PF08.15 | Uptake of seminal extracellular vesicles (EVs) subsets by cumulus cells-oocyte complex in pigs

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Introduction: Seminal plasma (SP) is a fluid rich in EVs that act as key modulators of reproductive processes. While, in pigs, SP supplementation can improve in vitro fertility outcomes, there are no studies evaluating the effect of SP on in vitro oocyte maturation (IVM). This study is the first attempt to (1) examine the uptake of seminal EVs by cumulus-oocyte complexes (COCs) during IVM and (2) evaluate if they affect the viability of cumulus cells (CCs).
Methods: SP samples from five ejaculates (one per boar) were pooled and EVs were isolated by the SEC-based procedure (Barranco et al., 2021) that allows isolate small (S-) and large (L-) EVs. The EVs were characterized by protein concentration (MicroBCA), DLS and TEM. The conventional porcine two-day maturation protocol in the presence or absence of 0.2 mg/mL S-EVs and L-EVs was carried out (groups of 40 COCs). An aliquot of S-EVs and L-EVs was labeled (PKH67; uptake analysis), and another one remained unlabeled (CCs viability assessment). For uptake analysis of EVs, COCs were assessed by confocal microscopy and CCs were stained (0.4% Trypan Blue) for viability assessment.
Results: TEM showed that S-EVs were mostly spherical membranous vesicles (~30-130nm), whereas L-EVs were morphologically heterogeneous (~100-350nm). DLS revealed that S-EVs were smaller ($P < 0.001$) than L-EVs (124.1±7.7nm vs 303.9±9.6nm, respectively). No differences in protein concentration were found between EV subsets. S-EVs and L-EVs were able to bound COCs during IVM, as green fluorescent spots were observed in the membrane of CCs and not observed in control COCs (i.e., absence of EVs). No differences were found in CCs viability when incubated with S-EVs and L-EVs compared to control (93.78±5.17% and 94.91±6.55% vs. 100%, respectively).
Summary/Conclusion: Seminal EVs can be integrated by CCs, without interfering in their viability. Further studies are required to evaluate if seminal EVs may influence both oocyte nuclear and cytoplasmic maturation and further fertilization.
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PF08.16 | MSC-derived secretome induced GLUT4 translocation in HepG2 insulin resistance model

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Introduction: Diabetes mellitus (DM) is one of the most common metabolic disorders. By 2030, it is expected that more than 300 million individuals will be affected by diabetes. Glucose transporter type 4 (GLUT4) is a transmembrane protein that is involved in the elimination of glucose from the bloodstream. In addition to GLUT4, several investigations have shown that interleukin-6 (IL-6) plays an important role in DM. Recent evidence has indicated that IL-6 is a key regulator that affects glucose homeostasis. Interleukin-6 (IL-6) is a pleiotropic cytokine that can be discovered in the secretome of MSCs. The aim of this study is to examine whether IL-6 that was found in the Mesenchymal Stem Cells (MSC)-derived secretome could increase the GLUT4 translocation through AMPK activation in the HepG2-insulin resistance model.
Methods: MSC-derived secretome was produced by culturing the umbilical cord mesenchymal stem cells in a normoxic conditioned until it reach 80% confluency using DMEM High Glucose and supplemented with 5% Human Platelet Lysate. Upon it reached the confluency, the growth medium was discarded and changed with serum-free media, and incubated for 24 hours. The medium was collected and centrifuged for 500xg for 5 minutes and filtered using a 0.2μm filter membrane. The IL-6 concentration was confirmed using the MACSPlex Cytokine 12 kit. The insulin resistance model of HepG2 cells is established by adding 100nM of insulin for 24 hours. To detect the GLUT4 translocation. The insulin resistance model was fasted for 12 hours prior to the treatment using MSC-derived secretome for 24 hours. GLUT4 translocation was detected using immunofluorescence. HepG2 insulin-resistant model was fixed using methanol and cells were incubated with GLUT4 antibody-conjugated with Alexa488.
Results: The treatment of MSC-derived secretome increased the GLUT4 translocation in the HepG2 insulin model compared to the control ($p < 0.05$). This result corresponds with previous studies that show the expression of the GLUT4 gene is downregulated in T2DM. The expression of GLUT4 is lowered in diabetic patients due to blood glucose buildup. IL-6 plays an essential role in regulating glucose homeostasis by activating the GLUT4 translocation through AMPK activation. Given the critical roles of IL-6 that were found in the MSC-derived secretome, it could be a treatment strategy against diabetes.