ng/ml LH) during passage 0 (P0) but declined soon after. Trial 2 resulted in loss of normal trophoblast organoid phenotype after P1, but eCG was detected at P0 and 1 (7,270 and 292 ng/ml, respectively). In Trial 3, the phenotype was maintained for 5 passages (6 weeks and counting). Trial 3 will be continued until loss of phenotype, and additional analyses will include quantification of eCG and IHC.
CONCLUSIONS
CG organoids maintain morphology best when using fresh CG tissue, providing a 3D in vitro model for early equine pregnancy and may be a long-term in vitro source of eCG.

W78
MATERNAL BLOOD ACTIVIN A CONCENTRATIONS IN LATE PREGNANCY OF THOROUGHBRED MARES

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BACKGROUND-AIM
Equine fetal loss during the last trimester of pregnancy causes big economical problems to the horse breeding industry globally. Diagnostic biomarkers are needed for detecting abnormal pregnancies at an early stage. In the present study, we were interested in activin A hormone which is secreted by uterine-placental tissue and modulates the release of pro-inflammatory cytokines. We hypothesized that activin A concentration might be useful for detecting abnormal pregnancy in mares. The aim of the present study was to compare plasma activin A concentrations in healthy pregnant Thoroughbred mares with pregnant mares that suffered fetal loss or showed abnormal symptoms during late gestation.
METHODS
Plasma activin A concentrations in normal and abnormal (dystocia, abortion, red bag delivery, premature udder development and other abnormalities) pregnant Thoroughbred mares (n=95) were investigated during late pregnancy. Mares were categorized into normal and abnormal groups based on the history, clinical symptoms and pregnancy outcome. For normal group, blood samples from 48 healthy pregnant mares were randomly selected at one time point during late pregnancy (Day 199 to 349). Inclusion criteria for abnormal group included vaginal discharge, premature udder development, miscarriage and abnormal delivery. For abnormal group, 47 blood samples taken from 33 abnormal pregnant mares around day of symptom onset and before abortion or parturition (Day 257 to 333) were selected. Plasma concentrations of Activin A were measured by ELISA kit (Human/Mouse/Rat Activin A Immunoassay, DAC00B; R&D Systems, Inc., Minneapolis, USA) according to the manufacturer's guideline. Data were presented as mean ± SEM.
RESULTS
Plasma activin A concentrations were higher in abnormal group (173.9 ± 8.5 pg/mL) compared to normal group (132.0 ± 14.5 pg/mL) in late gestation ($P < 0.001$). In samples taken on day of symptom onset (160.7 ± 10.5 pg/mL) and within 1 to 10 days prior to abortion (194.8 ± 21.8 pg/mL), plasma activin A concentrations were higher compared to gestationally age-matched normal groups ($P < 0.01$), while normal delivery group (180.8 ± 20.1 pg/mL) was not different from abortion group within 1 to 10 days before delivery or fetal loss.
CONCLUSIONS

The present study first demonstrated that plasma activin A levels are higher in abnormal pregnant mares compared to normal mares during the late gestation. Measurement of activin A may facilitate the diagnosis of abnormal pregnancy in mares.

W79

FIRST SEX MODIFICATION CASE IN EQUINE CLONING

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BACKGROUND-AIM
Somatic cell nuclear transfer (SCNT) is an asexual reproductive technique where cloned offspring contain the same genetic material as the original donor. However, the birth of sex-reversed offspring has been reported in some species. Here, we report for the first time a female equine foal born through SCNT of a male nuclear donor.
METHODS
Pasage-2 mesenchymal stem cells (MSCs) derived from a male horse were used as nuclear donors for a standard zona free-SCNT procedure (Olivera et al., 2018).
RESULTS
A total of 420 oocytes were in vitro matured, resulting in 53.6% maturation rate (225/420). One hundred and seventy cloned equine zygotes were produced, 125 cleaved (73.5%) and 17 blastocysts were obtained (10%). Sixteen embryos were transferred to 8 recipients, resulting in 3 pregnancies (37.5%) and the birth of 2 clones (25%): one male and one female. Both animals presented the same genetic profile, as observed in the analysis of 15-horse microsatellite marker panel from hair (male) and hair and blood (female) samples, which confirmed they are indeed clones of the same nuclear donor. The chromosome count was determined by conventional Giemsa staining, C banding and G banding, from fibroblast (30 metaphases) and blood samples (50 metaphases). In addition, the MSCs used as nuclear donors were analyzed (30 metaphases). The MSCs and the male foal showed the expected 64, XY chromosome set, whereas the female foal presented a 63, X0 chromosome set. The identity of the lost chromosome in the female was further confirmed through PCR of X- and Y-linked markers. The sample from the male foal presented the AMEL-X and AMEL-Y chromosome markers, whereas the sample from the female foal presented only the AMEL-X chromosome marker, without amplification of the SRY gene or any of the Y-linked STR markers.
CONCLUSIONS
The fact that the MSCs used as nuclear donors contained a euployd chromosome count suggests either that alterations occurred at a low rate and could not be detected through cytogenetic analysis, or that the Y chromosome was lost during embryo development. Although the cause of the spontaneous chromosome loss remains unknown, the possibility of equine sex reversal by SCNT holds great potential for the preservation of endangered species, development of novel breeding techniques, and sportive purposes.

W80
COMPARISON BETWEEN CASA ANALYSIS AND OTHER SPERM INTEGRITY TESTS AND THEIR CORRELATION WITH FERTILITY

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BACKGROUND-AIM
Prior to AI, semen assessment is required. In commercial practice, spermatic motility parameters are most frequently considered, though they neglect the functionality of plasma and acrosomal membranes and DNA integrity.
The aim of this study was to compare data obtained with Computer – Assisted Sperm Analysis (CASA) and data obtained by Hypoosmotic Swelling test (HOS), Eosin – Nigrosine staining (LIVE), SpermacStain (ACR) and Sperm Chromatin Dispersion test (DNA).
METHODS
We analyzed thirty - six semen samples cooled and shipped 24 hours after collection. All samples were used for artificial insemination and pregnancy diagnosis was carried out 14 days after ovulation. Thirty - four mares were included. Pregnancy diagnosis was compared with semen quality and mare's reproductive health.
Associations between variables were assessed with Pearson correlation coefficient. T – student test was used to analyze the effect of semen quality on pregnancy and predictive values for pregnancy diagnosis were defined using ROC analysis for the variable PMS.
Results were considered statistically different for $p<0.05$.
RESULTS
Significant correlations were obtained between MOT and ACR ($p=0.04$), MOT and head anomalies ($p=0.001$), PMS and HOS ($p=0.037$), PMS and ACR ($p=0.004$), ALH and LIVE ($p=0.009$), STR and morphologically normal sperm ($p=0.016$), STR and midpiece anomalies ($p=0.003$). PMS is the main parameter associated with pregnancy: indeed, with PMS values >31% the probability for the mare to be pregnant are 4.8 times higher.
With Pearson coefficients it was possible to find a correlation between pregnancy and sperm parameters: PMS values are positively correlated with pregnancy, while lower values of ACR are negatively correlated with pregnancy.
At last, a risk analysis was performed to assess if mare reproductive health was correlated to pregnancy, obtaining highly significant p – Yates (0.0007) with RR 6.50 (CI: 1.71 – 24.77): a mare with evidence of reproductive disease has a probability not to be pregnant that is 6.5 times higher compared to a healthy mare.
CONCLUSIONS
The importance of progressive motility and mare's health in the determination of pregnancy expectation could help owners to take the best decision about breeding.

W81
THE FIRST TRANSCRIPTOMIC PROFILE OF EPITHELIAL GLANDS AND STROMAL CELLS FROM THE EQUINE ENDOMETRIUM

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BACKGROUND-AIM
Early pregnancy loss has major economic implications for horse breeders, and despite there being many studies focusing on conception and embryo loss, the mechanisms by which the uterus is primed for pregnancy are yet to be elucidated in this species. Furthermore, there remains no long-term in vitro culture system capable of recapitulating the equine endometrium. To advance knowledge in this space, the present study aimed to characterise the transcriptomes of epithelial glands and stromal cells derived from the equine endometrium.
METHODS
Endometrial tissue samples were collected from two mares immediately post-mortem and dissociated via enzymatic digestion. Cell populations were purified by selective adhesion of the stromal cells. The epithelial cell suspension was pelleted and frozen at -80°C whilst stromal cells were cultured to confluence (approx. day 5). RNA was isolated using a Total RNA Isolation Mini Kit (Agilent) according to the manufacturer's instructions before sequencing using DNB-Seq technology. Sequencing data were analysed at SAHMRI and bioinformatics analysis was completed using Database for Annotation, Visualisation and Integrated Discovery (DAVID).
RESULTS
A total of 12,702 genes were identified in epithelial glands, while 11,482 were identified in the stromal cell population with >2 counts per million in each sample. 11,084 (84.6%) genes were identified in both populations, 1,618 (12.4%) were unique to the glands and 398 (3%) were unique to the stroma. A total of 910 genes were observed to be differentially expressed (Fold-change > log(2); FDR <0.05), 790 upregulated in the glands and 120 upregulated in the stroma. DAVID analysis revealed the epithelial enriched genes were associated with signalling, ATP-binding immunoglobulins and the immune response, while those enriched in the stroma were associated with extracellular exosomes, cytoplasm, metabolic pathways and glycolysis. Furthermore, genes associated with oxytocin, oestrogen and prostaglandin receptors were all observed to be significantly enriched within the epithelial gland population.
CONCLUSIONS
These findings establish a foundation for the development of novel in vitro models required for the advancement of knowledge surrounding uterine priming and early pregnancy in the mare.

TOPIC Imaging methods in reproduction

W82
VALIDATION OF THE ISPERM® PORTABLE DEVICE FOR THE ANALYSIS OF RAM SPERM MOTILITY

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BACKGROUND-AIM
The breeding ram selection in farms is often based on their behaviour and testicular anatomy, but may have a low accuracy. Semen evaluation of sires seems necessary, however, it requires samples to be transported to the andrology laboratory, which increases the costs and can compromise the analysis results. Recently, portable devices, as iSperm®, a mini/mobile computer-assisted sperm analyzer (mCASA), have been developed and can be very useful to identify infertile males. Thus, the aim of this work was to compare the sperm motility results analyzed by iSperm® and other CASA systems.
METHODS
Twenty-four semen samples were obtained from 9 rams using an artificial vagina. For analysis, 7.5 µL of the diluted samples (3x107 cell/mL) were loaded in a disposable microfluidic chip and inserted in the microscopic lens with a heating system in an iPad® with the iSperm® software. Simultaneously, a drop of the same volume was placed between a pre-warmed slide and coverslide and evaluated with two desktop CASA systems: one commercial (ISAS, Proiser, Spain) and one open-source (OpenCASA, Alquezar-Baeta et al., PLoS Comput Biol, 15(1), 2019). Four fields were evaluated in each sample. Total and progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), straightness (STR) and linearity (LIN) were analyzed using the Pearson correlation method and the Bland–Altman test (Graphpad Prism v.8).
RESULTS
The values for total motility obtained with iSperm®, but not for progressive motility, correlated (P<0.05) with those obtained with both CASA systems. The lack of correlation in progressive motility could be due to differences in the calculation method or recording velocity (frames per second) of the cameras. The correlation was also significant (P<0.01) in all kinematic parameters but STR. Nonetheless, despite the lack of correlation in progressive motility and STR, the Bland–Altman test revealed that the difference between methods in these parameters was below 10%.
CONCLUSIONS
The iSperm® system is suitable for the motility analysis of ram spermatozoa. It could be a valuable tool for a first on-field analysis of seminal samples and for identifying infertile males.
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W83
ECHOTEXTURE PARAMETERS OF THE TESTICULAR PARENCHYMA AND FERTILITY IN MALES OF THE MAIN DOMESTIC MAMMALS

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BACKGROUND-AIM
We have developed several algorithms to analyze testicle ultrasonograms. The aim of this work is to validate these algorithms as predictors of seminal quality in boars(n=112), bulls (n=74), stallions(n=32) and rams(n=54).
METHODS
Ultrasonograms (3 transverse sonographic images or videos of each testicle) were carried out using an EXAGO scanner (ECM, France) connected to a 5-7.5 MHz linear probe.
The echographic videos were analyzed by the algorithms, selecting a region of interest (ROI) and obtaining the following parameters: black pixels, white pixels, mean gray level of pixels, density of hypoechogenic areas, mean diameter of hypoechogenic areas and percentage of hypoechogenic areas. Fresh semen samples were collected and the relationship between the echotexture parameters and semen quality was analyzed, by means of correlation, ANOVA, and logistic regression analysis.
RESULTS
In all species, males producing subfertile ejaculates had significant differences in several echotexture parameters and mainly in the mean density of hypoechogenic areas of the testicular ultrasonograms (p<0.05). Logistic regression, including the density of hypoechogenic areas, could predict the fertility of a bull. Sensitivity was 97.1% and specificity was 63.6%. Rams and boars with more than 30% abnormal spermatozoa in their ejaculate have less density of hypoechogenic areas (p<0.05). Percentage of hypoechogenic area detects rams with more than 30% abnormal spermatozoa in their ejaculate, with a sensibility of 100% and a specificity of 77.4%. In boars, with a cut-off value of 80 hypoechogenic areas /cm2 algorithms detects a subfertile boar, with a sensitivity of 100% and a specificity was 83.5%.
CONCLUSIONS
This study demonstrates that testicular ultrasonography may be useful in veterinary practice to investigate testicular function in domestic species.

W84
FIBROTIC LESIONS IN THE TESTIS OF RAMS AND RELATIONSHIP TO SEMEN QUALITY

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BACKGROUND-AIM
This study aims to investigate the prevalence of fibrotic lesions in the parenchyma of ram testis and their relationship with semen quality.
METHODS
Transversal ultrasound scans were performed per testicle, in a total of 32 rams with ages between 6 and 105 months. Ultrasonograms were done using an EXAGO scanner (ECM, France) connected to a 7.5 MHz linear probe.
Ultrasound raw videos were analyzed with an Image analysis software (ImageJ) to determine the presence and amount (percentage of area) of fibrosis spots. We calculated the median of this parameter among 124 images per testicle. A semen sample was collected per ram and analyzed by computer-assisted sperm analysis (CASA) to investigate sperm motility and concentration, in a minimum of 500 spermatozoa. The eosin-nigrosin stain was used to investigate sperm morphoanomalies in a total of 200 spermatozoa.
The relationship between testicular fibrosis age, semen production and semen quantity/quality was analyzed, using correlation, Fisher test and ANOVA analysis.
RESULTS
In this study, testicular fibrosis appears in 44.8% of the males, in 100% of the cases as multifocal fibrosis. We discarded 2 males with other gross pathology in the testicle (intratesticular spermatocele). The amount of parenchymal area occupied by fibrosis spots ranged from 0 to 5.6%. The presence and amount of testicular fibrosis were highly related to the age of the ram (Pearson coefficient r=+0.571, p=0.001). Fibrosis was more prevalent and more developed in rams older than 33 months ($p<0.05$): 76.9% of these older males has fibrosis. Conversely, in younger rams the presence of fibrosis was scarce and no fibrosis was found in animals younger than 1 year.
Neither the presence nor the amount of multifocal fibrosis was related to any semen quantity and quality parameters.
CONCLUSIONS
This study suggests that the development of multifocal fibrosis could be related to the age of the ram and that this feature has no influence on sperm production and quality at least when the percentage of parenchyma affected is lower than 6%. This must be confirmed in a higher number of animals. Ultrasound video analysis could be a valuable tool to investigate objectively the percentage of parenchyma affected.

W86
MAGNETIC RESONANCE IMAGING (MRI) OF THE REPRODUCTIVE TRACT IN PIGS AS AN ANIMAL MODEL FOR EXPERIMENTALLY-INDUCED INSULIN RESISTANCE AND POLYCYSTIC OVARY SYNDROME.

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BACKGROUND-AIM
Pigs are widely used as a reference in translational medicine model in studies of reproductive and gastrointestinal tracts. MRI has a high spatial resolution and a great delineation of anatomical structures and has no radiation exposure, which might improves animal welfare in accordance with 3Rs rule for biomedical research.
The aim was to compare the structure of the porcine uterus and ovaries during follicular phase of the ovarian cycle between two groups of pigs.
METHODS
Six mature, clinically healthy, crossbreed Polish Landrace gilts with no abnormalities in reproductive organs as confirmed by ultrasonography were used. After weaning, half of them were fed with a standard commercial diet twice a day (SE) whereas the second group was fed with a high energy diet (HE) equivalent to 150% of the nutritional energy requirement, and achieved by the addition of sucrose and rape oil to the standard diet. MRI was performed during general inhalational anesthesia with a patient in sternal recumbence. Protocols were validated with MRI on Discovery MR750w 3.0T (GEHC, USA) using the 32-Channel Cardiac Coil to produce T2-weighted sequences without fat-suppression technique in two orthogonal planes. The T2W Propeller sequences were acquired in a sagittal plane using settings: field of view (FOV) [cm] 28x28; slice thickness/gap (S/G) [mm] 3/3.0; echo time (TE) [ms] 182-193; repetition time (TR) [ms] 7639-12551 in SE; FOV=28x28; S/G=2/2.0; TE=126-132; TR=9047-12592 in HE to increase accurate of images and a decrease the time for anesthesia and scanning. The selection of Cor T2W Propeller in SE and Cor T2W Cube sequences in HE allowed to provide the clearest opposing contrasts for follicles and ovarian stroma.
RESULTS
Images analysis was completed on Advantage Workstation (AW4.7, GEHC, USA) by the determination the same number and size of regions of interest (ROIs) on uterus and ovary in each individuals. The mean and median signal intensities were 238.7 and 207.9 vs 65.0 and 163.6 for the uterus; and 587.3 and 568.3 vs HE:650.2 and 653.0 for the ovaries, in the SE and HE groups, respectively.
CONCLUSIONS
MRI allows the qualitative evaluation of reproductive organs structure in swine. Our results strongly suggest that with the development of insulin resistance in HE pigs, we experimentally-induced polycystic ovary syndrome. Appropriate molecular research is conducting to directly confirm this.

W87
EFFECT OF PATERNAL EXPOSURE TO HIGH TEMPERATURE-HUMIDITY INDEX ON SUBSEQUENT IN VITRO EMBRYO MORPHOKINETICS IN BOS TAURUS

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BACKGROUND-AIM
Bulls exposed to high temperature-humidity index (THI) experience adverse effects on sperm quality and in vitro embryo production (IVEP) outcomes. However, detailed information about the influence of paternal high-THI exposure on in vitro embryo morphokinetics is missing. The aim of this study was to evaluate the effect of bull exposure to high THI on the subsequent in vitro embryo morphokinetics by means of time-lapse image monitoring (TLM).
METHODS
Twelve ejaculates from six mature Simmental bulls that were exposed to high (n=6) and low (n=6) THI during the epididymal maturation phase were used for IVEP. Briefly, 96 in vitro matured cumulus-oocyte complexes retrieved from slaughterhouse ovaries were inseminated with high (n=48) and low (n=48) THI sperm samples during six IVEP sessions. A total of 73 fertilized embryos were cultured in vitro (IVC) in a micro well group culture dish (Vitrolife, Denmark) and monitored with a TLM system (PrimoVision, Vitrolife, Denmark). Images were recorded every 10 minutes until day 9 of IVC. Insemination time was considered as time 0 and used to calculate the developmental events. The time of first (T1) and second (T2) cleavage, last cleavage before entering the lag-phase (T9), cleavage resumption after lag phase (RCl), length of lag-phase (LP), start time of blastocyst expansion (tSB) and time of hatching (tHB) were recorded.
RESULTS
Both groups of embryos were similar regarding T1, T2, T9, RCl, LP and tHB (P>0.05). However, the in vitro derived embryos from bulls exposed to high-THI presented a faster blastocyst expansion (P<0.05) when compared to low-THI (159 ± 11 and 169 ± 21, respectively).
CONCLUSIONS
Our results described for the first time the effect of paternal high-THI exposure on the morphokinetic parameters of in vitro produced bovine embryos and suggest a relationship between paternal high-THI exposure and the time of blastocyst expansion.

W88
THERMAL COMFORT AND THERMOGRAPHIC SCROTAL GRADIENTS OF BULLS RAISED ON SHADED TROPICAL PASTURES: PRELIMINARY RESULTS

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BACKGROUND-AIM
This study aimed to evaluate the thermal comfort and the scrotal temperature gradients of bulls of two breeds raised on natural shaded tropical pastures.
METHODS
Nelore (NEL, n=16; Bos indicus; 30 months; 478kg) and Canchim bulls (CAN, n=16; 5/8 Bos taurus x 3/8 Bos indicus; 32 months; 488kg) were raised on pastures with a 20% shaded area (Eucalyptus urograndis) in São Carlos, Brazil (21°57S, 47°50'W). Monthly, respiratory rate (RR, breaths/min), rectal temperature (RT, oC), and infrared thermographic scrotal surface patterns were evaluated (Testo 890-2), during the day´s greatest thermal challenge period (11:00 am to 2:30 pm). Thermal gradients (oC) were determined by the difference between the RT and the temperatures of the scrotum (G1), spermatic cord (G2), proximal testicular pole (G3), distal testicular pole (G4), tail of epididymis (G5), and between the temperatures of both testicular poles (G6). The data comprised the period from April to June (Mean air temperature: 20.2oC; relative humidity: 69.2%; THI: 66.8). Means were compared by ANOVA and Tukey´s post-hoc test.
RESULTS
The RR (34.1±1.1 vs 34.5±1.6) and RT (39.37±0.08 vs 39.43±0.07) of NEL and CAN did not differ and were within the physiological range, indicating no thermal stress. NEL and CAN bulls did not differ regarding G1 (5.75±0.14 vs 5.70±0.18), G3 (5.07±0.15 vs 4.70±0.16), G4 (6.64±0.12 vs 6.96±0.19) and G5 (8.14±0.18 vs 8.72±0.26). However, NEL bulls presented higher G2 (3.78±0.18 vs 3.18±0.17, P<0.05) probably due to the longer and narrower testes and a greater number of scrotal sweat glands, which benefit both sensible heat and latent heat transfers. CAN bulls presented higher G6 (2.26 ± 0.14 vs 1.57 ± 0.12, P <0.01) probably because of the volume of scrotal sweat glands, that increases from the proximal to the distal testicular poles both in Bos taurus and their crossbreeds.
CONCLUSIONS
Shaded pastures promoted thermal comfort to Nelore and Canchim bulls. Despite the breed, thermal gradients particularities of the spermatic cord and the testicular poles indicated that these anatomical regions play a critical role in scrotal thermoregulation. The use of infrared thermography may be an interesting complementary tool to the breeding soundness evaluation of zebu and composite bulls. (FAPESP Process 2019/04528-6).

W89
SPATIAL DISTRIBUTION OF DIFFERENT LIPIDS BY MASS SPECTROMETRY IMAGING MAY REVEAL SPECIFIC SIGNATURES OF FOLLICULAR DIFFERENTIATION IN OVINE OVARY

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BACKGROUND-AIM
Lipid metabolism is involved in the regulation of ovarian follicle growth, capacity to ovulate, corpus luteum (CL) formation, and luteolysis. During each estrous cycle, from the antral follicles emerging in a follicular wave, only the dominant one can ovulate, whereas the others undergo atresia. We aimed to map lipids through the whole ovary to explore their distribution according to follicle stage and the presence of blood vessels.
METHODS
Paraformaldehyde-fixed ovine ovaries were consecutively analyzed by ex vivo 3D Magnetic Resonance Imaging (MRI) and 2D/3D Mass Spectrometry Imaging (MSI). The whole organs were cut using cryostat to generate 10 µm-thick sections spaced with intervals around 100 µm. Slides were coated with DHB matrix using M5 sprayer (HTX Technologies). MSI measurements were done on a RapifleX MALDI-TOF spectrometer (Bruker) in positive ion mode with m/z 100-1200 to detect lipids, with a lateral resolution of 30-40 µm. MSI data were treated by SCiLS Lab software. Immunohistochemistry was performed on adjacent sections, and 20x light microscopy images were acquired using the AxioScanZ1 scanner (Zeiss).
RESULTS
From two ovaries, 72 and 123 MSI sequences were acquired and analyzed separately. From each section, 180-300 ion density maps were generated, revealing different ions enriched in either follicular cells, or fluid, or blood vessels, or CL. After hierarchical clustering, segmentation maps clearly discriminated blood vessels, CL, and cortex/interstitial tissue from antral follicles, due to specific lipid profiles.
Then, for each ovary, all sections were aligned, merged into a 3D data set, in order to topography lipids between the follicles of different sizes and atretic stages. 3D representations allowed in situ cartography of blood vessels and reconstruction of dominant and subordinated follicles by specific molecular signatures of follicular cells and fluids. In addition, a multimodal analysis combining immunodetection of Hemoglobin subunit A and Proliferating Cell Nuclear Antigen confirmed lipid mapping to definite ovarian structures.
CONCLUSIONS
2D and 3D-dimensional MSI of lipids allowed the spatial position of the follicles relative to blood flow and discrimination of follicular compartments that may help to enlighten the involvement of lipids in follicle differentiation.

W90
OVINE OVARY MULTIMODAL IMAGING COMBINING EX VIVO 3D MAGNETIC RESONANCE IMAGING, 2D HISTOLOGY AND MASS SPECTROMETRY IMAGING TO STUDY TERMINAL FOLLICULOGENESIS

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BACKGROUND-AIM
In ruminants, numerous antral follicles enter in final growth but only a dominant follicle will ovulate. Inside the follicle, follicular fluid (FF) and follicular cells have specific lipid contents, which change during follicular growth due to differential lipid uptake from blood and fatty acid metabolism of follicular cells. The objective was to develop a multimodal imaging approach to explore lipid distribution through the whole ovary.
METHODS
Ewe ovaries (Ovis aries) were fixed by 4% paraformaldehyde and analyzed using 3 Teslas Magnetic Resonance Imaging (MRI) instrument (Siemens). 3D images were acquired with an isotropic voxel size of 0.25 mm. 10 µm cryosections of the ovaries were analyzed by Mass Spectrometry Imaging (MSI) using RapifleX MALDI-TOF spectrometer (Bruker). The MSI sequences were acquired with a spatial resolution of 30 µm/pixel, in positive ion mode to detect lipids in 100-1500 m/z range. Light microscopy 20x images of either hematoxylin, or Oil red-stained sections were acquired using the AxioScanZ1 scanner (Zeiss).
RESULTS
MRI was performed on 28 whole ovaries. 15-71 antral follicles per ovary with inner diameter ranging from 0.25 mm to 10.5 mm were detected. Segmentation of 3D MRI images allowed measuring of FF volume in each follicle and determining their position. Five organs were sectioned, and either MSI of lipids, or histological staining were performed on ovary sections. Hierarchical clustering of lipid spectra, which discriminated antral follicles from blood vessels, luteal bodies, and cortex, generated MSI segmentation 2D maps. Hematoxylin staining revealed the morphology of ovary sections. Lipid accumulation sites were detected by Oil red staining. Single 2D histological images were then aligned to MSI of adjacent sections. Both could be integrated within the 3D MRI ovary volume. By such 2.5D representations, the topology of lipids through ovarian compartments, or between the follicles of different sizes could be analyzed.
CONCLUSIONS
The combination of 3D MRI to optical microscopy and to 2D MSI has allowed access to new anatomical and structural information within the whole ovary, with especially a more precise mapping of lipids, enlightening their involvement in follicle differentiation through terminal folliculogenesis.

TOPIC Neuroendocrine control of reproduction

W91
INVESTIGATION OF SPERM COLLECTION AS A STRESS FACTOR IN CHINCHILLA (CHINCHILLA LANIGERA)

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BACKGROUND-AIM
The reproductive properties of chinchillas have not been thoroughly investigated yet and the available data are contradictory. So far, the most common sperm collecting method has been the electroejaculation, although it does not meet the animal welfare criteria. Earlier we developed a massage technique for sperm collection in chinchilla. In the present study the effect of sperm collection by massage on stress was investigated by monitoring corticosterone and testosterone changes in the urine and their effect on the success of sperm collection and sperm quality.
METHODS
Urine samples were collected from all animals before the examined period to determine the basic values of corticosterone levels. 1-2 hours following sperm collection urine samples were collected once a week for 13 weeks from 30 trained, 2 year old males and 10 untrained, young males. The sperm motility and concentration were determined with CASA (SCA®). The ratio of live intact; live abnormal and dead spermatozoa was examined with aniline blue-eosin staining method. The corticosterone and testosterone levels in the urine were detected with ELISA.
RESULTS
The rate of successful semen collections of older males was 33.4%. Semen collection with massage method did not trigger stress on the trained chinchilla males, while the untrained ones responded with high corticosterone level following the first massage collection, however, at later stage it decreased. During the 13 week period the young males did not produce semen at all, even though, there was no detectable stress by the handling. The testosterone levels were high at the start of handling both in trained and untrained males, and then they decreased. There were large individual differences in the values of spermatological parameters of the trained males, that is, there could not be found connection neither between the amounts of corticosterone or testosterone and sperm quality.
CONCLUSIONS
The success or unsuccess of our semen collection method does not depend on the stress caused by the procedure. Hopefully, the ratio of successful sperm collection can be further improved by refining the technology and a conscious selection work. The reason of the large deviation in sperm quality needs further investigation.

W92
SYNTHETIC ETHANOLAMINE PLASMALOGEN (EPL) STIMULATE FSH SECRETION FROM BOVINE GONADOTROPH, BUT OLD BRAIN EPL INHIBITS SUCH STIMULATION

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BACKGROUND-AIM
Brain ethanolamine plasmalogens (EPIs), unique alkenylacyl-glycerophospholipids, are the only known ligands of G-protein-coupled receptor 61 (GPR61)—a novel receptor co-localised with gonadotropin-releasing hormone (GnRH) receptors on gonadotrophs. Unlike the young-brain EPI, the old-brain EPI have no stimulation effect on FSH secretion from gonadotrophs, probably due to the age-related changes in ratio of six different EPI molecular species. Although it is very difficult to synthesize EPI chemically, only a chemosynthesis one, 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphoethanolamine (PLAPE) is commercially available. Therefore, this study hypothesized that PLAPE can stimulate FSH secretion from bovine gonadotrophs in the presence of young, but not old,brain EPIs. Additionally, we tried to performed molecular docking simulation to estimate how PLAPE and other EPI molecular species bind onto bovine GPR61.
METHODS
We prepared bovine AP cells from postpubertal heifers and cultured the cells for 3.5 d. We treated the cells with increasing concentrations of PLAPE alone, or in the presence of EPI extracted from the young or old bovine brain. The medium samples were harvested 2 h after culture for LH and FSH assays.
RESULTS
As expected, PLAPE alone could stimulate FSH secretion from bovine gonadotrophs. PLAPE could stimulate FSH secretion also in the presence of EPI extracted from the brain of young heifers, but not old cows, suggesting presence of any antagonized EPI molecular species in old brain. Additionally, we could construct a 3-dimentional structure model of bovine GPR61 using, a recent, highest accuracy method, AlphaFold ver. 2. Furthermore, in silico molecular docking simulation clarified three binding sites, located in either extracellular, transmembrane, or cytoplasmic regions. The ethanol-amine head of PLAPE is likely attach onto the extracellular site while tip of SN-1 and SN-2 side chains are attaching outside membrane of gonadotroph.
CONCLUSIONS
Therefore, we concluded that PLAPE was a synthetic agonist of GPR61. Further studies are required to clarify EPL molecular species for age-related infertility.

W93
HORMONAL AND SEMINAL SPERM PARAMETERS OF SUBFERTILE STALLIONS TREATED WITH GNRH AGONIST LECIRELIN

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BACKGROUND-AIM
Testicular hormone secretion and sperm production are regulated by the hypothalamic-pituitary axis which relies on gonadotropin-releasing hormone (GnRH) stimulation of gonadotropin secretion and gonadal response. In stallions several studies have demonstrated a stimulatory effect of GnRH agonist treatment and testosterone release followed by a decrease in daily sperm production in long-term GnRH treatment using implants in normal stallions. Therefore, no studies were performed with low dose given daily in stallions with poor semen quality.
METHODS
The hypothesis in this study is that daily treatment with the GnRH agonist lecirelin improves reproductive functions in subfertile stallions. Stallions received 50 µg once a day (n=4) for 12 weeks and were re-evaluated 4 weeks after the end of the treatment. Testicular response was assessed by measuring testosterone and estrone sulphate (interstitial compartment) and AMH and inhibin-B (tubular compartment) and correlated with sperm kinetics and production at 2-week intervals. Sperm kinetics were evaluated by CASA (IVOS Version 12 Hamilton Thorne Research, MA, USA), serum concentrations of inhibin- B, AMH and estrone sulfate were determined by ELISA and testosterone by RIA. Data were analyzed by ANOVA for repeated measures over time and correlations were calculated all measured parameters.
RESULTS
Testosterone concentrations decreased from week 0 to 6 (667 vs 302 pg/ml, respectively P<0.05), but tended to recover after 1 month of treatment ceased (469 to 977pg/ml, respectively, P<0.08). Concentrations of all hormones were positively correlated with one another (P<0.01), the highest correlations being between and inhibin-B and estrone sulphate (R2=0.72) and inhibin-B and AMH (R2=0.41). Despite the decreases in testosterone, there was no change in sperm motility and production among stallions during the course of treatment (P>0.05). All hormones positively correlated with total sperm number (P<0.01).
CONCLUSIONS
In summary, the treatment did not improve hormonal and seminal parameters in subfertile stallions, but also, did not affect testicular function. The correlation between hormones and sperm output indicates these hormones are good markers for testicular function.

TOPIC Nutrition and reproduction

W94
EFFECTS OF DIETARY SUPPLEMENT CONTAINING LEPIDYUM MEYENII, LAMINARIA AND EQUISETUM EXTRACTS ON EQUINE FROZEN SEMEN

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BACKGROUND-AIM
Nutraceutical supplements including several botanicals, oils of both animal and plant origin, and vitamins have been tested with the aim of improving sperm quality in human and veterinary medicine. Among botanicals, Lepidyum meyenii (Maca) was found to have positive effects on male sexual functions and spermatogenesis in mammals. In particular, 60 days dietary supplementation in stallions was found to improve spermatozoa concentration, motility, and acrosome integrity on fresh and cooled equine semen. However, no information exists on possible effects of Maca supplementation on equine frozen semen.
METHODS
Eight adult Sicilian Oriental Purebred stallions were included in the study. Group A (n.4) was fed 50 g/day of powder containing Lepidyum meyenii, Laminaria and Equisetum extracts, for 60 days in addition to normal diet. Group B (n.4) received the same diet without supplements. Semen collection and freezing was performed on each horse before starting supplementation (D0) and then after 60 (D60) and 90 days (D90). Frozen/thawed semen samples have been evaluated by CASA system (Androvision, Minitube). Data were considered significant for $p \leq 0.05$.
RESULTS
No side effect was observed in experimental Group A during the study. Two-way ANOVA showed no significant effect of nutraceuticals and time between groups for total (MOT), progressive (PROG) motility, and percentage of rapid (FAST) and immotile spermatozoa. However, when considering the percentual differences in studied parameters at D0 vs D90, MOT (p=0.0002), PROG (p=0.0433) and FAST (p<0.0001) were significantly higher in Group A compared to Group B.
CONCLUSIONS
Frozen/thawed semen samples from horses receiving the above-mentioned botanical extracts had improved total and progressive motility and velocity of spermatozoa. These nutraceuticals seem to lessen the deleterious effects of cryopreservation on stallion semen by reducing the oxidative stress damage. Despite the small samples size, our results encourage to study the use of botanicals for improving equine frozen semen quality in more detail. Further research on artificial insemination using this semen should be performed to determine possible effects on fertility especially in poor freezers stallions.

W95
DEVELOPMENTAL PROGRAMMING BY GESTATIONAL AND LACTATIONAL SUBNUTRITION OF TESTICULAR FORM AND FUNCTION PERSISTS IN MATURE ONE-YEAR-OLD RATS

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BACKGROUND-AIM
Developmental programming by early life subnutrition alters testicular form and function with smaller Sertoli cell numbers (and thus, reduced sperm producing capacity) in young adult rats (i.e., 100 days old). Our aim was to study the effects of gestational and lactational subnutrition on testicular form and function of 1 year old male Wistar rat offspring.
METHODS
Eight dams (Underfed group: UG) were fed 50% of ad libitum standard chow during gestation, and after parturition their litter sizes were adjusted to 14 pups. On the other hand, 8 dams (Control group: CG) were fed standard chow ad libitum along all the experiment and after parturition they suckled litters with 8 pups. At 25 days of age, all pups were weaned and fed ad libitum chow until 365 days of age. Animals were weighed (BW), their body length was measured (BL), after eutanasia blood serum was immediately sampled, left testes were dissected, weighed (LTW), immersion-fixed (Bouin's), processed histologically for standard paraffin wax embedding and haematoxylin eosin stained sections. Quantitative histological variables were measured: seminiferous tubules diameter (STD), seminiferous tubules absolute volume (STV), number of Sertoli cells/seminiferous tubule cross section (NSST) and /testis (NST), as well as number of Leydig cells/intersticial space (NLI) and /testis (NLT). Serum testosterone (ST) concentrations were measured by RIA. Data (mean ± sem, UG vs CG in Results section) were analized by one way ANOVA.
RESULTS
BW (g) 531.6 ± 10.2*** vs 588.4 ± 12.7; BL (cm) 45.2 ± 1.77** vs 47.0 ± 2.18; LTW (g) 1.68 ± 0.03*** vs 1.79 ± 0.02; ST (ng/mL) 5.2 ±1.0* vs 2.9 ±0.45; STD (µm) 220.2 ± 3.4 vs 221.8 ± 4.0; STV (mL) 1.57 ± 0.03*** vs 1.70 ± 0.02; NSST 21.8 ± 0.1*** vs 23.4 ± 0.1; NST (x106) 149.5 ±6.3 *** vs 175.9±9.23; NLI 5.3 ± 0.3 vs 5.5 ± 0.1; NLT (x106)36.2±2.5 vs 41.4±2.8.
CONCLUSIONS
As far as we know, this is the study on developmental programming by gestational and lactational subnutrition on testicular form and function which has advanced farthest into mature age of any mammal. We demonstrate that early life subnutrition is followed by testicular changes strongly indicating reduced sperm producing capacity and increased serum testosterone levels well into mature life.

W96
INTERSTITIAL TESTICULAR LEYDIG (BUT NEITHER SERTOLI NOR MYOID) CELLS EXPRESSION OF IGF-1 RECEPTOR IS AFFECTED BY EARLY LIFE NUTRITIONAL PROGRAMMING

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BACKGROUND-AIM
IGF1 regulates important testicular functions such as testosterone production by Leydig cells and Sertoli cells survival in the developing testis. Little is known regarding subnutrition programming of testicular IGF1-R expression and its eventual effects on testicular activity in adult life.
METHODS
In order to test whether gestational subnutrition affects testicular IGF-1R expression, Wistar rat dams were assigned (n=6/group, except for TCC, where n=4), to either CCC (ad libitum), CTC (feed restricted at 45% of ad libitum from gestation day 13 until parturition), TCC (feed restricted at 45% of ad libitum from days 0-13 of pregnancy) or TTC (feed restricted from gestation day 0 to parturition). Pups were weighed at birth (BW0, g), fed ad libitum until 40 days of age, then weighed (BW40, g) and slaughtered. Their left testes were weighed (TW, g), processed for immunohistochemistry anti IGF-1R. A positivity index (PI, mean ± sem) was calculated for each experimental group and cell type by evaluating 300 cells of each type/animal. Data were analysed by anova. P presented are significant at 0.05, as compared to CCC group.
RESULTS
BW, TW and Leydig cells IGF-1R immunoexpression (LCI) were affected by treatment. BW0: CCC = 7.64 ± 1.09a, CTC = 7.04 ± 0.87ab (n.s), TCC = 6.31 ± 0.67b(*), TTC = 5.49 ± 0.75bc(**) (TTC p = 0.09 as compared to TCC); BW40: CCC = 200.0 ± 4.93a, CTC = 166.0 ± 2.47c(***), TCC = 181.1 ± 2.47b(**), TTC = 149.84 ± 4.8d(***); TW: CCC = 0.645 ± 0.024a, CTC = 0.563 ± 0.0314a(n.s.), TCC = 0.6245 ± 0.0267a (n.s.), TTC = 0.3642 ± 0.0289b(***); LCI: CCC = 2.20 ± 0.02a, CTC = 1.97 ± 0.04b(*), TCC = 1.83 ± 0.07b(***), TTC = 1.77 ± 0.06b(***) (TTC p = 0.07 as compared to CTC). Neither Sertoli nor myoid cells IGF-1R immunoexpression were affected by treatment. Sertoli cells PI (n.s.): CCC = 1.07 ± 0.06, CTC = 1.17± 0.08, TCC = 1.11 ± 0.15, TTC = 0.97 ± 0.24; myoid cells PI (n.s.): CCC= 0.87 ± 0.03, CTC = 0.85 ± 0.04, TCC = 0.90 ± 0.10, TTC = 1.00 ± 0.09.
CONCLUSIONS
In conclusion, in utero rat dam subnutrition affects body weight, testicular weight as well as interstitial testicular Leydig cells but neither Sertoli cells nor myoid cells IGF-1R immunoexpression in their offspring.

W97
EFFECT OF DIETARY SUPPLEMENTATION OF BY-PASS LINOLENIC ACID ON FOLLICULAR AND LUTEAL FUNCTION DURING EARLY STAGE OF PREGNANCY IN EWES

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BACKGROUND-AIM
It is well known that the quality and composition of the diet can influence reproductive performances in farm animals and contribute to reduce the reproductive failure during early stage of pregnancy. Thus, the aim of the present study was to assess the effect of dietary supplementation with by-pass a-linolenic acid (ALA) on ovarian function and pregnancy establishment in ewes.
METHODS
Thirty-nine dry Sarda ewes were assigned to the following groups: control ewes (CTR; N=20), which were fed with a control diet without ALA; and ALA ewes (ALA; N=19), which were fed with a diet supplemented with ALA (10.8 g/ewe/day). Both diets have similar crude protein and energy level and were offered for 5 weeks (-2 to +3 weeks after expected mating). Estrous synchronization was induced in all the ewes using intravaginal sponge (45mg fluorgestone acetate) for 14 days and equine chorionic gonadotropin (350 UI/ewe) at the end of the treatment. The ewes were mated using fertile rams 48 hours after pessaries withdrawal. Estradiol (E2) levels in plasma was determined on Days -2 (pessaries removal), -1 and 0 (mating). Also, progesterone (P4) levels in plasma were determined on Days 5, 8, 11, 14, and 16 after mating. Ovulatory rate and number of embryos were determined on Days 8, 14, 16 and 24 post-mating, using ultrasonographic scanning and uterine flushing. Body weight, body condition score and intake were similar between CTR and ALA group during experimental period.
RESULTS
Percentage of ewes in estrus (85 and 100%) and pregnancy rates (88.2 and 89.5%) were similar between CTR and ALA groups, respectively. Ovulation rate per mated ewe (1.88±0.15 vs 1.94±0.21) and per pregnant ewe (1.93 ± 0.15 vs 2.00 ± 0.21) did not differ in CTR and ALA groups. Number of embryos/ewes was not different between experimental groups (1.53±0.13 vs 1.71±0.17). E2 level during induced follicular phase was not different between the groups. Conversely, P4 levels were higher in ALA ewes at Days 5, 8 and 16 ($P<0.05$) with a statistical tendency at Day 14 ($P<0.10$).
CONCLUSIONS
In summary, dietary supplementation of ALA during preimplantation period can improve the luteal function needed to guarantee embryo survival.

W98
ANTIOXIDATIVE EFFECT OF DIETARY BY-PASS LINOLENIC ACID ON REPRODUCTIVE TISSUES AND PLASMA DURING EARLY STAGE OF PREGNANCY IN EWES

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BACKGROUND-AIM
Polyunsaturated fatty acids are well known for their antioxidative properties. This effect could be positive on maternal environment during embryo maternal recognition phase. Thus, the present study evaluated the effect of dietary supplementation with by-pass a-linolenic acid (ALA) on luteal, uterine and plasmatic oxidative status at Days 14 and 16 of pregnancy in mature-dry Sarda ewes.
METHODS
Twenty ewes (CTR) were fed with a control diet without ALA; and nineteen ewes (ALA), were fed with a diet supplemented with ALA (10.8g/ewe/day). Both diets have similar crude protein and energy level and were offered for 5 weeks (-2 to +3 weeks after expected mating). Estrous synchronization was induced in all the ewes using intravaginal sponge (45mg fluorgestone acetate) for 14 days and equine chorionic gonadotropin (350 UI/ewe) at the end of the treatment. Forty-eight hours after pessaries withdrawal ewes from both groups were mated using fertile rams. Eight ewes from each group were slaughtered at Days 14 (ALA, N=4; CTR, N=4) and 16 (ALA, N=4; CTR, N=4) after mating. Plasma, uterine and luteal tissues were sampled. Thiols, total antioxidant activity (TEAC) superoxide dismutase (SOD) activity, and Malondialdehyde (MDA) content were measured.
RESULTS
Thiol was not different between groups. At Day 14, TEAC in uterine tissue was higher in ALA group (53.42±4.47 vs 30.46±0.76 nmol/g of tissue for ALA and CTR groups, respectively; $P<0.01$). Also, SOD activity was higher in ALA group in luteal tissue (10.61±0.51 vs 7.46±0.76 U/g of tissue for ALA and CTR groups, respectively; $P<0.05$). At Day 16, TEAC was higher in ALA group in luteal tissue (223.39±17.81 vs 125.37±4.91 nmol/g of tissue for ALA and CTR groups, respectively; $P<0.01$). Likewise, the SOD activity was higher in ALA group on uterine tissue (0.57±0.02 vs 0.21±0.04 U/g of tissue for ALA and CTR groups, respectively; $P<0.01$) and plasma (15.53±0.62 vs 12.28±1.09 U/mg of protein for ALA and CTR groups, respectively; $P<0.05$). Finally, MDA content was lower for ALA group on uterine tissue (206.56±15.64 vs 429.81±11.50 nmol/g of tissue for ALA and CTR groups, respectively; $P<0.001$).
CONCLUSIONS
The systemic and local antioxidative effect of dietary ALA could contribute to improve the embryo survival during embryo-maternal recognition period of pregnancy.

W99
EFFECTS OF A GLUCOGENIC DIET ON REPRODUCTIVE PERFORMANCES AND FOETAL GROWTH IN DAIRY EWES

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BACKGROUND-AIM
Nutritional challenges during the periconceptional period and gestation can affect fetal growth and future development of the offspring. The aim of the experiment was to test, in dairy ewes, the effects of two different nutritional plans (high and low starch) fed from weaning to parturition on pregnancy rates, foetal development and morphometric parameters of lambs at birth.
METHODS
From 30 days of age until parturition, 118 Sarda ewes were randomly allocated to two different nutritional plans: the high starch group (HS; n= 56) and the low starch group (LS; n=62) were fed respectively diets consisting in 25% and 9% of starch and sugars on total dry matter. In the LS diet, part of the starch was substituted with beet pulp. At the onset of puberty, ewes were submitted to oestrus synchronisation and allowed to mate with rams of proven fertility wearing a marking harness. Non return to oestrus rates were recorded. Pregnancy diagnosis was confirmed by transabdominal scan (Mylab™ One, Esaote, Italy) at 25 days post mating. Ultrasound scanning was repeated at 30 days for the measurement of crown rump length (CRL) and assessment of twinning rates, and at 48, 68, and 88 days for the measurement of biparietal diameter (BPD) of foetuses. At birth, liveweights and withers heights were recorded. Data were analysed by x2 test or t-test using StataIC 11 (StataCorp LP, USA).
RESULTS
Non return to oestrus and pregnancy rates did not differ between groups (HS 66.1% vs LS 56.4%; P>0.05) and no effect of nutrition was observed for twinning rates (HS 37.8% vs LS 25.7%; P>0.05). In HS ewes, CRL of foetuses was higher compared to LS ones (2.16 cm ± 0.03 vs 2.05 cm ± 0.04; P<0.05) but BPD recorded up to 88 days post mating did not differ between groups (P>0.05). At birth, no effect of the diet was reported on lambs liveweight (HS 3.72 Kg ± 0.57 vs LS 3.67 Kg ± 0.56; P>0.05) and withers height (HS 36.6 cm ± 1.5 vs LS 36.2 cm ± 1.63; P>0.05).
CONCLUSIONS
The glucogenic diet fed from weaning to parturition did not have any remarkable effect on reproductive performances, foetal growth, and morphometric parameters at birth. Further investigations are needed to assess if exposing the foetus to these maternal nutritional challenges in a specific gestational window may have consequences on its development and growth.

W101
EFFECT OF PHYTOMELATONIN-RICH DIETS ON OXIDATIVE DAMAGE IN RAM SPERM

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BACKGROUND-AIM
Reproductive seasonality in sheep is regulated by nocturnal melatonin secretion, and subcutaneous implants of this hormone have been used to modulate it. However, it would be interesting to replace the synthetic melatonin with phytomelatonin, which is present in plants and can be included in the diet. Thus, the main objective of this work was to evaluate the effect of phytomelatonin-rich diets on ram sperm quality and seminal plasma composition.
METHODS
Nine Rasa Aragonesa rams were fed with a commercial diet and nine with a phytomelatonin-rich diet for five months. The phytomelatonin-rich diet consisted of a mixture of various sources of phytomelatonin (20%), such as pomegranate pulp, tomato pulp and grape pomace (all of them derivatives of the food industry), which was added to a commercial feed (80%). Semen samples were obtained by artificial vagina every 15 days and blood samples were collected once a month. Sperm motility, viability, intracellular levels of reactive oxygen species (ROS) and phosphatidylserine inversion were evaluated, as well as the melatonin concentration and the activity of antioxidant enzymes (glutathione reductase and peroxidase, catalase, and superoxide dismutase) in seminal plasma and blood plasma. Differences were analyzed using the mixed-model ANOVA.
RESULTS
The phytomelatonin-rich diet increased melatonin concentration in seminal plasma, which was statistically significant in the third month of feeding (P <0.05). From the second month, an increase in the percentage of viable sperm and viable sperm with low levels of ROS was observed compared to the control group (P <0.05). This increment was maintained until the end of the experiment. In addition, the lack of differences between groups in the analysis of the activity of the antioxidant enzymes in blood and seminal plasma suggests that phytomelatonin would not act modulating these enzymes.
CONCLUSIONS
Phytomelatonin-rich diet increases sperm viability, decreases intracellular ROS levels, and can exert this effect directly on sperm, not through the modulation of antioxidant enzyme activity. Grants: AGL-2017-83799-R, DGA-A07-17R and S M-J and V P-D have a grant from the Aragón Government.

W102
DOES UNDERNUTRITION ALTER DNA METHYLATION AND FOLATE METABOLISM IN SHEEP UTERUS?

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BACKGROUND-AIM
Undernutrition is able to modulate uterine physiology and gene expression. This could be achieved by inducing epigenetic changes, such as DNA methylation, since a lesser supply of precursors for the methionine/folate cycles, which provide methyl donors in cells, could lead to altered DNA methylation. The aim of this study was to assess if undernutrition influences the degree of DNA methylation, and the expression of some key components of the one-carbon metabolism pathway in the sheep uterus.
METHODS
Adult Rasa Aragonesa ewes were offered diets to fulfill all (control, n = 6) or half (undernourished, n = 6) their requirements for maintenance, and they were estrus synchronized with progestagen pessaries. On day 16 of the cycle (30 days of dietary treatment), ewes were euthanized and the ipsilateral uterine horns were processed for molecular analyses. Global DNA methylation (i.e., 5-methylcytosine) was determined by ELISA. Gene expression was assessed by real-time PCR.
RESULTS
By the end of the experiment, undernourished ewes had decreased body weight (P<0.01) and body condition (P<0.05), and no changes were observed in control ewes. No effect of undernutrition was observed on uterine DNA methylation. From the enzymes responsible of DNA methylation, undernourished ewes presented higher expression of DNA methyltransferase (DNMT) 3a (P<0.05), while DNMT3b and DNMT1 expression remained unchanged. Undernutrition reduced the expression of solute carrier SLC19A1, encoding the reduced folate carrier (RFC) (P<0.02), and folate receptor (FOLR)1 (P<0.05), whereas FOLR2 and FOLR3 were not affected. As for the enzymes involved in one-carbon metabolism, undernutrition did not affect the gene expression of methionine adenosyltransferase 2A (MAT2A) and methionine synthase (MTR), but it drastically reduced the mRNA expression of methylenetetrahydrofolate reductase (MTHFR, P<0.01) and betaine-homocysteine methyltransferase (BHMT, P<0.0001).
CONCLUSIONS
These preliminary results suggest that undernutrition could alter the availability of folate in uterine cells, and affect the interconnected methionine/folate cycles. This would lead to lesser cellular content of methyl donors, essential for several cellular processes, which could ultimately lead to altered uterine physiology.

W170
EFFECT OF ORAL CALCIUM BOLUSES AT CALVING IN PREPARTUM GRAZING COWS FED ANIONIC SALTS ON PLASMA CALCIUM, AND POSTPARTUM DISEASES.

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BACKGROUND-AIM
Hypocalcemia is a common metabolic disease affecting dairy cattle throughout the peripartum. It can be either clinical (milk fever) or subclinical, affecting muscle function, and becoming a risk factor for other diseases and infertility. Anionic salts during the prepartum period have been useful to prevent milk fever in dairy cows. However, older cows may still develop hypocalcemia regardless of the anionic strategy. As a result, calcium supplementation at parturition has been considered to complement the anionic programs. The objective of this study was to evaluate the effect of oral calcium boluses right after parturition in cows fed prepartum anionic diets on plasma Ca, and the incidence of postpartum diseases.
METHODS
The study was conducted on a grazing Holstein dairy located in the south of Chile. During fall 2019, 60 cows on their second lactation or higher were randomly assigned at calving in 2 groups. Both groups were handled in the same lot and fed an anionic supplement during the prepartum period. Treatment group received immediately after calving an oral bolus providing 44 g of Ca, 6 g of Mg and 50,000 IU of Vitamin D3. A second bolus was provided 24 h later. The control group did not receive any bolus. Blood samples were taken at day 1, 2, and 3 postpartum. Incidence of milk fever retained fetal membranes and metritis were assessed and treated accordingly. Plasma total Ca concentration was analyzed by mixed model ANOVA for repeated measures, and the incidence of diseases by chi-square.
RESULTS
Plasma total Ca was higher at day 2 (2.21 vs 2.04 mmol/L) and 3 (2.18 vs 2.01 mmol/L) in third lactation treated cows (P = 0.07), and at day 2 (1.98 vs 1.89 mmol/L) in fourth or more lactation treated cows (P = 0.15) than in controls, respectively. The incidence for diseases was: milk fever 10% vs. 0% (P=0.05); retained fetal membranes 13.3% vs. 6.7% (P=0.21), and metritis 6.7% vs. 0% (P=0.12), for control and treated groups, respectively.
CONCLUSIONS
In conclusion, oral calcium boluses at parturition in cows from 3 or more lactations under a prepartum anionic program tended to increase plasma total calcium during the first 3 days postpartum. In addition, calcium supplementation had a significant reduction on milk fever and tended to reduce the incidence of metritis and retained fetal membranes.

TOPIC Other

W106
CHARACTERISTICS OF PRION GENE KNOCKOUT JAPANESE BLACK CAWS PRODUCED TO PREVENT SPONTANEOUS BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) ONSET OR INFECTION

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BACKGROUND-AIM
In 1986, United Kingdom (UK)-type bovine spongiform encephalopathy (BSE; variant type Creutzfeldt- Jakob disease: vCJD) firstly occurred in UK. Cattle were given feed containing abnormal prion protein (pathogen of BSE) prepared from scrapie sheep. In 2006, it was confirmed that orally administered abnormal prion protein of BSE cattle causes vCJD infection in human. In 2003, spontaneous BSE was firstly found in Italy, then in many countries. Spontaneous BSE may be transmissible from cattle to human. BSE continues to be critical problem in animal and veterinary science area. To make prion gene knockout (KO) cattle is just one solution to prevent BSE onset or infection. The aim of this research was to make prion gene KO cows and to reveal their characteristics.
METHODS
Prion gene was knockouted in fibroblasts prepared from Japanese Black cow embryo. The somatic cell nucleus of prion gene KO fibroblasts was transferred into oocyte with removal of nucleus (somatic cell nuclear cloning). After activation and in vitro culture, the blastocysts were transplanted into the uterus. Eleven prion gene KO calves were born.
RESULTS
Their characteristics were examined as the follows: The deletion of prion gene on chromosome was confirmed. No expression of prion mRNA was detected in major organs (brain, spinal cord, tonsils, spleen, liver, kidneys, lungs, heart, skeletal muscles). No prion positive immunohistochemical staining was shown in major organs. Epigenetic modification or variation was not noted in the major organs, Noteworthy histopathological changes was not observed in major organs. Prion KO cattle specific protein spots in the brain and tonsils were detected by the two-dimensional electrophoresis performed to analyze the protein profiles. Then, comprehensively analyses of protein expression in the brain and tonsils were carried out by shotgun method using liquid chromatography-tandem mass spectrometry. Lower expression levels of proteins which are reduce and/or prevent the intracellular peroxidation in the brain and tonsils of KO cows were noted.
CONCLUSIONS
In conclusion, to produce the prion gene KO cattle is the only way to prevent BSE infection, but more detailed studies on prion gene KO cattle are needed.

W108
MORPHOMETRIC ANALYSIS OF ALPHA-1, 3-GALACTOSYLTRANSFERASE KNOCKOUT PIG KIDNEY AND HEART TO PROVIDE THE APPROXIMATE TIME OF GRAFT HARVEST TO MATCH THE SIZE OF THE RECIPIENT

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BACKGROUND-AIM
Finding out the ideal anatomical parameters of donor organs is one of the determinant factors for successful transplantation. However, estimation of organs size when the animal alive is complex. In this study, we performed a morphometric evaluation of a1,3-galactosyltransferase (GTKO) pig heart and kidney (n=9).
METHODS
After necropsy, we measured the weight, length (heart- base to apex; kidney- pole to pole), width (heart- transverse; kidney- lateral), and thickness (heart and kidney- anteroposterior) of the organs at the end of one, three and seven months.
RESULTS
All of the organ's parameters were gradually increased with age ($p<0.05$) and body weight ($p<0.05$) of the pigs. In addition, we performed a correlation analysis of organs morphometries with age and body weight of the animal. Significant correlation was observed between pig's age and the heart morphometries (weight, ($r= 0.983$); length, ($r=0.986$); width, ($r=0.872$)) as well as kidney morphometries (length, ($r= 0.898$); width, ($r= 0.944$); and weight, ($r= 0.830$)). Significant correlation was also observed between pig's body weight and heart morphometries (length, ($r= 0.972$); weight, ($r= 0.979$); and width, (0.832)) as well as kidney morphometries (length, ($r= 0.935$); weight, ($r= 0.885$); and width, ($r= 0.932$)).
CONCLUSIONS
From this morphometric cross-relation study, we were able to conclude the average size of GTKO pig heart and kidney based on age and body weight, which would be helpful in estimating these organs size non-invasively for xenotransplantation.

W109
METAGENOMIC ANALYSIS SHOWS SIMILAR ENDOMETRIAL MICROBIOTA COMPOSITION IN MARES AND JENNIES

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BACKGROUND-AIM

The aim of this study was to compare the endometrial microbiota composition in fertile mares and jennies and investigate if they can experience changes throughout the estral cycle.

METHODS

Nine mares (8-12 years old) and 6 jennies (3-7 years old) without clinical sings of endometritis and with negative cytology and culture were included in the study. For the microbiome sample uterine, flushing catheter was inserted connected to a 500ml bottle of sterile saline solution. Samples were obtained in estrus and in diestrus phases (same estral cycle). The endometrial microbiota was analyzed using 16S rRNA gene sequencing with the Ion Torrent™ (Thermo Fisher Scientific, USA) technology and the Ion Reporter™ software. Bioinformatics analysis was performed using QIIME2. Taxonomic characterization and a, b diversity were compared between species and in estrus and diestrus.

RESULTS

A total of 15 different bacterial genera were identified in endometrium of mares and 20 genera were found in jennies. The most represented bacterial genera in mares and jennies were similar: Pseudoxanthomonas (57.36 ± 6.46 vs 50.40 ± 13.37%), Xanthomonas (21.53 ± 2.23 vs 19.83 ± 5.80 %) and Pseudomonas (13.01 ± 3.8 vs 13.03 ± 4.84%). In estrus, 14 different genera were identified in mares and 13 in jennies and in diestrus, we detected 11 in mares and 19 jennies.

However, there were not significant differences in the frequency of the OTUs between follicular and luteal phases in both species. Assessing the alpha diversity (Simpson: 0.59 vs 0.61, Shannon: 1.13 vs 1.26, Evenness: 0.59 vs 0.61) revealed no significant differences between endometrial microbiota from mares vs jennies or between estrus and diestrus (Simpson: 0.57 vs 0.63, Shannon: 1.12 vs 1.28, Evenness: 0.60 vs 0.59). Beta diversity of the endometrial microbiota did not differ significantly between both groups.

CONCLUSIONS

Analysis of the endometrial microbiota using 16S rRNA sequencing revealed the presence of commensal bacteria in endometrium of fertile mares and jennies. Moreover, both species had similar endometrial microbiota composition (genera) and diversity, and this microbiota seem to be constant thought the estral cycle.

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W110
ISOLATION AND CHARACTERIZATION OF VESSEL-ASSOCIATED STEM/PROGENITOR CELLS FROM CANINE YOLK SAC

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BACKGROUND-AIM

Mesoangioblast is a vessel-associated stem cell lineage that can differentiate into several mesoderm lineages and it is used for Duchenne Muscular Dystrophy research. The yolk sac is a source of cell lineages free from immunological incompatibles and is ethically acceptable. Due to the high vascularization of this tissue, it is believed that vessel-associated stem cells may be present in the canine yolk sac. The aim of this study was to isolate and characterize Vessel-Associated Stem/Progenitor Cells from the canine yolk sac.

METHODS

Canine yolk sac tissue samples were obtained, plated and grown in two separate culture media supplemented with: Group 1 - The cells were cultured in Minimum Essential Medium Eagle - alpha with 15% defined fetal bovine serum and (2) Group 2 - The cells were cultured in Minimum Essential Medium Eagle - alpha with 2% defined horse serum. The cells were immunophenotyped by flow cytometry and RT-qPCR technique. We compared the expression of CD44+, CD13+, CD90+, CD31- (PECAM1) and CD117- (c-Kit). T test analysis was performed for RT-qPCR ($p<0.05$).

RESULTS

Both groups adhered to the plate and presented fibroblastoid shape; however, Group 1 presented higher results regarding plate dispersion and proliferation, with faster growth in culture. Both groups were CD44 positive and CD13, CD31 and CD117 negative immunophenotyping. For gene expression, Group 1 showed expression of CD44+, CD13+ and CD90+, CD31+, CD117+ and CD45-, while group 2 showed expression of CD44+, CD90+, CD13-, CD31-, CD45-, CD177-. There was no statistical difference between the groups.

CONCLUSIONS

It is believed that the canine yolk sac can present Vessel-Associated Stem/Progenitor Cells when grown in a supplemented culture medium. Therefore, it can contribute as a source of stem cells for future therapies.

W111
ISOLATION AND CHARACTERIZATION OF VESSEL-ASSOCIATED STEM/PROGENITOR CELLS FROM CANINE YOLK SAC

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BACKGROUND-AIM
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METHODS
Canine yolk sac tissue samples were obtained, plated and grown in two separate culture media supplemented with: Group 1 - The cells were cultured in Minimum Essential Medium Eagle - alpha with 15% defined fetal bovine serum and (2) Group 2 - The cells were cultured in Minimum Essential Medium Eagle - alpha with 2% defined horse serum. The cells were immunophenotyped by flow cytometry and RT-qPCR technique. We compared the expression of CD44 + , CD13 + , CD90 + , CD31 - (PECAM1) and CD117 - (c-Kit). T test analysis was performed for RT-qPCR ($p<0.05$).
RESULTS
Both groups adhered to the plate and presented fibroblastoid shape; however, Group 1 presented higher results regarding plate dispersion and proliferation, with faster growth in culture. Both groups were CD44 positive and CD13, CD31 and CD117 negative immunophenotyping. For gene expression, Group 1 showed expression of CD44 + , CD13 + and CD90+ , CD31+ , CD117+ and CD45- , while group 2 showed expression of CD44+ , CD90+ , CD13-, CD31- , CD45- , CD177- . There was no statistical difference between the groups.
CONCLUSIONS
It is believed that the canine yolk sac can present Vessel-Associated Stem/Progenitor Cells when grown in a supplemented culture medium. Therefore, it can contribute as a source of stem cells for future therapies.

TOPIC Pig reproduction

W112
CAFFEINE AND L-CARNITINE ADDED TO COOLED PORCINE SEMEN IN HOMOLOGOUS ZONA BINDING ASSAY

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BACKGROUND-AIM
The effect of enhancing in vitro fertilization (IVF) medium with metabolic regulators is still not clear. L-carnitine (LC) is an antioxidant, which transports fatty acids to the mitochondrial matrix for metabolic breakdown. Accumulation of reactive oxygen species in sperm causes ATP depletion, lipid peroxidation and axonemal phosphorylation, affecting sperm motility. In porcine IVF, caffeine (caf) exposure (1-5mM) is usually used to induce sperm binding to the zona pellucida (ZP). However, there is still polyspermy. In a previous pilot study, we tested LC concentrations to improve porcine sperm quality. The concentration of 10mM LC reduced hydrogen peroxide concentration and increased sperm functionality. This work aimed to evaluate whether caf and LC addition on day 6 to boar semen, cooled at 17°C, improves the capacity of porcine sperm binding to homologous ZP.
METHODS
At day 6 a pool of ten cooled insemination doses of boar semen was distributed in four following treatments: T1) Control: extended pool semen, T2) T1 + 2mM caf, T3) T1 + 10mM LC and T4) T1 + 2mM caf+ 10mM LC. To induce sperm ZP binding, after swim-up (37°C, 90 min), a concentration of 0.5x10^6 sperm from each treatment was co-incubated with a 200µl droplet (TCM199+10%SFB+ 100UI/ml penicillin) containing 30 oocytes at 38.5°C in 5% CO2 atmosphere (for 20 min). Three replicates were performed with 30 oocytes per treatement. As the number of zona bound sperm did not present a normal distribution, their medians were compared using the Kruskal-Wallis and Dunn Test. A probability of $p<0.05$ was considered significant.
RESULTS
The addition of 2mM caf and 10mM LC increased the number of sperm bound to ZP (median= 34 and 27 zona bound sperm) compared to the control (median=15 zona bound sperm, $p<0.05$). Although the number of ZP bound sperm did not differ significantly between caf and LC treatments, their combination showed lower numbers of zona bound sperm (median=24) compared to the caf treatment ($p<0.05$). Both metabolic enhancer substances were beneficial in increasing the number of zona bound sperm compared to the control, although 10mM LC was as effective as 2mM caf.
CONCLUSIONS
In conclusion, LC can be recommended as an alternative to caf in porcine sperm IVF. However, more studies are necessary to confirm these results in the in vitro production of porcine embryos.

W113
APPLICATION OF CASA-MOT TECHNOLOGY TO EXPLAIN FERTILITY OF BOAR SEMEN

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BACKGROUND-AIM
The capability assessment of the fertility of boar ejaculate is important for optimizing artificial insemination (AI) in swine production. The relationship between sperm kinematics, motile traits of semen, pregnancy rate, and litter size are of great profitable importance. The aim of the study was to determine the relationship between CASA-Mot variables and the fertilizing capacity of boar ejaculates.
METHODS
From June to November of 2019, were carried out experiments in boar semen to establish the relationship between semen variables and fertility in sows. ISAS®v1 (Proiser R+D, Spain) was the CASA system used. Boars used in this study were of two genetic lines from Agropecuaria Los Sagitarios S.A. (Río Cuarto, Costa Rica). The semen analyzed by CASA-Mot was used to inseminate sows of four genetic lines. The statistical methods include normality and homoscedasticity testing before ANOVA was run. ANOVA was used to determine differences in semen and the effect in sow genetic lines, tested by fertility parameters.
RESULTS
As results, the mean (±SEM) pregnancy rates of sows had a difference (P< 0.05) comparing Pietrain boars (71.88 ± 5.44 %) to Duroc x Pietrain boars (82.84 ± 5.59 %). The difference (P< 0.05) in total motility and progressive motility patterns showed that semen of Pietrain boars (62.90 ± 2.24 %; 52.39 ± 2.54 % respectively) had lower motility that Duroc x Pietrain boars (80.89 ± 1.66 %; 62.11 ± 1.89 % respectively). The kinematics values were significantly better (P< 0.05) in Duroc x Pietrain boars. The fertility parameters showed no difference (P< 0.05) between female lines, but YLP-50 (¼ York + ¼ Landrace + ½ Pietrain) with semen of Duroc x Pietrain boars had the highest value of piglets born alive (10.20 ± 0.58).
CONCLUSIONS
In summary, sows inseminated with semen of Duroc x Pietrain boars had a higher pregnancy rate. Boar genetic line Duroc x Pietrain showed more total motility. The female crossbreed YLP-50 with semen of Duroc x Pietrain boars had more piglets born alive and those piglets were the heaviest.

W114
DEFINITION OF TECHNICAL SET-UP FOR BOAR ASSESSMENT BY CASA-MOT IN TROPICAL CONDITIONS

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BACKGROUND-AIM
The multiple variations on the settings to assess the motility and kinematic variables of boar semen presents a nonuniformity on the results. The semen set-up is being evaluated under tropical conditions, therefore it's necessary to define standard conditions to homogenize the semen assessment on farms and compare results from different boars. The aim was to define the optimal conditions of CASA-Mot system from testing of boar sperm kinematic variables and achieve results that can be compared.
METHODS
During 2014 to 2019, were carried out experiments in boar semen to establish the video recording time, depth of counting chamber and optimal frame rate of image acquisition. ISAS®v1 (Proiser R+D, Spain) was the CASA system used. Mature boars from commercial genetic lines by pig farms from Costa Rica were used as semen donors using the double-glove semen collection technique. The statistical methods include normality and homoscedasticity testing before ANOVA was run. Therefore, non-parametric analyses were performed with a Kruskal-Wallis test.
RESULTS
As results, the video recording time recommended for boar semen assessment was two seconds to maximize the results of kinematics values because this capturing time provides more data and the results are more reliable, however one-half second can be used to determine only motility values. It´s recommended to use 20µm depth of counting chamber because of these provide the optimal amplitude and space for promoting the spermatozoa movement and it explains the increase in kinematics values. Also, the optimal image acquisition rate was 50 frames per second (fps) due to it provides consistent kinematics values. In comparison with experiments carried out in temperate climatic conditions for total motility with working up to 200 fps, the report indicates that had no significant difference compared with 50 fps, however, it depends on the computational storage capacity and camera specifications.
CONCLUSIONS
In summary, technical conditions work to assess the motility and kinematics of boar semen with CASA-Mot in the tropics must be developed on the basis of these results. Being two seconds of video recording time and 50 fps image acquisition rate, using 20µm depth of the counting chamber.

W115
ANALYSIS OF ENDOMETRIAL HOMEOBOXA10 (HOXA10) GENE EXPRESSION DURING THE LUTEAL PHASE OF THE ESTROUS CYCLE INDICATES THAT IT LACKS ASSOCIATIONS WITH REPRODUCTIVE TRACT MORPHOLOGY AND IS POORLY CORRELATED WITH FERTILITY IN SOWS

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BACKGROUND-AIM
Reproductive efficiency is key to maintaining profitability of commercial livestock-breeding operations. No procedure presently used allows for the accurate prediction of piglet productivity. One of the recently suggested solutions to improve reproductive efficiency of sows involves analyzing the expression of specific genes governing the growth and differentiation of the female reproductive organs and uterine receptivity. Homeobox (HOX) genes are responsible for proper development of female reproductive organs, and HOXA10 mutations or depressed expression lead to female infertility or subfertility. Hence, the main objective of this study was to examine endometrial expression of HOXA10 for correlations with morphological characteristics of the sow's reproductive tract and their lifetime fertility traits.
METHODS
The present study used clinically healthy crossbred sows of the Polish Landrace x Polish Large White. Animals were housed in a commercial facility and slaughtered in a certified local abattoir. Complete reproductive tracts from 23 sows in the luteal phase of the interovulatory period were retrieved immediately after slaughter and quantitatively analyzed for several morphological attributes. Endometrial samples collected from the middle portion of the left and right uterine horns were used to determine mRNA expression levels of the HOXA10 gene by real-time PCR using swine-specific primers TaqMan (assay ID: Ss03373365_g1).
RESULTS
There were no differences (P>0.05) in endometrial HOXA10 gene expression (expressed relative to the GAPDH reference gene expression) between the two uterine horns (0.05±0.01; mean±SEM). There were no significant correlations between HOXA10 gene expression and any of the morphological traits of sows' reproductive tracts. An overall and right-horn endometrial HOXA10 gene expression related directly to the number of stillborn piglets/first pubertal litter (r=0.52, P=0.02 and r=0.45, P=0.04, respectively), but there were no other correlations with the lifetime reproductive indices of the sows.
CONCLUSIONS
Endometrial HOXA10 gene expression during the luteal phase of the estrous cycle is not indicative of the current reproductive tract morphology and does not appear to serve as a useful metric of swine fertility.

W116
SYZYGIUM AROMATICUM ESSENTIAL OIL SUPPLEMENTATION DURING IN VITRO MATURATION IMPROVES THE RATES OF CUMULUS CELL EXPANSION AND METAPHASE II IN PORCINE OOCYTES

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BACKGROUND-AIM
In vitro embryo production requires cumulus-oocyte complexes (COCs) competent for embryonic development. This competence can be acquired during the in vitro maturation (IVM); nevertheless, at this stage, COCs may be exposed to adverse conditions such as oxidative stress. In this context, the essential oil of Syzygium aromaticum (EOSA), considered a potent antioxidant agent, could be used in porcine IVM, and it is still necessary to study the effects of EOSA on IVM of this species. Therefore, we evaluated the effects of the EOSA on porcine IVM and levels of reactive oxygen species (ROS).
METHODS
Then, viable oocytes were matured in vitro under five sets of conditions: EOSA0 (without antioxidants), EOSA10 (10 µg/mL of EOSA), EOSA15 (15 µg/mL of EOSA), EOSA20 (20 µg/mL of EOSA), and CYS (100 µM of cysteamine). Oocytes were evaluated for IVM according to the full expansion of cumulus cells, the presence of the first polar body (1PB), and metaphase II (MII). Moreover, denuded oocytes were evaluated for an antioxidant effect by labeling them with H2DCFDA (ROS levels). All data are expressed as the mean ± standard error and were analyzed using the StatView 5.0 software ($P < 0.05$).
RESULTS
A total of 58 ovaries were used to acquire 222 viable immature oocytes (3.8 viable oocytes/ovary) that were selected and distributed in four repetitions. Initially, for cumulus cell expansion, the EOSA15 (86.0% ± 2.8) and EOSA20 (84.1% ± 1.4) were increase to that of EOSA0 (66.7% ± 3.4) and CYS (53.2% ± 13.5). No difference was observed in the IVM rates obtained from 1PB ($P > 0.05$). Nevertheless, EOSA20 (95.2% ± 5.0) improved the IVM rates obtained from presence of metaphase II, when compared to EOSA0 (73.9% ± 14.6) and CYS (69.2% ± 3.8). Additionally, no difference has been observed in the ROS levels for all groups when evaluating ROS levels ($P > 0.05$).
CONCLUSIONS
Therefore, EOSA added to the IVM medium could be an interesting alternative for the increase the meiotic competence in porcine oocytes.

W117
PREVALENCE OF ANTIBODIES TO SELECTED LEPTOSPIRA SEROVARS IN POLISH PIG POPULATION

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BACKGROUND-AIM
Leptospirosis is a worldwide zoonotic disease caused by pathogenic serovars of Leptospira sp. Clinical symptoms such as abortion, stillbirths and sometimes infertility are the most frequently observed consequences of Leptospira sp. The aim of the study was to examine the seroprevalence of Leptospira sp. infections in Polish swine population.
METHODS
A total of 22165 swine serum samples were collected from 2014 to 2018. All the serum samples were tested by microscopic agglutination test (MAT) with the panel of 6 serovars representatives of 6 serogroups, Icterohaemorrhagiae (RGA), Grippotyphosa (Moskva V), Sejroe (M84), Tarassovi (Perepelicyn), Pomona (Pomona), Canicola (Hond Utrecht IV). The minimum sera dilution was 1:100.
RESULTS
During the last 5 years the seroprevalence of Leptospira in swine was as follows: 1.42%, 1.32%, 0.93%, 1.32%, 1.25% respectively. Yearly, the highest percentages of seropositive swine serum samples were found in the north and south parts of Poland, while a low seroprevalence was observed in the central and western regions. The most common serovars between 2014-2018 were: Pomona (0.58%, 1.04%, 0.93%, 0.47% and 0.77%, respectively) and Sejroe 0.29%, 0.14%, 0.21%, 0.55% and 0.50%, respectively).
CONCLUSIONS
Our observations from the last 5 years confirm that serological reactions with serovar Sejroe in pigs in Poland have a decreasing tendency. Although west and north-west part of the country represent a high pig population density and an intensive pig production system. Our investigations from the last years indicate a low seroprevalence to 6 tested serovars. Afore-mentioned regions with the highest Leptospira seroprevalence represent more extensive pig production systems as well.

W118
SEMINAL PLASMA INDUCES UP-REGULATION OF THE UNCHARACTERIZED HEAT-SHOCK FACTOR HSF5 IN THE SWINE FEMALE GENITAL TRACT

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BACKGROUND-AIM
Semen (sperm and seminal plasma, SP) affects gene expression in the female genital tract. Heat-shock proteins (HSPs) are involved in several metabolic and cellular functions beyond heat stress. This study examined changes in gene expression of HSPs and heat shock transcription factors, particularly the heat-shock factor 5 (HSF5, GO:0006355, regulation of transcription) in the pig female genital tract.
METHODS
Samples of the internal genital tract (cervix (Cvx), uterus (distal and proximal), utero-tubal junction (UTJ), isthmus (Isth), ampulla (Amp) and infundibulum (Inf)) were surgically removed from sows 24 h after natural mating (n=4), artificial insemination with 10 mL of sperm-peak fraction (P1-AI, n=4) or of sperm-free SP infusion from the sperm-peak fraction (SP-P1, n=4) or the whole ejaculate (SP-Ejac, n=4). Infusion with the extender Beltsville Thawing Solution (BTS) was used as control (n=4). RNA was isolated following a TRIzol-based modified protocol and analysed for global transcripts using specific microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix). The data was normalized (Robust Multiarray Average) and analysed with the Transcriptome Analysis Console (RMA-method, -1> fold changes >1, $p<0.05$). Molecular processes were identified by PANTHER.
RESULTS
Mating down-regulated HSPA13 expression (Biological process (BP): response to stimulus (GO:0050896) and molecular function (MF): binding (GO:0005488)) in all tissues. Mating also down-regulated the HSPA5 expression in Cvx, uterus, Amp and Inf (BP and MF equal to HSPA13). Conversely, all treatments upregulated the expression of the uncharacterized heat-shock transcription factor HSF5 in the sperm reservoir (UTJ).
CONCLUSIONS
Although other heat-shock transcription factors had been widely described, the findings on HSF5 as modulating the functional tubal sperm reservoir is novel and not only restricted to germ cell development and meiotic progression in zebrafish males. Further mechanistic studies are needed to validate the regulation-related processes apart from non-heat-shock response functions. Supported by The Swedish Research Council FORMAS (2017-00946 and 2019-00288), Stockholm, Sweden.

W119
ROLE OF AKT AND MTOR SIGNALING IN IGF-I-MEDIATED CELL PROLIFERATION IN PORCINE SERTOLI CELLS

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BACKGROUND-AIM
The critical role of IGF-I in promoting Sertoli cell proliferation both in vivo and vitro have been established in the past but its downstream signaling mechanisms remain unknown. In addition to mitogenic effects, a role for IGF-I in mediating cholesterol biosynthesis within the testes has been implied. Thus, our objectives were to investigate the roles of PI3K/Akt/mTOR signaling in IGF-I mediated Sertoli cell proliferation; and IGF-I in mediating cholesterol biosynthesis in Sertoli cells, using a porcine Sertoli cell culture model.
METHODS
Primary cultures of Sertoli cells were prepared from one-week old porcine testes. On Day3 of culture, Sertoli cells were serum starved for 24 h followed by treatment with IGF-I (300ng/mL) combined w/wo inhibitors of IGF-IR (PPP-2 µM), Akt (Wortmannin-1 µM) or mTOR (Rapamaycin-200 nM). Following IGF-I and inhibitor treatments, cells were cultured for 30 min and phosphorylation levels of Akt, mTOR, and p70S6K determined by immunoblotting. For cell proliferation and qPCR assays, cells cultured for 24 h were used.
RESULTS
IGF-I increased phosphorylation of Akt, mTOR and p70S6K and cell proliferation which was inhibited by IGF-IR, Akt and mTOR inhibitors. Furthermore, IGF-I upregulated the expression of cholesterol biosynthetic genes; HMGCR, HMCS1 and CYP5A1, but not SREBF1.
CONCLUSIONS
Increased phosphorylation of p70S6K, a major downstream target of mTOR and upregulated cholesterol biosynthetic gene expression is evocative of the key role played by IGF-I in regulating synthesis of cholesterol, the precursor for steroid hormones important for various reproductive functions.

W120
APPLICATION OF CANONICAL ANALYSIS FOR INTERPRETING SPERM CHROMATIN RESULTS IN AN EXPERIMENT TESTING LARGE-VOLUME SINGLE LAYER CENTRIFUGATION (SLC) OF BOAR SEMEN.

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BACKGROUND-AIM
The relationship among different variables of sperm quality and explanatory factors is usually performed using correlations or linear models. However, with a large number of variables, interpretation of results is challenging. Canonical correlation analysis (CCA) and canonical discrimination analysis (CDA) can be used while reducing the number of variables (roughly equivalent to principal component analysis for descriptive purposes). Here, we analyze a dataset from an experiment to test the effect of large-volume single layer centrifugation (SLC) with Porcicoll to remove bacteria on boar sperm.
METHODS
Semen doses (10 boars) were extended to 100 M/ml in BTS with no antibiotics. For SLC, 200 ml were centrifuged through 150 ml Porcicoll (300g, 20 min). Pellets were resuspended and stored at 17 °C, with an unprocessed control. On days 0, 3 and 7 aliquots were processed for: Microbiology (CFU/ml and species) and sperm chromatin analysis (SCSA for DNA fragmentation; monobromobimane, mBBr, for disulfide bridges status; chromomicyn A3, CMA3, for compaction). Data were analyzed with R by CCA and CDA (treatment and incubation effects).
RESULTS
Ten bacterial species (X-set) and 11 chromatin variables (Y-set) were used CCA analysis. Only the first pair of canonicals variates (X1Y1) is described. The first correlation (0.86, P<0.001; 44% variability), showed that most bacterial species were associated with lower chromatin status (mainly SCSA and CMA3, with increasing chromatin compaction by disulfide bridges). With CDA and MANOVA for microbiology, we could separate individual boars, and SLC from control, showing increasing loads with time. For chromatin status, the variate discriminated among boars depending on SCSA and mBBr, whereas treatments and time discriminated more on chromatin compaction.
CONCLUSIONS
Multivariate analysis such as CCA and CDA are a good alternative to conventional analyses in spermatology, especially with many variables which are difficult to interpret simultaneously, or with the risk of high type I error. These results must be carefully evaluated to establish the relationship of the original variables among them, or the effects of the tested factors.
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W122
SAMPLE PREPARATION METHOD DEVELOPMENT AND OPTIMIZATION FOR BOAR SEMINAL PLASMA PROTEOMIC PROFILING

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BACKGROUND-AIM
Semen of reliable quality must be used to achieve success in artificial insemination (AI), making the fertility predictions of boars a pivotal step. Differences in cryopreservation ability and fertility post-AI suggest that more sensitive methods beyond the use of sperm motility classification are required. This study aims to explore the proteome of boar seminal plasma (SP) using highly sensitive mass spectrometry-based methods.
METHODS
The use of Hydrophilic Interaction Liquid Chromatography (HILIC) method for sample clean-up, digestion and subsequent quantification of SP proteins was explored to improve reproducibility and possibly identify low abundant proteins that play a role in fertility. Semen pool from 8 boars was used for method optimization. The seminal plasma pool was diluted in 2% SDS buffer and protein concentration was determined using the BCA assay. For bead-based digestion, 20µg protein samples were spiked with casein and enolase as internal standards. Followed by reduction and alkylation with 10mM 1,4-dithiolthreitol and 40mM iodoacetic acid, respectively. Clean-up and digestion with trypsin were performed using KingDuo machine for 4 hours at 47°C. SDS quantification using methylene blue test was done to measure the effectiveness of on bead clean-up. The thermofisher calorimetric kit was used for peptide quantification. The peptides recovered from digestion were analysed by liquid chromatography using NanoLC ultra-connected to an AB SCIEX TripleTOF 6600 MS. Protein composition was determined using sequential window acquisition of all theoretical mass spectra analysis. Data were analysed using PeakView and Spectranaut software.
RESULTS
The HILIC method was an effective clean-up process for SDS. A total of 236 protein groups were identified at 1% false discovery rate (FDR). Spermadhesins and heat shock proteins known to affect fertility and cryopreservation were identified. Some less abundant proteins like the seminal plasma protein pB1 known to have interactions with spermadhesins were identified.
CONCLUSIONS
The results found in this study prompt further research on protein-protein interactions of low abundant proteins with the more abundant proteins. The investigation may help in the identification of putative biomarkers of fertility and cryopreservation.

W123
CHOLECYSTOKININ MODULATES BOAR SPERM KINETICS UNDER CONDITIONS OF LOW HCO3- CONCENTRATION DURING CAPACITATION

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BACKGROUND-AIM
Cholecystokinin (CCK) is a gastrointestinal hormone which also functions as an important neurotransmitter. Moreover, CCK has been detected in reproductive tissues and cells, but the role of the protein has not been totally elucidated. Recently CCK has been found in the porcine oviductal fluid being upregulated at the post-ovulatory stage during the oestrus cycle. Moreover, CCK has been reported as a modulator of bicarbonate (HCO3-) uptake of mouse spermatozoa, which is an essential fact for sperm capacitation and fertilization. The aim was to analyze the effect of the CCK on boar sperm function during capacitation using different concentrations of bicarbonate.
METHODS
Seminal doses (n=7) made from ejaculated sperm rich fractions were kept at 15ºC for 1 or 5 days. After storage sperm were subjected to 1-hour capacitation in TALP medium using different levels of HCO3- (0 mM, 5 mM, 25 mM) including CCK protein (25 µM, 50 µM) or not (control). Then, sperm total and progressive motility as well as kinetic parameters were analysed using Computer Assisted Sperm Analysis. For viability, acrosome integrity status and mitochondrial membrane potential evaluation, the fluorochromes propidum iodide, PNA/FITC and JC-1 were used respectively. Statistical analysis was performed using SPSS 24.0 software. The assumption of normality was evaluated by Shapiro Wilks test. When normality was fulfilled, one-way ANOVA test followed by a post hoc Tukey test was applied. For the variables which data were not normally distributed, the non-parametric Kruskal Wallis test was used.
RESULTS
When 0 mM HCO3- was used, no variances between groups (0, 25 or 50 µM of CCK) were observed. Interestingly, groups tested with concentration of 5mM HCO3- showed statistically significant variances ($p<0.05$) in terms of motion parameters being higher values for LIN, STR and WOB and lower values for VCL and ALH when CCK was used. In regard to 25 mM of HCO3- treatment, the only significant higher value was observed for sperm viability with 50 µM of CCK ($p<0.05$).
CONCLUSIONS
Ultimately, CCK protein modulates boar sperm kinetics

during capacitation under conditions of low HCO3- concentration. Supported by the Spanish Ministry of Science and Innovation PID2019 106380RB I00/ AEI/10.13039/501100011033 and PGC2018-09781-B-I00

W124
EFFECT OF ACCUMULATIVE EJACULATE FRACTIONS ON SPERM STORAGE AND REPRODUCTIVE PERFORMANCE IN PORCINE SPECIES

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BACKGROUND-AIM
Nowadays, artificial insemination (AI) is the most used reproductive method in porcine, due to the growing demand from this industry. A boar ejaculate, composed by sperm cells and seminal plasma (SP), is emitted in different fractions: 1) pre-spermatic fraction: SP; 2) spermatic-rich fraction: mostly sperm, less SP; 3) intermediate fraction: less sperm and SP; 4) post-spermatic fraction: few sperm, more SP. Seminal doses are prepared by diluting sperm-rich fraction or the whole ejaculate in commercial extenders. However, it is still not clear the right way to prepare seminal doses, due to the different SP composition and proportion between the fractions. Thus, our aim was to evaluate how the synergic effect of accumulative ejaculate fractions affects sperm quality during storage and in vivo reproductive performance.
METHODS
A total of 51 ejaculates (17 per group) from 6 fertility proved boars (Pietrain breed) were collected: 1) F1: sperm-rich fraction; 2) F2: sperm-rich + intermediate fractions; 3) F3: sperm-rich + intermediate + poor fractions. Semen samples were diluted in AndroStar Plus extender (final concentration of ~30x106 sperm/ml). Each dose was stored at 15ºC for up to 3 days, and sperm quality was analyzed. At day 3 sows were inseminated with the different groups (57 per group), and AI performance was evaluated. Statistical analysis was performed with SPSS 24.0 software package. Normality by Shapiro-Wilks test was applied, following by one-way ANOVA and post-hoc Tukey test or non-parametric Kruskal-Wallis test.
RESULTS
No differences were observed for sperm quality, which was high after storage (total motility: from 89.11±0.79% to 90.89±0.92%; mitochondrial activity: from 91.79±0.34% to 92.37±0.38%). Even after AI, no significant differences were observed for pregnancy and farrowing rate (from 92.98% to 96.55% and from 82.46% to 89.66%, respectively), and number of piglets (from 20.50±6.50 and 22.57±4.73).
CONCLUSIONS
This study highlighted that the whole ejaculate could be used for seminal doses preparation, being not affected by the synergic effect of its fractions and leading to successful AI. This could result in a more advantageous and time-efficient preparation of a greater number of doses. Supported by Ministry of Science and Innovation (PID2019-106380RB-I00 / AEI / 10.13039/501100011033).

W125
PIG PREGNANCIES AFTER TRANSFER OF ALLOGENEIC EMBRYOS SHOW A DYSREGULATED ENDOMETRIAL/ PLACENTAL CYTOKINE BALANCE: A NOVEL CLUE FOR EMBRYO DEATH?

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BACKGROUND-AIM
Pig embryo transfer (ET) is burdened by high embryo mortality, with cytokines playing a significant role in recruitment of immune cells during embryo attachment and placentation. We hereby tested if their levels in endometrium and placenta from sows carrying hemi-allogeneic (artificially inseminated sows; C+ positive control) or allogeneic embryos (sows subjected to ET; ET) during peri-implantation (D18) or post-implantation (D24) are suitable mirrors of embryo rejection or tolerance after ET. Non-pregnant sows (C-) were used as negative controls.
METHODS
A set of cytokines was assayed in the tissues through multiplexed microsphere-based flow cytometry (Luminex xMAP, Millipore. USA).
RESULTS
Fewer (58.7%. P<0.003) conceptuses were recovered at D24 after ET compared to C+ (80.9%); with more than 20% of the ET conceptuses being developmentally delayed. Cytokine levels shifted during implantation. Anti-inflammatory IL-10 levels were significantly (P<0.05) lower in ET sows compared to C+ at D24 of pregnancy. The C+ controls (carrying hemi-allogeneic embryos) consistently showed higher levels of pro-inflammatory TNF-a, IFN-γ and IL-2 cytokines at D18 and IL-1a at D24, compared to the ET group.
CONCLUSIONS
This clear dysregulation of pro- and anti-inflammatory cytokine levels in sows subjected to ET could be associated with an impaired maternal immune tolerance, explaining the high embryonic mortality of ET programs.

W126
THE OXIDATION-REDUCTION POTENTIAL IN BOAR STORED SEMEN IS POSITIVELY ASSOCIATED WITH MOTILITY IN GOOD-QUALITY SAMPLES.

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BACKGROUND-AIM
Oxidative stress control is key for sperm fertility and viability preservation. An excess of oxidative species can break the balance between antioxidants and prooxidants in the spermatozoon, causing lipid peroxidation, mitochondrial damage, and DNA fragmentation. Refrigeration or cryopreservation procedures and time of storage reduce antioxidants and increase oxidative species. sORP (semen oxidation-reduction potential) in humans has been proposed as a possible fertility predictor and fast diagnostic method of oxidative stress (PMID: 29124237). Our objective was to preliminary test the analysis of sORP in seminal doses which could be useful to optimize animal production in swine.
METHODS
Semen samples were obtained from proven fertility boars (n=10), diluted in a commercial extender up to 40×106 cells ml-1, and stored at 17°C for 7 days. Sampling was carried out at days 0, 1, 2, 3, and 6 of storage. Motility was measured by a Computer-Assisted Sperm Analysis (CASA) at 37°C obtaining the total and progressive motility (MOT and PROG). sORP was determined in volts by a Redox probe with platinum reference (Mettler-Toledo). Voltage data was converted to mV/106 cells ml-1. Statistical analyses were carried out by linear mixed-effect models considering boar as the random effect and motility, sORP, and storage time as main variables. Results are shown as mean ± SE.
RESULTS
Both MOT and PROG decreased with storage time (-5.3%±0.8 and -4.7%±0.7 per day), whereas sORP showed a linear trend only until day 2 (-0.04 mV/106 ml-1±0.03) stabilizing afterwards. The relationship of sORP with motility was positive and significant (MOT: 1.01±0.22×10-2, PROG: 1.32±0.25×10-2 per unit sORP; p<0.001). There was no significant linear relationship between sORP at day 0 and CASA parameters at days 2, 3, or 6.
CONCLUSIONS
Unlike humans, in the pig spermatozoa sORP was not related to a decrease in motility. Boar semen samples were of good quality, and therefore a higher sORP could indicate active metabolism within balanced oxidant/ antioxidant levels, which could also explain why sORP apparently lacks predictive power. Future studies should include samples of heterogeneous quality and relate sORP with fertility data from the studied males.
We thank Topigs-Norsvin España, AIM (Spain, León).

W127
ADDITION OF CAFFEINE AND L- CARNITINE TO COOLED SEMEN OF BOAR TO IMPROVE SPERM BINDING TO PORCINE OVIDUCT EXPLANT

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BACKGROUND-AIM
The oviduct reservoir lengthens the fertile lifespan of sperm and regulates capacitation to provide a supply of fertile sperm to the site of fertilization. In many species, sperm-oviduct binding assays shows the ability to select sperm that are morphologically and functionally viable. Caffeine (caf) and L-carnitine (LC) are metabolic regulators with the latter presenting antioxidant properties. This work aimed to evaluate the effect of the addition of caf and LC to boar semen, cooled at 17°C, for seven days in sperm oviduct binding.
METHODS
After slaughter, oviducts were collected and dissected from pre-ovulatory oestrus gilts and ovaries without corpus luteum (CL). The isthmus region was squeezed by pressure, the cells were disaggregated in medium by pipetting, followed by re-aggregation to form explants. After seven days, a pool of ten insemination doses of cooled boar semen was distributed into four treatments: T1) Control: extended pool semen, T2) T1 + 2mM caf, T3) T1 + 10mM LC and T4) T1 + 2mM caf + 10mM LC. The 10mM LC dose was selected based on our previous study to improve porcine sperm quality. Porcine in vitro fertilization procedures are regularly conducted using the above caf concentrations. As preliminary results, one replicate with twenty oviduct explants per treatment were performed. The explants were incubated in a 60µl droplets in Petri dishes (TCM199 + 10% FBS + 0.1 mg/ml streptomycin + 100 UI/ml penicillin) at 38.5°C with 5% CO2 atmosphere (24h) and after swim-up, co-incubated with a final concentration of 1x106 sperm (5h). The statistical analysis used was variance analysis (ANOVA) and Duncan test. A probability of $p<0.05$ was considered significant. The number of oviduct- bound sperm was calculated by measuring the perimeter of the explants with the Image J analysis program.
RESULTS
The combination of caf + LC decreased the number of sperm bound to the explants (7.6) compared to other treatments (T1: 16.8, T2: 15.6 and T3: 16.7, $P<0.05$), which did not differ among them ($p>0.05$).
CONCLUSIONS
It is possible when both caf and LC were added, that sperm lost affinity for receptors of oviduct epithelial cells. In conclusion, caf and LC did not increase the number of porcine bound sperm cooled for 7 days to the oviduct explants.

W128
GENETIC DIVERGENCE BETWEEN MATERNAL AND PATERNAL PIG BREEDS REVEALS REPRODUCTION AND GROWTH ASSOCIATED GENES DESPITE LOW WITHIN-GROUP GENETIC SIMILARITY.

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BACKGROUND-AIM
Commercial pigs can be broadly grouped into maternal (M) and paternal (P) breeds. M breeds are selected primarily for reproductive fitness (i.e. litter size) but score lower in production traits (i.e. lean growth rate), whereas P breeds are selected to achieve high productivity but have lower reproductive performance. Thus, a comparative genomic analysis between both groups could reveal candidate genes associated with fertility and growth.
METHODS
Whole genome sequencing data from 87 individuals was used to detect Single Nucleotide Variants (SNVs). Data sets originated from own sequencing data (n=20, ~57x coverage) and from the European Nucleotide Archive (PRJEB1683, PRJEB9326, PRJNA260763; n=67, ~6x coverage). Samples were assigned to the M group (Large-White, n=24; Landrace, n= 16) or the P group (Pietrain, n= 23; Duroc, n=24). Only properly genotyped SNVs (Genotype Quality ≥ 20, Genotype Depth ≥ 4) in min. 12 samples per breed were retained. SNVs were used to assess population genetic structure and differentiation (FST(M-P)). Extreme FST(M-P) scores (top 0.1% of the genome) were further studied through enrichment analysis and literature research of overlapping genes.
RESULTS
A total of 5,034,003 SNVs were detected, Duroc being the least polymorphic of the four breeds with ~3.1M SNVs (Large-White: ~4M, Landrace: ~4.1M, Pietrain: ~3.9M). Population structure analysis assorted samples by breed, but did not group breeds by their assigned groups (maternal, paternal). Though regions of extreme genetic differentiation appeared in 1/3 of autosomes, significantly enriched functions were not found. However, where genetic differentiation was strongest (FST(M-P) > 0.6), two genes of interest were found: CORIN (trophoblast invasion) and ATP10D (lipid metabolism).
CONCLUSIONS
Despite sharing similar selective pressures, M and P breeds exhibit poor within-group genetic similarity, demonstrating that selection response for complex traits can be achieved

through different polygenic routes and that other evolutionary influences need to be accounted for (i.e. genetic drift). Nevertheless, this comparative approach yielded two candidate genes whose relevance for genetic control of reproduction performance and leanness should be studied further.

W129
PRODUCTION OF GENETICALLY MODIFIED PORCINE EMBRYOS BY LIPOFECTION OF IN VITRO MATURED OOCYTES USING CRISPR/CAS9 SYSTEM

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BACKGROUND-AIM
Lipofection has been commonly used to introduce external molecules into cells since its development in 1987 by Felgner et al (Proc Natl Acad Sci USA). However, its use in porcine oocytes and embryos to produce genetically modified pigs using CRISPR/Cas9 has not been studied in deep. As this technique can facilitate the procedure of producing genetically modified animals, as it does not need specific machinery or trained personal, we compare the use of lipofectamine versus electroporation in porcine oocytes to produce mutations in CD163 gene using the CRISPR/Cas9 system.
METHODS
In vitro matured oocytes were electroporated (E) or lipofected (L) with Lipofectamine CRISPRMAX Cas9 at two concentrations [5%(v/v) -1x group-, 10%(v/v) -2x group-] with sgRNA and protein Cas9, in vitro fertilized and in vitro cultured up to 6 days. A group without treatment was used as control. Cleavage and blastocyst rate (blastocyst/oocyte) were evaluated and mutation rates were analysed by fluorescent PCR-capillary gel electrophoresis. 328 oocytes were evaluated.
RESULTS
Cleavage rate was significantly lower in L2x group (18.3%, $p<0.01$) in comparison with control (41.7%), E (62.1%) and L1x groups (36.6%). Furthermore, the blastocyst rate was also significantly lower in L2x group (5.5%) in comparison with control group (16.7%, $p=0,03$), finding no differences between the others (E: 13.8%; L1x: 12.9%). These results suggest that a high concentration of lipofectamine can be toxic to the embryos, but no detrimental effect was detected with a low concentration. No significant differences were found between groups on mutation rate (E: 50%; L1x: 38.5%, L2x: 50%), mosaicism rate (E: 12.5%; L1x: 7.7%, L2x: 0%) and on the overall efficiency (E: 6.9%; L1x: 5%; L2x: 2.75%).
CONCLUSIONS
Previously, Hirata et al (Animals) used this method successfully in zona pellucida (ZP) free oocytes and embryos but the use of ZP intact oocytes is an advantage for embryo culture and transfer to recipients. For that reason and considering these results, lipofection of in vitro matured oocytes can be an effective alternative to produce genetically modified embryos and animals. However, an optimization of the conditions and more analysis are still needed.
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W130
EFFECT OF POST-WEANING GNRH (BUSERELIN ACETATE) TREATMENT USING DIFFERENT COMMERCIAL FORMULATIONS ON REPRODUCTIVE PERFORMANCE IN SOWS

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BACKGROUND-AIM
The goal of this study was to evaluate the effect of different commercial buserelin-based (GnRH) products on the reproductive and productive performance in post-weaned sows.
METHODS
The trial was conducted in a commercial farm, DanBred genetic, in Spain, where 30 weaned sows were enrolled. Animals were checked not to show estrous (E) or CL by Ultrasound (US) between weaning (W) and up to 72 h. Based on parity (1 to 5), prolificity, BCS were homogenously distributed into three groups. A Control Group (GC) with no further treatment, and two groups to receive different GnRH products between 83 to 89 h from W: GG group (Gonaxal, Biogénesis Bagó) and GP group (Porceptal, MSD); both products as a single dose of 2.5 ml (buserelin 10.5 µg) by IM. E detection was performed twice a day (9 am and 5 pm) using 3 trained boars, while US check started 72 h after W, right before treatments, and continued every 12h since E until ovulation (O). Operators remained blind to the animals' group. AI followed the farm's routine: sows showing E 78-90h after W were AI at 106 and 120h; if E after 90h were inseminated 8h and 16h later. When tested for normality by Shapiro-Wilk test, all data resulted not normal. Thus, Kruskal-Wallis test was used for the analysis. Fertility (Fer) and Farrowing Rate (FR) were analyzed by Chi2. The software used was XLSTAT 2019 4.1.
RESULTS
One sow (GC) was excluded due to a cystic ovary. Wean-to-Ovulation Interval (WOI) was shorter for treated groups [GG 121±9h (114-144h), GP 121±4h (114-126h); P>0.4)] compared to GC 135±9 h (120-150 h; P=0.007). Similar results for EOI, GG 34±8h (24-54h), GP 35±7h (24-48h; P>0.6), while GC 49±9h (36-60h; P=0.004). WEI was not different among groups (mean 86±6h). Even though that, is worth saying that GnRH-treated groups showed a range of 78-90h while GC 78-102h. Probably, because of reducing the WOI, the E duration was shorter in treated groups (mean 53±7h against 65±11h for GC; P<0.03). Fer and FR were similar (90%; P>0.1). Litter Size was 19.6 for GG while 18.1 GP and 17.9 GC; Born Alive for GG resulted in 18.2 piglets while GP 16.6 and GC 16.7, with no difference (P>0.05).
CONCLUSIONS
In conclusion, different commercial GnRH agonist (buserelin-based) products achieved the same results and performance and were effective in shortening the WOI and synchronizing the ovulation.

W131
MATING INDUCES UP-REGULATION OF THE TOLL-LIKE RECEPTOR 2 (TLR2) IN THE PIG OVIDUCT AMPULLA AND INFUNDIBULUM

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BACKGROUND-AIM
The immunology of the female genital tract changes accordingly to reproductive events. It allows sperm transport and, after fertilization, the development of allogeneic embryos and placenta while keeping protection from pathogens. Toll-like Receptors (TLRs) are conserved pattern-recognition receptors (PRRs) that play a major role in the innate immunity but also in the regulation of events as ovulation and fertilization. This study evaluated changes in TLRs gene expression along compartments of the pig genital tract, following exposure to semen or seminal plasma.
METHODS
Tissue samples of cervix, distal & proximal uterus, utero-tubal junction (UTJ), isthmus, ampulla (Amp) and infundibulum (Inf) were surgically removed from oestrus sows 24h after natural mating (n=4), sperm-rich fraction AI (10mL; P1-AI; n=4), cervical infusion with sperm-free seminal plasma from the first 10 mL of the sperm rich fraction (SP-P1; n=4) or from the whole ejaculate (SP-Ejac; n=4). Beltsville Thawing Solution was used as negative control (n=4). Total RNA isolated by TRIzol modified protocol was analysed for whole transcripts by using species-specific microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix). The data was normalized (Robust Multiarray Average) and analysed with the Transcriptome Analysis Console (RMA-method, -1>fold changes>1; p<0.05 or FDR: q<0.05). Biological processes were identified by PHANTER.
RESULTS
In the Inf, mating significantly upregulated the expression of TLR4 and TLR2 (q<0.05), being the latter also upregulated in the Amp. In contrast, TLR2 was downregulated in UTJ after seminal plasma infusion (SP-P1). Moreover, TLR10 was upregulated by mating in the oviduct (Inf & Amp).
CONCLUSIONS
Since the pro-inflammatory factor of innate immunity TRL2 activates the NF-κB pathway, it is presumably involved in sperm clearance in the upper oviduct, whereas its down-regulation in the absence of spermatozoa may be related to signaling for immunotolerance in the UTJ. In addition, TLR10, unique for its anti-inflammatory action via suppression of NF-κB, may be associated with the oviduct preparation for gamete/embryo protection at this early stage of the fertilization process. Supported by The Swedish Research Council FORMAS (2017-00946 & 2019-00288), Sweden. MRC is supported by FPU15/06029, Spain.

W132
SEMINAL PLASMA SUPPLEMENTATION PRESERVES THE CHROMATIN STRUCTURE OF THAWED BOAR SPERMATOZOA BY ACTING ON DISULFIDE BRIDGES

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BACKGROUND-AIM
Sperm cryopreservation is still a challenge in the pig industry due to the low fertility results related to chromatin alterations. Seminal plasma (SP) could reduce these deleterious post-thawing effects (PMID: 23756043, 27543989). This work evaluated the SP role in the post-thawing dynamics of disulfide bonds between protamines by labeling the free thiols with the monobromobimane (mBBr) fluorochrome. This feature was studied in relation to the reactive oxygen species (ROS), the abundance of extracellular free thiols, and DNA fragmentation.
METHODS
Thawed spermatozoa (n=7 boars) and SP (n=11 boars) were incubated with 0, 10 or 50% SP at 37°C for 4 h. The samples were loaded hourly and analyzed by flow cytometry for ROS (1µM H2DCFDA), surface free thiols in live sperm (25 nM 5-IAF + 3 µM PI), Sperm Chromatin Structure Assay (SCSA, 6µg/mL acridine orange + 0.1% Triton solution), and disulfide bonds (5µM mBBr). The parameters obtained were the mean fluorescence intensity (MFI) of H2DCFDA, % live sperm with 5-IAF fluorescence, the % sperm with high DNA fragmentation index (%DFI) and high DNA stainability (%HDS, low chromatin compaction) in SCSA, the mBBr MFI, % sperm with low (%mBBrL), moderate (%mBBrM) or high (%mBBrH) fluorescence, and the relative abundance of disulfide bonds (PMID: 16210011). Data were analyzed by linear mixed-effects models and Pearson correlation.
RESULTS
Whereas 10% SP produced irrelevant changes, 50% increased %mBBrL at 1 h of incubation and reduced mBBr-MFI while increasing disulfide bonds at 4 h of incubation ($p<0.001$). ROS (MFI) correlated with %mBBrL ($r=0.44$) and with disulfide bonds ($r=0.53$). 5-IAF MFI negatively correlated with mBBr-MFI ($r=-0.41$). mBBr-MFI moderately correlated with %DFI ($r=0.38$), but not with %HDS. All correlations were significant at $p<0.001$.
CONCLUSIONS
50% SP preserved the integrity of the disulfide bonds in post-thawed pig spermatozoa, potentially contributing to maintaining the chromatin structure. This could be related to a ROS burst correlating to lower viability in previous studies. The application of SP should take these observations into account for optimal application in artificial insemination.
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W133
EFFICACY OF BIOMEDICAL TECHNIQUES ON BOARS' EVALUATION TO PRODUCE HIGH QUALITY SEMEN

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BACKGROUND-AIM
Male animals' health and stress status are reflected in behavior, which affects sperm quality and fertilizing ability. Biomedical measurements by modern technological equipment have been performed in farm animals to collect information about nutrition, behavior and welfare. This reproductive study investigated the relation of semen quality (CASA analysis, viability, morphology, membrane biochemical activity, DNA fragmentation) with boar behavior immediately before and during ejaculation.
METHODS
Sensors were placed on boar's body. Galvanic Skin Response (GSR) was recorded and movement features were collected using an Inertial Measurement Unit, IMU, comprising an accelerometer, a gyroscope and a magnetometer. Boar, scrotum, and dummy temperatures were measured by an infrared (IR) camera and an IR thermometer, while the face moisture of the boar was recorded by a moisture-camera. All signals and images were logged on a mobile device (smartphone or tablet) using a Bluetooth connection and then transferred wirelessly to the cloud. Files were then processed using scripts in Matlab 2021a (MathWorks, Natick, Massachusetts) to derive the necessary indices (biomarkers).
Five boars were involved, and 94 ejaculates were collected/analyzed in this study within the span of 12 months. The statistical analysis was performed in the Statistics and Machine Learning Toolbox of Matlab 2021a using a linear mixed effects model.
RESULTS
In the following, only the results which revealed strong correlations ($R2>0.5$, $p\leq0.05$) are presented. Strong negative correlations were observed between boar, dummy and scrotum temperature with sperm progressive motility and VCL, VSL, ALH kinematics. Dummy's temperature was negatively correlated with sperm viability, normal morphology and BCF, while the boar temperature was negatively correlated with BCF. Scrotum's temperature was negatively correlated with semen volume, while major physical parameters, such as Overall Dynamic Body Acceleration (OBDA) and raw acceleration, were positively related with total time of semen collection processing. In addition, raw acceleration was positively related with BCF.
CONCLUSIONS
In conclusion, biomedical techniques, such as OBDA and raw acceleration support the evaluation of boar semen production capacity, providing useful information about semen quality.

W134
ESSENTIAL OILS FOR PORCINE REPRODUCTION: ARE THEY REALLY SAFE?

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BACKGROUND-AIM
The possibility to use essential oils (EOs) in porcine reproduction has considerably risen. Amongst the different reasons, the strong antibacterial, antiviral and antioxidant capabilities can be addressed as the main drive for such interest. Addition of EOs to seminal material may indeed improve cryopreservation protocols and provide good alternatives to antibiotics. Data regarding the potential detrimental effects of EOs on spermatozoa and the female genital tract are still relatively poor. The aims of the present study were: 1) to evaluate the effects of previously identified non-spermicidal concentrations of M. alternifolia and R. officinalis EOs on porcine female reproductive tract; 2) to assess the toxicity and identify non-spermicidal concentrations of other six EOs on boar spermatozoa.
METHODS
EOs were characterized by gas-chromatography and added with emulsifiers to facilitate solubilization in fertilization media (SFM). For aim1, an in vitro model using uteruses collected at the slaughterhouse was set up. SFM added with essential oils was applied to the mucosal layer of horns sections (2x2 cm) and incubated for 1h at 38°C. An ex vivo model was also set up by filling uterine horns with the same solutions. Analyses included histology and Evans blue permeability assays. For aim2, different concentrations of M. piperita, L. angustifolia, O. Vulgare, S. montana, E. globulus and C. limon EOs were added to swine seminal doses (3x107 spermatozoa/ml) and stored at 16°C for 5 days. Analyses included viability, percentage of acrosome reaction and objective motility at 2 time points (3h and 5days).
RESULTS
Aim1: both essential oils, at non-spermicidal concentrations (<0.4 mg/ml), proved to be harmless on porcine uterine horns. Higher doses, used as positive controls, induced severe damage of the mucosa. Aim2: EOs showed concentration dependent effects on the cells, with worsening toxicity at 5 days, as expected. The lowest tested concentration (0.1 mg/ml) was non-toxic for all EOs
CONCLUSIONS
The study proves the necessity to perform accurate preliminary toxicity screening for each essential oil as they are capable of exerting negative effects on both spermatozoa and the female genital tract. Nonetheless, it is possible to identify non-toxic concentrations that may be then applied in porcine reproduction in light of their beneficial capabilities.

W135
EFFECTS OF LEPTOSPIRA NATURAL INFECTION ON REPRODUCTIVE TRAITS OF HOLSTEIN HEIFERS: PRELIMINARY RESULTS

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BACKGROUND-AIM
Studies have shown that Leptospirosis is associated with late puberty, failure to conceive, estrus repetition, and early embryonic death. The objective of this study was to describe the ovarian characteristics and reproductive parameters of naturally infected heifers by Leptospira spp. during rearing period.
METHODS
A previously identified positive farm to Leptospira spp. was used in this study. Heifers (n=93) with up to 3 months of age were screening to assure that all calves included were seronegative and noninfected. Heifers (n=60, 3 months of age) negative for Leptospira spp. prior to first vaccination were randomly selected and monthly evaluated serum by MAT to detect seroconversion and qPCR and urine by lipL32 qPCR to detect infection. At first vaccination, calves were divided between two groups: vaccinated (n=30) and non-vaccinated (n=30, placebo) following the immunization protocol, first vaccination at 3 months of age, 1st booster after 30 days and 2nd booster after 6 months. At 6 months of age, estrous detection started being checked daily to determine the first estrous. At 14 months, heifers were submitted to a timed-AI (sexed semen) and ovaries were evaluated using a color Doppler ultrasound (Sonoscape S9): total follicle counting (TFC; D0 and D7 of the protocol), diameter of the preovulatory follicle (DFOL), corpus luteum blood flow (D20 post AI), and conception rate (D30 post AI). Statistix v.9 was used for statistical analyses using the ANOVA and Tukey's test for comparing the means and the Chi-square test for the proportions.
RESULTS
Although vaccinated animals had leptospiruria, the number of infected heifers was similar (P>0.05) between vaccinated (12%) and non-vaccinated (15%) animals during the period of 3 to16 months of age. Animals did not present any disease symptom. Then, we compared noninfected versus infected heifers to the parameters abovementioned. The first estrous detection (9.6±0.3 and 9.9±0.5 months, respectively), the ovarian parameters (overall, TFC=19.5±1; DFOL=11.9±0.5), and the conception rate at the first service (24%) were similar (P>0.05) between groups.
CONCLUSIONS
The natural infection of Leptospira during the heifer's development did not cause changes in the ovarian structures regarding to its number, size and blood flow.

TOPIC Reproductive system diseases

W136
SEROLOGICAL AND BACTERIOLOGICAL DETECTION OF BRUCELLA CANIS IN KENNELS AND HOUSEHOLD DOGS IN CHILE

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BACKGROUND-AIM
Canine brucellosis caused by Brucella canis is a zoonotic disease that typically causes reproductive problems such as infertility, abortions, epididymitis, and spermatogenic disorders. Brucellosis in dogs is prevalent in South America and in most of the world. Since the disease is a major threat to the breeding capability of dogs, all dogs used for breeding purposes should be tested regularly. This study aimed to compare the prevalence of B. canis infection in kennel and household dogs by using blood culture and serology tests.
METHODS
Dogs (130 females; 108 males) from 142 household and 96 from five kennels in Santiago city, Chile, were sampled by venipuncture over 12 months period. All blood samples were analyzed for bacteraemia with trypticase soy broth enriched with sodium citrate (2% w/v) at 37 °C for 30 days. Every week, 100 µL were plated onto Brucella agar plates supplemented with 100 mg/L cycloheximide, 25,000 IU bacitracin, and 6,000 IU polymyxin B, for at least 72 h at 37°C. All suspicious colonies were identified by PCR with specific primers for B. canis using strain B. canis SCL as control strain (access number: NZ_LGAQ00000000.1). Serological evaluation was assessed by counterimmunoelectrophoresis (CIEF) with LPS-R of B. ovis as an antigen. Fisher´s exact test was used to compare prevalence rates.
RESULTS
From the 238 dogs analyzed, 21 (8.8%) were infected (12 females and 9 males), of which 20 were seropositive and only in five dog B. canis was isolated. In household dogs 16 positives (11.3%) were detected and only five (5.2%) in kennels. Three of the four kennels presented at least one positive animal. Seroprevalences were not different between household and kennels dog (p=0.16).
CONCLUSIONS
Both kennel and household dogs are reservoir of B. canis, influencing the reproductive performance and being a risk for human health. FOUNDED BY FONDECYT 1180544

W137
DETECTION OF HIGH ANTIBODIES TITERS AGAINST RAT LEUKEMIA VIRUS IN AN OUTBREAK OF REPRODUCTIVE DISORDERS AND LYMPHOMAS IN WISTAR RATS.

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BACKGROUND-AIM
Young female Wistar rats from an SPF breeding colony presented an outbreak of infertility along with neurological symptoms and malignant lymphomas. We evaluated the presence and the potential role of the rat leukemia virus (RaLV) in the disease because these clinical signs could be compatible with a retrovirus. RaLV is a mammalian type C endogenous retrovirus (ERV) initially isolated from in vitro Sprague-Dawley rat embryo cultures. There are no reports of clinical disease in rats associated with this virus, and little is known about its interaction with the host.
METHODS
Total RNA from the thymus and the uterus were obtained from clinically affected and healthy rats using RNeasy mini total RNA isolation kit (Qiagen, USA) according to the manufacturer's protocol. This was later converted into cDNA using the Superscript III Reverse Transcriptase (Invitrogen). Primers were designed from the rat leukemia virus cDNA sequence (Genbank access code: M77194.1) using Primer-BLAST bioinformatics tool. These covered regions of the three genes present in the virus: GAG (group-specific antigen), POL (polymerase), and ENV (envelope). The RaLV ELISA developed in our laboratory was used to measure antibody titers against this retrovirus. ELISA plates were coated with a envelop RaLV protein produced by China Peptides (China) from a cDNA sequence of enveloping genes of RaLV. ELISA plates assay conditions were optimized for measuring rat antibodies.
RESULTS
All Wistar rats produce continuously competent viral particles. Sequences of RaLV genes were amplified by PCR using cDNA (thymus and uterus) from healthy and affected Wistar rats. The bioinformatic analysis confirmed that the amplified fragments belong to RaVL.
All diseased rats had antibodies against RaLV; the sera of clinically healthy rats were negative. Interestingly, when the rat samples were grouped according to the clinical signs and manifestations, the RaLV antibody titers tended to be higher in rats with lymphomas, than the rats with ataxia-infertility symptoms (p=0.07).
CONCLUSIONS
This is the first report of a rat antibody response against RALV. Higher antibody titers correspond to higher RALV antigen stimuli; we suggest that diseased animals carried higher viral loads than healthy animals. Higher RALV loads could be the genesis of the clinical signs associated with this case.

W139

ASSOCIATION OF BVDV, IBRV, AND NEOSPORA CANINUM WITH LATE EMBRYONIC LOSSES IN A GRAZING DAIRY HERD

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BACKGROUND-AIM

Poor embryo survival and increased pregnancy losses are among the most important factors leading to poor reproductive performance in dairy cows. Some reports have found that Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR) and Neospora caninum (NEO) are related to fetal loss, but, surprisingly, no study has evaluated their association with the risk for late embryonic losses (LEL).

METHODS

A prospective cohort study was carried out in an Argentinian commercial dairy herd to identify the presence of BVD, IBR and NEO by PCR, and to assess the associations between seroprevalence and seroconversion to BVD, IBR and NEO with the odds for LEL. LEL was defined as cows having embryo with no heartbeat, or with detached membranes or floating structures including embryo remnants detected by ultrasonography (US) at 28-42 days post-AI, whereas cows with positive pregnancy by US 28-42 d post-AI were considered as non-LEL. A total of 92 cows were selected for the study (46/group). All the cows were bled twice, at pregnancy (and LEL) diagnosis (d 0), and 28 d later. Serological diagnosis to BVD, IBR and NEO infection was performed on all samples and progesterone concentration was measured in d 0. The conceptus from LEL cows were sampled (d 0) with an insemination pistol attached to a 10 mL syringe, stored in a vial with RNAlater, and transported to the lab. Subsequently, the aspirated conceptuses from LEL cows were processed for BVD, IBR and NEO identification by PCR. The associations of risk factors (serological titers, seroconversion, and progesterone) with the odds for LEL were assessed with logistic regression models.

RESULTS

The risk for LEL increased 3.44 times per 1 SD of increment in titer to BVD over the mean at d 28 (P = 0.03), and, also, increased 3.27 times in cows that seroconverted to BVD (P = 0.09) compared with herd-mates that did not. Conversely, neither the remaining seroprevalences and seroconversions nor progesterone concentration were associated with the odds for LEL. Finally, only BVD virus was identified in all the conceptuses from LEL cows that seroconverted (9/46).

CONCLUSIONS

We concluded that BVD is a risk factor for LEL in dairy cows. Conversely, IBR, NEO and progesterone concentration seem not to play any role.

W140

HASTENING FIRST POSTPARTUM OVULATION EARLY IN LACTATION AS A MEANS TO IMPROVE UTERINE HEALTH IN HOLSTEIN-FRISIAN DAIRY COWS

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BACKGROUND-AIM

Postpartum uterine inflammatory diseases are prevalent in bovine dairy herds, resulting in major economic losses. Previous studies suggest that cows spontaneously ovulate early in lactation have better reproductive performance later in lactation. Our objective was to evaluate the efficacy of two hormonal treatment regimens (Select-sync; Select-sync CIDR) to hastening ovulation in early lactation (treatment initiation at 24-27DIM), and to explore the associated incidence of cytological endometritis (CEM) later in lactation.

METHODS

Based on transrectal palpation and ultrasonographic examination (presence of corpus luteum, CL) combined by milk progesterone (mP4) cow-side test at 24-27DIM, postpartum Holstein-Friesian dairy cows (n= 450) were divided into four groups: Positive control Group: cows ovulated spontaneously by 24-27DIM, no treatment. Cows that did not ovulate spontaneously were divided randomly into the following (n= 101 cows/group): Select–synch Group: cows treated with GnRH analog, and PGF2a a week later; Select–synch-CIDR Group: cows treated as the previous group, but also with CIDR for that week; Negative control Group: no hormonal treatment was given (two saline injections one week apart). Ovarian function was evaluated five times by transrectal ultrasonography (Ex1, 24-27DIM; Ex2, 31-34DIM; Ex3, 38-41DIM; Ex4, 45-49DIM; Ex5, 66-69DIM). CEM diagnosis was performed based on endometrial cytobrush at 38-41DIM and 66-69DIM.

RESULTS

Of the 450 cows, 417 were clearly defined as ovulating or not at 24-27DIM.A total of 114 cows (27%) were defined as ovulated at Ex1; with a higher proportion among multiparous vs. primiparous cows (33% vs. 17%; OR= 2.44, 95% CI 1.49-4.00; P=0.0003). Both hormonal protocols efficiently induced ovulation early in lactation and increased the number of cows ovulating during the 70DIM voluntary waiting period (Select-synch 92.1%, Select-synch-CIDR 89.1%; Negative control 71.3%; P=0.0466). Furthermore, the Select-synch protocol was associated with a decreased risk for CEM compared to the negative control, particularly in cows without ketosis in early lactation (18.6% vs. 35.4%; OR=0.42, 95%CI 0.18-0.90; P=0.0295).

CONCLUSIONS

We concluded that hormonal treatments could induce ovarian activity in early lactation and subsequently improve uterine health.

W141
DEVELOPMENT OF A HIGH-RESOLUTION MELTING ASSAY FOR THE MOLECULAR DIAGNOSIS OF BOVINE GENITAL CAMPYLOBACTERIOSIS

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BACKGROUND-AIM
Bovine Genital Campylobacteriosis (BGC) is a worldwide spread venereal bacterial disease of cattle caused by Campylobacter fetus subsp. venerealis (Cfv). Diagnosis is hampered by the existence of two C. fetus subspecies, C. fetus subsp. fetus (Cff) and Cfv. Due to the fastidious growth and limited survival of Cfv, real-time PCR assays have been developed for Cfv identification, mostly directed to mobile genetic elements parA gene and ISCfe1. These targets may be horizontally transferred to other Campylobacter species, leading to a false positive diagnosis.
This study aimed to develop a novel real-time PCR method coupled with high-resolution melting (HRM) analysis, to allow the differentiation of C. fetus subspecies and application in BGC diagnosis.
METHODS
The HRM assay was designed to target a single nucleotide polymorphism (SNP) and its performance was assessed with 59 C. fetus strains (Cfv=39; Cff=20). The specificity was evaluated in 50 preputial samples previously tested as negative for C. fetus and in 24 Campylobacter spp. strains. The analytical sensitivity was determined through standard curves built with Cfv genome copies and preputial samples spiked with Cfv. Differences in melting temperatures (Tm) were analysed with student's t-test using a significance level of 0.05.
RESULTS
The HRM assay differentiated Cfv from Cff based on a Tm of 73.08 ± 0.07 °C and 73.60 ± 0.06 °C, respectively (P<0.001). The assay detected 10^2 genome copies of Cfv, showing an efficiency of 93.16%. For preputial samples the limit of detection was 10^3 CFU/mL. The HRM assay showed a specificity of 100% since no amplification curves were obtained in the negative preputial samples and with other Campylobacter spp. For the 59 isolates tested, subspecies identification by the HRM assay totally agreed with results provided by ISCfe1 amplification, for which only Cfv strains are positive. However, Cfv strain CCUG 34111 was genotyped as Cff by HRM analysis, which is also in agreement with the absence of ISCfe1 amplification and tolerance to 1% glycine.
CONCLUSIONS
In conclusion, the HRM assay directed towards an SNP in the core genome, precluding its horizontal transfer, proved to be effective for differentiating Cfv from Cff.
Work funded by PTDC/CVT-CVT/30145/2017 (FCT and FEDER) and UIDP/CVT/00276/2020 (FCT).

TOPIC Spermatology and sperm quality

W142
EVAULATION OF SPERM PARAMETERS BY A NEW SEMEN COLLECTION METHOD IN CHINCHILLA (CHINCHILLA LANIGERA)

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BACKGROUND-AIM
Studies on reproductive properties of chinchillas have two important points: (a) the economic relevance of the domesticated stocks, (b) conservation of the genetic material of the species. In Hungary, chinchilla breeding has a history of 50 years. Artificial insemination has not been elaborated in the species so far and the available data about their reproduction properties are contradictory. Generally, semen is collected exclusively by electroejaculation with or without anesthesia. However, it is not the most appropriate method in terms of sperm quality and animal welfare.
METHODS
The objective of the study was firstly to develop a semen collection technique that is safe and makes the routine long-term collection of semen possible throughout the year, as well as can meet the animal welfare requirements. Additional purpose was to determine the basic spermatological parameters such as motility, concentration, morphological abnormalities and live/dead cell ratio by a CASA (SCA®) under the northern hemisphere conditions, in Hungary.
A special massage technique was developed in the study and sperm parameters of 46 males were analyzed weekly for one year.
RESULTS
Approximately 29% of chinchillas responded positively to this technique. However, we believe that with a thorough selection of the males and by refinement of the collection technique the success of semen collection can be improved. The presence of females during the training and collection period slightly, but not significantly influenced the success of semen collection.
The average sperm concentration was 873 million/ml. On average, the total motility was 87%, the rapid, advanced cells were 62%, while the immotile cells 13%. The standard deviation among the parameters was high, without a definite seasonal peak, but with fluctuating values throughout the year. The ratios of live intact, live abnormal and dead cells were 80, 6 and 14%, respectively, during the examined period.
CONCLUSIONS
Summarized, with adequate routine, semen can be successfully collected without electroejaculation and anesthesia. Although, spermatological parameters show fluctuations throughout the year, considering this, semen is useable throughout the year in artificial insemination.

W143
MOTILITY FEATURES OF BOAR AND STALLION SPERMATOZOA AFTER TREATMENTS WITH MITOCHONDRIAL COMPLEXES INHIBITORS: A COMPARISON

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BACKGROUND-AIM
Mammalian sperm cells rely at various degrees on mitochondria to obtain energy for their metabolism; porcine spermatozoa preferably use the glycolytic pathway, while equine ones the oxidative phosphorylation.
This work was aimed at delineating sperm kinematic features after treatment with different mitochondrial function inhibitors.
METHODS
Porcine and equine sperm cells (30x106 spz/mL in Androhep and Tyrode's medium respectively) were incubated 1 h at 37°C with: Rotenone 5 µM (ROT) complex I inhibitor; Dimethyl-malonate 10 mM (DMM), complex II inhibitor; Carbonyl cyanide m-chlorophenyl hydrazone 5 µM (CCCP) uncoupling agent; Antimycin A 1 µg/mL (ANTI), complex III inhibitor; Oligomycin 4 µg/mL (OLIGO), ATP synthase inhibitor and 2 µl DMSO (CTR: control vehicle). Sperm kinematics (VAP, VCL, VSL, LIN, STR, ALH, BCF) were assessed by Hamilton IVOS 12.0 and subsequently analysed by principal components and cluster analysis.
RESULTS
In both species four subpopulations were delineated by cluster analysis. In boar two rapid (progressive and non-progressive), one average and one slow clusters were shown while in stallion one rapid progressive, one average and two slow (progressive and non-progressive) clusters were observed.
In both species CTR and DMM treated cells were mainly distributed in rapid clusters (52% and 60% in porcine, 57% and 54% in equine sperm, respectively). In equine cells the other treatments induced a shift toward slow cluster (80% in ROT and ANTI, 75% in OLIGO; 70% in CCCP); in porcine spermatozoa, 33% of OLIGO and CCCP, 28% of ANTI and 38% of ROT treated cells belonged to slow cluster and 30% cells in the same groups belonged to rapid (non-progressive) cluster.
CONCLUSIONS
In conclusion, equine and porcine sperm motility is dependent on mitochondrial activity and it responds differently to inhibition of mitochondrial complexes, thus confirming that different metabolic strategies are involved in sperm function.

W144
OUTCOMES FROM 1220 FROZEN BULL SEMEN STRAWS SUBMITTED TO AN ANDROLOGY LABORATORY FOR QUALITY TESTING

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BACKGROUND-AIM
Success in AI depends on many factors, including semen quality.
METHODS
1220 frozen bull semen straws were submitted for quality testing to andrology lab at UNA-Costa Rica from 1998-2021 (1 straw per batch). The same operator performed the evaluation after thawing the straws in water at 38°C/30 secs. Motility was determined by observation under phase contrast microscopy (200x) using prewarmed (38°C) slides and cover glasses. Sperm number per straw (NTS) was calculated after diluting the sample (1:100) with Hancock solution (HS) in a Newbauer hemocytometer. Sperm head abnormalities (size and shape) were determined in smear stained with carbol fuchsin (200 cells/slide-1000x). Other defects were assessed in a wet smear fixed with HS (phase contrast, 200 cells/slide-1000x). Semen quality was categorized as sound for AI when ≥7.5x106 viable spermatozoa (VS) and ≤15% uncompensable sperm defects (USD). The unsound were disclosed as C1: <7.5x106 VS and >15% USD, C2: <7.5x106 VS and ≤15% USD, and C3: ≥7.5x106 VS and >15% USD. Data were analyzed (SAS) using T test for independent means of interest (IS vs. LS), and Chi-squared test to compare sound vs. unsound classes.
RESULTS
Control testing before AI accounted as the main submission reason (57.1% n=697), followed by low pregnancy rate after AI (28.2% n=344) and warning of low N2 in the tank (14.7% n=179). Imported semen (IS) (mainly from Europe, North and South America), and frozen locally (LS) accounted 75% (n=915) and 25% (n=305) respectively. Motility (%), USD (%) and NTS (x106) for IS and LS straws were respectively 56.9±23.3 (0-95) vs 37.3±22.7 (0-85) (P<0.0001), 14.3±18.4 (0-100) vs 21.2±27.1 (0-100) (P<0.0001) and 27.0±17.8 (0.6-154) vs 27.8±16.4 (1.3-122) (P>0.05). 68.2% (n=832/1220) of straws were ranked as unsound for AI, being from C1, C2 and C3 24.9% (n=304/1220), 39.4% (n=480/1220) and 3.9% (n=48/1220) respectively (P<0.0001). Disclosing the unsound straws by origin, 62.4% (n=571/915) and 85.6% (n=261/305) belonged to IS and LS respectively (P<0.0001). Unsound IS vs. LS accounted as C1: 21.2% (n=194/915) vs. 36.1% (n=110/305) (P<0.0001), C2: 36.4% (n=333/915) vs. 48.2% (n=147/305) (P<0.0001) and C3: 4.8% (n=44/915) vs. 1.3% (n=4/305) (P<0.01).
CONCLUSIONS
Regardless the straw origin, the results emphasize the need to improve quality controls -underlying testing sperm morphology- before freezing, giving the impact on the conception rate caused by semen of questionable quality.

W145
EFFECT OF THE INDIVIDUAL INBREEDING VALUES ON THE SPERM MOTILITY PATTERNS IN ANGUS CATTLE

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BACKGROUND-AIM
Inbreeding can affect the sperm motility average and subpopulation structure in cattle. However, it has been suggested that their effect is not the same among breeds. In this study, we aimed to evaluate the effect of individual inbreeding on the sperm kinematic patterns in Angus cattle.
METHODS
We analyzed frozen sperm samples of 35 individuals (two replicates, n=70) using a Computer Assisted Sperm Analysis system (CASA, AndroVision®). Eight parameters were determined: curvilinear (VCL), straight-line (VSL) and average path (VAP) velocities, linearity (LIN), straightness (STR) and wobble (WOB) trajectory indexes, and the amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF). Thereafter, we selected VAP, LIN, ALH using a principal component analysis, which was submitted to a two-step (non-hierarchical – hierarchical) clustering procedure that determined the pertinence of each sperm to four different subpopulations (Sp). In addition, we determined the individual inbreeding value (Fped) of each bull using a pedigree of 250.000 records. Finally, we performed a Spearman correlation between the six sperm traits and the proportion of each Sp with the respective individual Fped.
RESULTS
Clustering analysis revealed the existence of four different subpopulations: Sp1 included hyperactivated sperm, showing high VCL and ALH but low VAP and LIN. Sp2 showed low velocities and reduced head displacement. Sp3 encompassed progressive and medium velocity sperm (intermediate VCL, VSL and VAP, high LIN, and low ALH). Sp4 included the fast and progressive sperm (high velocities and LIN, and intermediate ALH and BCF). The analysis showed significant correlations ($p<0.05$) on VCL (0.15) and ALH (0.25) (positives) and LIN (-0.39), STR (-0.35), WOB (-0.37) and BCF (-0.22) (negatives) with individual inbreeding values. Additionally, Sp1 (0.36) and Sp3 (-0.33) showed also significant correlations with the inbreeding.
CONCLUSIONS
This study suggests that inbreeding could affect the sperm kinetic patterns, particularly increasing the hyperactive-like and non-progressive movements in Angus cattle. We also detected a similar effect of inbreeding in the motility subpopulation patterns. Further studies are necessary to validate the effect of these findings in the field fertility of Angus bulls.

W146
UNRAVELLING JAGGED1 ORIGIN AND ROLE IN BULL SPERM ACROSOME REACTION

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BACKGROUND-AIM
Sperm proteins are involved in acrosome reaction (AR), a process of membrane fusion and lytic enzyme release that confers sperm's ability to fertilize. Previous work identified JAGGED1, a Notch ligand, in ejaculated bovine spermatozoa (Spz) and its relocation during AR. This study investigated the origin and role of JAGGED1 in bull sperm AR.
METHODS
The presence of JAGGED1 was evaluated in bovine testicular, epididymal and ejaculated Spz (immunofluorescence- IF), and in seminal plasma (Western-blot). JAGGED1 localization pattern was evaluated in frozen-thawed Spz obtained following swim-up and incubation (capacitation conditions). Samples were either treated with Calcium Ionophore (CaI) to induce AR or left untreated to evaluate spontaneous AR, and then processed for IF using Peanut agglutinin (PNA) and anti-JAGGED1 antibody, allowing the localization of JAGGED1 in non-reacted (NR), reacting (R) and acrosome reacted (AR) Spz (n= 500 from each of 3 bulls). The role of JAGGED1 on AR was evaluated in frozen-thawed Spz treated with JAGGED1 either during swim-up (0, 10 and 50 ng/ mL, non-capacitating conditions) or following swim-up (0, 10, 50 and 100 ng/mL, capacitation conditions). Samples incubated in capacitation conditions were either treated with Cal or left untreated, and processed for IF (n= 300 PNA stained Spz, from each of 3 bulls, in each treatment). IF images were analyzed using ImageJ.win64 software. Data were analyzed using chi-squared test. Values of $p< 0.05$ were considered statistically significant.
RESULTS
Results evidenced that JAGGED1 is absent in testicular Spz, but present in epididymal (corpus and cauda), ejaculated and in seminal plasma. JAGGED1 pattern was related with acrosome status. As the AR (spontaneous and induced) proceeds, JAGGED1 is re-localized from the apical to the post-equatorial region ($p<0.05$). Sperm JAGGED1 stimulation, in non-capacitation conditions, decreased the proportion of NR Spz ($p<0.001$). In accordance, JAGGED1 stimulation in capacitation conditions, both during the spontaneous and induced AR, increased the proportion of RA Spz ($p<0.05$).
CONCLUSIONS
In conclusion, Spz JAGGED1 is acquired during epididymal maturation, its dynamic relocation pattern is associated with AR, and exerts a stimulatory effect on this event. Results prompt for a role on the regulation of AR, and may also suggest that JAGGED1 is associated with a positive regulation of sperm fertilization capacity.
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W147
CORRELATION BETWEEN ROS GENERATION AND MEMBRANE CHANGES IN EQUINE SEMEN

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BACKGROUND-AIM
Reactive oxygen species (ROS) are products obtained from oxygen metabolism. There is a wide range of tests to evaluate the generation of ROS and can even evaluate various types of radicals. This study aims to determine which types of ROS are harmful to sperm quality.
METHODS
Two ejaculates were collected with artificial vagina from 4 stallions. After collection, they were filtered, extended in milk-based extender at a concentration of 50x106 sperm/mL, and split into 5 groups. Different concentrations of 2-hydroxyestradiol (0mM, 125mM, 250mM, 375mM and 500mM) were added to 1mL of extended semen and incubated for 15 minutes at 37°C for ROS induction. Samples were centrifuged 2x (300xg) for 5 minutes and the pellet was suspended in the same concentration. One aliquot of each was diluted in TALP-PVA sperm medium (final concentration of 2x106 sperm/mL) and split again into 5 tubes with 500µL each, added 7µM Hoeschst 33342 to exclude debris and prepared for flow cytometer analysis. For mitochondrial superoxide anion were added 2µM of MitoSox Red and 25nM of YoPro for membrane stability; for cytoplasmic anion superoxide 2µM Dihydroethidium and Yopro were added; for hydrogen peroxide 10µM CM-H2CDFDA and 1.5µM propidium iodide for plasma membrane integrity were added; for nitric oxide 10µM of DAF-FM and propidium iodide (samples were incubation at 37oC for 20min); for lipidi peroxidation 1µM of C11-Bodipy and incubated at 37oC for 1h. Correlation of ROS generation and membrane changes were determined by Sperman or Pearson tests according to the distribution of samples, only correlations with P<0.05 were described in the results
RESULTS
Hydrogen peroxide and nitric oxide were positively correlated with plasma membrane integrity (r=0.78; r=0.92) and cells with stable membrane (r=0.81; r=0.90) but did not correlated with lipid peroxidation (r=-0.31; r=-0.41). Mitochondrial and cytoplasmic superoxide anion were negatively correlated with membrane integrity (r=-0.87; r=-0.64) and stable membrane (r=-0.86; r=-0.67), only intracellular superoxide anion was correlated with lipid peroxidation (r=0.69).
CONCLUSIONS
These results shown that both mitochondrial and cytoplasmic superoxide anion were the anions with major deleterious effects on seminal quality, cytoplasmic anion superoxide promotes lipid peroxidation and it is suggested that hydrogen peroxide and nitric oxide anions may be related to healthy sperm metabolism.

W148
L-CARNITINE SUPPLEMENTED SKIMMED MILK-BASED EXTENDERS SUCCESSFULLY PRESERVES THE CRYOPRESERVED PERUVIAN PASO HORSE SPERM QUALITY

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BACKGROUND-AIM
The Peruvian Paso horse is an original breed of Peru and it is distributed by Andean countries constituting a flagship product especially in Peru and Ecuador. Thus, is very important to work on promoting and preserving their genetic material through semen. However, the post-thaw sperm quality has been discouraging, leading to the use of additives to improve sperm cryosurvival. L-carnitine (LC) plays a key role in sperm metabolism based on energy production (ß-oxidation) and reduction of lipid availability for peroxidation. In this sense, this research evaluated the effect of LC supplemented to two skimmed milk-based extenders on cryosurvival of chilled and frozen-thawed Peruvian Paso horse spermatozoa.
METHODS
A first experiment assessed the optimal LC dose (0, 1, 5, 10, 25, and 50 mM) added to INRA-96®- and UHT-(skimmed milk plus 6% egg yolk) extenders in 9 individual semen ejaculates from three Peruvian Paso horses. A second experiment evaluated the effect of 25 mM LC supplemented to freezing mediums (INRA-Freeze® or UHT [plus 5% glycerol]) in 16 pooled semen ejaculates (n=8 pools, 2 ejaculates/pool) from four Peruvian Paso horses
RESULTS
In the first experiment, better results were obtained with INRA than with UHT extender. The results demonstrated that 25 mM LC successfully preserved the kinetics y and integrity of plasmatic and acrosomal membranes for up to 48 to 96 h of cold storage. In the second experiment, post-thaw sperm quality was reduced for two extenders ($P < 0.05$) compared with fresh semen. Like chilled semen, better cryosurvival results were obtained with INRA diluent compared to UHT. After the freezing-thawed process, LC supplementation to INRA-Freeze extender improved ($P < 0.05$) motility (LC= 46.6 ± 3.44% vs. control= 34.2 ± 3.75%) and plasma membrane integrity (LC= 51.4 ± 1.71% vs. control= 39.0 ± 1.53%). However, when UHT extender was supplemented with 25 mM LC only improved ($P < 0.001$) post-thaw acrosome integrity (LC= 77.5 ± 2.77 vs. control= 67.7 ± 2.68%).
CONCLUSIONS
In conclusion, the addition of L-carnitine to skimmed milk-based extenders has a positive effect on the kinetics and integrity of plasma and acrosomal membranes of chilled and frozen-thawed Peruvian Paso horse sperm.
Keywords: L-carnitine, semen, Peruvian Paso horse, sperm cryosurvival.

W149
EFFECT OF FRUCTOSE, TROLOX AND ATP ON FROZEN-THAWED SPERM QUALITY IN BUFFALO

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BACKGROUND-AIM
Buffalo sperm are extremely sensitive to oxidative damages during freezing-thawing, due to the high concentration of long chain polyunsaturated fatty acids in the sperm membrane. Several additives have been used to protect frozen-thawed sperm from thawing-induced oxidative stress, to improve sperm fertility parameters. The aim of this work was to evaluate the effects of the combination of the antioxidant Trolox and supplementary energy sources, like fructose and ATP, on frozen-thawed sperm quality in buffalo.
METHODS
Four ejaculates from 4 bulls were used for the trial. After thawing, Percoll-separated spermatozoa were incubated in Tyrode's Albumine Lactate Pyruvate (TALP) alone (control) and in the presence of 2.5 mM of ATP, 5 mM of Fructose and 0.1 mM of Trolox (Mix) for 1 h. Sperm motility was evaluated by phase contrast microscopy, viability by Trypan blue-Giemsa staining and membrane integrity by the hypoosmotic swelling test (HOS). The DNA fragmentation was evaluated by Tunel. Data were analyzed by Student's T test and reported as means ± SEM.
RESULTS
The treatment with the mix of additives increased ($P < 0.05$) the motility of frozen-thawed buffalo sperm compared to the control (69.4 ± 0.2 vs 63.8 ± 0.1, respectively). Moreover, the mix increased ($P < 0.01$) the percentage of spermatozoa with intact membrane compared to the control (65.9 ± 1.5 vs 59.6 ± 1.2, respectively). However, no differences were reported in both sperm viability (84.1 ± 1.2 and 86.1 ± 0.8, respectively in the control and mix groups) and in the percentage of spermatozoa with DNA fragmentation (5.5 ± 1.0 and 3.2 ± 0.7, in the control and mix groups, respectively).
CONCLUSIONS
In conclusion, the preliminary results of this study showed that the combination of fructose, ATP and Trolox improves motility and membrane integrity of frozen-thawed buffalo sperm without affecting DNA integrity. The latter parameter is particularly relevant as plasma membrane is the primary site of injury in cryopreserved sperm. Further studies are required to clarify the potential effect of the mix on the in vitro fertilization procedure and subsequent embryonic development.

W150
PRINCIPAL COMPONENT ANALYSIS ON EQUINE SPERM MOTILITY AND OXIDATIVE PARAMETERS IN RELATION WITH MITOCHONDRIAL FUNCTION

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BACKGROUND-AIM
Equine spermatozoa highly rely on oxidative phosphorylation for their energy management; the aim of the present work was to delineate the mitochondrial activity of equine spermatozoa after incubation with specific inhibitors of the different mitochondrial complexes.
METHODS
Equine sperm cells (30x106 spz/mL diluted in Kenney extender) were incubated 1h and 3h at 37°C with: Rotenone 5 µM (ROT) complex I inhibitor; Dimethyl-malonate 10 mM (DMM), complex II inhibitor; Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) 5 µM uncoupling agent; antimycin A 1 µg/mL (ANTI), complex III inhibitor; Oligomycin 4 µg/mL (OLIGO), ATP synthase inhibitor and 2 µl DMSO; (CTR) vehicle. Sperm kinematics (VAP, VCL, VSL, LIN, STR, ALH, BCF) were assessed by Hamilton IVOS 12.0. Mitochondrial activity by Mitotraker (MT), mitochondrial superoxide anion (O2-) production by MitoSOX (MS) probe and H2O2 intracellular content by DCFDA were evaluated by BD FacsCalibur flow cytometer. Principal components analysis (PCA) on viable cells was performed.
RESULTS
The first five principal components (PC) explained the 84.79% of the total variance. The first PC is positively correlated with motility parameters and spermatozoa with high mitochondrial membrane potential and it represents the treatment effect. Indeed, CTR and DMM treated cells have positive scores on first PC; while ROT, ANTI, OLIGO and CCCP treated cells have negative scores on this variable. The second PC positively correlates with spermatozoa with high intracellular ROS production (DCFDA+) and without mitochondrial activity and O2- production (MT-MS-) and it represents the time effect. Indeed, it divides spermatozoa analysed after 1h storage (negative scores) from cells analysed after 3h storage (positive scores). Spermatozoa with high mitochondrial ROS production (MS+) positively correlate with the third PC, ; ANTI treated cells present negative scores on this PC.
CONCLUSIONS
In conclusion, depression of mitochondrial function by ROT, ANTI, OLIGO and CCCP results in a significant decrease in sperm motility; storage time induces a decrease in sperm mitochondrial function with an accumulation of intracellular H2O2.

W151

ACROSIN IS ESSENTIAL FOR SPERM PENETRATION THROUGH THE ZONA PELLUCIDA: A KNOCKOUT HAMSTER STUDY

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BACKGROUND-AIM

During natural fertilization, mammalian spermatozoa must pass through the zona pellucida before reaching the plasma membrane of the oocyte. It is assumed that this step involves partial lysis of the zona by sperm acrosomal enzyme(s), but there has been no unequivocal evidence to support this view. For example, acrosin, a major acrosomal protease, is thought to be dispensable because of the lack of distinct phenotypes in acrosin-deficient mice and rats. In this study, we reinvestigated the role of acrosin in fertilization using the golden hamster (Mesocricetus auratus) as a model, because this species has provided invaluable information on the mechanisms of mammalian fertilization. However, hamster embryos are highly vulnerable to in vitro conditions. Recently, an in vivo genome editing system (improved genome-editing via oviductal nucleic acids delivery system; i-GONAD) has been developed, which enables us to bypass all the in vitro embryo-handling steps.

METHODS

We synthesized 6 sgRNAs for the hamster Acrosin gene and performed i-GONAD using female hamsters which were mated with males one day before. Of the 15 pups born, 5 pups (33%) carried mutated alleles. One was used for generation of an Acrosin knockout (KO) hamster line.

RESULTS

Homozygous KO males (N =8) were proven to be sterile by mating with wild-type (WT) females for 2 weeks. Next we examined the behavior of the acrosin-KO spermatozoa in vitro. We confirmed normal sperm motility by computer-assisted sperm analysis and the ability of spermatozoa to undergo the acrosomal reaction in vitro. Finally we analyzed the fertilizing ability of acrosin-KO spermatozoa in vitro. About 90% oocytes were fertilized by WT spermatozoa, whereas none were fertilized by acrosin–KO spermatozoa. Spermatozoa could attach to the zona surface irrespective of the genotype. Interestingly, when the zona pellucida was removed before insemination, all oocytes were fertilized by KO spermatozoa.

CONCLUSIONS

These results clearly indicate that acrosin is essential for sperm penetration through the zona. Our study also implies that the knockout hamster system would substitute for mouse models in identification of new gene functions or analysis of human diseases because of its technical easiness, reproducibility, and the phylogenically distant position to murine rodents.

W152
ON THE CHALLENGES OF CRYOPRESERVING BAT EJACULATES

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BACKGROUND-AIM
The more than 1400 extant bat species play crucial roles in most ecosystems, providing pollination, seed dispersal, and insect control. In the face of the ongoing sixth mass extinction, many species are threatened by habitat degradation, pollution and pathogens, such as white nose disease in North America.
Given their low reproductive rates, assisted reproductive technologies (ART) will become important tools to store and sustain genetic diversity of endangered bat species. So far, ART protocols are largely lacking, except for a cryopreservation protocol for Carollia perspicillata epididymal sperm (Hermes et al. 2021), which may be obtained by castration or post mortem.
METHODS
To implement cryopreservation protocols for sperm from live specimen, we captured microbats in Südharz, Germany, between June and October of 2018-2021, using mistnets. Ejaculates were obtained by electroejaculation under isoflurane anesthesia, using a custom-built probe to test different cryopreservation protocols. Bats were released shortly after the procedure.
Ejaculates of Brandt's bat (Myotis brandtii, n=8) and Daubenton's bat (Myotis daubentonii, n=8) were halved and diluted in an egg-yolk based extender containing either glycerine or DMSO as cryoprotectant. After equilibration for 120 minutes at 4-6°C, sperm was slow-frozen in straws over liquid nitrogen and thawed after > 1 month for assessment.
RESULTS
Ejaculate obtainability, quality and quantity differed largely across species and seasons. In Brandt's bats, ejaculate volumes averaged 5.6±1.3 µl. Pre-freeze total sperm motility averaged 74.8±15.7%, and post-thaw total motilities 27.5±10.7% (18.1±12.6% progressive) for glycerine, and 19.0±8.2% (17.2±6.8% progressive) for DMSO, respectively.
Daubenton's bats had an average ejaculate volume of 4.9±1.3 µl, with a pre-freeze total sperm motility of 68.8±10.9%. Post-thaw total motilities averaged and 12.9±6.8% (6.4±3.5% progressive) for glycerine,
and 17.3±3.1% (13.5±9.5% progressive) for DMSO, respectively.
CONCLUSIONS
Here, we report the first successful cryopreservation of microbat ejaculates with a total post-thaw sperm motility of 27.5±10.7% for glycerine in Brandt's bat, which is considerably higher than in the related Daubenton's bat, indicating high inter-species variability.

W153
FERTILITY-ASSOCIATED COMPREHENSIVE PROTEOMICS OF BOVINE SPERM HEAD PLASMA MEMBRANE

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BACKGROUND-AIM
Bull fertility significantly impacts herd fertility whether breeding is done naturally or by artificial insemination, but sperm characteristics are poor predictors of male fertility once extremes are removed. The head plasma membrane (HPM) of sperm impacts motility, sperm-egg binding, capacitation and ion transport, among other processes.
Objective: Identify, quantify, and compare HPM proteins in sperm from bulls of differing fertility by subcellular fractionation-guided novel proteomics.
METHODS
Methods: An international multi-factor bull fertility index (BFI; Semex; average BFI=100 based on >1000 inseminations per bull) identified Holstein bulls with BFI significantly > or < 100 (high or low fertility; HF, LF; n=3 each). Computer Assisted Semen Analysis (CASA) analysed one ejaculate per bull (sperm number, concentration, morphology, motility kinetics). HPM was isolated and proteolytically digested with trypsin. Peptides were fractionated by strong cation exchange and analysed by tandem mass spectrometry (LC-MS/MS). SpectrumMill (SM) aligned MS spectra to UniProt Mammals protein database and Mass Profiler Professional (MPP) characterized proteins.
RESULTS
Results: BFI did not affect CASA parameters (% motile 41±8 vs 42±3; VAP 84±11 vs 80±12 µm/sec; ALH 4.6±0.2 vs 4.7±0.4µm; mean±SE, HF vs LF; $p>0.3$), nor total number of proteins from SM (4126 HF, 4243 LF). MPP identified 67 proteins differing by >two-fold [fold change (FC)>2; $p<0.05$] between HF and LF. Of these FC proteins, 48 were up-regulated (up-FC) and 19 were down-regulated (dn-FC) in HF vs LF. Association of fertility groups to BFI was confirmed by meta-analysis. UniProtKB assigned up-FCs' functions to sperm motility, spermatogenesis, sperm-oocyte binding, signal transduction, sperm capacitation and/or acrosome reaction functions, and dn-FCs' to catalytic and transporter activity. Linear regression positively correlated up-FCs to BFI ($r2=0.65$ to $r2=0.97$; $p\leq0.05$) and negatively correlated dn-FCs to BFI ($r2=0.76$ to $r2=0.96$; $p\leq0.05$).
CONCLUSIONS
Conclusion: In vivo bull fertility correlates to specific sperm head membrane proteins that impact signal transduction pathways. Identifying such proteins may support selection of semen and/or bulls for better fertility in artificial insemination.
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W155
RECOMBINANT PORCINE JUNO BEADS SUPPORT SPECIES-SPECIFIC SPERM BINDING

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BACKGROUND-AIM
Juno protein is localized on the egg's membrane and it is essential in mammals fertilization due its binding to the sperm receptor IZUMO1. Knock out for some of these two proteins show absence of fertilization. So, we propose the use of beads coated with recombinant JUNO protein to bind those spermatozoa with the greatest capacity to fertilise.
METHODS
We used two plasmid vectors pcDNA3.1 to cloning the predicted sequence encoding JUNO gene with a 6-histidine tag added to the C-terminus, from Sus scrofa and Homo sapiens. Human Embryonic Kidney (HEK) 293T cells were transiently transfected with the plasmid vectors to produce the recombinant porcine and human JUNO protein (rpJUNO and rhJUNO). Secreted proteins were purified and conjugated to commercial magnetic beads (His Mag Sepharose Excel GE Healthcare) to generate the 3D models pJUNO-beads and hJUNO-beads. 3D models were co-incubated for 1, 2, 3 and 4 hours with fresh ejaculated boar semen from fertile males to assess the number of spermatozoa bound per bead and the number of bound spermatozoa reacted acrosome. Beads without protein (Ctrl-beads) were used as internal control for the assay. Beads were fixed in glutaraldehyde and stained with Hoechst and PNA-FITC. We studied a total of 605 beads and statistical analysis was performed by one-way ANOVA and when P-value was <0.05, differences among groups were analysed by a Tukey's.
RESULTS
Expression of proteins and its conjugation to commercial beads was confirmed by SDS-PAGE and Western-Blot. The greatest significant differences in the number of spermatozoa bound were observed at 4 hours (P= 0) with 12,58±1,38 for pJUNO-beads, 7,96±0,98 for hJUNO-beads and 5,69±0,61 for Ctrl-beads. The number of reacted spermatozoa at 4 hours was significative higher (P= 0) in pJUNO-beads with 5,68±0,67 while in human and control models had 3,43±0,39 and 2,64±0,23 respectively.
CONCLUSIONS
These preliminary data show that JUNO proteins were successfully expressed and secreted by HEK cells and conjugated to commercial beads. The generated pJUNO-beads model binds more reacted sperms than control and human models showing that porcine sperm specifically recognize porcine JUNO protein hence, indicating that the Juno-sperm binding is species-specific.
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W156
IMPACT OF GLYCEROL ON SPERM FERTILIZING CAPACITY IN CHICKEN

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BACKGROUND-AIM
Glycerol is the most common cryoprotectant of vertebrate semen because it provides an excellent protection of sperm against cryodamages. In chicken, 11% glycerol is the standard concentration for freezing sperm, but it needs to be removed before inseminating hens, otherwise the fertility is dramatically decreased. This phenomenon has been discussed since 1950s, but the mechanisms involved remain unclear. Here we hypothesize that glycerol preserves sperm capacities at low temperature whereas interfere with sperm biology at physiological temperature (i.e. 41°C). Thus, we firstly investigated the effect of increasing glycerol concentrations on fertility. Secondly, we explored how sperm biology, especially sperm motility and membrane integrity, could be impaired by glycerol presence at 41°C within 60 min (physiological time of sperm evolving from vagina to infundibulum).
METHODS
Semen of 10 adult T44 roosters was collected, pooled, and diluted with glycerol-Lake PC diluent to final concentration of 0, 1, 2, 6 and 11% glycerol at 4°C. For fertility test (n=2), 80 Isabrown hens were inseminated immediately after dilution with 100×106 pooled glycerolized sperm/female. For in vitro tests (n=5), sperm mass and individual motilities were assessed at 0, 10, 20 and 30 min at 41°C by microscope and computer-assisted sperm analysis (CASA), respectively, and membrane integrity was evaluated at 0, 30 and 60 min by fluorescence flow cytometry. Data were analysed by ANOVA tests.
RESULTS
Results revealed that 2% glycerol led to 50% decline of fertility, and infertility was observed with 6 and 11% glycerol. No impact of glycerol on sperm mass motility was revealed but 1% glycerol significantly reduced sperm individual motility after 10 min, and more severe reduction was observed with higher concentrations. Moreover, whereas no impact was observed with 1 and 2% glycerol, 6 and 11% glycerol induced sperm membrane integrity failure after 30 min of incubation at 41°C.
CONCLUSIONS
Collectively, the presence of glycerol in semen samples at very low concentration impacted fertility and sperm motility, whereas higher concentrations are also associated with membrane defects, supporting the need to remove glycerol before insemination to stop the disturbances of sperm transportation and integrity in female tract.

W158
PRESERVE RABBIT SPERM AT 4°C FOR 48H: EFFECT OF CHOLESTEROL-LAODED-CYCLODEXTRINS, α-TOCOPHEROL-LAODED-CYCLODEXTRINS AND VITAMINE C

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BACKGROUND-AIM
Refrigeration deteriorates sperm motility in rabbits and limits its usefulness over 48h of storage. The oxidant stress associated with the diminution of temperature leads to the high production of Reactive Oxygen species (ROS) and harmful alterations to the cell membrane. In this context, the objective of the present study was to supplement the extender with Vitamin C (VitC) and Cholesterol (CLC) or a-tocopherol (TLC) loaded in Cyclodextrins, to explore the quality of rabbit semen preserved at 4°C for 48h.
METHODS
Rabbit semen was collected, pooled and treated with CLC (2.5 mg), TLC (0.625 mg) and VitC (0.125 mg) for 80-100 million spz/ml ; and by different duals (CLC+TLC), (CLC+VitC), (TLC+VitC) and (CLC+TLC+VitC). We analyzed motility kinematic parameters by a Computer assisted semen analysis (CASA SPA®); and the oxidative status by measuring the amount of lipid peroxidation (TBARS).
RESULTS
Results showed a diminution of motility during preservation. However, when compared to the control, CLC and TLC treatment significantly (P<0.05) improved kinematic parameters after 24 h of preservation at 4°C. Curvilinear velocity (VCL) and average pathway velocity (VAP) in TLC (56.0±23.9 and 32.2±15.3 µm/s, respectively) and CLC-TLC (VCL 57.9±22.5, VAP 36.4±15.3 µm/s) treatments were significantly higher (P<0.05) than the control (VCL: 46.9±24.0, VAP: 24.8±14.7 µm/s). After 48 h, VAP (34.9±17.0 µm/s) was significantly higher (P<0.05) in TLC-TLC-VitC treatment than in the control (22.9±11 µm/s). With regard to the oxidative status of refrigerated semen, TBARS levels at 0 h were similar in all treatments. In addition, no significant increases in TBARS levels were observed between treatments and the control after 24 h or 48 h of storage.
CONCLUSIONS
We observed that cyclodextrins complexes (CLC-TLC) ameliorated the quality of refrigerated semen after preservation at 4°C for 24 h. This effect was highly attributed for higher solubility of cholesterol and a-tocopherol by mean of cyclodextrins and to the complementary protection mechanisms of these two active molecules against the deterioration caused by low temperature shock.

W159
EFFECT OF DIFFERENT CRYOPROTECTANTS AT DISTINCT COOLING TIMES AT 5 ° C ON STALLION SEMEN CRYOPRESERVATION

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BACKGROUND-AIM
The aim of the present study was to evaluate the effect of different penetrating cryoprotectants on sperm characteristics at distinct cooling times at 5 °C.
METHODS
Stallions (n=9) were collected 3 times by artificial vagina and the ejaculates were divided into 4 aliquots. Each aliquot was diluted 1:1 with the extender BotuSemen Special®, centrifuged at 600g-10 min, then the pellet was resuspended with egg yolk extender (BotuCrio®) and added 4 different cryoprotectants: (GLY) glycerol, (MF) methyl formamide, (DMF) dimethyl formamide and (DMA) dimethyl acetamide, at a concentration of 5%. Semen was packed in 0.5mL straws at a concentration of 100x106 mobile sptz/mL, cooled at 5°C inside a refrigerator (Minitube®), where they remained at the following times: T1=0, T2=5, T3=10, T4=20, T5 = 60 and T6=90 min. Subsequently, the straws were placed 4 cm above the liquid nitrogen level for 20 min, then immersed in the liquid nitrogen and thawing was performed at 46° C for 20s. Sperm kinetics were analyzed by CASA (IVOS Version 12 Hamilton Thorne Research, MA, USA) and membrane integrity (PMI) by fluorescent carboxyfluorescein diacetate and propidium iodide.Data were analized by SAS followed by Shapiro-Wilk and Chi-Square test using Proc GLM.
RESULTS
The result of total and progressive motility post-thawing did not differ (P <0.05) between the cooling times for the same cryoprotectant. However, there was a difference (P <0.05) in total motility in T6 GLY (59 ± 18A) vs DMA (51 ± 14B). Likewise, progressive motility significantly differed between GLY vs MF, DMF and DMA respectively at T1 (25 ± 14A; 14 ± 9B; 14 ± 3B; 13 ± 5B), T2 (28 ± 13A; 15 ± 7B; 15 ± 2B; 12 ± 4B), T3 (26 ± 14A; 14 ± 9B; 13 ± 2B; 11 ± 5B), T4 (27 ± 14A; 14 ± 8B; 14 ± 3B; 12 ± 5B), T5 (26 ± 14A; 13 ± 8B; 13 ± 3B; 11 ± 4B) and T6 (27 ± 13A; 17 ± 17B; 14 ± 6BC; 9 ± 4C) and MF vs DMA in T6. There was a significant difference in PMI at T1 MF (34 ± 10B) vs DMF (44 ± 9A) and at T4 DMF (46 ± 7A) vs DMA (38 ± 10B).
CONCLUSIONS
In conclusion, the freezing extender with GLY presented better progressive motility, while DMF presented the best membrane integrity compared to the other cryoprotectants.

W160
EFFECT OF CONCENTRATION DECREASING ON EQUINE SPERM FREEZING: ANALYSIS OF PERFORMANCE FOR LOW-DOSE INSEMINATION TECHNIQUES.

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BACKGROUND-AIM
Cryopreserved equine semen has been traditionally stored using high sperm concentrations (100-400 x 106 sperm/ml) for conventional artificial insemination (AI), requiring a large number of sperm. However, novel assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), do not require such a large number of sperm, and lower concentrations are more efficient.
The objective of this study was to evaluate the effect of decreasing sperm concentration in cryopreserved equine sperm to 5 x 106 sperm/ml (CON 5), compared to 100 x 106 sperm/ml (CON 100). The ejaculate performance index (EPI) was also calculated in both sperm concentrations.
METHODS
A total of 12 ejaculates from two stallions were frozen at both sperm concentrations. Total (TMOT) and progressive (PMOT) sperm motility (by computer-assisted sperm analysis), intact-membrane sperm (IMS, by fluorescence microscopy) and normal sperm morphology (NF, by bright-field microscopy) were evaluated in fresh semen, CON 100 and CON 5. Ejaculate performance index (EPI), i.e. the number of potential inseminations per ejaculate and IA technique, was also calculated for each freezing concentration. All values were expressed as mean ± standard error of the mean (SEM). The differences were considered statistically significant when $P < 0.05$.
RESULTS
No significant differences ($P > 0.05$) were observed between freezing treatments (CON 5 vs. CON 100) for any of the sperm parameters evaluated: TMOT = 77.90 ± 1.73 % vs.76.68 ±. 2.91; PMOT = 34.99 ± 4.59 % vs. 35.01 ± 5.82 %; IMS = 84.59 ± 3.89 % vs. 84.55 ± 3.33 %; NF = 87.57 ± 2.24 % vs. 83.13 ± 4.14 %; respectively for CON 5 vs. CON 100. EPI was significantly higher ($P < 0.001$) for CON 5 (3843.67 ± 347.96) than CON 100 (192.18 ± 17.40) in low-sperm AI techniques, such as ICSI.
CONCLUSIONS
We can therefore conclude than it is possible to freeze equine sperm at 5 x 106 sperm/ml without decreasing the sperm quality. This concentration also yielded in a more efficient use of each ejaculate for novel low-dose AI techniques.

W161
COMPARING THREE EXTENDERS FOR CHILLED STORAGE OF EQUINE SPERM

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BACKGROUND-AIM
The objective of this study was to compare three semen extenders for their ability to protect sperm cells against various shocks and maintain motility of stallion spermatozoa during storage and transportation in 4°C used for artificial insemination.
METHODS
Semen was collected using artificial vagina from three stallions (4 replicates from each stallion; total: 12 ejaculates). Following gel extraction, the semen samples were assigned into three equal fractions and diluted (1:1) with commercial Z extender (VMD, Belgium), ASB extender (ASB means Horse in Persian language; an extender based on cow skim milk powder) and fresh cow skim milk. Diluted samples were kept in dark place for 30 minutes and then centrifuged at 600 x g for 20 minutes. Precipitated pellet was suspended in the same extender. Next, the samples were gradually cooled within one hour to 4°C and then transferred to refrigerator (4°C). Semen evaluation including total and progressive forward motility, livability using Eosin B Fast green stain and plasma membrane integrity using HOS test, was carried out after pellet suspension and 24 hours after maintaining at 4°C. Data were analyzed using GLM procedure following arcsine transformation in SAS.
RESULTS
There was no interaction between extender type and time of evaluation ($P>0.05$). At the time of pellet suspension, there was no difference between different extenders ($P>0.05$). The pooled data for total motility, progressive forward motility, live percentage and plasma membrane integrity of sperm were: 74.5±1.69, 52.3±2.43, 79.1±1.35 and 63.5±1.57, respectively. There was no significant difference between Z and ASB extenders in total motility (73.1±1.52 vs 72.9±1.5%), progressive forward motility (49.5±1.72 vs 48.2±1.72), live percentage (80.9±1.07 vs 78.7±1.22) and plasma membrane integrity (53.8±1.66 vs 58.4±1.68; $P>0.05$). Whereas, both latter extenders were superior to fresh cow skim milk (total motility: 64.8±2.64, progressive forward motility: 39.5±2.48, live percentage: 73.5±2.1 and plasma membrane integrity: 52.4±2.2; $P<0.05$).
CONCLUSIONS
In conclusion, ASB extender could be considered as suitable extender to substitute Z extender for short-term preservation of stallion semen.

W162
INFLUENCE OF THE POLYMORPHISMS OF THE MELATONIN RECEPTOR 1A (MTNR1A) GENE ON RAM SPERM RESPONSE TO MELATONIN DURING THE NON-REPRODUCTIVE SEASON. A PRELIMINARY STUDY.

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BACKGROUND-AIM
Melatonin is a hormone mainly synthesized by the pineal gland, which is involved in regulating many biological processes, such as seasonal reproduction in ovine. This hormone can exert some of its functions by binding to specific membrane receptors, MT1 and MT2. The melatonin receptor 1A (MTNR1A) gene exhibits several polymorphic sites, and certain RsaI (g.17355458G>A) genotypes have been related to an advance in the mating activity of ram-lambs or a more intense reproductive behaviour in adult rams. However, the influence of those genotypes in sperm quality and sperm response to melatonin remains unknown. The aim of this work was to evaluate whether RsaI polymorphisms of the MTNR1A gene influence the ram sperm quality after in vitro capacitation and the response to in vitro added melatonin.
METHODS
During the non-breeding season, spermatozoa from 6 previously genotyped Rasa Aragonesa rams were selected by swim-up and incubated for 3 hours under capacitating conditions (39 °C, 5% CO2 and 100% humidity) in TALP medium with cAMP-elevating agents, alone (control) or with 1 µM melatonin. After incubation, motility, viability, intracellular levels of reactive oxygen species (ROS), apoptotic markers (phosphatidylserine (PS) inversion and caspase activation) and capacitation state were assessed. Statistical analysis (chi-square test) was performed using IBM SPSS Statistics v.26.
RESULTS
After the incubation with the hormone, only G/A and G/G genotypes showed the decapacitating effect of 1 µM melatonin, previously described by our group, with an increased percentage of non-capacitated sperm cells and a decreased capacitated spermatozoa rate, compared with the control samples. On the other hand, the A/A genotype showed higher rates of motile, viable cells without PS translocation and viable cells with low levels of ROS in samples incubated with 1 µM melatonin.
CONCLUSIONS
This preliminary work suggests that the spermatozoa carrying the A/A genotype of the RsaI polymorphisms of the MTNR1A gene show no response to the decapacitating effect of 1 µM melatonin. However, this hormone maintains its antioxidant and antiapoptotic effect on these cells.
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W163
THE PRACTICABILITY IN VITRO OF USE MODIFIED EQUIPURTM COLLOIDAL CENTRIFUGATION PROTOCOL BEFORE AND AFTER EQUINE SEMEN CRYOPRESERVATION.

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BACKGROUND-AIM
This study aimed to verify in vitro practicability of using modified EquipurTM colloidal centrifugation protocol equine semen cryopreservation.
METHODS
Ten stallions were used, 2 ejaculated from each animal (n=20). Ejaculates were diluted 1:1 and previously divided into two groups Control Group (CG) and Equipur Group (EG). For EG, 5 mL de EquipurTM were pipetted into a centrifuge tube and an aliquot (30 mL) of extended semen containing 2 billion sperm, centrifuged at 400g for 25 min, the supernatant was discarded and the sperm pellet was resuspended in cryoprotectant extender Botucrio® in a concentration of 70 million sperm/mL. For CG, the semen was centrifuged at 600g for 10 min, supernatants were discarded, and pellets were resuspended to obtain 70 million sperm/mL. After filling, the straws (0.5 mL) were kept at 5 °C for 20 min; transferred to a thermal box containing liquid nitrogen kept in nitrogen vapor for 20 min, 6 cm above the level of liquid nitrogen and immersed in liquid nitrogen, stored in racks, and stored in a cryogenic cylinder at –196 °C. After thawing in a water bath at 37°C for 30 seconds, 1 straw from each group (CG and GE) was analyzed and another 15 from each group were pipetted in 2mL de EquipurTM and centrifuged at 400 x g for 25 min and re-suspended in a concentration of 70 million sperm/mL. forming two new groups: CG post equipure (CG+E) and EG post equipure (EG+E). Sperm concentration was realized to verify the sperm recovery rate (%) after the procedures with the Equipur about the initial concentration of 2 billion.
RESULTS
In the experiment, group EG+E showed higher (P>0.05) total % (80±2.8) and progressive motility %(51±2.5) when compared to other groups (CG-55±2.2 and 29±1.6; EG–66±2.4 and 37±1.4;CG+E-68±3.8 and 44±2.8, respectively total motility and progressive motility) and the recovery rate for equipur groups were EG=64±4.5, CG+E=38±3.9 and EG+E=37±3.5.
CONCLUSIONS
Thus, it can be concluded that the modified EquipurTM protocol used before and after the equine semen freezing procedure has in vitro viability, as it demonstrated a positive response in sperm kinetics, and even if the recovery rate is low about the total ejaculate, it is expected that the improvement in sperm quality will increase the fertility rate.

W164
SPERM CLUSTERING BASED ON MORPHOMETRIC PARAMETERS INDICATES BETWEEN-BULL VARIABILITY IN DROMEDARY CAMEL

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BACKGROUND-AIM
The objectives of this study were to describe sperm morphometrics in dromedary camels and investigate their variations among bulls.
METHODS
Sixteen ejaculates were collected from 4 fertile bulls,stained and studied under 1000X magnification.A minimum of 100 morphologically normal sperms in each slide was used for measurements by CellSense Dimensions software. The measurements included Head area (µm2),head length and width (L and W),tail length, midpiece length, total length, ellipticity(L/W), elongation(L-W/L+W), regularity(πLW/4A),tail/head area and Total length/area.Comparison among subjects was carried out by a GLM procedure and the variance component was used to calculate the repeatability (0 to 1) of parameters in different collections, defined as the proportion of variance attributed to the subjects.A multivariate K-means cluster analysis was carried out to classify sperms into 3 distinct subpopulations.The frequency distribution of sperms in clusters was compared among the bulls by chi-square test.
RESULTS
Results indicated high overall repeatability in sperm morphometrics within subjects over collections (>89.1% in all parameters).There was a significant inter-individual variation in all studied parameters with the length of the midpiece and total length showing difference among all four bulls (P<0.05). Characteristics of sperms classified in clusters were as follows:Cluster 1 included sperms with the highest average head area (18.87;P<0.001),shortest midpieces(5.88µm;P=0.01),and least elliptical heads (1.61;P=0.033) while Sperms in cluster 2 had the lengthiest tails (34.61;P<0.004), lengthiest midpieces(7.59;P< 0.02) and the most elongated heads(25.12;P<0.003). Sperms in cluster 3 showed similar head area to cluster 2, similar tail length to cluster 1 but had longer midpieces and more elongated heads as compared to cluster 1(P<0.05).The highest proportion of short and round-headed sperms was observed in bull 1(P<0.000) while the proportion of long-tailed and elliptical sperms was significantly higher in bulls 3 and 4(P<0.05).The lowest proportion of sperms in cluster 3 was observed in bull 2 (P<0.001).
CONCLUSIONS
In conclusion,dromedary camel sperm seems to show consistent morphometric characteristics within each bull which can be applied to the identification of between bull variations.

W166
DYNAMICS OF CATTLE SPERM SNCRNAS DURING MATURATION, FROM TESTIS TO EJACULATED SPERM

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BACKGROUND-AIM
During epididymal transit, spermatozoa go through several functional maturation steps, resulting from interactions with epididymal secretomes. In particular, the sperm membrane is in constant interaction with epididymosomes, which also deliver sncRNA cargo to sperm. As a result, the payload of sperm sncRNAs changes during the transit from the epididymis caput to the cauda. This work was designed to study the dynamics of cattle sperm sncRNAs from spermatogenesis to final maturation.
METHODS
Comprehensive catalogues of sperm sncRNAs were obtained from testicular parenchyma, epididymal caput, corpus and cauda, as well as ejaculated semen from three Holstein bulls, by small RNA-seq.
RESULTS
The cattle sncRNA sperm content is markedly remodeled as sperm mature along the epididymis. Expression of piRNAs, which are abundant in testis parenchyma, decreases dramatically at epididymis. Conversely, sperm progressively acquires miRNAs, rsRNAs, and tsRNAs along epididymis, with regional specificities. For instance, miRNAs and tsRNAs are enriched in epididymis cauda and ejaculated sperm, while rsRNA expression peaks at epididymis corpus. Beyond the bulk differences in abundance of sncRNAs classes, K-means clustering was performed to study their spatiotemporal expression profile, highlighting differences in specific sncRNAs and providing insights into their putative biological role at each maturation stage. For instance, Gene Ontology analyses using miRNA targets highlighted enriched processes such as cell cycle regulation, response to stress and ubiquitination processes in testicular parenchyma, protein metabolism in epididymal sperm, and embryonic morphogenesis in ejaculated sperm.
CONCLUSIONS
Our findings confirm that the sperm sncRNAome does not simply reflect a legacy of spermatogenesis. Instead, sperm sncRNA expression shows a remarkable level of plasticity resulting probably from the combination of multiple factors such as loss of the cytoplasmic droplet, interaction with epididymosomes, and more surprisingly, the putative in situ production and/or modification of sncRNAs by sperm. Given the suggested role of sncRNA in epigenetic trans-generational inheritance, our spatiotemporal analysis may stimulate a study of sperm sncRNAs role in embryo development.

W168
EFFECT OF HETEROLOGOUS, HOMOLOGOUS AND AUTOLOGOUS SEMINAL PLASMA ON THE SPERMATIC QUALITY OF THE SEMEN OF STALLIONS SUBMITTED TO REFRIGERATION

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BACKGROUND-AIM
The aim of this study was to evaluate the effect of heterologous, homologous and autologous seminal plasma after cooling, on the semen of tolerant and sensitive stallions to refrigeration.
METHODS
Two stallions, classified as tolerant and one as sensitive, five ejaculates from each one was used, being divided into five groups; G0: semen not centrifuged; G1: semen centrifuged without addition of plasma; G2: semen centrifuged with addition of autologous seminal plasma; G3: centrifuged semen with addition of homologous seminal plasma; G4: semen centrifuged with the addition of heterologous seminal plasma (Equus asinus). After centrifugation the pellets were resuspended with milk-based extender or 20% of seminal plasma and 80% extender; setting the concentration of 30 x 106 sptz/ml for all groups. Samples were subjected to passive cooling in Botu-flex® model boxes for 24 hours at 5°C. Analyzes of sperm kinetics were performed by CASA (IVOS Version 12 Hamilton Thorne Research, MA, USA), whereas plasma membrane integrity (MPI), high mitochondrial potential (HPM) and superoxide generation by flow cytometry. Statistical analysis was performed by Kolmogorov-Smirnov, test ANOVA and Tukey test, P<0.05 was considered.
RESULTS
Overall, the G1 group (58.8±2.1; 41.9±2.7, respectively total motility and percentage of fast sperm) presented lower values compared to G0 (73.8±4.3; 65.9±4.3), G2 (76.8±2.1; 66.7±3.3), G3 (75.2±3.8; 67.5±4.2) and G4 (77.9±2.1; 72±2.3). Progressive motility was reduced in G2 (42±3.2) compared to G4 (49.3±2.3) whereas G0 (42.5±3.2) and G3 (44.9±4.1) were similar to G2 and G4. However, G4 had lower IMP values (60±2.8) compared to G0 (70.8±3.8) and G3 (70.6±4.2), while G1 (67.3±4.7) and G2 (67.3±4.4) were similar each other and all groups; for HPM, G4 (63.6±3.6) had the lowest value and the others that did not show any difference between them (G0=80.6±2.3; G1=77.5±2.3; G2=76.8±2.4; G3=76.8±2.6); The superoxide generation G4 (32.9±2.2) presented higher values than the others (G0=18.5±2.2; G1=19.3±2.3; G2=22.6±2.7; G3=22.7±2).
CONCLUSIONS
The removal of seminal plasma was detrimental to sperm kinetics, the addition of heterologous plasma was prejudicial to MPI and HPM due to high superoxide generation, and adding homologous and autologous plasma did not improve seminal quality.

W169
THE EFFECT OF PROPOLIS EXTRACTS ON BOAR SEMEN VIABILITY AND MOTILITY PARAMETERS DURING LIQUID STORAGE FOR 72 HOURS

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BACKGROUND-AIM
Propolis is a mixture of substances used by honeybees to defend the hive. It has different types of biological activity in various cells and tissues of animal models, exhibiting a strong pharmacological effect, serving as an antioxidant source. The current study was carried out to investigate the protective effects of the propolis extracts supplementation on the sperm kinematics and plasma membrane functionality during the liquid storage of boar semen at 16 °C for 72 hours.
METHODS
At semen station 11 semen samples were collected from 11 boars, by routine semen collection procedure for AI, diluted with a long-term extender and supplemented without (control) or with different concentration of propolis (5 % and 10 % ethanolic (5EPE and 10EPE), 5 % and 10 % aqueous (5APE and 10APE) solutions) at a final concentration of 50 × 10 6sperm/mL. In the laboratory, viability (eosin-nigrosin staining) and subjective motility and objective sperm motility by sperm class analyzer (SCA) were assessed in all semen samples after one hour after dilution and 72 h of storage at 16 °C. In total, 110 tests for sperm viability and motility were performed.
RESULTS
The longer storage time showed the lower sperm motility and viability results in the all treated and non-treated samples. The results showed that aqueous propolis supplementation (5APE and 10APE) for storage time at 16 °C temperature resulted in a protective effect on cell membrane integrity, however the liquid storage of semen supplemented with 5EPE and 10EPE has a negative effect on sperm plasma membrane integrity. Sperm viability values in 5APE and 10APE were higher (61.64±11.32 % and 60.82±8.76 %) than in control group (49.18±12.71 %) at 72 h of storage (P<0.05). Objective sperm motility values in 5APE and in 10APE at 72 hrs also were found higher (82.61±11.72 % and 84.06±8.42 %) than in control group (82.59±8.81 %; P>0.05).
CONCLUSIONS
Our data showed that the aqueous propolis supplementation at 5 % and 10 % concentrations can improve boar semen motility and viability parameters during liquid storage at 16 °C for 72 hours and no protective effect was observed when 5 % and 10 % ethanolic propolis solution were used.

WORKSHOP 1 - OPU: HORMONES, TIME AND FOLLICULAR SIZE

USING FSH AND TIME TO OPTIMIZE OOCYTE QUALITY PRIOR TO OPU.

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The success of IVF depends mainly on oocyte quality which is highly associated with the follicular status at collection. Using OPU allows to prepare and select follicles of different physiological conditions and offers the possibility to optimize oocyte quality. FSH plays a role that is unique during folliculogenesis and this role is different from LH although some functions at the cellular level are similar. FSH must rise for the selection of follicles but physiologically decreases before ovulation which is a signal associated with the dominant status and the beginning of LH supported growth. Once the role of FSH is well understood, the way to use it to maximize the number of follicles that can be harvested with a competent oocyte inside is a matter of creating multiple dominant follicles. The timing and duration of FSH support depend on the initial population which can be manipulated by GnRH or DFR (Dominant follicle removal) and the size of the follicles present on the ovary. Recently pre-puber animals were also used OPU in bovine and such different hormonal environment requires an adaptation of the protocol prior to OPU. New evidences indicates that FSH timing is a clear determinant of oocyte an embryo quality and potentially the health of the offspring. Oocyte quality is dependent of follicle size and status and therefore sensitive to hormonal pre-treatment creating a link between the treatment and the outcome.

FOLLICULAR WAVE SYNCHRONIZATION PRIOR TO OVUM PICK-UP

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The in vitro embryo production (IVEP) is a useful tool when performing the selection and breeding of genetically superior animals. The number of in vitro-produced embryos has grown exponentially and, for the first time, recently was significantly higher compared to the number of embryos produced in vivo worldwide. With the advent of genomic technology, shortened breeding intervals, and increased selection accuracy, IVEP has attracted increasing attention for commercial use. Laboratories and companies have been seeking strategies that serve to optimize methods to use IVF in large-scale programs. Previous studies have shown that the quantity and quality of aspirated oocytes are essential factors for successful IVEP. Many alternatives can be employed to improve the process-seeking methods that assist in the recovery of better-quality oocytes and higher competence in OPU. In this context, the moment of the follicular growth wave during oocyte recovery may influence oocyte quality and competence, which is gradually acquired with follicular development. Thus, characterizing the physiology of the bovine estrous cycle, as well as the synchronization of the emergence of follicular growth wave pre-OPU, can help the outcome from IVEP due to the absence of atresia events. The follicular wave synchronization prior to OPU was recently observed to improve the embryo production rates, as well as the post-transfer conception rates, for the recipients. Moreover, other strategies such as the evaluation of the influence of antral follicle count (AFC), use the pre-OPU gonadotrophic stimulus and applying non-hormonal methods for selecting female donors, can be used in OPU-IVEP to obtain better results. Finally, there are a series of alternatives to be devised regarding the multifactorial interactions that interfere with the quality of bovine COCs to contribute to the practical application of OPU-IVEP programs.

FOLLICLE PRIMING BY FSH AND PRE-MATURATIONAL CULTURE TO IMPROVE OOCYTE QUALITY IN VIVO AND IN VITRO

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Nowadays there is strong demand to produce embryos from premium quality cattle, and we can produce embryos using oocytes collected from living premium animals by ovum-pick up (OPU) followed by in vitro fertilization (IVF). However, the developmental competence of IVF oocytes to form blastocysts is still not sufficient. The developmental competence of oocytes depends on the size and stages of follicles. Administration of FSH prior to OPU (FSH-priming) can induce the growth of multiple follicles, which would degenerate during the natural estrous cycle, and can promote oocyte growth and improve the developmental competence of oocytes. Furthermore, from the induction of ovulation using an injection of luteinizing hormone or gonadotropin stimulating hormone after FSH-priming, we can collect in vivo matured oocytes from ovulatory follicles, which show higher developmental competence into blastocysts than oocytes matured in vitro. However, the conventional protocols for FSH-priming consist of multiple FSH injection for 3 to 4 day, which is stressful for the animal and labor-intensive for the veterinarian. In addition, these techniques cannot be applied to IVF of oocytes collected from bovine ovaries derived from slaughterhouses, which are important sources of oocytes. In the presentation, we will review previous research focused on FSH-priming, especially for collecting in vivo matured oocytes and a simplified method for superstimulation using a single injection of FSH. Further, we will highlight further prospective to develop those treatments to improve oocyte quality. We also introduce the previous achievements in pre-in vitro maturation culture, which can improve the developmental competence of oocytes derived from non-stimulated animals.

WORKSHOP 2 - REPRODUCTION OF CULTURED FISH

REPRODUCTIVE PHYSIOLOGY OF ATLANTIC SALMON (SALMO SALAR) – APPLICATIONS FOR SUSTAINABLE AQUACULTURE.

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Aquaculture of Atlantic salmon (Salmo salar L.) has grown enormously, from the production of several thousand tons in the 1970´s to 1.4 million tons in 2020. At present, the total revenues from salmon culture exceed those from fisheries, which makes aquaculture one of the major sources of income in Norway. Research on basic and applied reproductive physiology has been crucial in providing optimal conditions for broodstock management and the production of large salmon in sea cages. Early efforts to enhance production protocols included successful attempts to use photoperiod to control the timing of maturation to obtain eggs out of season. The discovery that continuous light applied to sea cages in winter both improved growth and blocked sexual maturation was also a major contribution with significant impacts on feed economy and fish welfare. Today, new production facilities using recirculating aquaculture system technology are increasingly used to produce larger "smolts". However, an increased incidence of early puberty in males has been observed in these systems. As such, it is still crucial to understand the physiological mechanisms triggering puberty to develop new methods to prevent it.
Another important point related to reproduction is the potential for genetic interactions between escapees from salmon farms and wild salmon stocks. Domestic escapees are genetically maladapted for a life in the wild but still participate in natural spawning events leading to dilution of the wild gene pool. In addition, those wishing to use/develop genetically modified strains require methods to ensure they are not inadvertently transferred to wild stocks or utilized by competitors. A possible solution to mitigate all these issues is to produce sterile salmon.
To approach the above mentioned issues, we have worked extensively with potential approaches to delay maturation, by manipulating environmental conditions in combination with genetic selection to delay maturation. To induce sterility in salmon, we have studied and tested a range of methods, including the use of triploidy, vaccination and gene editing. We have also developed single sex lines, surrogacy, and clonal lines as new breeding techniques to help address the current challenges around salmon reproduction.

THE REPRODUCTION OF EUROPEAN EEL IN CAPTIVITY: A SCIENTIFIC CHALLENGE WITH MULTIPLE FACETS

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An intense decline of the European eel (Anguilla anguilla) populations has caused its inclusion as "Critically Endangered" on the Red List of Threatened Species, by the International Union for Conservation of Nature (IUCN). The EU categorized the eel as critically endangered and established measures for the recovery of the stock in 2007. In the same year, it was included into the Appendix II of the CITES, prohibiting international trade of European eel, including the exportation of glass eels to Asia, to "avoid utilization incompatible with their survival''.
At the same time, the European eel is a demanded species with high market values, which is intensively fished at all its life stages, including glass, yellow and silver eels. Moreover, wild glass eels are still the base of the eel aquaculture, which supplies most of the eels consumed.
Nowadays, reproduction in captivity seems the only realistic alternative to reduce the pressure on natural populations and supply glass eels to eel farms. During decades, many different research aspects, approaches and techniques have been used in the attempt of reproducing eels. Results have evidenced the high complexity of the reproductive physiology of the eels, but many positive results have arrived from the improvement of broodstock selection, the design of specific diets (for breeders and larvae), the assay of many different hormonal treatments, the use of environmental factors, the development of techniques for the gamete evaluation and handling, or specific breeding and hatchery methods. The development of biotechnology techniques, such as production of recombinant hormones, germ cells xenotransplantation, or spermatogonia and sperm cryopreservation, can be considered as alternative ways, or at least complementary tools.
However, despite the evident interest in this species, many improvements are still required to reach a sustainable commercial scale production of glass eels in captivity. This is still an exciting open scientific challenge for fish researchers.
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THE MALE CONTRIBUTION TO REPRODUCTIVE SUCCESS: PATERNAL TRANSMISSION OF ENVIRONMENTAL EFFECTS TO THE SPAWN

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The ability to control the reproductive cycle of fish is a challenging task. The success of the process requires engendering good quality spawns which generate healthy larvae. The gametes provide the zygote with the genetic and epigenetic programs that enable them to deal with development. Nevertheless the sperm role has been systematically minimized, ignoring that the spermatic information has a huge impact on the health of the progeny. Factors such as nutrition, water quality or some industrial practices may have an effect on the sperm genome and epigenome with further consequences on the reproductive success.
Studies on the paternal transmission of environmental effects to the progeny will be summarized. Most of the assayed factors (cryopreservation, UV radiation, or presence of endocrine disruptors in the water), both in the ejaculated sperm or in the adult males during spermatogenesis, increased DNA strand breaks in the spermatozoa. DNA damage response enhanced in the embryos after fertilization is dependent on the extent of spermatic damage and on the egg quality. Moreover, fish show a remarkable capacity to tolerate unrepaired genetic damage during development. This tolerance results in increased rates of malformed larvae at hatching, revealing the importance of sperm DNA damage on the reproductive outcome (Fernandez-Díez et al 2018, Lombó et al 2019). In addition, the presence of endocrine disruptors -such as the plastic's component bisphenol A- in the water, modifies the epigenetic profile of spermatozoa, which is inherited by the non-exposed embryos (Lombó et al 2019, 2021) leading to changes in the expression of developmental genes and to increased ratios of malformations (Lombó et al 2015, 2021).
The performed studies confirm the vulnerability of the sperm genome and epigenome to the male breeding conditions. These effects are undetectable by the standard methods of sperm quality assessment, highlighting the importance of developing more accurate biomarkers.
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A STATE-OF-THE-ART REVIEW OF SPERM MOTILITY SIGNALING IN FISHES

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Background-Aim
Broodstock management is a prerequisite for seed production in fish farming. In case of male broodfish, understanding physiology of sperm motility provides valuable information to ensure successful oocyte fertilization, and to develop methods for artificial insemination and for sperm storage. This is a state-of-the-art review in sperm motility signaling in major groups of freshwater and marine fishes.
Methods
Sperm of various freshwater and marine fish species were collected, and the effects of osmolality, ions, and pH were investigated on motility initiation.
Results
Osmolality of the seminal plasma is a key factor to maintain sperm in the quiescent state in fishes. However, K+ ions prevent sperm motility in salmonid and sturgeon fishes. At spawning, sperm motility is initiated in hypo-osmotic and hyper-osmotic environments in freshwater and marine fishes, respectively. Duration of sperm motility is short, lasting for a few seconds to few minutes in most fishes due to rapid depletion of energy required for the beating of the motility apparatus called axoneme. In the osmotic-activated sperm, K+ and water effluxes occur in freshwater and marine fishes, respectively, which trigger sperm motility signaling. In general, initiation of axonemal beating is associated with an increase in intracellular Ca2+ ions in sperm of both freshwater and marine fishes and a post- or pre-increase in intracellular pH, while cAMP remains unchanged. However, axonemal beating is cAMP-dependent in demembranated sperm of salmonid and sturgeon fishes. Calcium from extracellular environment or intracellular stores supply required Ca2+ concentration for axonemal beating. Several axonemal proteins have been so far identified in fishes that are activated by Ca2+ and cAMP, directly or mediated by protein kinase C and protein kinase A, respectively.
Conclusion
Our current knowledge shows that environmental osmolality is a key factor to either maintain spermatozoa in the quiescent state in the reproductive organ, or to trigger motility initiation at spawning. However, regulatory signals in sperm motility initiation are diverse in fishes. In this context, physiological functions of ion channels and intracellular messengers in sperm motility signaling await further elucidation.

WORKSHOP 3 - PREGNANCY AND PERINATAL CARE

MATERNAL TRANSMISSION OF MICROBIOME IN MAMMALS

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Different animals have specific microbiomes that colonize niches within and on their body. Microbiomes have coevolved with their host to increase its fitness and performance. Microbial components are transmitted from parent to offspring by a variety of methods, strictly related to the anatomy and physiology of the host, including eggs contamination, coprophagy, direct contact during and after birth, lactation, and other ways through the environment. Vertical transmission from the mother to the offspring during and after delivery, as well as during milk suckling, is the most studied microbiome transmission process in mammals, especially in humans. For instance, it is estimated that half of the infant's microbiome at birth derive from the gut, vagina, skin, mouth and colostrum of the mother, with the gut having the most relevant impact on the newborn microbiome composition. Such portions increases during the following months, especially if the newborn is breastfed. The process of microbiome assembly during the first phases of life is crucial for ensuring the appropriate mutualism between host and microbial components during the whole life, contributing to health maintenance as well as, in the case of production animals, high productivity. This has brought to an increasing research interest towards early microbiome acquisition and subsequent development, as well as potential opportunities for intervention, both in humans and animals. Indeed, the likelihood of manipulating the gut microbiome, which is the most studied in relation to the host's health, is higher in early life because of its flexibility, and has more chances to exert profound effects along all subsequent developmental stages. Potential strategies for gut microbiome modulation in production animals cannot withdraw attention from a deep knowledge on the adaptation of maternal microbiomes to offspring production and feeding, which is functional to mother-to-offspring transmission of microbial components and to a successful acquisition and development of offspring microbiomes. The gaining and exploitation of such knowledge, still sparse in animals, is deemed crucial in a global scenario in which animal health and productivity must be ensured but the usage of antibiotics should be diminished.

WORKSHOP 4 - PLURIPOTENT EMBRYONIC STEM CELLS: PAST, PRESENT AND FUTURE

HISTORICAL PERSPECTIVES ON ESC AND IPSC IN COMPANION AND DOMESTIC ANIMALS

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Pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) provide great potential as cell sources for gene editing to generate genetically modified animals, as well as in the field of regenerative medicine. Stable, long-term ESCs have been established in laboratory species such as mice since the 1980's. Yet, isolation of true pluripotent ESCs in companion and domesticated animals, in spite of considerable efforts by many laboratories worldwide, was not successful until recently; forty years after mouse ES cells were first isolated! It was only with the increased understanding of the complexity of early embryonic stem cells, and the identification of distinct pluripotent states, from naive to primed, that more generally applicable systems were developed. The combination of our greater understanding of signaling pathways, advances in reprogramming using exogenous transcription factors, and the utilization of small chemical inhibitors of key biochemical pathways, have led to the isolation of various states ES and iPSCs from a wide range of domestic animals including cattle and pigs. This presentation will cover the key milestones that have led us to this point and will act as an introduction to the state-of-the-art presentations.

CAPTURING PLURIPOTENCY IN BOVINE EMBRYONIC STEM CELLS: PERSPECTIVES FOR IN VITRO BREEDING

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We recently reported on the efficient derivation of embryonic stem cells (ESCs) from in vitro produced bovine blastocysts. Conditions for successful derivation of bovine ESCs were based on FGF2, MEF feeders and tankyrase/Wnt inhibition by IWR-1. Bovine ESCs were obtained in 3–4 weeks, showed high proliferation capacity and could be propagated by simple trypsinization. These ESCs retained stable karyotype, population doubling time and transcriptome profile over extended culture, and were able to form teratomas in vivo that contained tissues from the three germ layers. We have further optimized culture conditions to a more chemically defined, feeder-free, culture system; which should facilitate the use of bovine ESC for pluripotency studies and biotechnological applications. The availability of bovine ESCs combined with the recent developments in mouse pluripotent cell in vitro differentiation to functional gametes, open the door for novel breeding schemes that when combined with genomic selection, have the potential to significantly accelerate genetic progress. One such scheme, which we termed In Vitro Breeding (IVB), would use high genetic merit embryos to derive ESCs, which can be subjected to genomic selection so that cell lines with superior genetics are used to produce gametes in vitro, so that in vitro generated gametes would be used for producing the next generation of embryos. Based on results from mice, and IVB cycle could be competed in just a few months, instead of the current 2.5-year generational interval for cattle. The IVB cycle could be repeated multiple times, which given the highly reduced generational interval, could result in genetic progress accelerated by a factor of 7-10. For this scheme to be feasible, it is important to generate fertile gametes from bESCs. We have tested multiple conditions for the in vitro differentiation of bESCs towards primordial germ cells (PGC), which are the progenitor of both sperm and oocytes. Successful conditions so far have resulted in upregulation of PGC markers such as NANOS3 and PRDM1. Future studies will characterize the in vitro differentiated cell population and test conditions for their differentiation to more advanced germline stages.

ESTABLISHMENT OF PORCINE EXPANDED POTENTIAL STEM CELLS

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Embryonic stem cells derived from the inner cell mass of preimplantation blastocysts differentiate into all embryonic lineages of any adult cell type. While mouse, monkey and rats ESCs were established, the derivation of stable, well-characterized ESCs from pigs remain problematic. The domestic pig is an excellent large animal in biomedical medicine and holds great potential for testing the clinical safety and efficacy of stem cell therapies. Most of the reported porcine ESC lines showed only some features of pluripotency, had limited proliferation potential, loss of pluripotency markers expression during passaging and had only limited differentiation capacity. Thus, published line usually do not meet with the stringent criteria for pluripotency and are frequently called "ES-like" cells. Recently we reported establishment of novel porcine ESC lines (Gao, Nowak-Imialek, Chen et al. 2019). The conditions containing small molecule inhibitors, the SRC inhibitor and the Tankyrase inhibitor, as well as additionally supplementation with activin A, LIF and vitamin C (Vc) were necessary for establishment of porcine expanded potential stem cells (pEPSCs). Porcine EPSCs can be generated from parthenogenetic, in vitro produced, in vivo derived and cloned transgenic embryos during 3 weeks. These cells formed compact colonies with high nuclear/cytoplasmic ratios, express key pluripotency genes and are genetically stable. Porcine EPSCs belongs to the state of 'expanded pluripotency' and demonstrated a broad propensity for extra-embryonic and embryonic lineage differentiation. In vitro differentiation and generation of mature teratomas showed the presence of derivatives of the three germ layers and contained trophoblast-like cells. Following injection into porcine blastocysts, pEPSCs demonstrated enhanced potential and incorporated into both trophectoderm and the inner cell mass of blastocysts. The chimeric embryos were implanted and derivatives of pEPSCs could be found in both embryonic tissue and in the placenta of fetuses day 26-28. Importantly, PGC-like cells (PGCLCs) can be induced from pEPSCs in vitro. The establishment of in vitro gametes and synthetic embryos 'blastoids' from porcine EPSCs will offer an exciting setup for In vitro-breading system in the future.

WORKSHOP 6 - REPRODUCTION IN CAMELIDS

NEW INSIGHTS TO PREDICT AND ACHIEVE BETTER SUPEROVULATORY RESPONSES IN DROMEDARY CAMEL

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Superovulation has been used for in-vivo (1, 2) and in-vitro (3) production of camel embryos. This could be achieved via a single injection of eCG (4), two injections of FSH, 48 hrs apart (5), and multiple injections of FSH or hMG (1, 2) in camel. The negative impact of using eCG and/or high constant doses of FSH is continuous recruitment of different generation of follicles resulting in the formation of follicles with different diameters that could interfere in the process of ovulation followed by the production of unovulated follicles, which in turn, interrupt with the process of uterine flushing and embryo recovery. In contrast, multiple injections of the right amount of FSH and/or hMG in 5.5 days program, starting with the high dose of gonadotropin at the time of follicle emergence, followed by a sudden decline in the next day, and then gradual decreasing of gonadotropin terminated 36 hrs prior to mating could result in predictive and consistent results (1, 2). We have demonstrated positive correlations between the ratio of follicles >6 mm/follicles ≤6 mm, detected on Day 4 of 5.5 days program, and the number of CLs and embryos in FSH treated camel ($r=0.9$; $P<0.05$). As a result, a great percentage of follicles >6 mm, detected on Day 4 of superovulation, ovulated and established corpora lutea (90%), and embryos (70 %). In conclusion, if we select the just protocol for superovulation in camel, considering the right amount, duration, and the way of gonadotropin administration, it is possible to predict and achieve better superovulatory response in camel. This, in turn, enables us to program the right number of required recipients prior to the day of embryo recovery.

Keywords: Superovulatory response; Prediction; Gondadotropin

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UNVEILING THE LUTEOTROPHIC ROLE OF BETA - NGF PRESENT IN THE SEMINAL PLASMA OF LLAMAS

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The well-established ovulatory effect of NGF mediated by the release of the preovulatory LH surge from the pituitary gland is determinant for the initial stage of luteinization and Corpus Luteum (CL) formation and function in llamas. The formation of the CL by intrauterine infusion or intramuscular administration of ®-NGF consistently results in higher progesterone output from early stages of CL development than those induced after GnRH administration. Moreover, it has been described a positive relationship between the magnitude of the LH peak and the following luteal function when females are treated with either ®-NGF purified seminal plasma or whole seminal plasma. In addition, we have observed that systemic concentration of NGF and LH increased during the first 3.5 h after administration of ®-NGF. Along with the LH effect, we cannot rule out a local effect of ®-NGF at the ovarian level that could potentiate progesterone production. In fact, the high affinity trk-A NGF receptor has been recently detected in granulosa cells of preovulatory follicle and in luteal cells of CL in both induced and spontaneous species. Indeed, the luteotrophic effect of ®-NGF was associated with enhanced tissue vascularization during the preovulatory period and early stages of CL development enhancing steroidogenesis. These vascular changes have been associated to a direct effect of ®-NGF on Vascular Endothelial Growth Factor in llama granulosa cells collected from preovulatory follicles. Supplementation of purified NGF to a llama primary culture of granulosa cells collected by ultrasound-guided follicular aspiration from preovulatory follicles lead to an increase of genes related to progesterone and angiogenesis synthesis, but also progesterone release which can be prevented by blocking a specific intracellular signaling pathway. We conclude that the luetotrophic effect of ®-NGF in llamas is potentially exerted by a central direct effect on the magnitude of LH secretion and by a local ovarian effect in the granulosa cells of preovulatory follicle enhancing corpus luteum formation and progesterone secretion.

WORKSHOP 7 - ADVANCES IN SPERM CRYOPRESERVATION TECHNOLOGY FOR CONSERVATION OF AVIAN GENETIC RESOURCES

RELATION BETWEEN CRYOPROTECTANT CONCENTRATION, SPERM FUNCTIONAL INTEGRITY AND FERTILIZING ABILITY OF FROZEN/THAWED CHICKEN SPERM

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Cryoprotectants and their concentration still are key choices for the success of sperm cryopreservation in birds. Permeant Cryoprotectants (PerC) are usually preferred in chicken semen in order to prevent sperm cryo-damages; at the same time, PerC must avoid any toxic effect on sperm and embryos, therefore the study of its concentration, dependent by the type of molecule used, is fundamental. The effect of different concentrations (0, 2, 4, 6%) of the PerC dimethylacetamide (DMA) and N-methylacetamide (NMA) on sperm quality, functions and fertilising ability was studied in chicken semen. According to analysis of variance, the progressive increase of the PerC concentration was associated to a concomitant significant progressive increase in sperm membrane integrity (0=12.3%, 2=22.3%, 4=28.7%, 6=39.4%), motility (0=18.1%, 2=29.8%, 4=38.0%, 6=52.9%), and progressive motility (0=0.9%, 2=3.8%, 4=7.1%, 6=11.2%). With DMA, the highest proportion of progressive motile sperm recovered after thawing was 51% achieved with 6%, whereas with NMA was 35% achieved with 4%. Acrosomal integrity in thawed sperm was not affected by the presence and concentration of PerC, whereas the proportion of viable and dead sperm with active mitochondria was significantly improved in presence of PerC (0=22.7%, DMA=43.6%, NMA=42.3%) with no difference between PerC concentration. Principal component (PC) analysis showed that PC1 and PC2 reported for 81.08% of total variance; the most relevant variables in PC1 were sperm motility (60.62%) and membrane integrity (46.33%) and in PC2 were viable (59.23%) and dead (59.23%) sperm with active mitochondria; these variables were clear discriminant between DMA and NMA frozen semen samples, being NMA the PerC most effective. Viable embryos were recorded after artificial insemination of semen frozen with 4 and 6% DMA and with 2, 4 and 6% NMA. PerC concentration significantly affected fertility and embryo viability in DMA semen, and 6% inclusion provided the best fertility (15%) and embryo viability (43%) values. In contrast, PerC concentration did not affect fertility and embryo viability in NMA semen, providing 12% and 50% mean values respectively.

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GENETIC RESOURCES ROLE OF ENDOGENOUS AND EXTERNAL FACTORS ON POULTRY SPERM CRYORESISTANCE

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Different endogenous and environmental factors may affect the sperm response to freezing-thawing process. Recent reports have showed that there was an influence of breed ($P<0.05$) on the percentage of viable sperm after freezing-thawing. Thus some breeds return the best freezing-thawing response (good freezers) unlike other with lower cryoresistance (bad freezers). The pattern of certain amino acids of seminal plasma seems play a role on sperm cryoresistance; there was a positive correlation between seminal plasma concentrations of some of them (e.g. valine) with sperm viability and DNA integrity. The presence of seminal plasma also affected sperm response to freezing-thawing process, and thus removal of seminal plasma decreased the variability of the results and DNA fragmentation damages. Environmental factors, firstly photoperiod but also temperature, influence on the seasonal variation in freezing damage in free range rooster sperm. No seasonal effects on sperm viability and motility fresh sperm quality were found. Neither did season affect the percentage of viable frozen-thawed spermatozoa nor the percentage with an intact acrosome. However, the collection season influenced ($P<0.05$) most frozen-thawed sperm motility values: the percentage of sperm showing progressive motility was higher in spring-collected sperm compared to winter-, summer- or autumn-collected samples ($P<0.05$); the curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP) values of spring-collected sperm were also higher ($P<0.05$). Social interactions are also important in the control of sperm quality. Compared to roosters with no-female-contact, the roosters living with hens showed higher ($P <0.05$) percentages of progressive motile sperm and increased ($P <0.05$) VCL and VSL values; these sperm samples with better initial quality returned higher post-thaw motility.

MEMBRANE LIPID REGULATION OF CRYOPRESERVED SPERM FUNCTION IN POULTRY

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Cryopreserved avian sperm often undergo serious damages which results in impaired fertilization ability. Due to the complex nature of functional deterioration, the initial trigger for cryodamage still remains elusive. It is widely accepted that cellular membranes are a primary site cryodamage of sperm. Previous reports from other and our groups have showed that sperm from different species experience phospholipid and sterol loss from the plasma membranes during cryopreservation, resulting in diminishment of freezing tolerance of sperm via increase in abnormalities associated with membrane structure and signal transduction pathways.

Sterol is the predominant lipids in sperm membranes and has diverse roles in physico-biochemical properties and structural toughness of membranes. Based on these protective effects along with the important finding of sterol loss from the plasma membrane, numerous studies have been performed in several species to test the effects of cholesterol addition to the sperm membranes before cryopreservation, and showed contrasting effect on post-thaw semen quality between species. Recent our study has showed that loading sterols with appropriate amount into chicken sperm enhanced their quality by inhibiting structural damages as well as apoptotic response and premature acrosome reaction, despite the adverse effect occurred in response to overloading.

How is the membrane alteration translated into functional deterioration? Membrane rafts are specific membrane microdomains enriched in sterols, ganglioside GM1 and functional proteins, and play important roles in regulation of diverse cellular processes. Sperm are no exception. Our results have shown that sterol loss induces membrane rafts disruption concomitantly with activation of apoptotic cascades and spontaneous acrosome reaction via inherent cascades, together suggesting the lipid regulation of cryo-injuries in poultry sperm. The presentation will describe a potential of membrane lipid regulation of cryopreserved sperm function in chickens towards improvement of cryopreservation methodology.

ADVANTAGES AND LIMITS IN THE IMPLEMENTATION OF A SPERM CRYOBANK FOR AVIAN GENETIC RESOURCES.

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During the last twenty years, progress in the avian reproductive biotechnologies allowed the constitution of germ plasm cryobanks developed in national programs. They are very important for the conservation and the management of genetic diversity.
Since the avian egg structure makes it impossible to cloning or freezing embryos in the current state of knowledge, three main types of avian germplasm are stored in cryobanks: semen, primordial germ cells (PGCs), and gonadal tissues.
The semen freezing success is a higher challenge in birds than in most mammals or fishes. Indeed, the avian sperm must show very high quality after thawing in order to keep the fertilizing capacity during a long in vivo storage in the female sperm storage tubules (weeks/ months depending on the species) before reaching the fertilization site. The high sensitivity of avian sperm and/or female tract to deleterious effects of usual cryoprotectants such as glycerol seems also to be quite specific of birds. For these reasons, and because artificial insemination is not routinely employed in poultry (except in turkeys), the progress in the constitution of semen collections for cryobanking has been slower than in many other domestic species. Conversely, a major interest of bird semen cryobanking is that high numbers of sperm are available in one ejaculate by non-invasive massage methods, and many ejaculates are available, allowing the constitution of very complete collections.
PGCs culture and freezing are a growing alternative approach, also used for transgenesis. It involves highly specific laboratory skills, is very expensive, invasive, and its efficiency stays lower than semen methodology. The freezing and transfer of gonads is another alternative method that encounters a certain efficiency, but faces specificities between breeds and needs high surgical and technical skills.
Consequently, semen cryopreservation is and will stay the most available, less expensive and less invasive approach for avian reproductive cryobanking. We suggest the constitution of wide semen collections as basis of all bird reproductive cryobanks, and complementary addition of more focused restricted collections of PGCs or gonads, depending on the availability and characteristics of the breed/line to be stored.

WORKSHOP 8 - IMPROVED MAMMALIAN EMBRYO PRODUCTION BY NOVEL BIOMIMIC-DESIGNED 3D AND MICROFLUIDIC CELL CULTURE APPROACHES

ENGINEERING THE FEMALE REPRODUCTIVE TRACT USING MICROFLUIDIC DEVICES PROVIDES NEW INSIGHT INTO OVULATION AND OVARIAN CANCER

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We have developed a dynamic microfluidic platform comprised of an integrated network of tissue-engineered organ models relevant to female reproductive biology. The fundamental biology of individual and multi-organ systems as well as the physiology of ovulation can be studied. High grade serous cancer (HGSC) originates in the fallopian tube and the risk of developing this cancer is related to the lifetime number of ovulations, such that things that reduce ovulation are protective. HGSC typically invade and colonize the ovary as part of primary metastasis. We have defined factors that contribute to ovarian specific metastasis of fallopian tube HGSC in response to ovulation. Physical tearing of the ovarian surface to mimic ovulation resulted in more murine oviductal epithelium (MOE) and HGSOC cells adhering to the ovary. MOE cells also adhered more to three-dimensional (3D) collagen and primary ovarian stromal cells than to ovarian surface epithelia, indicating that FTE cells adhered to the extracellular matrix (ECM) exposed during ovulation. In order to define the chemical landscape of cellular communication we have optimized an IMS protocol for incubating explant murine ovarian tissue in 3D agarose cell culture. Our findings indicate that the developed MALDI-IMS method is capable of detecting diffusible low mass chemical signals in 3D cell cultures at sites of interaction between ovarian tissue and fallopian tube cells. Compared to normal murine oviductal epithelial, we have found several upregulated mass signals that are only present when an ovary is embedded with cells that represent a tumorigenic cell model. Lastly, elevated testosterone in tubal function may explain an additional contribution to subfertility in women with polycystic ovarian syndrome and other but also contribute to transformation. Human fallopian tube epithelium exposed to the 2nM testosterone displayed slower cilia beating and decreased expression of the ciliary marker FOXJ1 when compared to stimulation with 0.8nM testosterone. Our tissue engineering platform and novel metabolomics measurements will allow us to test the hypothesis that small molecules may regulate pathways that influence tumorigenesis and metastasis from the fallopian tube to the ovary in the context of ovulation.

AIR-LIQUID INTERFACE MODELS OF THE OVIDUCT EPITHELIUM: ADVANTAGES, APPLICATIONS, AND CHALLENGES

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The lumen of the oviduct provides the optimal environment for fertilization and early embryonic development and is lined by a simple, columnar shaped, ciliated epithelium. The polarized phenotype of the epithelium is crucial for the formation of the oviductal fluid as well as for the transport and barrier functions of the tissue.
Cultivation of oviduct epithelial cells (OEC) under standard adherent submersed conditions, however, does not support proper basal-apical polarization. Compartmentalized culture systems in which polarization is promoted by growing the cells on porous membranes or gel cushions with nutritive supply from the basal cell side are a straightforward, easily applicable alternative. Differentiation can be enhanced by modifying the culture media in the apical compartment or keeping the apical cell side entirely free of growth medium, which is known as air-liquid interface (ALI) culture. OEC (even though not exposed to ambient air in vivo) demonstrate excellent polarization, barrier formation, and long-term cell survival under these culture conditions. ALI-OEC maintain hormone responsiveness and produce oviductal fluid surrogates (including extracellular vesicles) in the apical compartment. ALI cultures are experimentally accessible from both apical and basal compartments, and therefore allow the analysis of effectors regulating the early embryonic milieu.
It should be considered, though, that the characteristics and composition of the cell population at seeding have a major impact on the quality of the resulting in vitro-epithelium. When using primary cells, the number of input cells needed and biological variation affect reproducibility. Passaged cells and cell lines, however, often lack competence for full differentiation and might not represent the original cell composition. Progenitor cell expansion and organoid pre-culture can be used to overcome these limitations, but at the expense of the ease of the method.
In summary, ALI cultures of the oviduct epithelium have higher physiological relevance than standard 2D approaches. They are straightforward tools for exploring the microenvironment experienced by gametes and early embryos and can give insights on the optimization of media and conditions currently applied in assisted reproduction.

MICROFLUIDIC CELL CULTURE APPROACHES OVIDUCT-ON-A-CHIP: CONSIDERATIONS FOR DESIGN, FABRICATION, CELL CULTURE, AND THE IMPACT ON EMBRYO DEVELOPMENT

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Organ-on-a-chip approaches are of equal interest to basic researchers and clinical embryologists. They are promising tools for elucidating the intricate interaction of gametes and embryos with the female reproductive tract as well as for improving the quality and quantity of embryos derived from assisted reproductive techniques. However, the merge of established static air-liquid-interface cultures with 3D printing and microfluidic perfusion is not straightforward, and only a handful of oviduct/endometrium and uterus-on-a-chip platforms have been reported so far.
The biocompatibility of materials for the fabrication of the device is of primordial importance. Gametes, pre-implantation embryos and oviduct epithelial cells respond sensitively with developmental arrest or cell death to compounds leaching from acrylate/methacrylate-based resins that are routinely used for stereolithography. Toxicologic screening identified polystyrene and polydimethylsiloxane (PDMS) as viable alternatives for device fabrication.
An essential feature of an oviduct model is the presence of trapping structures for retaining the oocyte/embryo in close vicinity to the oviduct epithelium. Pillars are challenging due to the required high aspect-ratio and the flexibility of the so far used material, PDMS. Alternative trapping structures which allow for loss-free loading and retrieval of gametes and embryos for group or individual culture are currently being designed and tested.
Establishing a fully differentiated, secretory active epithelium in the microfluidic devices is yet another key aspect. To do so, flow rates must be optimized for the perfusion with media containing hormone mixtures and factors which steer the differentiation of the epithelium towards an in vivo-like mixture of ciliated and secretory cells.
A first generation of oviduct-on-a-chip devices already supported the idea of a more in vivo-like epigenetic reprograming of early bovine embryos. Yet, the crucial impact of oviduct epithelial cells and their secretions on metabolic and epigenetic reprogramming of the developing embryo is still to be fully demonstrated. Future improvements in platform design, perfusion and cell culture are ongoing to add more biomimetic features and yield the next oviduct-on-a-chip generation.

CRISPR-CAS9 EDITING OF ORGANS-ON-A-CHIP TO CREATE REPRODUCTIVE MODELS: A SEROUS TUBAL INTRAEPITHELIAL CARCINOMA-ON-A-CHIP.

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With approximately 152,000 annual mortalities ovarian cancer is the most lethal gynecological cancer worldwide. The serous tubal intra-epithelial carcinoma (STIC) is the initial oviduct transformation that is the precursor of the ovarian high grade serous carcinoma (HGSC) and modeling transformation of the oviductal epithelium in vitro may be key to advancing our understanding of the genesis of ovarian cancer. The use of organ-on-a-chip (OoC) technologies, make it possible to rapidly create and refine sophisticated in vitro models of various organs to study organ-specific physiology and/or examine aspects of disease and toxicology. Although the first OoC was created 12 years ago, the use of this promising technologies to create reproductive OoC started a decade later and only a handful of publications are currently presented. Besides, CRISPR-Cas9 revolutionized the field of genome editing, allowing the precise manipulation of the genome. The use of CRISPR for knocking in and out target genes offers a great opportunity to improve the efficiency of genetically engineered in vitro models. To expand the use of promising gene edited OoC disease models, we introduce a process for fabricating complex compartmentalized microfluidics devices using equipment which is ubiquitous in biological laboratories (scale, oven, and desiccator). By partially curing PDMS parts casted from 3D printed molds, we were able to bond and create multi-compartmentalized microfluidics devices. These devices were used to create a biomimetic oviduct-on-a-chip. Next, CRISPR-Cas9 was used to knock out the oncogene p53 in the dog oviduct-on-a-chip and create an in vitro model that recapitulated human STIC. The edited STIC-on-a-chip morphologically (loss of cell polarization and ciliation, and increased cell atypia and proliferation) and genetically (increased Ki67, PAX8 and Myc with a decreased PTEN and RB1 mRNA expression) mirrored the human STIC and HGSC. This platform will open new opportunities for understanding STIC progression to HGSC and to the identification of biomarkers for the early diagnosis of this disease. Moreover, gene editing the oviduct-on-a-chip can be used to improve our understanding of the gamete/embryo-maternal communication and, consequently, improve the outcomes of IVF.

WORKSHOP 9 - REPRODUCTIVE TOXICOLOGY IN MARINE MAMMALS

REPRODUCTION IN BALTIC SEALS - HISTORY OF ENVIRONMENTAL POLLUTION EFFECTS AND PRESENT STATUS

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The populations of both the Baltic ringed seals (Phoca hispida botnica) and the Baltic grey seals (Halichoerus grypus) experienced a collapse during the 20th century. Uterine stenoses or occlusions that caused sterility were common in both ringed seals and grey seals, which slowed down the recovery of the populations. Also, leiomyomas were not an uncommon finding in older females. Environmental pollutants are believed to trigger these reproductive lesions.

At the Swedish Museum of Natural History, Baltic seals have been necropsied for monitoring purposes since the 1970s. Difficulties in gathering ringed seal carcasses were mitigated when hunting was allowed again in a larger scale in 2015. The reproductive health of ringed seal females was assessed and compared with older data on bycaught ringed seals, grey seals and monitoring data on environmental pollutants.

The frequency of occlusions and stenoses has decreased since the 1970s, but at different rates in ringed seals compared to grey seals. In the ringed seal, occlusions are still found occasionally in old females. They are related to pregnancy and could be the result of fetal death and/or abnormal involution processes. Occasionally there are signs of metritis and bacteria can sometimes be cultured from uterine fluids. Corpus luteum activity could potentially also be a factor.

Pregnancy rates have increased over time, but during 2008-2018 it has been lower in ringed seals (76%) than in the grey seal (86%). The reason for this is unknown, but could be related to year skipping. Ringed seal females with post partum signs had thinner blubber layer than barren females ($p=0.001$, t-test), and during the first 3 months of the post-implantation period, non-pregnant females were thinner than pregnant ones ($p=0.05$, t-test) indicating that body condition is an important factor.

Trends of environmental pollutants such as PCBs and DDTs in juvenile ringed seals show mostly slowly decreasing trends, but since around the year 2000, some pollutants (for example dioxins) show stable concentrations. Recent data from adult ringed seals indicate that the pattern of the pollutant concentrations have changed since the 1970s and 1980s. Emerging pollutants and climate change calls for further monitoring of these species.

UNDER PRESSURE - THE SCIENTIFIC CHALLENGE OF STUDYING EFFECTS OF ENVIRONMENTAL POLLUTANTS ON THE REPRODUCTIVE SUCCESS OF HARBOUR PORPOISES (PHOCOENA PHOCOENA)

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All over the world, marine mammals as top predators face adverse anthropogenic threats such as e. g. the contamination of the oceans with harmful substances. Some of these influences have recently been proven to harm the health and reproduction of marine mammals. Although it is an area with a high amount of human interference with the aquatic ecosystem, the North and Baltic Seas serves as a natural habitat for the harbour porpoise (Phocoena phocoena). Since the reproductive biology of this species is still poorly understood yet, it is important to achieve a scientific baseline of reproductive parameters that might serve as reference values in the future. This study was designed to investigate baseline parameters of reproduction such as age at sexual maturity, seasonal pattern of reproduction and spermatogenesis. The gonads of 111 females and 115 males from the German and Dutch North and Baltic Sea were macroscopically and microscopically investigated using diverse histological techniques like H&E (haematoxylin and eosin), Masson's Trichrome - and PAS- (Periodic acid-Schiff-) reaction staining.. Furthermore, the reproductive capacity of the populations in the study areas was calculated by the integration of the results into demographic data. The results reveal an age at sexual maturity of five years in females and first signs of sexual maturity in males with an age of three years. The peak breeding season was found to occur in June and July, where most of the females showed tertiary follicles and ovulations and the testes of male individuals showed full spermatogenic activity with eight stages of spermatogenesis, which were reflected in a multi-stage-arrangement. During the peak-breeding season, missing germ cell generations have been detected in the seminiferous epithelium. This phenomenon might be related to physiological or pathological changes due to toxic influences and has to be investigated further, since no data about the toxicant load in the studied individuals were available. With this study, data about the reproductive biology of harbour porpoises has been generated and holds the potential to serve as a baseline for future studies that shall investigate anthropogenic influences on the reproductive success of this species.

WORKSHOP 10 - THE GAIT OF ASSISTED REPRODUCTION IN THE EQUIDS

AN EXTENSIVE CHARACTERIZATION OF HORSE OOCYTES SUGGESTS THE USE OF DIFFERENT CULTURAL STRATEGIES IN ORDER TO IMPROVE IN VITRO EMBRYO PRODUCTION

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Thanks to in vitro embryo production (IVEP), oocytes isolated post-mortem or by ovum pick-up (OPU) from live animals can be used to expand the offspring of valuable livestock. Such technology is especially useful in horses, due to the unreliable responsiveness of mares to superovulation. Our aim was to improve the success of horse IVEP to better exploit the female genetic resources. First we defined the oocyte meiotic and developmental competence and status of the chromatin within the germinal vesicle (GV) [1] according to the diameter of the follicle of origin. Notably the condensation of the GV chromatin is a marker of oocyte differentiation and developmental competence in most mammals [2]. Then we sought to improve the developmental competence of oocytes by culturing them in meiosis arresting conditions before undergoing in vitro maturation (IVM).
We observed that oocytes isolated from small antral follicles (SAFs, <1 cm) had lower blastocyst rate than gametes from medium antral follicles (MAFs,1-2 cm) (15 vs 29% P<0.05). However the ability of successfully undergoing meiosis I in vitro was not different. This observation suggests that the last step of oocyte differentiation, critical for the acquisition of developmental competence, was unaccomplished in oocytes from smaller follicles. In line with this hypothesis, at the time of retrieval oocytes from SAFs showed significantly less Condensed GV than oocytes from MAFs (28 vs 54%, P<0.05).
Cumulus enclosed oocytes (CEOs) from SAFs and MAFs were then cultured for 6 h with cilostamide, an inhibitor of phosphodiesterase 3, before undergoing IVM, intracytoplasmic sperm injection (ICSI), and embryo culture.
This cultural approach did not rescue the blastocyst rate of oocytes from SAFs. However we observed that embryos from prematured CEOs had more blastomeres in both the follicular classes (402±196 vs 283±169 in SAFs and 401±45 vs 243±80 in MAFs), even though a statistical significance was only achieved in MAFs.
These results suggest that prematuration might improve embryo quality and indicate that customized cultural approaches should be devised for different oocyte subpopulations.
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[1] Franciosi et al. Mol Hum Reprod 2012;18:243-52
[2] Luciano and Lodde. Oogenesis 2013;93-108

EMBRYO AND RECIPIENT MARE CYCLE CHARACTERISTICS AFFECT THE LIKELIHOOD OF PREGNANCY AND FOALING AFTER TRANSFER OF ICSI EMBRYOS

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Over the last decade, in vitro production of equine embryos via ovum pick-up (OPU) and intracytoplasmic sperm injection (ICSI) has become an increasingly popular way of producing foals from sport horse mares. OPU-ICSI is used to produce embryos from mares with poor fertility or in active competition, and to enable efficient use of stallions for which spermatozoa is in short supply. One major advantage of OPU-ICSI is that it can be performed year round, with the resulting blastocysts cryopreserved for transfer to a recipient mare during the following breeding season. However, there is variation in the time that individual embryos take to reach the blastocyst stage (6 to 12 days) and uncertainty as to what constitutes the ideal stage of cycle to transfer the embryo to a recipient. During 2016-2018, 264 embryos produced in a commercial OPU-ICSI program were transferred to recipient mares on days 3-6 after ovulation. Embryos were subdivided by age of oocyte donor and the time taken to reach the blastocyst stage (7 versus 8 days). The effects of embryo and recipient mare parameters on both initial (7 days after transfer) and ongoing (end of season) pregnancy rates were examined. Overall pregnancy rates were 72.3% (day 7 after transfer) and 59.1% (end of season) with 12.7% of pregnancies lost before day 42 of gestation and 6.8% at later stages. The preferred day to transfer a frozen-thawed ICSI embryo was day 4, with high initial (78.5%) and ongoing (69%) pregnancy rates; the more ideal uterine environment was also reflected by a larger embryonic vesicle at day 7 after transfer. Transfer to mares on day 3 after ovulation resulted in a similar initial pregnancy rate (76.6%) but was more affected by early embryonic losses such that the ongoing pregnancy rate fell to 53.1%. Day 5 and 6 recipients yielded significantly lower initial (49.2%) and ongoing (37%) pregnancy rates. The likelihood of establishing pregnancy also tended to be higher for embryos that reached the blastocyst stage at 7 days (compared to 8 days: p=0.06); this was compounded by a higher risk of pregnancy loss for the more slowly developing embryos (8 days) culminating in a 10% lower likelihood of yielding a live foal.

FATTY ACID COMPOSITION OF THE EQUINE SPERM PLASMA MEMBRANE AND ITS IMPLICATION FOR SPERM FUNCTION

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Understanding the mechanisms involved in changes of the lipid profile of spermatozoa during spermatogenesis and sperm maturation may be crucial for improving assisted reproductive technologies not only in horses but also in other species. In present projects, we characterized the expression of enzymes involved in fatty acid synthesis in the testis and epididymis of stallions at the RNA and protein level, hypothesizing that testis and epididymis contain an active lipid biosynthesis. We also determined sperm membrane lipid patterns in stallions in response to a dietary supplementation with linseed oil that is rich in the Ω3 polyunsaturated fatty acid (PUFA) linolenic acid. Key enzymes involved in PUFA synthesis were determined by mRNA expression (fatty acid synthase, Δ9-, Δ6-, Δ5- and Δ4-desaturases, and elongases 6, 5 and 2) in testis and epididymis collected from 2 year old healthy warmblood stallions (n=10). All enzymes were present in testicular tissue and along the epididymis, but mRNA expression differed among localisations. Protein localisation of FASN, FADS1, FADS2 and ELOVL5 was also determined by immunohistochemistry. In testes, FASN was present in Leydig and Sertoli cells, FADS1 in germinal cells and ELOVL5 in germinal and Leydig cells. In epididymis, FASN, FADS1 and FADS2 were expressed in the principal and basal cells, whereas ELOVL5 was only present in the principal cells of the caput. Enzymes were also detected in epididymal vesicles that are secreted from epididymal tissue by apocrine mechanisms. When the daily diet of fertile Warmblood stallions was supplemented with either 100 ml linseed oil (group LSO; n=6) or sunflower oil (group SFO; n=6) from January to August, sperm content of the Ω3 PUFA linolenic acid and Ω6 PUFA docosahexaenoic acid (C22:6 n-3) increased continuously ($p<0.05$) in LSO but not SFO stallions. Palmitic acid content was influenced by month and treatment ($p=0.01$). Results demonstrate an active PUFA metabolism in the testes and epididymis of stallions. PUFA content of stallion diet influences the fatty acid metabolism. Modulation of PUFA metabolism in equine testicular and epididymal tissue may be a key to improve sperm function and fertility in horses.

ASSISTED REPRODUCTION IN DONKEYS: A NEW CHALLENGE

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Donkey breeding activities in Europe are increasing, requiring the use of newer technologies in this species. Artificial insemination (AI) in donkey is used mostly for fresh semen, and only one paper reported the outcome of timed AI with fresh diluted semen stored up to 6 hours. AI with frozen semen has been studied in this species, but the pregnancy rates were very low, ranging from 0 to 36% while inseminating mares with donkey frozen semen results in much higher pregnancy rates: many research groups are trying to understand why. The re-extension of the thawed semen in seminal plasma (SP) resulted in a 61.5% pregnancy rate in one paper, but this result was never repeated. For preserving donkey genetics, it is possible to cryopreserve small sized in vivo produced embryos. Few studies are reported and are mostly focused on in vitro trials in order to assess the effects of different techniques on embryo quality. In a study conducted in our laboratories, embryos after vitrification were more damaged compared to fresh or refrigerated ones. Recently, studies showed that vitrification with Fibreplug devices using 7 mol/L ethylene glycol lead to better results than slow freezing, and that Cryotop vitrification after addition of non-permeable cryoprotectant agents seemed to improve survival rates. The first alive foals born after vitrification were reported by our laboratory with the birth at term of two healthy fillies out of 11 vitrified embryos transferred. Research into new cryoprotectants and timing, and their effect on survival rate after transfer needs to be continued, and new techniques for preserving large embryos should be investigated. In vitro maturation (IVM) and different media were tested for donkey oocytes. Metaphase II stage maturation was higher using TCM199-F12 and CR1aa media (69.1% and 62.2%, respectively). Transvaginal aspiration can be performed in jennies for ovum pick up (OPU). Literature on that is at his first steps, but it is reported that donkey oocytes seems to need a longer maturation time than horses (34 and 30 hours, respectively). The development of assisted reproductive techniques for donkeys remains a challenge and more studies are needed to better understand the possible applications of ARTs in this species.

WORKSHOP 11 - GONADOTROPIN SECRETION IN CATTLE AND SHEEP: NEW DISCOVERIES

INTRA-PITUITARY MECHANISMS UNDERLIE ANNUAL TIME MEASUREMENT TO REGULATE SEASONAL REPRODUCTION

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Seasonal reproduction is a fundamental strategy for the perpetuation of the species in animals living in temperate zones. This physiological adjustment to predictable annual changes in the environment requires the ability to measure time of year as a means to anticipate, and thus prepare for, the impending season in order to ensure that the offspring is born when conditions for survival are optimal. Daily changes in day length (photoperiod) are the primary external cue used by seasonal photoperiodic breeders to synchronise their annual reproductive cycles to the changing environment. The pattern of nocturnal melatonin secretion from the pineal gland acting in the pars tuberalis (PT) region of the pituitary is the translator of photoperiodic information.

We have identified that in sheep the PT decodes the melatonin signal through alternative splicing of the vascular endothelial growth factor A (VEGF-A) gene.

During the short days of winter (breeding season) the long duration of nocturnal melatonin secretion induces the production of anti-angiogenic VEGF-A isoforms (VEGF-A164b), which will act as paracrine factors in the pituitary pars distalis (PD) to stimulate gonadotrophin production and suppress prolactin release. In contrast, during the long days of summer (non-breeding season) the short duration of nocturnal melatonin secretion induces pro-angiogenic VEGF-A isoforms (VEGF-A164a) that will cause suppression of gonadotrophin production and stimulate prolactin release. The melatonin duration-induced differential expression of VEGF-A isoforms also controls the vascular plasticity of the hypothalamic-pituitary portal system, thus enhancing or reducing the humoral connection between the brain and the pituitary gland throughout the year. Additional work has shown that a similar system operates in the equine and hamster pituitaries, providing evidence that this is a conserved mechanism of adaptation across species.

Collectively, our results show that an angiogenesis-reliant, intra-pituitary system for annual time measurement conveys the photoperiodic signal readout from the melatonin sensitive PT to the endocrine cells of the PD to regulate seasonal reproduction.

DISCOVERIES OF NEW RECEPTORS ON BOVINE GONADOTROPHS: POSSIBLE NEW MECHANISMS TO CONTROL REPRODUCTIVE FUNCTIONS.

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Gonadotrophs, in anterior pituitary (AP), secrete LH and FSH to control reproduction. Classic mechanisms regulating gonadotropin secretion in females are: (1) stimulation by gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus and (2) suppression by nuclear-localized estrogen receptors genomically activated by estradiol. However, there is accumulating evidence that gonadotroph functions are regulated by a complex cross-talk among several membrane receptors. Indeed, utilizing RNA-seq, we showed that bovine AP expresses 259 different receptor genes; roles of most of them are still unknown. Our extensive knowledge on the panel of receptors present on the bovine gonadotrophs might help unravelling the pathophysiological mechanisms underlying reproductive disorders. Our studies demonstrated that four new receptors of interest (G-protein-coupled receptor 30 (GPR30), AMH receptor (AMHR2), and orphan G protein-coupled receptors 61 and 153 (GPR61 and GPR153)) are expressed on bovine gonadotroph plasma membrane. Briefly, we showed that: (i) GPR30 mediates rapid, nongenomic, and negative feedback of GnRH-induced LH secretion by estradiol via decreasing cAMP and activating the ERK pathway. Remarkably, GPR30 can also bind to other endogenous estrogens, estrone and estriol. Further, GPR30 can bind to zearalenone and its metabolites; thus, it might be involved in the mechanism inducing reproductive disorders associated with these feed contaminants produced by fungi worldwide; (ii) Binding of AMH to AMHR2 stimulates LH and FSH secretion. AMH secreted from follicles may, therefore, have actions in gonadotrophs, where, as other TGF-® family members, inhibin and activin, it can affect FSH secretion; (iii) GPR61 and GPR153 expression changes stage-dependently in gonadotrophs. Ligand of GPR61 alters LH and FSH secretion significantly; (iv) AMRH2, GPR61, and GPR153 co-localize with the GnRH receptor in lipid raft of bovine gonadotrophs membrane, providing a possible mechanism of integrating various signals from the hypothalamus and peripheries to modulate reproductive functions. In conclusion, the discovery of new receptors on bovine gonadotrophs opens a new paradigm of the mechanisms controlling reproduction in mammals.

CENTRAL BLOCKING OF ESTROGEN RECEPTORS USING ICI-182780 EXTENDS THE NEUROENDOCRIINE BREEDING SEASON IN SHEEP

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We tested the hypothesis that central blocking of estrogen receptors in mature, seasonally anestrous ewes will prevent the negative feedback of estradiol on the secretion of LH, thus lengthening the neuroendocrine breeding season. Ewes that had been ovariectomized (OVX) for at least two months were administered basal levels of estradiol (3-5 pg/ml) continuously via subcutaneous silastic implants. Estrogen receptor antagonist ICI 182780 (ICI) or vehicle was administered directly into the lateral ventricle of the brain of seasonally anestrous ewes for 60 days via Alzet Osmotic Minipump. Ewes were treated with ICI beginning about 2 weeks prior to the expected onset of anestrus to see if the breeding season could be extended (EXT), or approximately 6 weeks prior to the expected onset of the breeding season to see if the breeding season could be induced sooner (IND). Blood samples were collected at 15-min intervals for 6 h beginning prior to infusion of ICI and repeated every 14 days until the end of the infusion. Treatment with ICI extended the length of time that pulses of LH were secreted in the EXT OVX ewes compared to controls ($P<0.05$). Likewise, in IND OVX ewes treated with ICI, pulsatile secretion of LH began sooner than in control ewes ($P<0.05$). Increased secretion of LH was associated with a higher number of kisspeptin immunoreactive cells ($P<0.05$) and levels of mRNA ($P<0.05$) in the arcuate (ARC) nucleus of the hypothalamus compared to the contemporary OVX ewes during anestrus and similar to levels in OVX ewes during the breeding season. Expression level of estrogen receptor 1 (ESR1) mRNA in the ARC and other hypothalamic regions were similar among anestrous ewes, breeding season ewes, and ICI-treated ewes. We concluded that in mature ewes, central blocking of estrogen receptors reverses the negative seasonal feedback effect of estradiol on the secretion of LH. The mechanism by which the neuroendocrine axis becomes refractory to the negative feedback of estradiol during the breeding season is unclear but does not appear to be associated with changes in the expression of ESR1 mRNA.

WORKSHOP 13 - TISSUE INVASION MECHANISMS OF TROPHOBLAST IN VETERINARY SPECIES

MOLECULAR MECHANISMS OF EQUINE TROPHOBLAST DEVELOPMENT AND INVASION

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Chorionic girdle development in the mare requires a series of developmental events that transform a single layer of uninucleate trophoblast into a multi-layered tissue with increasing numbers of binucleate cells on the tips. Histological studies suggest that only these terminally differentiated cells enter the uterus indicating regulatory molecules exist to tightly co-ordinate differentiation and invasion. In our functional studies we have demonstrated roles for RhoA signalling in chorionic girdle trophoblast movement, BMP4 in differentiation of trophoblast and GCM1 in regulation of CGB, a key molecular marker and secretion product of differentiated cells. Whether any these molecules also have a role in co-ordinating movement and differentiation is not known. GCM1 is a cAMP regulated transcription factor, a pathway shown in other species to contribute to cell motility. We have tested the hypothesis that the cAMP pathway acts to co-ordinate differentiation and invasion of primary chorionic girdle trophoblast. Forskolin, which induces cAMP, significantly decreased total cell number ($p<0.001$) but had no impact on binucleate cell number. Forskolin induced movement of multinucleated cells to the edges of a uninucleate cluster (25 μM) or to move and detach into discrete zones (≥50 μM) but did not affect uninucleate cell movement. Forskolin induced changes were reversed in the presence of cAMP/PKA pharmacological inhibitor H-89. These results suggest that cAMP signalling regulates cell movement in a morphologically dependent manner. Further studies are required to determine if cAMP co-ordinates movement of binucleate trophoblast via GCM1 and/or other molecules.

GENETIC REGULATION OF EQUINE CHORIONIC GIRDLE DEVELOPMENT: LINKING INVASION WITH IMMUNOMODULATION.

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Background: In early horse pregnancy, the invasion of the endometrium by the chorionic girdle trophoblast cells results from the explosive transformation of non-invasive trophoblast cells of the equine chorion to a rapidly dividing, highly motile phenotype over the course of only a few days. Transcriptional profiling has identified a large number of genes that are either up or down regulated during the development of equine invasive trophoblast, but relatively little is known about the molecular mechanisms governing these dramatic changes in gene expression.
Methods: Recently, new methods have been developed for identifying regulatory elements in complex genomes. Chromatin run-on and sequencing (ChRO-seq) is a variant of global run-on and sequencing (GRO-seq), a technique that can precisely capture active transcription events in a cell population by focusing on engaged RNA polymerases at sites throughout the genome. A single ChRO-seq assay can reveal the location of thousands of non-coding functional elements, and in addition generate information on nascent transcripts equivalent to conventional RNA-seq.
Results: We performed ChRO-seq assays on biological replicates of equine invasive and non-invasive trophoblast samples recovered at day 34 of gestation from normal horse mares. We also tested equine CD4+ T-cells and liver using the same assay. This large combined dataset allowed us to simultaneously compare gene expression across these four tissues and to identify putative transcription factor binding sites associated with genes that were highly expressed in placenta, and also genes that were differentially expressed between invasive and non-invasive trophoblast.
Conclusions: In our experience the ChRO-seq assay is robust, reproducible, and sensitive, producing data equivalent to that acquired using the traditional methods of the ENCODE Project, but with greater precision, at lower cost, and with reduced effort. ChRO-seq also has the potential to identify new regulatory elements not detected by earlier assays. Our data on expression of immune system genes in the invasive trophoblast may provide insights into tolerogenic mechanisms that are essential to pregnancy maintenance and survival.

TALKING TO THE MOTHER'S IMMUNE SYSTEM: INVASIVE EQUINE TROPHOBLAST CELLS SECRETE PREGNANCY-SPECIFIC GLYCOPROTEINS WHICH ACTIVATE LATENT TGF®

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Pregnancy-specific glycoproteins (PSGs) are a group of closely related immunoglobulin superfamily members, which are specifically expressed by trophoblast cells. PSGs belong to the carcinoembryonic antigen (CEA) family, and there is growing evidence that murine and human PSGs have an immune modulating function. Interestingly, PSGs did only evolve in certain mammalian species i.e. various primate and rodent species, several microbat species and the horse. With the exception of the horse, these species have a hemochorial placenta which contains highly invasive trophoblasts. Therefore, we and others have speculated that a highly invasive phenotype of trophoblast cells foster the evolution of PSG genes. Indeed, in the horse, PSGs are expressed by chorionic girdle and endometrial cup cells, which are specialized trophoblast cells effectively invading the endometrium. The size and protein domain structures of PSGs differ enormously between mammalian lineages ranging from up to 9 Ig-like domains in rodents to only a single Ig-like domain in horses. This together with the specific genomic organization of PSG genes strongly indicate that PSGs evolved independently in each of the four mammalian orders. Thus, similar and overlapping functions of PSGs across species would point to a convergent evolution. Since human and murine PSGs activate latent TGF®, we hypothesized that equine PSGs may have a similar activity. We found that equine PSG CEACAM49 can directly bind to the latency-associated peptide (LAP), which then leads to the activation of TGF® Based on our results, we hypothesize that activation of latent TGF® in the environment of endometrial cup cells by equine PSGs secreted by invasive trophoblast cells, could contribute to the generation of regulatory T cells to maintain maternal immune tolerance.

WORKSHOP 14 - BOVINE REPRODUCTION

RECENT ADVANCES IN DAIRY RESEARCH THAT AFFECT REPRODUCTIVE MANAGEMENT IN CONFINEMENT AND GRAZING SYSTEMS.

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The dairy industry adopts new technologies derived from dairy research to improve production efficiency. Advances during the past decade include genomic technologies, in vitro embryo production, sex-selected semen, sensor technologies for detection of estrus, and timed AI programs. Genomic technologies enable genetic evaluation of animals from the embryonic through adult stages, accelerating the selection of cattle that possess desirable traits. Selection indexes rank bulls based on expected daughter profit, taking cognizance of local opportunities and constraints. A correctly weighted selection index can quickly improve reproduction without sacrificing production. Sex-selected semen enriched in X-chromosome bearing sperm facilitates breeding replacement females from the existing females with the best genetic merit. The carbon footprint of beef production can be reduced if dairy cows with poorer genetic merit are inseminated with beef semen, and the resulting calves enter the beef market. Oocytes can be collected from the best genetic merit heifers, fertilized in vitro with sex-selected Y-bearing sperm from elite sires, and embryos used to create the next generation of elite sires. Sex-selected semen has potential benefits in both confinement and grazing systems, but the short duration of the breeding period in grazing systems requires that either conception rates for sex-selected semen are similar to conventional semen or a strategy to mitigate reduced conception rates is implemented. Confinement systems are less constrained when using sex-selected semen because cows calve year-round and inseminations with poorer conception rates can be accommodated. Labor shortages and larger herd size are making robotics, sensor technologies, and timed AI desirable for efficient reproductive management. Public perception of acceptable practices can have a large effect on dairy herd management. Consumer concerns for the welfare of dairy bull calves may increase the use of sex-selected semen. Detection of estrus with sensors may replace the hormone injections used in timed AI. Future reproductive management programs will need to be highly effective, profitable, and sensitive to the concerns of consumers who are increasingly aware of management practices on dairy farms.

THE FINE LINE BETWEEN CLINICAL AND SUBCLINICAL ENDOMETRITIS IN DAIRY COWS

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Half of dairy cows develop one or more types of reproductive tract inflammatory diseases within 5 weeks after calving. Among them, clinical endometritis (CE) results upon uterine dysbacteriosis with increased relative abundance of Trueperella pyogenes that via the production of pyolysin evokes endometrial cell lysis, massive polymorphonuclear neutrophil (PMN) migration, and pyogenesis. As a result, CE is clinically defined as endometrial inflammation accompanied by purulent vaginal discharge. On the other hand, subclinical endometritis (SCE) is asymptomatic and has not been linked with bacterial infection. It is believed that SCE is a product of metabolic and inflammatory dysfunction that impairs the innate immune function and the ability of PMN to undergo apoptosis, necrosis, and ultimately abandon the uterine cavity. Interestingly, CE and SCE overlap in the time of diagnosis that is between 3 to 5 weeks postpartum. Thus, it is clear that CE and SCE are distinct manifestations of reproductive tract inflammatory disease. Negative energy balance, systemic inflammation, immune dysfunction, and changes in the composition of the uterine microbiota are all common phenomena that occur in the transition period of high-yielding dairy cows. These events are finely regulated in healthy cows, while maladaptation may result in reproductive tract inflammatory disease. Hence, avoidance of CE or SCE largely depends on the management of potential risk factors, but fundamental mechanisms behind the pathogenesis of those uterine diseases remain obscure.

WORKSHOP 15 - ADVANCES IN THE USE OF DOPPLER ULTRASONOGRAPHY IN CATTLE REPRODUCTION

APPLICATION OF COLOR DOPPLER ULTRASONOGRAPHY TO IMPROVE EFFICIENCY OF HORMONAL TREATMENT: ESTIMATION OF FOLLICLE AND LUTEAL ACTIVITY AND PREDICTION OF ESTROUS CYCLE.

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Backgraund:

Color Doppler ultrasonography (CDS) is a useful technique to evaluate ovarian blood flow (BF). In cattle, since the evaluation of ovarian BF can assess the function of corpus luteum (CL) and the atresia of follicle, estrous cycle can be estimated by CDS. Fixed-time artificial insemination (FTAI) programs were developed to increase insemination rate for cattle. Conception rate (CR) in FTAI are affected by estrus cycle at beginning of treatment. Therefore, prediction of estrous cycle by CDS may improve the efficiency of hormonal treatment in FTAI. The objective of this study was to clarify the effects of the prediction of estrous cycle using CDS on CR in FTAI.

METHODS:

Beef cows having large CL (>20mm in diameter) and large follicle (>10mm) were chosen for treatment. Cows were treated with Ovsynch (OVS) or Shortsynch (SS) for FTAI. The cow, having a CL and one follicle (estimated as first follicular wave), was treated with OVS. The cow, having a CL and two follicles (estimated as second follicular wave), was treated with SS. OVS was performed as follows; 1) cows were received GnRH, 2) PGF2< were injected at 7 days after GnRH, 3) GnRH were administrated on 56 hours after PGF2<, 4) cows were inseminated at 16-20 hours after 2nd GnRH. In SS, first GnRH in OVS was omitted, and then PGF2<, GnRH treatment and FTAI were performed same as OVS. Both OVS and SS treated cows were divided into three groups as follows; 1) CDS group in which estrous cycle were estimated based on CL function and follicular health by CDS, 2) USG group in which estrous cycle were estimated based on changes in size of CL and follicles measured by ultrasonography (USG) weekly, 3) unknown group (UN) in which size of CL and follicles were measured in only one examination with USG. Cows inseminated with estrus detection were considered as control group. Pregnancy diagnosis was performed at 30 days after FTAI.

RESULTS:

All cows with OVS or SS showed similar CR (65.9%) compared with control group (59.2%). CR in CDS groups (76.0%) and USG group (70.7%) were higher than UN groups (54.2%) or control group (59.2%).

CONCLUSIONS:

Prediction of estrous cycle by confirming the presence of a functional CL and healthy follicles having BF using CDS improve CR in FTAI.

LUTEAL AND UTERINE BLOOD FLOW AFTER ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN HEIFERS

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The objective of this study was to investigate if luteal (LBF) and uterine blood flow (UBF) could be used to predict a successful embryonic development in heifers after embryo transfer (ET) and to compare alterations in LBF and UBF between heifers after ET and AI. In 20 heifers AI was carried out on the day of estrus (= Day 0) and in 35 heifers ET was performed on Days 6/7. Doppler sonography was carried out on Days 7/8, 9/10, 11/12, 14/15, 16/17, 18/19 and 21/22. LBF was quantified using the coloured area of the CL and UBF was determined using the time averaged maximum blood flow velocity in the uterine artery ipsilateral to the CL. According to the sonographic pregnancy diagnosis (PD) on Day 25 heifers were allocated in 11 pregnant (PREG) and 9 non-pregnant (NPREG) AI-heifers and in 17 PREG and 18 NPREG ET-heifers. In contrast to PREG AI-heifers LBF decreased (P≤0.05) on Day 18/19 in NPREG AI-heifers. UBF was higher (P≤0.05) in PREG than in NPREG AI-heifers on Days 14/15. On the day of ET a successful embryonic development could neither predicted by LBF nor by UBF (P>0.05). In NPREG ET-heifers LBF decreased (P≤0.05) already on Days 16/17, while no alterations of LBF were observed in PREG ET-heifers (P>0.05). UBF was lower (P≤0.05) in PREG compared to NPREG ET-heifers on Days 14/15. On Days 21/22, PREG ET-heifers showed a lower UBF (P≤0.05) compared to NPREG ET-heifers. No difference (P>0.05) could be observed in LBF between PREG heifers after AI and ET, whereas LBF on Day 21/22 was twice as high (P≤0.05) in NPREG ET-heifers compared to NPREG AI-heifers. PREG ET-heifers showed a higher UBF than PREG AI-heifers on Days 9/10 (P ≤ 0.05) and UBF was higher (P≤0.05) on Day 14/15 in NPREG ET-heifers compared to NPREG AI-heifers. In conclusion, the success of an ET could not be predicted by LBF and UBF at the time of ET, but changes in LBF as well as UBF differed between PREG and NPREG heifers in the first weeks after ET and AI.

POTENTIALS AND FLAWS OF EARLY DIAGNOSIS OF NONPREGNANCY BASED ON CORPUS LUTEUM BLOOD FLOW

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Optimal calving interval is one of the main goals in reproductive management. In this regard, it is critical to identify as soon as possible which animals failed to conceive after breeding. Recently, our research group demonstrated that the evaluation of corpus luteum blood flow (CLBF) using color Doppler ultrasonography can be used to detect changes in CLBF related to luteolysis as early as on day 16 after AI, before any alterations in other CL morphological (size, echotexture) or functional (plasma progesterone) parameters. Moreover, the likelihood of successful pregnancy outcome was higher in cows ranked in the upper quartile for CLBF at day 16 (i.e., higher CLBF values) compared to those in the lower quartile, by an odds ratio of 32.8 (Siqueira et al. JDS 102:5612-22, 2019). In a previous study we were able to identify nonpregnant dairy cows at day 20 after AI using the CLBF (Siqueira et al. JDS 96:6461-72, 2013). This diagnostic approach had an overall accuracy of 74.8%. Yet, considering only the identification of non-pregnant cattle, both sensitivity and negative predictive values were high (99.0% and 98.5%, respectively). Thus, CLBF evaluation is a reliable diagnostic test to identify non-pregnant cattle at an early stage of pregnancy and provide consistent results (excellent inter-evaluator agreement). Later, studies from other groups observed similar results in beef cattle and embryo recipients. Nonetheless, a limitation of CLBF was the low accuracy in diagnosing truly pregnant cattle. In our study, the positive predictive value (PPV) was 65.1%. We estimate that more than half of the false-positive females were actually pregnant during the CLBF exam, but had early embryonic loss (EEL) before pregnancy confirmation 10 days later. Thus, PPV is likely to differ between dairy and beef cattle herds, depending on the EEL rates. Another important source of false-positives were asynchronous ovulation due to failures of the synchronization protocol, resulting in younger than expected corpora lutea. In conclusion, CLBF evaluation is useful to assess luteal function and for early pregnancy diagnosis between days 16 and 22 post-breeding. Anticipating CLBF evaluations to timepoints before day 20, however, would result in a progressive increase in false-positives.
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APPLICATIONS OF COLOR DOPPLER ULTRASONOGRAPHY IN COMMERCIAL FIXED-TIME PROGRAMS IN CATTLE

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The close relationship between the corpus luteum (CL) function and its blood perfusion determined by Doppler ultrasonography allowed the development of powerful strategies to improve reproductive efficiency in fixed-time artificial insemination (FTAI) and embryo-transfer (FTET) programs. We aim to discuss the novel prospects of using Doppler imaging and to explore the potential of its inclusion in fixed-time reproductive programs in cattle industry. Early resynchronization programs starting 12 to 14 days after FTAI or 5 to 7 days after FTET coupled with Doppler imaging for diagnosis of non-pregnant animals based on CL function were developed for beef and dairy cattle and have been implemented in commercial programs, mainly in South America. These strategies allow a reduction, mainly in beef herds, in the interval between two FTAI or FTET from ≈ 40 to ≈24 days and may improve the gains in reproductive efficiency when compared to traditional programs starting resynchronization after the pregnancy diagnosis at 30 days. Several hormonal combinations have been tested and indicated a reasonable cumulative pregnancy rate after three FTAI or FTET, in both nulliparous and parous females. The Doppler ultrasonography is also an innovative tool to be used for selection of suitable embryo recipients based on CL blood perfusion at the time of embryo transfer. The CL blood perfusion has a positive and linear association with pregnancy outcomes after FTET in beef recipients. This may increase fertility in FTET, as embryos would not be transferred to females with non-functional CL, and in cases with recipient surplus, females with higher receptivity would be prioritized. Therefore, the real-time determination of CL function by Doppler imaging can be an interesting tool for the veterinarian practitioners to reduce the interval to second service in non-pregnant animals and to enhance the pregnancy rate in fixed-timed programs

WORKSHOP 16 - EMBRYONIC DIAPAUSE

PUTTING PREGNANCY ON HOLD DOWN UNDER

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Embryonic diapause is a period of developmental arrest in which the embryo is maintained in a dormant state for an extended period of time, and then reactivates and continues development with no ill effects. Over 130 species of mammals undergo embryonic diapause but the molecular mechanisms that control it remain unknown. The tammar wallaby, Macropus eugenii, has the longest diapause of any mammal where the blastocyst normally remains in embryonic diapause for 11 months without cell division or apoptosis occurring. Progesterone regulates reactivation of the wallaby blastocyst by inducing active secretion from the endometrium, but how the endometrium controls the diapause and reactivated blastocyst is unknown. Initial transcriptomic analysis of the endometrium and blastocyst during diapause and reactivation from diapause has revealed a number of previously unexplored mechanisms of interest for further study. The diapause blastocyst transcriptome has provided clues towards how it could maintain viability in the absence of uterine secretions. For example, there is an increase in branched chain amino acid metabolism and in the levels of proteases, peroxisomes and lysosomes which could support blastocyst metabolism during diapause. Unexpectedly, despite the quiescent state of the diapause uterus, both the Epidermal growth factor (EGF) family and the Notch signaling pathway appear to have important roles during diapause in the endometrium, along with Wnt5a, but the effects on the blastocyst are unknown. Finally, ornithine decarboxylase 1 (ODC1), the rate-limiting enzyme for polyamine synthesis, was significantly upregulated at reactivation from diapause in the endometrium and multiple other polyamine pathway enzymes were detected in the endometrium and blastocyst. However, inhibition of polyamines in the wallaby did not completely prevent reactivation from diapause at the concentrations tested. Despite this, there did appear to be a role for polyamines at later stages of pregnancy at attachment, similar to their role at implantation in the mouse. Therefore, while polyamines still appear to have a role in reactivation of the tammar embryo from diapause, they do not appear to be the primary factors controlling reactivation in this species.

MOLECULAR CUES CONTROLLING EMBRYONIC DEVELOPMENTAL PACE

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The European roe deer (Capreolus capreolus) displays a prolonged period of embryonic diapause. This phenomenon decouples fertilisation and implantation, which take place in July/August and January, respectively. After diapause, the embryo rapidly elongates and implants. The pre-implantation uterine fluid (UF) supports embryo development, and contains species-specific diapause regulatory factors. In the roe deer, the key factors required for maintenance of diapause and resumption of embryo development at fast pace are still unknown. We hypothesised a regulation of cell proliferation via UF cues. Roe deer samples (endometrium, embryos, UF and blood plasma) were collected during regular huntings between September and January, the period of diapause and reactivation (n = 360 roe deer). Endometrial luminal epithelial cells (LE) (n = 56) extracted by laser-capture microdissection and single embryos (n = 87) were subjected to time-course transcriptome analyses. The UF amino acids (AA) (n = 186) were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The embryonic transcriptome analysis showed dynamic changes, while LE transcriptome changes occurred almost exclusively at elongation. UF mTORC1 activating AA increased with developmental progression. We hypothesise that this drives embryonic developmental pace as evidenced by a striking increase in embryonic mTORC1 activity prior to the resumption of embryo development. The embryonic transcriptome reflected the increased mTORC1 signalling by expression of genes related to the glycolytic and phosphate pentose pathway, the TCA cycle, and one carbon metabolism. The LE transcriptome changes coincided with embryo elongation and are indicative of increased uterine estradiol-17®-signalling. We emphasise the role of nutrient signalling in preimplantation embryo development and propose selective mTORC1 inhibition as the physiological regulatory mechanism regulating slow cell cycle progression during the period of decelerated embryo development in the roe deer.

EMBRYONIC DERIVED SIGNALS FOR COMMUNICATING WITH THE UTERUS: CLUES FROM STUDYING EMBRYONIC DIAPAUSE

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Implantation of the blastocyst into uterus is the gateway for further embryonic development in mammals. Programming of blastocyst to an implantation-competent state known as blastocyst activation is the determining factor for implantation into the receptive uterus. The implantation compete blastocyst can crosstalk with the uterine cell, especially the luminal epithelium, to initiate the attachment reaction. The molecular mediating this dialoged was not extensively explored. Based on the expression profile of blastocyst with different implantation competence and dynamic gene expression in the uterine, several potential candidates were identified for this communication between the uterus and blastocyst. The protein-soaked beads transfer is a well-used approach to define the effect of embryonic derived signals for communicating with the uterus. In the presentation, secreted factors such as TNFa, S100A9, Igf2, which mediated the crosstalk between the embryo and uterus will be discussed.

WORKSHOP 17 - NUTRITIONAL STRATEGIES TO IMPROVE FERTILITY IN SMALL AND LARGE RUMINANTS

MANAGING FOR OPTIMAL DAIRY COW FERTILITY: THE OOCYTE DESERVES CENTER STAGE

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Dairy industry is rapidly evolving, characterized by an increasing milk production per cow, growing herd sizes, inclusion of computer assisted herd management devices, environmental pressure and growing public opinion and awareness. Optimal dairy cow fertility guarantees cost-efficient milk production, a next generation with improved genetics and a limited environmental impact.
A very long list of management and animal factors impacts the odds of being fertile. Due to this complexity, many fertility recommendations are available, but they are rarely based on solid research and impact may vary between farms. However, the capital importance of hygiene, calving conditions, uterine health, metabolic health and oestrus detection in affecting fertility outcome are generally recognized as critical control points.
Within the complex reproductive physiology, the quality of the oocyte and the resulting embryo takes center stage. The oocyte proper senses the metabolic health state of the mother. Metabolic stress is reflected in the follicular environment and can directly lower the oocyte's developmental capacity. If impacted oocytes result in a day 7 embryo, quality is reduced. We performed embryo transfer experiments with these disabled embryos. Elongation is significantly retarded and interferon tau production on day 14 is reduced. Gene transcription analyses of these day 14 embryos revealed many aberrations. Thus, adverse metabolic conditions during oocyte maturation will lead to embryo mortality and thus to disappointing fertility results (Desmet et al., 2019).
30 years ago, Britt (1992) suggested that follicles developing during the period of NEB early post-partum could be affected and may contain an incompetent oocyte several weeks later at ovulation. This long-term 'carry-over' effect has now been substantiated in heat-stressed dairy cows (Roth 2008) and in cows suffering from metabolic stress early postpartum, affecting embryo quality three months later (Carvalho et al., 2014). We could mimic similar effects in vitro using long-term mouse follicle cultures.
These findings create a strong need for a different way of thinking when it comes to fertility management: the chance for successful pregnancy is pre-programmed many months earlier in the cow's life.

ROLE OF FATTY ACIDS ON FERTILITY IN DAIRY COWS

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Dairy cows are fed diets with moderate content of long chain fatty acids (LCFA) and, in most diets, LCGA represent 4 to 5% of the total dry matter, 9 to 13% of the digestible energy and 12 to 16% of the net energy for lactation consumed by dairy cows. One of the desired effects of supplementing diets of dairy cows with LCFA is a potential improvement in reproduction. Indeed, the literature supports increases in pregnancy per AI and maintenance of pregnancy with fatty acid supplementation, and a meta-analysis showed that supplemental LCFA starting in the transition period resulted in a 27% increase in the relative risk of pregnancy to an artificial insemination, which tended to reduce the interval to pregnancy. Nevertheless, fatty acids are functional molecules and changes in chain length, degree of saturation, and the stereochemistry of double bonds in the acyl chain of unsaturated fatty acids can markedly change their functionality and cellular effects. Numerous potential mechanisms have been described by which supplementation with LCFA might influence reproduction, and some of those effects are linked with specific fatty acid groups. Fatty acids impact ovarian follicle development, luteal secretion of progesterone, endometrial function, embryo quality, and conceptus elongation. Some of the mechanisms observed with supplementing LCFA to lactating dairy cows are not always replicated by experimental models using nonlactating animals or in vitro models. The effects of feeding LCFA seem to be irrespective of caloric provision because supplementation in early lactation do not improve energy balance or reduce losses of body weight or body condition. In fact, the beneficial effects of LCFA on reproduction are likely related to the type of fatty acid absorbed. The polyunsaturated fatty acids of the n-6 and n-3 families seem to have the most biological effects on reproduction in cattle. These fatty acids or products of their cellular metabolism can act through nuclear receptors such as peroxisome proliferator-activated receptor-gamma and influence elongation of the conceptus. Furthermore, n-3 fatty acids likely alter T-lymphocyte lineage in reproductive tissues which might result in more immune-tolerant phenotypes that favor maintenance of pregnancy

THE CRYOSURVIVAL OF BLASTOCYSTS SIGNIFICANTLY IMPROVES AFTER EXPOSURE TO OLEIC ACID DURING THE FIRST FIVE DAYS OF IVP

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Blastocysts originating from in vitro produced (IVP) embryos show a reduced quality in comparison to in vivo derived blastocysts. Developmental competence has primarily been related to oocyte quality, but embryo quality was demonstrated to be affected by embryo culture conditions. In the current study it is investigated whether quality of IVP embryos and thus cryosurvival is influenced by supplementation of free fatty acids (FFA) during the first 5 days of embryonic development, i.e. the physiological period that the embryo would reside in the oviduct.
Cumulus-oocyte-complexes (COCs) were collected from bovine slaughterhouse ovaries and 23h matured and fertilized (IVF=day 0; according to standard protocol). Embryos were cultured from day 1-5 in fatty acid free (FAF) SOF without (control) or with 25µM stearic acid (C18:0) or oleic acid (C18:1; n≥805 COCs per group), and from day 5-8 embryos in fresh FAF SOF. At day 8, grade 1 and 2 blastocysts (IETS) were slow frozen. Cryosurvival, expansion blastocoel 24h post-thawing, was compared with in vivo blastocysts (donated by CRV). By confocal microscopy the number of lipid droplets (LD540), total (Hoechst 33342) and damaged cells (EthD-1 and TUNEL) of blastocysts was determined. Statistical analysis (R version 4.0.5) was performed with a binomial logistic regression model for blastocyst rate, cryosurvival and proportion of damaged cells. A mixed effect regression model was used for number of lipid droplets and cells.
Blastocyst rates were lower after embryo culture in FAF SOF (23.7 ± 7.2%) compared to C18:1 (30.8 ± 8.4%). Interestingly, cryosurvival blastocysts of the C18:1 group with a high lipid content and the FAF SOF (respectively 70.1% and 67.4%), likewise those of in vivo blastocysts (68%), was significantly higher than the cryosurvival of C18:0 exposed embryos (17.6%). The number of damaged cells after thawing was in all blastocysts higher in comparison to fresh, but significantly higher in the group exposed to C18:0 (43.2%) in comparison to C18:1 (26.0%) and FAF SOF (26.5%).
The current study demonstrates higher cryosurvival for embryos exposed to C18:1, despite their high lipid content, in comparison to C18:0 and stresses the importance to identify specific fatty acids in order to improve embryo quality

MATERNAL NUTRITION AND DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHAD) – RELEVANCE TO MALE FERTILITY IN RESEARCH AND LIVESTOCK PRODUCTION

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We address the effects of nutrition during pregnancy on the development of the reproductive system in male fetuses, with potential effects on adult male fertility. The proliferation of Sertoli cells is an obvious target for DOHAD because Sertoli cell number is fixed at puberty, and adult fertility is largely determined by the Sertoli cells:germ cells ratio.
In rats, maternal undernutrition changes the balance between cell proliferation and apoptosis in developing testes, through processes that affect intratesticular IGF-I, heat shock proteins 70 and 90 in germ cells, and transcription factors that affect caspase-3, Bax and Bcl-2 in Sertoli cells. The equilibrium between apoptosis and proliferation in spermatogonia and spermatocytes is essential in the control of spermatogenesis, so maternal undernutrition is likely to have major and persistent effects on the fertility of male offspring (10.1071/RD20260).
In sheep, maternal undernutrition can affect the development of the testis in the fetus (10.1002/mrd.22974), apparently through processes mediated by changes in STAR protein as well as intratesticular IGF-I, with consequences for the numbers of Sertoli cells (10.1071/RD02046; 10.1530/rep.0.1240033; 10.1016/j.theriogenology.2017.12.023). Indeed, maternal undernutrition from w10 of gestation to birth reduces the number of Sertoli cells at birth in ram lambs (10.1071/RD02046), and maternal undernutrition from d 31 to 100 of pregnancy reduces the number of Sertoli cells in 10-month-old ram lambs (10.1111/j.1439-0531.2007.01046.x). Importantly, these effects can be evoked in very early pregnancy (10.1155/2012/123610).
On the other hand, in a study of ewe undernutrition from mating to d 110 of gestation, there was no effect on the number of Sertoli cells or on apoptosis pathways in the developing testis (10.1186/1477-5751-12-2). The inconsistency with other reports is perhaps because undernutrition was imposed earlier in pregnancy. Clearly, more research is needed with respect to the timing of nutritional constraints.
In conclusion, maternal undernutrition during pregnancy affects testis development in ram lambs, contributing to between-ram variation fertility. Selection of rams for experimentation and for mating programs needs to be informed by their DOHAD history.

WORKSHOP 18 - GENETICALLY TAILORED PIGS AS DISEASE MODELS AND ORGAN DONORS FOR XENOTRANSPLANTATION

MODELING DUCHENNE MUSCULAR DYSTROPHY IN PIGS - RECENT PROGRESS

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Pig disease models offer numerous advantages in terms of their high physiological and anatomical similarity to humans, which allows for more accurate preclinical research on disease mechanisms and the development of new therapies. Here, we provide an overview of our recent progress in developing and utilizing a pig model of Duchenne muscular dystrophy (DMD). The founder dystrophin gene (DMD)-deficient male pigs originally generated via somatic cell cloning had limited lifespans while recapitulating similar symptoms to those of DMD patients. Therefore, we next developed a means to compensate the juvenile lethal traits of the DMD-KO cloned pigs using blastocyst complementation technology. Thus, a production system incorporating a DMD-XKOY↔XWTXWT chimeric boar enabled us to efficiently and sustainably obtain a supply of the DMD models.
The chimeric pigs showed the disease pathology in their body tissues in a mosaic pattern without juvenile lethality. Moreover, sperm production could be maintained, thereby enabling the generation of progeny, including F1 mutation carrier females, further allowing for the maintenance of stable male disease models through natural reproduction. A manifesting carrier female showed critical decrease in DMD expression in skeletal/cardiac myocytes, which reflected a significant clinical issue. The short life-span of founder cloned DMD pigs have been inherited by F2 progeny, while some F2 DMD-XKOY pigs survived longer than the founder clones and, similar to DMD patients, developed heart failure as they aged. Our preliminary study indicated that the short lifespan of the cloned DMD pigs may be prolonged by introduction of the human artificial chromosome carrying 2.4 Mb DMD gene.
The development of viable model pigs with severe symptoms offers a new direction for animal reproduction technology, as well as a valuable research tool for basic research and development of novel therapies. This example highlights the importance of new developments in animal reproduction technology for medical breakthroughs.

A PIG MODEL FOR HUTCHINSON-GILFORD PROGERIA SYNDROME

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Progerin, an aberrant protein that accumulates with age, causes the rare genetic disease Hutchinson-Gilford progeria syndrome (HGPS). Patients exhibit accelerated aging and die in their early teens mainly from myocardial infarction or stroke. The mechanisms underlying HGPS progression and complications are incompletely understood in part due to the lack of appropriate animal models. We recently generated knockin Yucatan minipigs carrying in heterozygosis the LMNA c.1824C>T HGPS-causing mutation (HGPS minipigs), a powerful animal model with invaluable translational potential (Dorado et al. Cell Discov. 5:16e2019). Like HGPS patients, HGPS minipigs express endogenous lamin A/C and progerin ubiquitiously, and recapitulate the main hallmarks of the disease: severe growth retardation, lipodystrophy, skin and bone alterations, cardiovascular disease, and premature death. However, they do not reproduce, preventing the establishment of a transgenic colony via conventional breeding. To circumvent this problem, we are generating 2 new transgenic Yucatan minipig lines whose cross progeny will include HGPS minipigs: 1) LMNALCS/+ minipigs engineered to express lamin A/C but allowing progerin synthesis upon Cre-recombinase activity; and 2) CAG-Cre+/- transgenic Yucatan minipigs with Cre-recombinase expression under the ubiquitous hybrid promoter of cytomegalovirus enhancer fused to chicken beta-actin promoter (CAG). These pigs will be generated using CRISP/Cas9 gene editing in male minipig fibroblasts, followed by somatic cell nuclear transfer by handmade cloning to enucleated oocytes from Large White sows. Breeding both lines will generate progeroid heterozygous LMNA c.1824C>T minipigs like the HGPS minipigs we recently generated and characterized. Colonies of LCS/+ and CAG-Cre+/- Yucatan minipigs will be maintained for crossbreeding to generate HGPS minipigs for preclinical studies. Results obtained in preclinical trials with HGPS minipigs should facilitate decision-making about which therapeutic strategies should advance to clinical trials and potentially expedite the development of effective therapeutic applications for HGPS patients.

THE ROLE OF ADULT STEM CELLS IN HOMEOSTASIS AND REPAIR. THE EXPANDING STORY OF LGR5 STEM CELLS.

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LGR5, a known potentiator of Wnt signaling, is involved in developmental processes, tissue homeostasis and tissue regeneration, and often marks key progenitor populations in multiple organs. To date, most, if not all, observations on LGR5 have been generated using transgenic mouse models due to lack of reliable anti-LGR5 antibodies. Thus, at this time essentially most, if not all, we know about LGR5 stem cells comes from a single mammalian species; a species that, while invaluable for basic research, has significant limitations for translational applications. Swine, in contrast, have a long history of use in the biomedical community as outstanding models for translational research. To address the lack of a large animal model with relevance to humans that could be used to study LGR5 stem cells, we developed a gene edited pig with an H2B-GFP marker gene inserted into the LGR5 locus by homology-directed repair. Cells expressing LGR5 are then identified by their intense nuclear GFP signal. We will present results generated from the LGR5-H2B-GFP line from two organs systems, the skin and the lungs. These organs were chosen due to greater similarities between humans and pigs than mouse and humans. With a sparse hair coat, asynchronous hair cycling, thick dermis and epidermis, and tight skin attachment, pig skin is highly similar to human. In lungs, mouse and human morphology is drastically different with respect to distribution of key airway cells as well as structures such as submucosal glands. Pigs, thus, represent a more physiologically relevant and translational model for developing wound healing therapies for humans. Our findings to date include: In the skin we made novel observations of LGR5 expression during development and show that it differs from mice in both timing and location of appearance, as well as extent of expression during hair follicle neogenesis. Similarly, in the lung we have identified, and spatially and functionally characterized, previously unreported LGR5 mesenchymal and epithelial cells, and our collaborators have been able to confirm their presence in humans but not in mice. In both organs, our results drastically differ from those in mice and further reinforce the power of the pig as a more relevant translational model.

RECENT BREAKTHROUGHS IN PIG-TO-PRIMATE XENOTRANSPLANTATION

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The number of donated human organs and tissues for patients with terminal organ failure falls far short of the need. Alternative sources, such as organs and tissues from animals are therefore urgently needed. For a number or reasons, including size, anatomical and physiological similarities with humans, the pig is the preferred donor species. Importantly, pigs can be optimized by genetic engineering as a source of cells, tissues and organs for xenotransplantation.
Recent advances in gen(om)e editing are speeding up progress in this field. Numerous genetically (multi-)modified pig lines have been generated to prevent immune rejection of xenotransplants, to overcome physiological incompatibilities, and to reduce the risk of transmitting zoonotic pathogens.
After heterotopic abdominal xenotransplantation into baboons, genetically multi-modified pig hearts (alpha1,3-galactosyltransferase deficient, human CD46 and human thrombomodulin transgenic) survived for up to 945 days (Mohiuddin et al., Nat Commun 7:11138, 2016). While this model demonstrated long-term acceptance of discordant cardiac xenotransplants with safe immunosuppression, their life supporting function remained to be proven. Therefore, we used the same genetic background of donor pigs to perform a series of orthotopic heart transplantation experiments in baboons, finally resulting in consistent long-term success with survival times up to 195 days. The most essential improvements were i) perfusion preservation of the xeno-hearts after explantation and during implantation with 8 °C oxygenated hyperoncotic cardioplegic solution containing nutrition, hormones and erythrocytes; and ii) post-transplantation growth control of the xeno-hearts by early weani ng of glucocorticoids, lowering the recipients' blood pressure, and inhibition of mTOR (mechanistic target of rapamycin) activation to counteract cardiomyocyte hypertrophy (Längin et al., Nature 564:430-433, 2018). Recently, four additional recipients supported the efficacy of this procedure (Reichart et al., J Heart Lung Transplant S1053-2498(20)31556-4, 2020).
Consistent life-supporting function of xeno-hearts for up to 195 days in the most relevant and stringent pre-clinical animal model is a milestone on the way to clinical cardiac xenotransplantation.

WORKSHOP 19 - OVERVIEW ON EQUINE CHORIONIC GONADOTROPIN (ECG, PMSG) USE IN BREEDING PROTOCOLS: RESULTS OF USE OF THE NATIVE PRODUCT AND INTRODUCTION OF A RECOMBINANT ALTERNATIVE

EQUINE CHORIONIC GONADOTROPIN (ECG) IN SHEEP AND GOATS: PAST, PRESENT AND FUTURE

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Equine chorionic gonadotropin (eCG) has been used in sheep and goats to control ovarian function. The application of assisted reproductive technologies in small ruminants would be difficult without the contribution of this essential tool. This hormone is used for estrus synchronization in insemination programs and in recipients for embryo transfer programs, in donors for multiple ovulation and embryo transfer (MOET) programs, and in donors subjected to follicular aspiration for in vitro embryo production. Discovered in the 1930s in the serum of pregnant mares, this hormone administrated in ruminants acts like LH and FSH due to its afinity to their receptors. For estrus synchronization protocols, eCG is used worldwide in low dosages (e.g., 200-400 IU depending of the breed, age, season and latitude) at the end of a progesterone priming (5-7 days in short-term protocols, or 10-14 days in long-term protocols). Out of breeding season, administration of eCG is necessary to induce ovulation in anestrous females, followed by natural mating or insemination. During the breeding season, the use of eCG is required for fixed time artificial insemination (FTAI) programs. In addition, eCG is used to improve twin rate in goats and in meat or dual purpose sheep. The use of eCG is required for the synchronization of recipients for fixed time embryo transfer (FTET), both for in vivo- and in vitro-produced embryos. For in vitro embryo technology, eCG is currently used for ovarian superstimulation in a single dose 36-48 h before follicular aspiration. For superovulation in MOET programs, the use of eCG made a relevant contribution in the early days of embryo transfer technology. A high dose of eCG (often greater than 1000 IU) is effective in inducing superovulation by using a single im injection instead of several doses required with FSH. However, this indication usually results in excessively long follicular stimulation and variable embryo yield, mainly due to the long half-life of eCG; solving this issue would be an oportunity for R&D in the development of new eCG-like compounds. Recent efforts in the production of synthetic eCG-like compounds have provided promising results in several species, including sheep, which offers new perspectives for the future of this tool that continues to be essential for the control of reproduction in livestock.

THE USE OF ECG IN REPRODUCTIVE PROGRAMS IN CATTLE: PHYSIOLOGICAL BASIS AND PRODUCTIVE IMPACT

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Equine chorionic gonadatrophin (eCG) is a large molecular weight glycoprotein produced by endometrial cups in the mare. In the mare, eCG has primarily an LH activity, but in the cow it has either FSH or LH activity, depending on the receptor populations in the ovary. Although eCG has been been used to induce superovulation in a variety of species, the application of eCG at the time of removal of a progestin device in has been used extensively in fixed-time AI (FTAI) programs in Bos indicus (300 IU) and Bos taurus (400 IU) beef cattle. The most important effect of eCG is the stimulation of the growth of the dominant follicle which increases ovulation rate, especially in cows in postpartum anestrus and/or with low body condition scores (BCS). However, treatment with eCG also increases circulating progesterone concentrations in the subsequent luteal phase with an associated increased diameter of the CL and increased progesterone production. It has been shown that treatment with eCG increases the expression of steroidogenic enzymes (P450scc, 3β-HSD and StAR) in the CL. Other studies have shown beneficial effects of eCG in fixed-time AI (FTAI) programs in Bos taurus (200-300 IU) and Bos indicus (200 IU) prepubertal heifers, and in grazing dairy cattle, administration of 400 IU eCG resulted in increased pregnancy rates after the first and second breeding, especially in anestrous cows. In addition, 300-400 IU of eCG has been used extensively in embryo transfer recipients, and several studies have shown increased pregnancy rates following transfer of in vivo-derived and in vitro-produced embryos. This presentation will review some of these data and explore strategies to utilize the benefits of eCG in improving bovine reproductive management.

MULTI-GLYCO ENGINEERING OF RECOMBINANT A-B ECG CONFERS IN VIVO PMSG-LIKE ACTIVITY TO THE RESULTING PROTEIN.

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Equine chorionic gonadotrophin (eCG) is a hormone produced by the endometrial cups of the pregnant mare. Formerly known as pregnant mare's serum gonadotropin (PMSG), this hormone is used routinely to improve pregnancy rates in artificial insemination protocols in livestock. It is a highly glycosylated glycoprotein composed of two different amino acid chains. In equidae (horses, donkeys and zebras), placental CG and pituitary LH are expressed from the same genes and therefore, obtain the same sequence of proteins, which are differentiated only by their lateral carbohydrate chains, particularly located in the β subunit, so the consensus also calls them eLH/CG. The eLH and eCG have many O-linked glycosylation sites and a N-linked glycosylation site in their β subunit, in addition to two N-linked glycosylation sites in the a subunit. As they are expressed and secreted in different tissues, they differ strongly in their side carbohydrate chains N and O, which gives them different biological activities in vivo due to a longer half-life of eCG compared to eLH, as well as different thermal stability.

The commercial production of eCG is currently carried out by bleeding pregnant mares that secrete the active hormone between 40 and 130 days of gestation. Once purified, formulated and controlled, it is marketed to be used in the artificial induction of estrus in female goats, cattle, pigs and other non-equid species. eCG has two outstanding characteristics for extensive use in the livestock industry. Unlike in horses where it has only luteinizing activity, it possesses the activities of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the other species. In addition, because of its quaternary structure and its multiple sites of glycosylation, its circulating half-life is lengthened.

The current production procedure suffers all the problems related to the production and purification of an extractive hormone and for this key reason, we decided to develop a production system based on CHO cells, capable of generating a recombinant eCG with multiple N-linked glycosylation sites and the same biological activity as extracted eCG.

RESULTS OF FIELD TRIALS EVALUATING A SYNTHETIC ECG-LIKE GLYCOPROTEIN PRODUCED BY SYNTEX FOR USE IN DIFFERENT SPECIES

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Equine chorionic gonadatrophin (eCG) has been used for more than 50 years in reproductive management several species. The use of natural eCG is currently under review due to the natural origin of the product and animal welfare issues associated with its production. Thus, we have developed a synthetic substitute. A first trial determined the biological activity of a synthetic eCG-like glycoprotein as an alternative to native eCG in cattle, using a superovulation model. All cows were treated with an intravaginal device (IVD) plus 2 mg of estradiol benzoate on Day 0. On Day 4, cows were divided into two groups and received 2000 IU of natural eCG (n=13) or 2000 IU of eCG-like substitute (n=14). On Days 6.5 and 7, 500 µg of cloprostenol was administered. On Day 7, IVD were removed and on Day 8, 100 µg of gonadorelin was administered. No differences were found in number of ovulations (eCG group-13.7±2.54 vs eCG-like group-13.3±1.79; P=0.83). In a second trial, ewes were treated with an IVD for 7 days during seasonal anestrus. On Day 5, ewes were assigned to the following three experimental groups: Control group (n=10) received a placebo (5 ml), eCG group (n=10) received 1000 IU of natural eCG, and eCG-like group (n=11) received 1000 IU of a synthetic eCG-like substitute. On Day 8, all ewes received 100 µg gonadorelin. Six days after GnRH the ovulatory response was evaluated by laparoscopy. The number of CL in ovulating ewes was higher in the eCG and eCG-like groups than in the Control group (5.2±0.7; 5.2±0.7 and 1.0±0.0, respectively; P< 0.05), but no difference was found between the eCG group and the eCG-like group (P>0.05). We are currently conducting a trial to compare pregnancy rates in anoestrous beef cows treated with 400 IU of eCG, 400 IU of eCG-like substitute or placebo (Control group) in a fixed-time AI protocol. Although the trial is not completed, preliminary results indicate that the eCG-like substitute results in a pregnancy rate that is similar to native eCG and that both result in higher pregnancy rates than in the Control group. Based on results obtained and partial results of ongoing trials, we conclude that the eCG-like substute produced by Syntex could be a very acceptable alternative for native eCG in breeding management of farm species.

WORKSHOP 21 - STEM CELLS AND THEIR PRODUCTS: APPLICATIONS IN REPRODUCTIVE DISEASES OF DOMESTIC ANIMALS

ENDOMETRIAL MESENCHYMAL STEM/STROMAL CELLS – REGENERATIVE PROPERTIES

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Mesenchymal stem/stromal cells (MSCs) are used commonly in veterinary regenerative therapies, namely in the horse and dog. Their therapeutic potential was initially attributed to cell multipotency, but subsequent studies, including our work, evidenced that MSCs produce trophic factors that are key mediators of immune, anti-infective and angiogenic responses, fundamental during tissue repair. MSC preparations are classically obtained from bone marrow and adipose tissue, but this requires the use of surgical procedures. Because the uterus can be accessed in a relatively non-invasive manner, is highly immunologically active and undergoes periodic remodelling/repair, we explored the endometrium as an alternative source of therapeutic MSCs.
Endometrial stromal cell preparations were obtained from horses (Eq-eMSCs, n=3) and dogs (Ca-eMSCs, n=3), and were compared with the respective adipose-derived MSCs (adMSCs) for MSC profile, immune and antimicrobial properties.
Eq- and Ca-eMSCs displayed typical MSC characteristics and produced numerous trophic immune-factors (IL-6, IL-8, MCP-1, CCL5) in vitro. Moreover, intrauterine infusion of autologous Eq-eMSCs to mares, during early diestrus, attenuated neutrophil responses compared to PBS. In addition to immune factors, Ca- and Eq-eMSCs expressed antibacterial peptides, with Ca-eMSCs and - adMSCs showing similar levels of BDF1, Elafin and LL37. However, Lipocalin-2 levels were higher in Eq-eMSCs when compared to Eq-adMSCs, and Eq-eMSCs condition medium attenuated Escherichia coli growth in vitro. Indeed, both Eq- and Ca-MSCs were responsive to bacterial products such as LPS, showing increased expression of cytokines IL-6, IL-8, MCP-1 and TLR4 ($p<0.05$); which levels were higher in Eq-eMSCs compared to Eq-adMSCs ($p<0.05$), but similar for Ca-eMSCs and -adMSCs.
Our studies show that eMSCs have distinct properties compared to adMSCs and may provide a useful alternative for veterinary regenerative medicine. eMSCs could be obtained from routine uterine biopsies or neutering procedures, and may be particular beneficial for combatting infections, particularly those caused by antibiotic-resistant bacteria.

MESENCHYMAL CELL SECRETOME APPLICATIONS IN LARGE ANIMAL REPRODUCTIVE REGENERATIVE MEDICINE

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Chronic endometritis can impair production of endometrial mediators (soluble factors or extra-cellular vesicles, EVS) and result in malfunction of the endometrium-embryo 'dialogue', with consequent infertility.
Since it is suggested that EVs or soluble factors contained in the conditioned medium secreted by mesenchymal/stromal/stem cells (MSCs), are mediators of cell-to-cell communications that ultimately lead to tissue repair, regenerative medicine using cell free products from MSCs is proposed as a new approach to restore embryo-maternal dialogue.
In this context, the role of amniotic-derived EVs on the rate on vitro bovine embryo production, on in vitro embryo hatching after cryopreservation and on recipient pregnancy rates in vivo were investigated. All results were compared to control embryos (CTR) produced in vitro without EV supplementation. Moreover, microRNA profiling of embryos produced in vitro, with or without EV supplementation, was also evaluate d and compared to in vivo produced embryos.
Our results show that the rate of blastocysts and the percentage hatching of cryopreserved in vitro+EVs embryos is higher ($P<0.05$) than in in vitro-CTR. The in vivo recipient pregnancy rate is also higher ($P<0.05$) both with fresh and cryopreserved in vitro+EVs embryos compared to in vitro-CTR embryos. The analysis of expressed microRNAs shows that embryos produced in vivo are different from in vitro embryos, but EV supplementation counteracts the adverse effect of in vitro culture and partially modulates the expression of specific microRNAs involved in successful embryo implantation.
To test the efficacy of EVs in vivo, a case of a mare with chronic endometritis is reported. The 11-year-old mare had regular and obvious heats but numerous artificial inseminations (IA) had failed. Grade IIB chronic endometritis was diagnosed on uterine biopsy. After two intrauterine infusions (at 5 and 9 days post-ovulation), each of 20 billion EVs, repeated at the following ovulation, the endometritis decreased to grade IA and, after insemination, a viable pregnancy occurred.
These studies provide new insights into the mechanisms of acellular treatments and could lead to the development of new therapeutic approaches in reproduction.

MESENCHYMAL STEM CELLS IN LIVESTOCK CATTLE: POTENTIAL APPLICATIONS IN REPRODUCTION FOR GERMLINE DERIVATION

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Germ cell (GC) in vitro derivation and transplantation has potential applications in bovine reproduction, as an alternative method for dissemination of elite animal genetics, production of transgenic animals, and conservation of endangered breeds. Our group has recently reported that bovine fetal mesenchymal stem cells (bfMSC) may be candidates for GC derivation and transplantation mainly based on their simplicity for isolation and culture, reduced immunogenicity and in vitro potential for transdifferentiation. We used plastic adherence and standard culture conditions for isolation of bfMSC from bone marrow (BM) and adipose tissue (AT). The in vitro GC differentiation potential of bfMSC was firstly evaluated under the effect of retinoic acid (RA), bone morphogenetic protein 4 (BMP4), and transforming growth factor ®1 (TGFß1). Treatment with exogenous BMP4 and TGFß1 induced a transient increase (P<0.05) in DAZL and NANOG mRNA levels, respectively. However, exposur e to RA was more effective in increasing (P<0.05) expression of DAZL and regulating expression of OCT4 and mRNA levels of NANOG. The in vitro GC differentiation potential of bfMSC was also evaluated under the effect of coculture with bovine Sertoli cells (SC). Cocultures of bfMSC with SC had higher (P<0.05) proportion of cells positive for Oct4, Nanog, and Dazl and higher (P<0.05) levels of mRNA of PIWIL2 and SCP3 compared to monocultures. The in vitro GC differentiation potential of bfMSC was also assayed using polycistronic vectors containing combinations of GC genes including DAZL, STRA8, and BOULE followed by exposure to BMP4 or RA. Overexpression of DAZL and STRA8 in bi-cistronic and DAZL, STRA8, and BOULE in tri-cistronic vectors resulted in the upregulation of OCT4, NANOG, and PIWIL2 in bfMSC. BMP4 and RA treatments increased (P<0.05) DAZL and c-KIT and activated FRAGILIS expression in bfMSC. Thereafter, the potential for in vivo transplantation was determined usi ng bfMSC stained with PKH26 probe in 14-month-old bulls. Scattered bfMSC were observed in testicular histological sections after 1 month after transplantation. Overall these data strongly suggest that bfMSC have the potential for in vitro GC differentiation and may be suitable candidates for GC transplantation in cattle. Fondecyt 1191114

WORKSHOP 24 - NEW TRENDS IN SWINE PRODUCTION MANAGEMENT

TECHNOLOGICAL AND FORMULATIVE INNOVATION FOR REVITALIZING A MATURE BUSINESS SUCH THAT OF THE SEMEN EXTENDERS FOR PIG ARTIFICIAL INSEMINATION

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Pig farming represents one of the most developed food chains worldwide. Its maintenance and development is, however, difficult to sustain, due, among other things, to the massive use of antibiotics in products such as the extenders for artificial insemination or hormones in the practice of chemical castration of male piglets. It is therefore imperative to take urgent measures to reduce the environmental burden of these products and reconvert them on a biological basis.
To do so, it is mandatory to set up and develop innovative formulation strategies and platforms suitable for introducing new paradigms more oriented towards the use of substances acting specifically on sperm cells biology.
These goals can be achieved through a cross-sectorial and interdisciplinary approach which brings together veterinary, biological, biotechnological, and pharmaceutical delivery knowledge.
This presentation offers a perspective view on possible scenarios of evolution in the field semen extenders for pig artificial insemination.

PATHWAYS TO ANTIBIOTIC-FREE PRESERVATION OF BOAR SPERMATOZOA

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With worldwide increased use of artificial insemination (AI) in swine reproduction, the contribution of antibiotics in semen extenders to the global bacterial resistance threat came into focus. Evolving multi drug resistances in AI centers caused by overuse of antibiotics together with institutional bans of the remaining efficient antibiotics are driving forces to search for alternative solutions. Risk associated with insufficient control of bacteria were identified in low fertility, transmission of diseases in sow farms, increase of antimicrobial resistances and loss of market position. On this basis, typically, the aim is complete eradiation of bacteria in the extended semen portions. In the process of risk assessment, however, this goal should be reconsidered. An array of studies demonstrated that moderate amounts of bacteria (for most strains greater than 106 colony forming units/ml) do not harm sow fertility or health. Moreover, bacteria are now increasingly recognized as a natural cellular component of ejaculates which promotes fertility chances by immunogenic interaction with the female tract. Nonetheless, effective antimicrobial control is necessary to maintain microbial growth under the identified bacteria-specific thresholds for sperm damage during several days of semen storage. Alternatives to conventional antibiotics must not be toxic to sperm or to the environment, should have a high antimicrobial effectivity without evoking resistances, and should be easy and economical useable in AI centers. In this workshop contribution, alternatives to antibiotics in extended boar semen are reviewed, including novel evolving concepts with the use of antimicrobial effective semen extenders and low temperature storage. Strategies of antibiotic-free preservation of boar semen will be discussed in context of the principal steps of risk assessment process.

INNOVATIVE SEMEN EXTENDER FORMULATIONS WITHOUT ANTIBIOTICS

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The demand for animal protein increases every year. For decades antibiotic substances have been used indiscriminately as growth promoters in different animal species; this, among other factors, places us today in front of the most critical global health problem, namely antibiotic resistance. The main objective of this work was to develop new semen extenders formulation containing alternative substances to antibiotics that are safe from the biological point of view and economically viable for industrial production.
The effects of biomolecules included in a formulation used to dilute boar semen were analyzed, the experimental input variables were planned under a D-optimal design experimental approach. Two substances coded as EL1 and EP1 applied at three concentration levels (-1, 0, and +1) were analyzed, and their effect on sperm quality and their ability to decrease the initial bacterial burden was evaluated. A negative correlation between progressive motility and total bacterial count at 0 h was observed. The tested formulations showed good quality in terms of motility (93%± 1.5) and progressive motility (86% ± 1.2) in the substance concentration groups of the experimental design. It was possible to identify a combination that obtained the best results in motility and acrosome integrity and decreased initial bacterial load. The formulation containing EP1 at lower concentration and EL1 at higher concentration provided values higher than 95% of motility with only 2% of damaged acrosomes and a reduction of the bacterial load even after three days. These values were statistically significant($p < 0.005$) values compared to the control, namely semen extender without antibiotics.
We can conclude that using the proposed biomolecules as components of boar semen extender represents a suitable alternative to control the bacterial burden in boar semen doses for artificial insemination. This work offers the basis for a new product and generates new alternatives for an innovative concept of green production.

WORKSHOP 25 - FLOW CYTOMETRY, A NECESSARY TOOL FOR SPERM VIABILITY ASSESMENT

MULTIPARAMETRIC FLOW CYTOMETRY: A POTENT TOOL FOR THE STUDY OF SPERM BIOLOGY

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While the integrity of the plasma membrane is rapidly lost when necrotic cell death occurs, subtle changes characterize other forms of sperm demise. The combination of the permeable DNA binding probe SYBR-14 and the impermeable DNA binding probe propidium iodide is widely used. This assay become very popular since its introduction in the early 90s of the past century. During the first decade of the present century, more sophisticated assays started to be introduced. One probe that has gained popularity is YoPro-1. Initially, this probe was used as an alternative to red emission viability dyes. However, YoPro-1 can stain not only necrotic cells, but this dye can also use specific channels to cross intact membranes, moreover live spermatozoa may pump out YoPro-1, while depletion of ATP may result in accumulation of YoPro-1 in the spermatozoa. This property allows the identification of spermatozoa, with intact membranes, but in a compromised physiological situation, this dye is used to identify apoptotic cells and identifies increased membrane permeability due to lipid peroxidation. The identification of the transposition of specific phospholipids from the inner to the outer membrane constitutes a classical assay for the detection of apoptotic cells. The Annexin-V assay is a popular technique to identify the presence of phosphatidylserine (PS) in the outer leaflet of the plasmalemma. Under normal conditions, PS and phosphatidylethanolamine (PE) are present in the inner leaflet of the plasmalemma, when the cell enters apoptosis, PS translocates to the outer membrane constituting an" eat me" signaling, the presence of PS signals the cells that will be phagocytosed in a silent, non-inflammatory manner. This is especially important for the elimination of redundant spermatozoa from the female genitalia, and failure in this mechanism may lead to an uncontrolled inflammatory reaction to semen and posterior endometritis. The presence of all these different forms of sperm dagame can be identified in the ejaculate using multiparametric flow cytometry, with the aid of computational analysis, these changes can be identified at the single sperm level to understand the complex nature of the ejaculate. Junta de Extremadura-FEDER (GR 21060 and IB 20008).

DETECTION AND IMMUNOPHENOTYPIC ANALYSIS OF SEMINAL FLUID EXTRACELLULAR VESICLES BY FLOW CYTOMETRY

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Extracellular Vesicles (EVs) are small (~50-150 nm) vesicles generated by fusion of a specialized endosome, the multivesicular body (MVB) (1), with the plasma membrane. EVs are released from all cell types and found in body fluids, including semen. They have been for intercellular exchange of macromolecules, allowing the transfer of proteins, lipids, mRNA, miRNA and DNA contributing to intercellular communication in relevant biological processes, including apoptosis, antigen presentation, angiogenesis, inflammation, coagulation and fertilization; playing therefore an important role in the development of several diseases, and specifically, modulating cancer microenvironment and the immune response (2,3,4). Accurate characterization EVs is critical to explore their diagnostic and therapeutic applications (5). EVs from seminal liquid can be isolated by different methods but the capacity or analyze them and study their heterogeneity is constrained by their size and technology available. Is the reason why flow cytometry become the technology of election due to its capacity of analyze individually the sample components in a multiparametric way. New developments in FCM allow particles smaller than 100 nm detection and study their phenotype. Also, is possible to sort them and analyze their cargo.

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FLOW CYTOMETRY AS EMERGING TECHNOLOGY IN VETERINARY APPLICATIONS

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Flow Cytometry as a technology to analyze multiple parameters of particles in suspension has been significantly further developed in the recent years with respect of detectors, laser options and throughput. The CytoFLEX platform is as an example using Avalanche Photodiode Detectors (APDs), which are known to be highly sensitive and allow accordingly the detection of even dim fluorescence expressions. In this presentation we will discuss a) the general concepts of Flow Cytometry, b) the CytoFLEX platform in particular and c) possible applications in Veterinary

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WORKSHOP 22

Reproduction technologies of the future in the data driven bovine industry

ROOM VERDE - Monday June 27, 17.00-17.40

Lee CAIRNS

IMV Technologies Group has developed software and equipment that covers the collection and use of data in the bovine lifecycle.

IMV Collection Lab Management Software to help:

- Streamline collection
- Improve accuracy
- Increase efficiency

When semen cost increases, particularly with the use of sexed semen, how IMV insemination and diagnosis tool will allow:

- Save semen
- Make insemination safe and easy
- Improve animal and human wellbeing

Ultrasound image overlay with cow Side KPIs and Feedback loop for Farm Management system Integration.

When assessing a cow during pregnancy check, there are useful parameters to have at hand when making a cull decision or next course of action and it takes time to get this information to the vet.

An open cow costs a farm money and ultrasound diagnosis is not always correctly recorded when the vet directly tells the farmer or when the farmer goes to put the recording into their farm management system.

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WORKSHOP 23

Bacterial control in swine semen

ROOM VERDE - Wednesday June 29, 11.00-11.40

Perrine NOGUES, PhD - Aranxta ECHEGARAY, DVM, PhD

Improving the semen quality of swine doses is the main purpose of swine centers. This quality includes a good motility, good progressive motility, good morphology, and viability for a long-term storage of doses. Several factors could influence this semen quality including packaging, storage temperature, transport, and mostly bacterial contamination. High concentrations of bacteria in swine semen doses may reduce drastically the quality of semen and subsequent fertility after insemination. Nowadays, the main challenge for 75% of boar studs is to control bacterial contamination in semen doses.

- What are the main sources of contamination and how control them?
- What are the process and the tools at your disposal?

This is the topic of this presentation.

We will review the main critical points where contamination occurs in pig insemination centers and the preventive recommendations to be implemented.

Finally, we will discuss about existing process and products to better control the bacterial contamination in swine semen doses. Indeed, IMV Technologies has developed protective media and packaging to ensure the high preservation of the semen quality.

WORKSHOP 20

Strategy to improve bovine fertility: use of an animal free-protein medium

ROOM VERDE - Tuesday June 28, 17.00-18.00

Lucie GAVIN-PLAGNE, PhD

Roundtable with specialists in bovine industry

Among the various techniques of Assisted Reproductive technologies, animal insemination (AI) is mainly done with frozen semen and allows the increase of the genetic value, the dissemination of selected semen while reducing health risks. In spite of efforts to produce efficient freezing media guaranteeing good sperm survival, the freezing process remains a step affecting semen quality.

Moreover, nowadays, these genetic resources are cryopreserved in media containing animal protein-derived products. In the bovine insemination industry, the use of egg yolk in freezing media is still a common method worldwide. However, using these products raises concerns. Proteins of animal origin can represent a barrier to international trade, notably due to the contamination risks, as well as a barrier to standardization due to the variability that they present.

In this context of improving sanitary guarantees, IMV Technologies is bringing together industrialists and scientists to discuss future strategies for improving bovine fertility through media without animal proteins.

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