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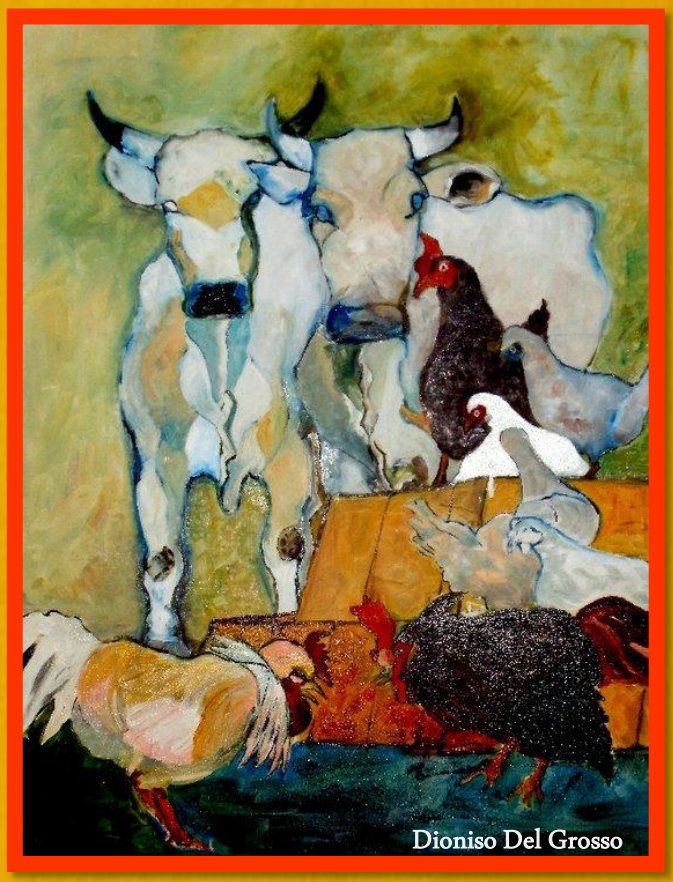

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CITTA' DI TORINO

REGIONE PIEMONTE

IN VITRO DEVELOPMENTAL COMPETENCE OF HORSE OOCYTES WITH DIFFERENT CUMULUS MORPHOLOGY

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In vitro production of equine embryos by intracytoplasmic sperm injection (ICSI) is gaining interest, and both commercial and research applications have rapidly increased worldwide. Oocyte collection from excised ovaries is generally performed using one of two methods: aspiration and scraping. Aspiration has been found to give a lower recovery rate [1] and to yield oocytes largely denuded of cumulus, when compared to scraping [2]. For commercial programs, oocytes are typically recovered from live mares using transvaginal ultrasound-guided aspiration. Despite the high number of oocytes with only corona radiata (CR) collected by aspiration (in vivo or ex vivo), most studies classify horse oocytes simply as having a compact (CP) or expanded (EX) cumulus, without considering oocytes with only CR. In the only study [3] classifying horse oocytes as having CP, EX, or partial cumulus investments, no data on embryo production were available. The aim of this study was to investigate the embryo developmental ability after ICSI of horse cumulus-oocyte complexes (COCs) presenting only CR as compared to CP and EX COCs. Horse oocytes were collected by follicular aspiration of abattoir-derived ovaries. After classification into EX, CP or CR COCs, they were in vitro matured for 26 h in DMEM-F12 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 ng/ml epidermal growth factor, 100 ng/ml insulin-like growth factor 1, 0.1 IU/mL pFSH-LH (Pluset). At the end of the maturation period oocytes were denuded and classified as mature, immature or degenerate. MII oocytes were fertilized by piezo-drill ICSI using frozen-thawed semen from the same stallion, and in vitro cultured in SOF medium for 7.5 days. Culture medium was refreshed every 3 days and on day 6 of culture 5% FBS was added. At day 7.5 of in vitro culture, embryos were stained with 1 µg/mL bisbenzimide fluorescent dye (Hoechst 33342) to assess the number of nuclei and classify them. Maturation rate, cleavage rate and morula/blastocyst rates were recorded; data were statistically analysed using a Chi Square test (IBM SPSS Statistics 23) and significance was assessed for $P<0.05$. The experiment included 14 replicates. A total of 611 oocytes were used. Overall maturation rate was 60.2%. MII, immature and degenerate oocyte rates were not statistically different ($P>0.05$) among different COC morphologies (MII 61.7% vs. 57.6% vs. 59.3% for EX, CP and CR COCs respectively). Cleavage rate was lower ($P<0.05$) for CR (42.1%) compared to CP (55.6%) but not significantly different from EX (54.3%), while morula/blastocyst development after 7.5 days of culture was similar ($P>0.05$) among groups (12.0% vs. 8.9% vs. 14.7% of injected oocytes for EX, CP and CR COCs respectively). In conclusion, even if CR COCs show a lower cleavage rate after ICSI, their developmental ability is similar to CP and EX COCs, demonstrating that they can be used as a useful source of embryos in the horse.

[1] Hinrichs K. The relationship of follicle atresia to follicle size, oocyte recovery rate on aspiration, and oocyte morphology in the mare, Theriogenology, 36:157-68, 1991. [2] Alm et al. Comparison of different methods for recovery of horse oocytes, Eq Vet J Suppl, 25:47-50, 1997. [3] Dell'Aquila et al. Influence of oocyte collection technique on initial chromatin configuration, meiotic competence, and male pronucleus formation after intracytoplasmic sperm injection (ICSI) of equine oocytes. Mol Reprod Dev, 60:79-88, 2001.