Animal Reproduction

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In vitro model to study the maternal-embryonic communication mediated by extracellular vesicles in cattle.

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Resumo

In ruminants, the maternal recognition of pregnancy (MRP) involves the production of interferon tau (IFNT) by the embryonic trophectoderm cells in order to prevent luteolysis. Besides the well characterized IFNT signaling, the production and release of extracellular vesicles (EVs) arose as a potential mechanism of cellular communication between the mother and the embryo during MRP. The purpose of this study was to create an in vitro model to understand the EVs roles in the regulation of critical biological processes such as maternal recognition of pregnancy and to investigate other mechanisms of maternal-embryonic communication. To test this, we generated cultures of endometrial cells (epithelial and stromal origin) and trophectoderm cells from in vitro fertilized blastocysts, and isolated EVs from their culture media. Thus far, stromal and epithelial cells lines (n = 5) were isolated, grown until the 4th passage, and characterized by immunofluorescence using anti-vimentin antibody (marker of stromal cells). Small EVs of the culture medium were obtained from two sets of ultracentrifugation at 120 000×g for 70 min (Optima XE-90 Ultracentrifuge; 70 Ti rotor; Beckman Coulter, Brea, California, USA). Isolated EVs were characterized based on size and concentration of particles using Nanoparticle Tracking Analysis (NTA). As a result, only stromal cells were positive to mesenchymal vimentin as expected. EVs showed an average size of 131.92 nm and 153.46 nm, and concentration 6.64 x108 particles/mL and 8.15 x108 particles/mL, for epithelial and stromal cells, respectively. There was no significant difference ($P<0.05$) between the cells groups. Further characterization using western blot analysis confirmed the presence of ALIX, and the absence of GRP78 protein in the EVs. In addition, transmission electron microscopy (TEM) showed EVs in the expected shape and size (<150nm). To isolated TC cells (n = 4 lines), we carried out in vitro fertilization, and Day 8-hatched blastocysts were single cultured on Matrigel (1.5 mg/mL). EVs obtained from the culture media showed an average size of 167.77 nm and concentration of 2.70 x 108 particles/mL. The EVs size and concentration of particles were similar ($P<0.05$) among the lineages. To in vitro simulate the maternal-embryonic crosstalk and investigate if cells from one source can modulate transcripts in the target cells, we treated the TC with EVs from the endometrial cells, and the endometrial cells were treated with EVs from the TC. Cells were collected and stored at -80 °C and they will be submitted to RNAseq for gene expression analysis. In this project we intend to better understand the internalization and modulation of EVs produced by endometrial and trofectoderm (TC) cells cultured in vitro and their effects in the trasncryptome in each target cell.

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