Luteal Fibroblasts Produce Prostaglandin F2α in Response to IL1β in a MAPK-mediated Manner

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The corpus luteum is a temporary endocrine gland that is crucial for pregnancy, as it produces the progesterone needed to maintain optimal uterine conditions for embryo implantation. In the absence of a conceptus, the corpus luteum becomes non-functional and undergoes rapid tissue remodeling to regress into a fibrotic corpus albicans, in a process initiated by prostaglandin F2α (PGF2α) in ruminants. Early luteal regression is characterized by elevated levels of luteal cytokines. Because the luteal tissue micro-environment is poorly understood. Further, the contribution of luteal fibroblasts to luteal regression remains to be elucidated. The aim of this study was to determine the response of bovine luteal fibroblasts to inflammatory cytokines. Because such cytokines are known to induce prostaglandin production, we hypothesized that bovine luteal fibroblasts produce PGF2α in response to tumor necrosis factor α (TNFα) and interleukin 1β (IL1β). To test our hypothesis, we established primary cultures of bovine luteal fibroblasts and treated them with TNFα (10 ng/mL) and IL1β (10 ng/mL). Protein and mRNA were collected for western blot and RT-PCR analysis, and culture medium was collected for PGF2α ELISA. Secreted arachidonate metabolites were also analyzed by LC/MS. Statistical analysis was performed by two-way ANOVA with a Tukey's post hoc multiple comparison test, or one-way ANOVA as appropriate. Short-term treatment (2-120 min) revealed that both cytokines induced canonical mitogen activated protein kinase (MAPK) signaling in luteal fibroblasts by stimulating the phosphorylation of ERK1/2, p38 MAPK, JNK. However, IL1β induced more robust activation of these kinases than TNFα ($p < 0.05$). Treatment for 24 h revealed that TNFα increased PGF2α, cytosolic phospholipase A2 ($cPLA_2$) protein, and prostaglandin-endoperoxide synthase 2 (PTGS2) protein nearly 2-fold, but not at a statistically significant level. IL1β elevated PGF2α levels in the culture

medium over 20-fold ($p < 0.0001$), as well as mRNA and protein for enzymes involved in prostaglandin synthesis, $cPLA_2$ (4-fold, $p < 0.0001$) and PTGS2 (22-fold, $p < 0.01$). Pre-treatment of luteal fibroblasts with Vehicle (DMSO), MEK inhibitor, U0126 (10 µM), p38 inhibitor, SB203580 (20 µM), or JNK inhibitor, SP600125 (10 µM) for 1 h prior to 24 h treatment with IL1β (10 µg/mL) abrogated the stimulatory effects of IL1β. IL1β-induced PGF2α secretion was diminished in U0126 ($p < 0.001$), SB230580 ($p < 0.001$) and SP600125-treated ($p < 0.05$) fibroblasts. Inhibition of ERK and p38 MAPKs also decreased IL1β-induced $cPLA_2$ protein ($p < 0.001$). However, inhibition of JNK induced an increase in both $cPLA_2$ and PTGS2 protein. IL1β still elevated PTGS2 protein in response to U0126; however, that response was blunted compared to the vehicle control (4-fold in U0126-treated fibroblasts vs 20-fold in vehicle control, $p < 0.01$), and p38 MAPK inhibition diminished the IL1β-induced PTGS2 response ($p = 0.148$ vs inhibitor control without IL1β). Mass spectrometry analysis of arachidonate metabolites following treatment with IL1β revealed significant increases in other prostaglandins (PGE2, PGD2, 6-Keto-PGF1). All together, we have identified luteal fibroblasts as potential inflammatory mediators operating in the luteal microenvironment during luteal regression. This study was supported by funding from the American Heart Association (AHA 23PRE1018741, to CFM), as well as funding from USDA NIFA Grant 2023-67015-40795 (JSD).