Animal Reproduction

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Embryology, developmental biology, and physiology of reproduction

In vivo and in vitro-produced bovine blastocysts secrete small extracellular vesicles with different miRNAs content

Alessandra Bridi[1], Gabriella Mamede Andrade[1], Júlio C. B. da Silva1, Maite del Collado[1], Ana Clara F.C.M de Ávila1, Igor G. Motta[2], Guilherme Pugliesi[2], Luciano A. Silva[1], Flávio V. Meirelles[1], Juliano Coelho da Silveira[1], Felipe Perecin[1]

[1]FZEA/USP - Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo (Pirassununga, Brazil); [2]FMVZ/USP - Department of Animal Reproduction, Faculty of Veterinary Medicine and Animal Science, University of São Paulo (São Paulo, Brazil).

In vivo and *in vitro*-produced bovine embryos present different metabolic profiles, gene transcription, and distinct ability to establish and maintain the pregnancy. Pregnancy losses may occur due to communication failures between embryo and mother. Small extracellular vesicles (sEVs) are part of the embryo-maternal crosstalk and carry bioactive molecules, such as microRNAs (miRNAs). These are small non-coding RNA molecules involved in post-transcriptional regulation and may play a role in modulating embryo-maternal communication during early pregnancy. Our hypothesis is that sEVs secreted by *in vivo* and *in vitro*-produced bovine embryos have different miRNA profiles. Nellore cows previously synchronized were super-stimulated with FSH to produce *in vivo* and *in vitro* embryos. On day 7 after fertilization, embryos from both groups were individually cultured for 48 hours in 30 µL of modified SOFaaci to obtain the conditioned medium (CM). Only CM in the presence of hatched embryos were used. Four pools of CM from 8 embryos each (240 µL of CM each pool) per group were used to isolate sEVs using Exoquick-TC (1:1). sEVs pellets were used for total RNA extraction. MiRNA reverse transcription was performed using miScript II RT Kit (HiFlex). Relative abundance of 382 bovine miRNAs were determined by RT-PCR data normalized by the geometric mean of miR-99b and Hm/Ms/Rt U1 snRNA. Differences in relative abundance were determined by Student's t-test. A total of 106 miRNAs were identified in both groups of sEVs. In sEVs from *in vivo* embryos, 14 miRNAs were upregulated, while two miRNAs were increased in sEVs from *in vitro* embryos. Enriched pathways modulated by these miRNAs were determined by bioinformatics analysis using mirWalk software (version 3.0). The miRNAs (miR-92b, miR-296-5p, miR-323, miR-382, miR-421, miR-541, miR-669, miR-935, miR-940, miR-1225-3p, miR-1249, miR-1281, miR-1296 and miR-1343-3p) were increased in sEVs from *in vivo* embryos and are predicted to regulate MAPK (158 genes), Ras (148), chemokine (101), oxytocin (89) and cell adhesion molecules (CAMs) (87) pathways. Furthermore, miR-494 and miR-1246, which were upregulated in sEVs from *in vitro* embryos are predicted to modulate Wnt (10), CAMs (9), hypoxia-inducible factor 1 (HIF-1) (7) and lysine degradation (5) signaling pathways. These results demonstrate that embryos produced under different conditions (*in vivo* vs. *in vitro*) secrete sEVs with different miRNA profiles. Moreover, miRNAs carried by *in vivo* embryonic derived sEVs are predicted to regulate important endometrial pathways, like oxytocin, MAPK (ERK1⁄2) and Ras, while miRNAs present in sEVs secreted by *in vitro* derived embryos can be involved in regulation of lysine degradation. Based on these findings we suggest that bovine embryo sEVs could modify embryo-maternal crosstalk during early pregnancy and consequently affect pregnancy establishment. Funding: FAPESP 2017/19681-9, 2014/22887-0 and 2018/13155-6. Acknowledgments: CRV Lagoa.