Animal Reproduction

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Embryology, developmental biology, and physiology of reproduction

Conjugated linoleic acid supplementation reduces prostaglandin E2 and F2a secretion from bovine trophoblastic cells in vitro

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Early embryonic mortality represents a major cause of reproductive failure in cattle. Strategies to decrease synthesis of prostaglandin F_{2alpha} (PGF_{2a}) and increase synthesis of prostaglandin E_2 (PGE_2)can benefit the establishment of pregnancy. Conjugated linoleic acid (CLA) supplementation in cell culture medium affectsthe synthesis of prostaglandins; however the effect of CLA supplementation on cultured bovine trophoblast cells (CT1) has not been determined. The hypothesis of this study is that CLA supplementation on *in vitro* culture medium of CT1 cells increase synthesis of PGE_2 and decrease synthesis of PGF_{2a}, benefiting the establishment of pregnancy. The aim was to determine the effects on PGE_2 and PGF_{2a} synthesis with supplementation of varying concentrations of CLA (Sigma-Aldrich, USA, Cat N°. O5507) on *in vitro* culture of CT1 cells. The CT1 cells were cultured for 22 days in a humidified incubator at 38.5□C with 5% CO_2 until they reached 100% confluence. On the 23th day they were transferred to six-well plates with DMEM (1X) + GlutaMAX medium supplemented with 10% of fetal bovine serum (FBS), 1% of non-essential amino acids, 1% of antibiotic-antimycotic and 0.001% of □-mercaptoethanol, to be cultured for another 5 days until reaching 50% confluence. Twenty-four hours before CLA supplementation (day 6 of culture), the medium was replaced with a new medium without FBS, and on day 7 medium without FBS was supplemented with varying CLA concentrations (0, 10, 20, 50 or 100 µM) for a 24-hour culture period. Collected medium was stored at -20□C until analysis. A total of five culture replicates were performed. Concentrations of PGE_2 and PGF_{2a} on day 8 were determined by enzyme-linked immunosorbent assay. Statistical analyzes were performed using the PROC MIXED of SAS program (version 9.2, SAS Institute Inc., Cary, NC, USA) considering the main effect of treatment group and the random effect of culture replicate. Concentration of PGE_2 was greater (P = 0.04) for control (89.74 ± 4.39 ng/mL) in comparison to 10, 20, 50 and 100 µM of CLA (63.65 ± 8.51; 56.28 ± 9.66; 58.61 ± 9.31 and 64.77 ± 11.59 ng/mL, respectively). Concentration of PGF_{2a} was also greater (P□ 0.0001) for control (66.67 ± 7.89 ng/mL) in comparison to 10, 20, 50 and 100 µM of CLA (33.49 ± 5.01; 24.86 ± 4.41; 26.22 ± 4.53 and 25.73 ± 3.23 ng/mL, respectively). A significant effect (P = 0.0007) on PGE_2/PGF_{2a} ratio was also observed, reflecting a greater (P< 0.05) ratio in CLA-treated groups (1.99 ± 0.21; 2.39 ± 0.31; 2.35 ± 0.26 and 2.55 ± 0.39, respectively) compared to the control group (1.41 ± 0.13) and a greater (P< 0.05) ratio in CTl cells treated with 100 µM compared to 10 µM of CLA. We conclude that CLA treatment for 24 hours on *in vitro* culture medium of CT1 cells decreased PGE_2 and PGF_{2a} synthesis, but a CLA dose-dependent effect was observed on PGE_2/PGF_{2a} ratio. Acknowledgement:grant #2018/24168-1 and #2019/00637-5, São Paulo Research Foundation (FAPESP).