The Role of IVF Culture Media mRNAs in Embryo-Mother Communication in Cattle

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Embryo mortality and suboptimal reproductive performance are major causes of economic loss in dairy production systems. Though there are many factors leading to pregnancy loss, insufficient embryo-mother communication is assumed to be one of the major factors. Our objective was to identify extracellular vesicle (EV) mRNAs expressed in the culture media of IVF derived bovine embryos and investigate their roles in embryo-mother communication. Following isolation of EVs from six IVF media samples, RNA extraction and amplification, and RNA sequencing, three genes were identified to have reads across all exons in all samples: SLBP2, ACCSL, and DPPA3. We selected DPPA3 for further investigation, and in-vitro transcription was utilized to produce mRNA for this gene. This mRNA was then transfected into bovine endometrial epithelial cells (BEnEpCs). Transfected and control cells were subsequently analyzed for RNA sequencing to examine the effects of DPPA3 transfection on gene expression in these cells. A total of 24 genes were found to be upregulated and 1 gene was downregulated (FDR < 0.01) following DPPA3 transfection. Out of the 18 annotated genes, 17 have been discovered to possess known functions in pregnancy recognition in ruminants or have been previously identified to be upregulated in pregnant animals. In addition to RNA sequencing, transfected and control cells were also subjected to proteomic analysis. In total, 34 proteins were found to be differentially expressed (FDR < 0.01, $p<0.05$, fold change > 1.5) with 19 being upregulated and 15 being downregulated. Two proteins, ISG15 and MX1, were found to overlap with the differentially expressed mRNAs. Overall, our results demonstrate that DPPA3 mRNA secreted from IVF embryos may have a role in early embryo-mother communication and maternal recognition of pregnancy prior to major interferon tau secretion.