Pro-inflammatory cytokine gene expression in endometrial cytobrush samples harvested from cows with and without subclinical endometritis

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A detailed understanding of the postpartum cow’s uterine immune responses is lacking due to difficulties in obtaining endometrial tissue. Our objectives were to develop a minimally invasive cytobrush technique to collect endometrial cells in postpartum cows (28 to 41 days in milk; DIM) for the isolation of mRNA, and to characterize the expression of key regulatory cytokines including: IL-6, IL-8 and TNFα. Cows without clinical signs of endometritis were subdivided into those with a negative or positive (defined as >18% neutrophils) endometrial cytobrush cytology. Thirty Holstein cows were sampled from six commercial dairies. A modified cytobrush was double guarded and advanced into the cervix, passed through the cervical canal and advanced into the uterine body, rotated 360 degrees and retracted into its sheath before removal from the reproductive tract. The cytobrush was then gently rolled onto a clean slide for cytology, and then transferred to a tube with 1 ml Trizol® reagent (Invitrogen, Carlsbad, CA) and stored at -80°C until mRNA isolation. Slides were air-dried and stained with modified Wright Giemsa stain. Each slide was examined at 400x magnification, and three differential counts of >100 cells were averaged for percentage of neutrophils. Total RNA from cytobrush samples was extracted, isolated and reverse transcribed to make cDNA which was used to perform real time (RT)-qPCR analysis of IL6, IL8, TNFα and β-Actin gene expression. Validated gene primers were used to amplify cDNA targets using a two-step qRT-PCR kit with SYBR® Green, in a real time thermocycler. The RT-qPCR amplification data were normalized to β-Actin (ΔCT). Variables were percentage of neutrophils in cytobrush cytology and quantified mRNA expression levels for IL-6, IL-8 and TNF α. Data were analyzed using STATA version 10. Twelve cows were categorized as endometritis positive and 18 were categorized as endometritis negative. Cytobrush sampling provided sufficient material for endometrial mRNA extraction (mean 0.96 µg total RNA per sample). Cytokine expression varied with IL-6 showing a 30-fold higher expression level (P=0.01) and IL-8 showing >50-fold higher expression level (P=0.0001) in subclinical endometritis positive versus negative cows. The TNFα mRNA expression level in subclinical endometritis positive cows was 20-fold higher (P=0.001) versus the disease-negative cows. Regression analysis between mRNA expression levels (ΔCT) of cytokines and percentage neutrophils in endometritis positive cows showed that for every threshold cycle increase in IL-8 expression, the number of neutrophils decreased by 3.3% (P= 0.00001). Similarly, for IL-6 and TNF-α, the number of neutrophils counted in endometritis positive cows decreased by 2.3% (P=0.015) and 2.4% (P=0.054) respectively. In conclusion, the cytobrush technique can be used to obtain sufficient endometrial material for both cytology and RNA extraction and the current analysis confirms that cytology accurately reflects endometrial inflammation.

Keywords: Cytobrush, postpartum endometritis, inflammation, mRNA expression