Endometrial cytokine gene expression in normal versus endometritic postpartum dairy cows
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Our objective was to better understand the inflammatory changes associated with uterine involution during the postpartum period and compare the changes that occur during normal involution versus those with endometritis. We hypothesized that distinct, time-dependent changes occur in the expression of both pro- and anti-inflammatory cytokines during uterine involution and the expression of these cytokines is altered in uterine mucosal inflammation -endometritis. Postpartum dairy cows (n=41) from a closed herd were included in our observational cohort study to assess the changes in gene expression (using qRT-PCR) in endometrial samples harvested from the uterine body using a modified cytobrush assembly. Cows were sampled first between 29-35 and then again between 49-56 days in milk (DIM). Cows were classified as normal (n=15) or endometritic (n=12) based on >18% neutrophils on cytological smears at 29-35 DIM. In addition, another set of cows with normal involution of uterus from the same herd were either sampled at 29-35 DIM (n=8) or 49-55 DIM (n=6) as procedural controls. Endometrial cytobrush samples obtained from animals from all groups were processed for qRT-PCR to quantify mRNA abundance for interleukin (IL)-1α, IL1β, IL6, IL8, IL13, IL17, IL18, colony stimulating factor (CSF)1, tumor necrosis factor alpha (TNFα), IL1 receptor antagonist (IL1Ra), IL10, transforming growth factor (TGF) β1, TGFβ2 and TGFβ3 genes relative to gene expression of β-actin (reference gene). Gene expression in uterine samples collected from the clinical cohorts was compared by Wilcoxon Rank Sum test. Expression of β-actin gene was similar (P>0.05) in samples collected from normal and endometritic cows. Gene expression data for procedural control groups did not differ from the time-matched cohorts of the Normal cows. Therefore, these data were combined with the Normal group data (with the respective time points) for further comparisons. At 29-35 DIM, uterine samples from the Endometritic cows had higher median expression of pro-inflammatory cytokines, including IL1α (17 fold, P<0.01), IL8 (16.8 fold, P<0.01), IL1β (7.6 fold, P<0.01), CSF1 (4.4 fold, P=0.03) and TNFα (2 fold, P=0.04) than uterine samples from the Normal cows. Furthermore, uterine samples from the Endometritic cows displayed greater expression of some anti-inflammatory cytokines, including IL1Ra (12.6 fold, P<0.01) and IL10 (2 fold, P=0.04), but lower expression of one anti-inflammatory cytokine - TGFβ3 (2.6 fold, P<0.01), than uterine samples from the Normal cows. By 49-55 DIM, the only difference was the higher median expression of the IL6 (9.4 fold, P=0.01) and IL17 genes (6.6 fold, P=0.04) in uterine samples from the Endometritic cows. In conclusion, the gene expression of pro- and anti-inflammatory cytokines differed between cows (at 29-35 DIM) with endometritis (≥18% neutrophils) and those involuting normally (<18% neutrophils). Differences in gene expression changed with increasing DIM.

Keywords: Cytokines, endometritis, qRT-PCR, postpartum cows.