

Developing a 3D Long-Term Trophoblast Culture System to Study Fetal-Maternal Interactions in Cattle

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The greatest limitation of in vitro 2-dimensional trophoblast cell culture systems used to study placental physiology is the inability to accurately mimic the complexity of tissue structure and physiology of in vivo placentation. Three-dimensional (3D) long-term cultures have the potential to represent the in vivo microenvironment of placental tissue more accurately, with cell-cell, and cell-extracellular matrix interactions. Our goal is to establish and characterize a 3D long-term bovine trophoblast cell culture system that accurately recapitulates bovine placental physiology and can be used to study placental function and embryonic loss in cattle. We hypothesize that the 3D culture will resemble the morphology, gene expression, and protein expression of placental tissue. Eight Angus cows were estrus synchronized using OvSynch + CIDR (controlled internal drug-releasing dispenser), superovulated, and artificially inseminated. Embryos were flushed transcervically at sixteen and twenty-one days after artificial insemination. Concepti retrieved from two day-16 pregnancies and one day-21 pregnancy were identified under a dissecting microscope, mechanically separated from mucus and uterine secretions, the embryonic disk was removed, and incubated for 2 minutes in 0.25% of trypsin in Dulbecco's Modified Eagle Medium (DMEM). The tissue was placed in T25 flasks with Trophoblast Organoid Medium containing DMEM/F12, N2 supplement, B27 supplement minus vitamin A, primocin, N-acetyl-L-cysteine, L-glutamine, recombinant EGF, CHIR99021, recombinant R-spondin-1, recombinant FGF-2, recombinant HGF, A83-01, prostaglandin E2, and Y-27632. Tissue was cultured in an incubator at 37°C, 5% CO₂. Trophoblast tissue was collected at 0, 3, 6, and 12 months in culture. Day 40 and 80 fresh placental tissue and cultured fibroblast cells were used as controls for gene expression analysis. Cellular viability and proliferation rates were assessed using the CyQuant XTT cell viability kit over time. Expression of genes related to trophoblast development, function, and differentiation was assessed. The presence of placental alkaline phosphatase (PLAP) was assessed using a sandwich ELISA. Cell morphology was evaluated by a pathologist. Collagen and extracellular matrix deposition were evaluated using picosirius red and Masson trichrome stains. Cell viability and proliferation results showed proliferation in all culture groups throughout the 48-hour assay. Histological analyses demonstrated that some cells differentiate into binucleate cells and that these cells form spheroid structures that resemble trophoblast organoids. Gene expression analyses have demonstrated that these cells maintain the expression of trophoblast-specific genes such as PD-L1, PRP-VII, PAG11, PAG 12 and PTSG2 up to 12 months after the start of culture. In addition, placental alkaline phosphatase (PLAP) was secreted in the supernatant of cultured trophoblast cells. There was no evidence of extracellular matrix deposition. These results indicate that this 3D long-term trophoblast cell culture model is

morphologically and molecularly similar to placental tissue. Further characterization of this 3D model is needed to better understand if it could be a useful tool to study fetal-maternal interactions in cattle. This project was supported by the Utah Agriculture Experiment Station Project no. 1459.