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Uptake evaluation of bta-miR-181d present in extracellular vesicles from bovine oviductal and uterine fluids by in vitro produced embryos

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression through post-transcriptional mechanisms. We have found that bta-miR-181d was more abundant in extracellular vesicles (EVs) isolated from uterine fluid during mid-luteal phase (Days 5-10 of the estrous cycle) than in EVs from the oviductal fluid recovered during the early luteal phase (Days 1-4 of the estrous cycle) in cattle. In addition, bioinformatic analysis indicated that this miRNA is related to Hippo and WNT biological pathways, both critical for lineage segregation and blastocyst formation. Therefore, we aimed to determine whether bta-miR-181d is uptaken in bovine in vitro produced embryos by passive transfection (gymnosis). Presumptive zygotes (PZ) produced by in vitro maturation and fertilization were cultured in SOF (Control) or supplemented with 1 µM miR-181d mimics (miRCURY LNA miRNA Mimics; Qiagen, Maryland, USA); or 1 µM control mimics fluorescently labeled (miRCURY LNA miRNA Mimic 5'FAM, Nº 339173, Qiagen). Embryos were collected at ≥16-cell (≥16C) and D7 blastocyst (BD7) stages and snap-frozen in LN_2 (3 pools n=10/ group) to examine the expression pattern of miR-181d by qPCR using miRCURY LNA miRNA PCR Assay. To confirm the uptake of control mimics fluorescently labeled, BD7 (n=10/group) were fixed, stained with Hoechst 33342, and observed under a widefield fluorescence microscope. Data were transformed by arcsine square root and tested for normality prior to One Way ANOVA. Embryo development was not affected by the presence of miR-181d or control mimics in the media (cleavage rate/PZ: 88±6.0%, 86±1.6%, 87±1.5% and blastocyst yield/PZ on Day 7: 25±0.8%, 25±2.2%, 24±0.1% for miR-181d mimics, control mimics and control respectively, P>0.05). Fluorescent staining showed that the control mimics can be taken up in blastocysts by gymnosis; however, expression of miR-181d mimics did not differ between groups, suggesting that embryos failed to incorporate this miRNA via this mechanism. Consequently, a second experiment was conducted to test Lipofectamine RNAiMAX Transfection Reagent (Life Technologies, Carlsbad, USA) for the delivery of miR-181d mimics. Hence, presumptive zygotes were cultured in SOF (Control) or supplemented with 50 nM miR-181d mimics and 1.5 µL Lipofectamine; or 50 nM control mimics and 1.5 µL Lipofectamine. Embryos (≥16C and BD7) were snap-frozen in LN_2 for future miR-181d expression analyses. Supplementation of Lipofectamine to the culture media did not have any deleterious effect on embryo development (cleavage rate/PZ: 82±4.0%, 81±2.1%, 82±4.1% and blastocyst yield/PZ on Day 7: 25±8.5%, 18±6.4%, 25±3.1% for miR-181d mimics, control mimics and control respectively, P>0.05). In conclusion, despite the fact that the control mimics is uptaken, the miR-181d mimics was not able to internalize in bovine embryos by gymnosis. Lipofectamine does not impair embryo development, hence, it could potentially be used as a carrier for miR-181d. Ongoing work will confirm whether miR-181d is internalized in embryos via this system.

Keywords: mimic miRNAs, early embryo development, bovine