Interactions between addition of serum and type of culture medium on gene expression of bovine blastocysts produced in vitro.

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Serum is commonly added to culture medium used for producing bovine embryos in vitro. It is often suspected that serum can cause abnormal calf development. Here we tested 1) whether addition of bovine serum to embryo culture medium increases the percent of embryos becoming blastocysts and alters blastocyst gene expression and 2) whether actions of serum depend upon culture medium. The media used were synthetic oviduct fluid medium bovine embryo 2 (SOF) or a commercial medium from IVF-Biosciences (IVFB). Three experiments were performed using embryos produced by in vitro fertilization with abattoir-derived oocytes. The design for each experiment was a 2 x 2 factorial design with 10% (v/v) serum or control medium as one main effect and medium type as the other. Fetal bovine serum was added on day 5 of culture in Exp 1 (6 replicates). Adult bovine serum was added at day 5 in Exp 2 (3 replicates) and on day 1 in Exp. 3 (4 replicates). Data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Effects of medium and serum on development were similar for each experiment so data were combined for statistical analysis. The percent of oocytes that cleaved was not affected by medium or serum but the percent of putative zygotes becoming blastocysts was greater for IVFB than SOF (P=0.0010) and greater for serum than no serum (P=0.0004). Least-squares means were 44.1 (SOF), 54.5 (SOF+serum), IVFB 51.9 (IVFB), and 58.2% (IVFB+serum) (SEM=0.04%). Gene expression for pools of 10 blastocysts collected in Exp. 1 was evaluated by RNA-Seq (n=4 pools per treatment). Differentially expressed genes (DEG) were those with an adjusted p-value of < 0.01 and an absolute log2 fold change > |2|. In the absence of serum, there were 99 DEG for IVFB vs SOF (22 upregulated and 67 downregulated). Serum resulted in 80 DEG (21 upregulated and 59 downregulated) for embryos cultured in IVFB and 108 DEG (71 upregulated and 37 downregulated) for embryos cultured in SOF. A total of 11 DEG were regulated by serum similarly for both media including transcription factors *ASCL2* (downregulated) and *ZSCAN4* (upregulated). *ASCL2* is imprinted in the mouse and plays a role in placental formation and fetal growth. Among the DEG downregulated by serum for IVFB only were *NANOG*, involved in epiblast formation, and three interferon-tau genes (maternal regulation of pregnancy signals). Four genes downregulated by serum in IVFB are imprinted in the cow (*MEG3*, *MEG8*, *MEG9,* and *RTL1*) but none of the DEG for SOF are known to be imprinted in the cow. In conclusion, actions of serum on gene expression are modest in extent, dependent on culture medium, and include an overrepresentation of imprinted genes. One DEG, *ASCL2*, has been implicated in fetal growth. Future experiments to evaluate the impact of disruptions in its expression on fetal development are warranted.

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