Animal Reproduction

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Embryology, developmental biology, and physiology of reproduction

The bovine embryo modify the miRNA profile within the ovidutal environment at early diestrus

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The oviduct provides the environment for early embryonic development. Its lumen is composed mainly by ciliated and secretory cells, which secrets the oviductal fluid (OF). Among the components of the OF, small extracellular vesicles (sEV) can play a role in cellular communication and through miRNAs modulate important metabolic pathways in oviduct cells and embryos. Recently, it has been shown that the embryo can modify the microenvironment in the female tract to its own favor. Herein, we determined the miRNA content of oviduct epithelial cells (OECs) and of sEV from oviducts exposed or not to the embryo. For this, Nelore cows were synchronized by hormonal protocol and divided in two groups, normal artificial insemination (pregnant) and semen diluent (cyclic). Cows were slaughtered 120 hours after ovulation induction. The isthmus portion of the oviduct ipsilateral to the corpus luteum was dissected and flushed for OF and epithelial cells collection. The embryo presence in the OF was confirmed using a microscope. The sEV were isolated from OF by serial centrifugations and by ultracentrifugation. The profile of 383 miRNAs was evaluated in sEV and OECs from pregnant (n=6) and cyclic (n=6) cows. Total RNA was extracted according to Trizol protocol (Thermo Fisher), reverse transcription was performed with HiFlex Buffer using miSCRIPT II RT kit and RTq-PCR with SYBR Green PCR kit (QIAGEN). Real time PCR data were normalized by the geometric mean of Hm/Ms/Rt U1 snRNA and bta-miR-99b. Statistical analysis was performed by Wilcoxon test considering $p<0.05$ for statistical difference. Comparison between miRNA contents of sEV and OECs within the same group demonstrated a total of 358 miRNAs in sEV and OECs of the pregnant group. Among these, 200 miRNAs were detected in both samples, 52 miRNAs were up and 50 down regulated in sEV. Similarly, in sEV and OECs in the cyclic group 364 miRNAs were detected, among them 239 miRNAs in common. Of these, 37 miRNAs were up and 86 were down regulated in sEV. Functional enrichment analysis performed with miRWalk software (version 3.0) demonstrated that miRNAs differently expressed in sEV compared with OECs in both groups are predicted to regulate pathways such as endocytosis, Ras and MAPK signaling. However, cytoskeleton regulation and focal adhesion pathways were predicted to be strongly modulated by miRNAs down expressed in OECs from the cyclic group, but by up expressed miRNAs in OECs from pregnant cows. These pathways are crucial for intercellular communication, signal transduction, proliferation and survival and could be strongly modulated in OECs with the embryo presence. Thus, we suggest that normal expression of these pathways could favor the early embryonic development and transport during their passage through the oviduct. In conclusion, the embryo presence can modulate the ovidutal environment, more specific the miRNA profile within sEV and OECs. Funding: FAPESP 2014/22887-0, 2019/04981-2 and CNPq 420152/2018-0.