Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Conjugated linoleic acid supplementation alters prostaglandin synthesis and transcript abundance in bovine trophoblast cells cultured *in vitro*

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Early embryonic mortality, caused by failures in maternal recognition of pregnancy (MRP) in the first three weeks after fertilization, represents the major cause of reproductive inefficiency in beef cattle females. Modifications in prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) concentrations may benefit MRP. The addition of conjugated linoleic acid (CLA) in endometrial and fetal cell culture affected prostaglandin synthesis, however such effect on bovine trophoblastic cells (CT-1) is unknown. We aimed to determine the effects of CLA (mixture of cis- and trans-9, 11- and -10,12-octadecadienoic acid Sigma- Aldrich, USA, O 5507) on PGE2 and PGF2α synthesis and the expression of transcripts involved with MRP in bovine trophoblast. The CT-1 were cultured for 24 days, incubated at 38.5°C in a humidified atmosphere with 5% CO2 in culture bottles. After this step, the CT-1 were transferred to culture plates containing medium added with 10% fetal bovine serum (FBS), where they were cultivated for 5 days. Next, the CT-1 received culture medium free of FBS for 24 hours. The CT-1 were supplemented with different concentrations of CLA (0, 10, 20, 50 or 100 µM) in an SFB-free medium for 24, 48 and 72 hours. After collection from the culture medium, the CT-1 were lysed with 1 mL Trizol for 5 minutes and stored at -80ºC. The transcript abundance was determined by qRT-PCR and PGE2 and PGF2α were quantified by ELISA. Statistical analysis was performed using PROC MIXED from SAS (summer 9.2, SAS Institute Inc., Cary, NC, USA) considering the well the experimental unit. In all groups treated with CLA the concentrations of PGE2 (P = 0.0285) and PGF2α (P = 0.0001) were reduced with 24 and 72 hours of culture compared to control. In addition, CLA at all doses tested increased the PGE2 /PGF2α ratio (P = 0.0001) with 24, 48, and 72 hours and determined a quadratic effect on the relative expression of PTGER4 (P = 0.0026), PTGES2 (P = 0.0273), and MMP9 (P = 0.0256) transcripts. The relative abundance of PTGER4 was reduced in CT-1 cultured with 100 µM CLA when compared to the control group and supplemented with 10 µM CLA (0.0470 ± 0.0054 control vs. 0.0532 ± 0.0054 10 µM -CLA *vs*. 0.0223 ± 0.0061 100 µM -CLA). It is concluded that treatment with CLA decreases the synthesis of PGE2 and PGF2α and increases the PGE2:PGF2α ratio in a dose-dependent manner. The CLA supplementation determines a quadratic effect on the expression of transcripts related to prostaglandin metabolism (PTGES2, PTGER4) and on extracellular matrix remodeling (MMP9).