Animal Reproduction

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# Day 7 in vivo- or in vitro- produced bovine embryos induce distinct molecular alterations in the endometrium

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## Resumo

The first interactions between embryo and endometrium are important for pregnancy establishment and maintenance. On day 15, conceptuses produced from different biotechnologies altered the expression of interferon–tau dependent and independent genes in endometrial cells (Mathew et al., 2019. Biol Reprod. 100:365-380). However, endometrial molecular modifications caused by a single day 7 in vivo or in vitro blastocyst after the embryo transfer are still elusive. Thus, we hypothesized that a single day 7 in vivo or in vitro-derived bovine embryo modify endometrial global transcriptomic response when co-cultured without contact with endometrial explants. For this, Day 7 embryos were produced in vivo or in vitro. The endometrial explants were collected from the uterus of cows (n=3) previously synchronized to be on day 7 of the estrous cycle. Endometrial explants from the same uterus were cultured with medium (without embryo; EE), or a single day 7 in vivo–produced bovine embryo (EE-AI), or a single day 7 in vitro-produced bovine embryo (EE-IVF). The co-cultured was performed using inserts with pores of 0.4 µm size to retain the embryo directly above the endometrial surface for 24 hours. Total RNA extraction from endometrial explants was done using miRNeasy Mini Kit (QIAGEN) following the manufacturer's instruction. The RNA library preparation was performed using Illumina TruSeq Stranded mRNA Sample Prep kit. The sequencing was performed in 1 lane of Hiseq 2500 V4 (2x100pb). Compared to EE, 273 differently expressed genes (DEGs) were identified in EE-AI and 409 in EE-IVF (P-adjust.≤ 0.1 and log2FoldChange>0.6) groups. Of these, 142 DEGs were expressed in EE-AI and EE-IVF groups, among these DEGs, some traditional IFNt responsive genes (ISG15, MX1, MX2, RSAD2, and OAS1Y) were more expressed in these groups than EE. The tops three pathways identified in enriched KEGG analysis were influenza A, measles, and herpes simplex infection (P-adjust.≤ 0.1). Between the EE-AI and EE-IVF groups, 156 genes were differentially expressed (P-adjust.≤ 0.1). The top five enriched pathways identified were calcium signaling pathways, ABC transporters, renin secretion, focal adhesion, and complement and coagulation cascades (P-adjust.≤ 0.1). Of these 156 DEGs, 87 were exclusively altered when compared EE-AI with the EE-IVF group. The GO analyses identified steroid hormone mediated signaling pathway, and positive regulation of JNK cascade as the biological processes associated with these 87 DEGs (P-value.≤ 0.1). In conclusion, the present study showed that the presence of a single day 7 blastocyst was able to induce DEGs in endometrial explants in an origin-dependent fashion (in vivo or in vitro). Curiously, we identified endometrial DEGs modulated by the embryo presence, demonstrating that these DEGs possibly have an important role during the first embryo-maternal interactions.

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