Bovine Follicular Fluid Extracellular Vesicles mRNAs Contents and Effects on Oocyte Maturation and Development When Supplemented During In Vitro Maturation, Preliminary Results.

Fernanda Schneberger[1]; Alessandra Bridi[1,2]; José Roberto Q. de Oliveira[1]; Ludimila C.Z. Janini[1]; Lorena C. Munhoz[1]; Cláudia L.V. Leal[1]

[1]Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, Pirassununga, Brazil

[2]Universidade do Oeste de Santa Catarina, Xanxerê, Brazil

Oocytes develop and mature in vivo as the cumulus-oocyte complex (COC) which is bathed by fluid within the ovarian follicle (follicular fluid, FF). The FF contains extracellular vesicles (fEVs), which are known to function as a communication system between follicular cells and the oocyte, as they carry several molecules as RNAs, which can regulate the function of cell uptaking such vesicles. However, during in vitro maturation (IVM), the COCs are placed in culture medium without fEVs. The present study aimed to assess effects of fEVs during IVM on oocyte maturation and developmental competence and to determine their transcript contents related with lipid metabolism, antioxidant enzymes and epigenetic regulators, which are important for oocyte maturation. Bovine FF and COCs were obtained from slaughterhouse ovaries by aspiration of 3-6mm follicles from separate collections. fEVs were isolated from 1 ml FF by size exclusion chromatography (SEC, qEV1 columns 35 nm Gen 2, Izon) followed by ultracentrifugation (100,000 x g for 70 min at 4°C, Beckman Coulter), and used for mRNA analysis content by RT-PCR or for medium supplementation during IVM. Bovine COCs were subjected to IVM in TCM199 containing 0.4 mM glutamine, 0.2 mM pyruvate, 50 mg/mL gentamicin, EGF (20 ng/ml) and supplemented only with 10% fetal calf serum depleted of its own EVs (dFCS, control) or with dFCS plus fEVs (fEVs group), for 24h, at 38.5ºC and 5% CO_2 in air. At the end of IVM, cumulus cells (CC) were removed from part of the COCs, and the denuded oocytes evaluated for maturation rates by first polar body extrusion (1st PBE, 5 replicates). CC were assessed, as also the fEVs, for lipid metabolism, antioxidant enzymes and epigenetic regulation transcripts by RT-PCR (3 replicates). The other part of the COCs was submitted to in vitro fertilization and culture, and blastocyst rates on day 7 (D7) were recorded (5 replicates). The Wilcoxon two group test was used to analyze the rates of maturation, blastocyst and ΔCt values. Maturation rates were not affected by treatments (P>0.05) and 1st PBE rate was 77.2 (n=169) and 79.5 % (n=170), for control and fEVs, respectively. From 224 and 245 COCs matured without or with fEVs, D7 blastocyst rates were also unaffected (47.0 and 44.5%, respectively, P>0.05). fEVs were shown to contain transcripts related with lipid metabolism (PLIN2 Ct=29.05; ACACA Ct=29.92; LDLR Ct=27.93; PLIN2 Ct=29.05), antioxidant enzymes (GPX1 Ct=28.65; GPX4 Ct=29.08; SOD1 Ct=29.04; SOD2 Ct 28.59) and epigenetic regulation (DNMT1 Ct=30.53; DNMT3A Ct=31.29; MAT2A Ct=30.11; SHMT2 Ct=29.05). CC also expressed the same genes found in fEVs, but their expression levels were not affected by maturation with fEVs (P>0.05). In conclusion, fEVs carry mRNAs that could impact important functions for COCs, including lipid metabolism, antioxidant enzymes and epigenetic regulators. Although fEV during IVM did not improve maturation and embryo development and did not affect transcript levels in CC, effects on oocytes cannot be ruled out. These are preliminary results, and more replicates are being performed as well as analysis of expression in oocytes and of other transcripts and miRNAs. Future assessments may bring further knowledge to the area. Financial support: FAPESP (SPEC Grant # 2021/09886-8; AR Grant #2021/06760-3); FS - DS Scholarship (Capes 88887.694635/2022-00); AB - PD Scholarship (FAPESP 2023/01524-5); JRQO - ScI Scholarship (FAPESP 2023/12424-1); LCZJ DS Scholarship (Capes 88887.836321/2023-00); LCM - ScI Scholarship (PUB-USP 2023/83-1).