IFNT-Dependent and IFNT-Independent Changes in the Corpus Luteum of Early Pregnancy

Camilla H. K. Hughes[1], Adelaide C. Hellmers[1,2], M. Isabel da Silva[1,2], Troy L. Ott[1], Joy L. Pate[1]

1. Center for Reproductive Biology and Health, Department of Animal Science, The Huck Institutes for the Life Sciences, Pennsylvania State University, University Park, PA, USA
2. Graduate Program for Integrative and Biomedical Physiology, The Huck Institutes for the Life Sciences, Pennsylvania State University, University Park, PA, USA

The effects of interferon tau (IFNT) on the uterus are critical for luteal rescue during maternal recognition of pregnancy in ruminants. In addition, during the time of IFNT secretion, the corpus luteum (CL) of pregnancy is transiently more resistant to the actions of prostaglandin (PG) F2A than the CL of the cycle. We and others reported changes in the CL of early pregnancy, including mechanisms related to cell survival, immune pathways, PGF2A signaling, and matrix remodeling (527 mRNA changed temporally in day 14, 17, 20, and 23 CL, Padj<0.05. An additional 630 tended to change, 0.15 > Padj > 0.05; Hughes et al., 2020). The objective of this study was to determine the extent to which the changes observed in the CL of pregnancy are directly regulated by IFNT. To address this, we performed two experiments. In experiment 1, IFNT (10 ug/ml) in 20 ml of PBS containing BSA (200 ug/ml) or BSA vehicle alone (control) was infused into uteri of nonbred heifers 2X per day from day 14-16 of the cycle. CL were collected on the morning of day 17 and RNAseq was performed (n=4/group). Relative to BSA alone, IFNT infusion resulted upregulation of 87 mRNA and downregulation of 30 (Padj < 0.05). As expected, these genes were primarily involved in interferon signaling and antiviral response. In experiment 2, CL were collected from nonbred, day 10-12 cows, luteal steroidogenic cells were cultured for 24 hours with or without 1 ng/mL of IFNT, and RNAseq was performed (n=4/group). Relative to control, treatment with IFNT resulted in upregulation of 1733 mRNA and downregulation of 1153 (Padj < 0.05). These genes were primarily involved in immune response, cytokine signaling, and antiviral response. In total, 75% of changed genes from experiment 1 also changed in experiment 2, confirming the effectiveness of intrauterine IFNT infusion in inducing interferon-stimulated genes in the CL. Remarkably, only 31% of the genes that changed during early pregnancy were regulated by IFNT treatment in vitro or by IFNT infusion in vivo (Padj < 0.05). Gene ontology analysis revealed, as expected, that these genes were regulators of antiviral and immune responses. Among these genes were three Poly(ADP-Ribose) Polymerase (PARPs), PARP9, 12, and 14, which promote DNA damage response and cell survival, and CD55, an inhibitor of the complement-mediated cell death pathway. In contrast, 50% of the genes that changed (Padj < 0.05) during early pregnancy were neither regulated by IFNT in vivo nor in vitro (padj > 0.2 and log2 fold change <|1| in both IFNT datasets). These mRNA are associated with cellular proliferation and extracellular matrix organization. Predicted upstream regulators included estradiol, ESR1, and prostaglandin E receptors. A final 19% of transcripts could not be conclusively grouped as either regulated or not regulated by IFNT. In summary, this integrative analysis suggests that most of the temporal changes in the CL during early pregnancy that we previously reported are not regulated directly by IFNT, drawing into question identities of other luteal regulators during early pregnancy or the effect of age of CL independent of embryonic signaling. Supported in part by USDA NIFA grant # 2017-67015-26455 to TLO, NIH-T32 Fellowship grant #T32GM108563, and C. Lee Rumberger and Family Chair in Agricultural Sciences endowment to JLP.