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Abstract

Two experiments were designed to study endometritis in postpartum dairy cows. In the first experiment, 30 cows 28 to 41 days in milk (DIM) and without evidence of clinical endometritis were sampled using cytobrush cytology. Cytobrush sampling provided sufficient endometrial material to prepare cytologic specimens and to extract endometrial mRNA. Pro-inflammatory cytokines were analyzed in harvested endometrial tissue taken from cows with and without endometritis. Cytokine expression varied between experimental groups with 30-fold higher IL-6 expression levels ($P=0.01$), greater than 50-fold higher IL-8 expression levels ($P=0.0001$), and 20-fold higher TNF- α expression levels ($P=0.001$) in endometritis-positive versus negative cows. Regression analysis of cytokine expression levels (Ct) and the percentage of PMNs in subclinical endometritis-positive cows showed that for each additional threshold cycle required for IL-8 detection, which corresponded to two-fold less mRNA, the percentage of PMN decreased by 3.3% ($P=0.00001$). Similarly, for each additional threshold cycle required to detect IL-6 and TNF- α , the percentage of PMNs in endometritis-positive cows decreased by 2.3% ($P=0.015$) and 2.4% ($P=0.054$), respectively. Cows with > 18% PMNs required significantly fewer amplification cycles to detect IL-6 ($P = 0.01$), IL-8 ($P = 0.0001$) and TNF- α ($P=0.053$) mRNA than cows with <18% PMNs (endometritis-negative). There was a highly significant positive correlation between the expression of individual pro-inflammatory cytokines when comparing IL-8 and IL-6 ($P=0.0001$), IL-8 and TNF- α ($P=0.00001$), and finally IL-6 and TNF- α ($P=0.0002$).

In the second experiment, 340 cows 28 to 41 days in milk were examined using cytobrush cytology and transrectal ultrasonography of the uterus and ovaries. One-half of the cows were treated with benzathine cephapirin uterine infusion to determine the lowest PMN percentage where a significant improvement in reproductive performance occurred. Subclinical endometritis-positive (>15%) cows in this study were defined as those with the lowest percentage of PMNs that was associated with a significant positive treatment effect. Treated cows with >15% PMNs required 31 fewer days ($P=0.041$) to become pregnant and had 2.5 times fewer services per conception ($P=0.0001$) than untreated cows with >15% PMNs. The likelihood of there being CLs at the time of examination in cows with >15% PMNs in endometrial cytobrush cytology was 2.3 times significantly higher ($P=0.04$). The treatment of cows with

ultrasonographically detectable fluid in the uterine lumen with benzathine cephapirin had no effect on days open compared to treatment of cows without fluid in the uterus ($P=0.39$). Cervical diameter and endometrial thicknesses did not differ between groups of cows with $>$, $<$ 15%PMNs ($P=0.46$, $P=0.36$, respectively).

In summary, based on the response to a single treatment with benzathine cephapirin, and the analysis of pro-inflammatory cytokine gene expression, we recommend that a threshold of $>18\%$ PMNs be used to define endometritis-positive disease status in cows 28 to 41 DIM. Cervical diameter, ultrasonographic evidence of uterine fluid and ultrasonographic measurement of endometrial thickness were not useful for diagnosing benzathine cephapirin responsive endometritis.

Key words: Endometritis, Cytokines, Treatment, Dairy cows

DEDICATION

To Oksana, Lina and my parents

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
cDNA	Complementary DNA or Copy DNA
CL	Corpus luteum
cm	Centimeter
DIM	Days in Milk
FSH	Follicle-stimulating hormone
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IU	Intrauterine
LH	Luteinizing hormone
LPS	Lipopolysaccharides
mg	Milligram
mL	Milliliter
mRNA	Messenger RNA
ng	Nano gram
ON	Ontario
PBS	Phosphate Buffer Saline
PGF2 α	Prostaglandin F2 α
PGFM	metabolite of PGF2 α
PMNs	Polymorphonuclear leukocytes
qRT-PCR	Quantitative real-time polymerase chain reaction
SD	Standard deviation
TLRs	Toll-like receptors
TNF- α	Tumor necrosis factor-alpha
μ g	Microgram
μ L	Micro liter
WCVM	Western College of Veterinary Medicine