Immunoglobulin G, white blood cell, and fibrinogen concentrations among dairy cows with and without endometritis during transition period

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Abstract

Postpartum reproductive performance closely depends on uterine health. Under normal circumstances, almost 100% of cows have uterine contamination within first 2 weeks after calving. Whereas the innate immune and reproductive systems usually eliminate most offending microbes, persistent infection still reportedly occurs in ~ 20% of postpartum cows. Our purpose was to estimate concentrations of systemic immune indicators (IgG, white blood cells, and fibrinogen) during the transition period in dairy cows with and without endometritis. Fifty-nine multiparous cows were systematically and consecutively enrolled during the dry period and examined 6 times from 40 days before to 40 days after calving. Cows in the diseased group (n = 11) were identified based on 4 criteria (presence of *Trueperella pyogenes* grade > 2 in the uterus, clinical endometritis, subclinical endometritis, and cervicitis). Cows in the control group (n = 11) were negative for all 4 criteria. Prevalence of *Trueperella pyogenes*, clinical endometritis, subclinical endometritis, and cervicitis was 25, 16, 23, and 31%, respectively. Concentrations of IgG, white blood cells, and fibrinogen did not change over the period or vary among control and diseased cows. In conclusion, systemic indicators of inflammation were not good markers for diagnosing or monitoring endometritis in postpartum dairy cows.

Keywords: Diary cattle, inflammatory markers, uterine disease

Introduction

Reproductive health of cows is the foundation of productivity in the dairy industry. Although entire transition period is critical for dairy cows, postpartum reproductive performance is closely dependent on uterine health. For cattle, the transition period is essentially a period of stress management that requires metabolic adjustments in the face of a substantial negative energy balance and immune system modulations.[1] These adjustments enable a smooth transition from pregnancy state to restoration of uterine condition, ovarian cyclicity, and establishment of subsequent pregnancy.[2-4] Under normal circumstances, almost 100% of cows have uterine contamination within the first 2 weeks after calving.[5,6] Although the innate immune system usually eliminates all offending microbes, persistent infection or inflammation reportedly occurred in 15 - 40% of postpartum cows.[6] Postpartum uterine disease is commonly associated with *Escherichia coli (E. coli)*, *Trueperella pyogenes (T. pyogenes)*, *Fusobacterium necrophorum (F. necrophorum)*, and *Prevotella* species.[4] Numerically, the most common pathogens were *E. coli* (37% of pathogenic bacteria isolated) and *T. pyogenes* (49%).[7] When contamination of the uterus persists beyond 4 weeks postpartum, the uterine infection is labelled as endometritis. Clinical and subclinical endometritis are prevalent conditions in postpartum dairy cows, causing economic losses due to decreases in both milk production and fertility.[8] Clinical endometritis is defined as inflammation of the endometrium with purulent vaginal discharge (PVD) and in the absence of systemic clinical disease at ≥ 21 days postpartum,[4] whereas subclinical endometritis is defined as the presence of > 18% polymorphonuclear neutrophils (PMNs) in uterine cytosmears (21 - 33 days postpartum) or > 10% PMNs (34 - 47 days postpartum).[9]

Cellular and humoral mechanisms of nonspecific and specific immunity have important roles in the resolution of postpartum endometritis. First and most substantial cell type recruited during uterine inflammation is the PMN.[10] These cells, along with monocytes, are recruited from the blood circulation into uterine lumen to eliminate bacteria.[11] Like PMNs and macrophages, endometrial cells also have Toll-like receptors (TLRs) that recognize pathogens present in the uterus. For example, TLR4 recognizes lipopolysaccharides in the cell wall of gram-negative bacterial species, initiating an innate immune response that triggers the release of antibacterial substances from responding cells.[12] This inflammatory response results in other inflammatory cells attracting PMNs, amplifying an inflammatory loop.[13] Endometrial cells also secrete diverse molecules, including cytokines, whose presence in uterine secretions reflected the intensity of endometrial inflammation.[14]

Fibrinogen response is a useful indicator of the presence of inflammation, bacterial infection, and surgical trauma in cat-

tle. Fibrinogen binds specifically to CD11/CD18 integrins on the cell surface of migrated phagocytes, triggering a cascade of intracellular signals that leads to degranulation, phagocytosis, cellular cytotoxicity, and delayed apoptosis. Integrins, a group of adhesion molecules that are expressed on leucocytes, have an important role in immune function.[15] Plasma fibrinogen concentrations were higher in cows with subclinical and clinical mastitis than in control cows.[14]

Immunoglobulins (Ig) also have a protective role against pathogens in the bovine uterus.[16] However, the specific functions of the isotopes IgA, IgG, and IgM in the genital tract are still not clear. Innate and adaptive immunity, both local and systemic, have an essential role in defending the uterus against postpartum infection. We hypothesized that clinical endometritis is associated with an increase in systemic indicators of inflammation in dairy cows. The hypothesis was tested by comparing concentrations of IgG, white blood cells (WBC), and fibrinogen during the transition period among dairy cows with and without endometritis.

Materials and methods

Animals

Procedures were carried out in compliance with Canadian Council on Animal Care Guidelines, and the animal care committee of the Université de Montréal approved the experimental protocol. Multiparous cows (n = 59) were selected based on sequential date of calving (June 2016 - February 2017) from 3 commercial dairy herds in Quebec (Canada). Herd size was from 70 - 130 lactating cows. Reproductive and health data were compiled in a databank using health record management software (DSAHR, Saint-Hyacinthe, Québec, Canada). Rolling herd average milk production was ~ 9000 kg. Cows were housed in tie stall barns and milked twice daily. Cows were fed a total mixed ration consisting of mainly corn silage or hay silage formulated to meet the dietary requirements of lactating dairy cows at each stage of production.[17] Farms were visited weekly by the same veterinarians. Cows were vaccinated intramuscularly against *E. coli* on day 40 and on day 26 before calving (2 ml, J-VAC, Merial Inc., Athens, GA), and against types 1 and 2 BVD, IBR, PI-3, and BRSV on day 15 - 40 after calving (2 ml, Bovi-Shield GOLD® FPTM 5 L5, Zoetis, Parsippany, NJ). Cows were injected with 5 ml of selenium on day 60 after calving (MU-SE, Intervet Canada Corp., a subsidiary of Merck and Co. Inc, Kirkland, QC, Canada).

Experimental design

This was an observational prospective cohort study in which cows (n = 59) were systematically and consecutively enrolled during the dry period and then examined 6 times from 40 days before to 40 days after calving. Six examinations (EXAM 1 - 6) were performed: on days 40 ± 4, 26 ± 4 and 12 ± 4 before calving (DBC), and on days 7 ± 4, 21 ± 4 and 35 ± 4 after calving (DAC). Examinations included assessment for lameness, cyclicity, and body condition. Milk, blood, and endometrial samples (via cytobrush) were collected, and transrectal and vaginal examinations were performed. Before calving, each cow had a transrectal examination to confirm pregnancy. After calving, the reproductive tract was examined transrectally and ultrasonographically to determine cervical and uterine horns' diameters, assess fluid in uterus, and characterize ovarian structures (corpus luteum, dominant follicle, and follicular cyst). Additionally, a uterine endometrial cytobrush examination (described below) and vaginoscopy were performed.

Cows were enrolled in a systematic synchronization protocol with 2 injections of prostaglandins given at a 14-day interval starting at the end of the voluntary waiting period (60 DIM) with estrus observation. Reproductive data were collected until at least 300 days in milk (DIM). Throughout the sampling period, none of the 22 cows in the control and diseased groups received any antibiotics. Diseased cows (n = 11) were identified based on 4 criteria. In decreasing order of importance, these were: 1) cows with *T. pyogenes* grade ≥ +2 in the uterus (culture-based method only on EXAM 5); 2) cows with PVD of grade ≥ 2;[18] 3) cows with > 18% PMNs on endometrial cytology (number of PMNs/number of total cells);[19] and 4) cows with grade 2 cervicitis.[20] Control cows (n = 11) were negative for all 4 criteria (*T. pyogenes* grade < 2, PVD < 2, PMNs < 5%, and cervicitis grade < 2).

Vaginal and transrectal examinations

Cervicitis was assessed[20] and vaginal discharge was examined.[18] Following transrectal palpation, vulva was cleaned of feces with a wet paper towel and then wiped with gauze soaked in isopropyl alcohol. For vaginoscopy, a multiple-use vaginal speculum (50 cm long) was inserted through the vulva and into vagina up to the outer cervical os. No lubricant was used. With a light source, the vaginal cavity and cervical os were examined visually for discharge and categorized using a 4-point classification system.[18] Vaginal content was classified as follows: 0 = no discharge, 1 = clear and translucent mucus, 2 = cloudy mucus with or without flecks of pus (< 50% pus), 3 = mucopurulent discharge (> 50% pus), and 4 = purulent discharge with fetid smell.[7]

Transrectal examination was performed first for the sake of convenience. However, detection of vaginal discharge by vaginoscopy was not enhanced by a preceding transrectal palpation of the uterus.[9] Cervix was assessed and classified[20] as follows: C0 = cervix without abnormality; C1 = second cervical fold swollen with no redness, and C2 = second cervical fold swollen with redness.

Blood sampling

At each examination, blood samples were collected from the coccygeal vein using 10-ml Vacutainer K2 EDTA blood collection tubes for hematology analysis, and without an anticoagulant for biochemistry analysis (Becton, Dickinson and Company, Franklin Lakes, NJ). Collected samples were immediately placed on ice for further processing at the laboratory within < 3 hours. Once clotting was complete in the red top tube, the serum was separated by centrifugation (3,000 revolutions per minute for 10 minutes). Beta-hydroxybutyrate concentration was then measured using a Freestyle Precision NEO Kit (Abbott Laboratories Ltd, Abbott Park, IL). Finally, 3.0-ml aliquots of serum were labelled and placed at – 80 °C for long-term storage and further analysis. After 30 minutes at room temperature and gentle agitation, the anticoagulant-containing tube was subject

to a complete hematology analysis (VetScan HM5 hematology analyzer, Abaxis Global Diagnostics, Union City, CA).

Quantification of serum IgG and fibrinogen concentrations

Bovine IgG concentrations were measured[21] by ELISA using a commercial Bovine IgG ELISA Quantitation Set (E10-118, Bethyl Laboratories, Montgomery, TX).

Endometrial cytology sampling

Uterine cytobrush samples (n = 59) were collected for routine endometrial cytology and bacteriology assessment. Briefly, a sterile cytobrush (CytoSoft, Camarillo, CA) was screwed onto a stainless-steel rod (65 cm x 4 mm) and was then protected within a sterile stainless-steel tube. The rod-guard apparatus was inserted into a hard protective plastic sheath (IMV Technologies, L'Aigle, France) before being inserted into a second protective sheath (30-cm long Sani-Shield Rod Protector, Agtech, Manhattan, NY) to reduce the risk of vaginal and cervical contamination. Instrument was pushed through the cervix and the sterile cytobrush gently rotated clockwise (360 degrees) on the endometrium to collect cellular material. Cytobrush was then retracted into the stainless-steel tube and hard plastic protective sheath, and withdrawn. The bristles of the brush were rolled over a sterile microscope slide (Fischer Scientific, Toronto, ON, Canada), and then the brush tip was cut off and packaged for bacterial culturing.

Slide preparation and staining

The smear on the slide was allowed to dry at room temperature for 10 -15 minutes. Cytology smears were stained with modified Wright Giemsa stain using a Hematek automated slide stainer (Miles Scientific, Napierville, IL). Slides were examined under the microscope at 250 and 400 x magnifications and various inflammatory cell types (PMNs, lymphocytes, and monocytes), and endometrial epithelial cells were counted. For each slide, a total of 300 cells were counted by a single observer. Cows were classified as endometritis positive if > 18% PMNs was present at 5 weeks after calving.

Uterine bacterial culturing and identification

Uterine cytobrush samples for bacteriology testing were collected only at 21 days after calving (EXAM 5) for routine bacterial culturing (aerobic and anaerobic) using standard methods for bacteriological testing (API system, bioMérieux, Marcy l'Étoile, France). Cytobrush samples were stored in a culture tube (Starplex Scientific Inc., Etobicoke, ON, Canada) and transported at room temperature to the Faculty of Veterinary Medicine's diagnostic laboratory within 3 hours. For microbiological analysis, the brushes were scraped onto sheep blood agar (soy agar with 5% sheep blood; Becton, Dickinson and Company, Sparks, MD). Plates were incubated for 48 hours at 35°C under aerobic conditions and then examined. When growth was observed, colony types were identified based on morphology, pigmentation, and hemolytic patterns. Tiny beta-hemolytic, catalase-negative colonies demonstrating coliform gram-positive rods were identified as *T. pyogenes*, *F. necrophorum* and *P. melaninogenicus.* These were then isolated using the standard procedure at the Faculty of Veterinary Medicine's diagnostic laboratory (PON-BAC-019). For other bacterial species, cytobrush samples were scraped directly onto Brucella agar containing neomycin (100 g/ml) and incubated anaerobically at 35°C for 5 days. When gram-negative rods were observed, colonies were examined using the API 20 A gallery system for identifying *F. necrophorum* and *P. melaninogenicus*. For isolation of *E. coli*, cytobrush samples were scraped directly on blood agar and MacConkey agar (Oxoid Inc., Ottawa, ON, Canada) at 37°C. At the OIE Reference Laboratory for *Escherichia coli* (EcL; Faculty of Veterinary Medicine, University of Montreal), 5 typical lactose-positive *E. coli* colonies from the MacConkey agar plates were streaked with blood agar for isolation and further identification. Isolates were submitted to 3 biochemical tests (indole spot, Simmon's citrate, and motility) for confirmation of *E. coli*. Isolates of *E. coli* were stored in tryptic soy broth containing 30% glycerol at – 80°C (Becton, Dickinson and Company, Sparks, MD).

Data analyses

Reproductive data were obtained from the health records databank. We calculated descriptive statistics and then analyzed the data using SAS v. 9.2. The model used herd as the random effect. Statistical power and sample size calculations were performed before analysis. Based on reports,[18,21] the following values were deemed indicative of endometritis: 18% PMNs on endometrial cytology, > grade 2 PVD, cervicitis score > 2, and 15 - 35 mg/ml of IgG in the serum. We determined that 11 cows per group were required based on a 10% difference between the control and disease groups, and using a one-sided test with 95% confidence interval and 80% power (G*Power, version 3.1.9.2, Germany).[22]

Data were not normally distributed and so were transformed using logarithm base 10 to normalize the distribution. A linear mixed model was used, with farm and cow nested within farm as random effects, and health status (control and diseased animals) and period (1 - 6) as fixed effects. Means for each health status at each time were compared. Alpha level was adjusted downward using the Benjamin-Hochberg procedure. For ordinal scores, the Cochran-Mantel-Haenszel test was used to examine potential differences in the distribution of score values between control and diseased cows. Significance was set at $p \leq 0.05$.

Results

Descriptive statistics for the study population

Initial potential sample size was 250 multiparous (2^{nd} - 5^{th} lactation) cows. From the original group of cows (n = 83), individuals were eliminated from the dataset because of culling (n = 5), use of uterine antibiotics (n = 4), missing data (n = 10), and metabolic diseases (n = 5). Mean day of sampling for EXAMS 1 - 6 were: 40 (45 - 37 DBC), 28 (31 - 24 DBC), 13 (17 - 10 DBC), 4 (0 - 7 DIM), 17 (14 - 21 DIM), and 31 (28 - 35 DIM), respectively. The remaining 59 cows had the following prevalence: clinical endometritis (EXAM 6) 23%; cytological endometritis (EXAM 6) 16%; *T. pyogenes* positive on culture (EXAM 5) 25%; and cervicitis (EXAM 6) 31%. There were no differences (p > 0.5) among 6 sampling times in blood concentrations of WBCs (Figure 1) and fibrinogen (Figure 2).

Descriptive statistics for the study groups (control versus diseased cows)

Body condition score, lameness score, and cyclicity status were not different (p > 0.05) between control (n = 11) and diseased groups (n = 11). Control cows were free of *T. pyogenes* (score ≤ 1) at EXAM 5; clinical

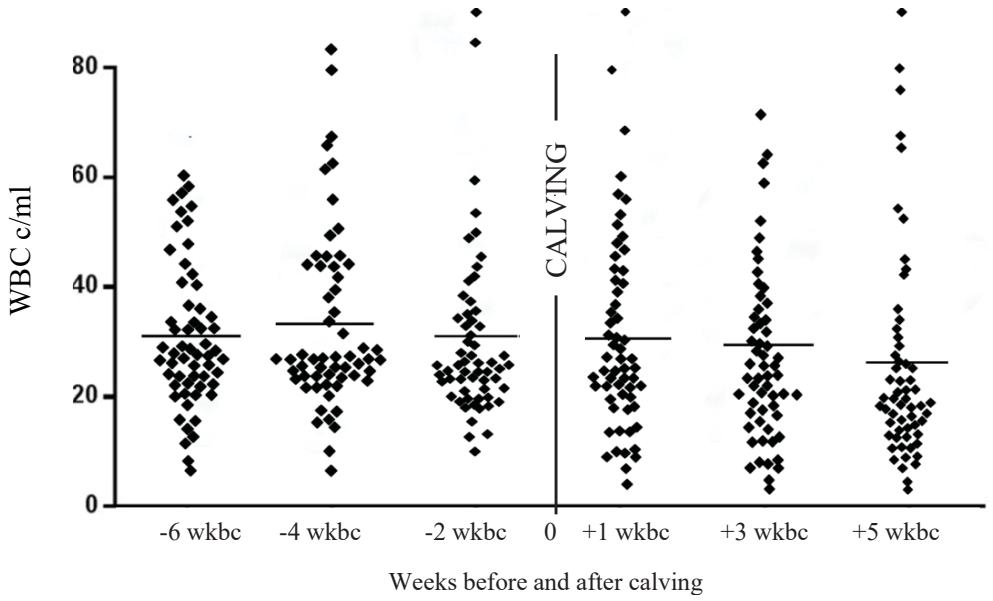

Figure 1. WBC (c/ml) in cows (n = 59) overtime of examination; note the lack of difference at examinations.

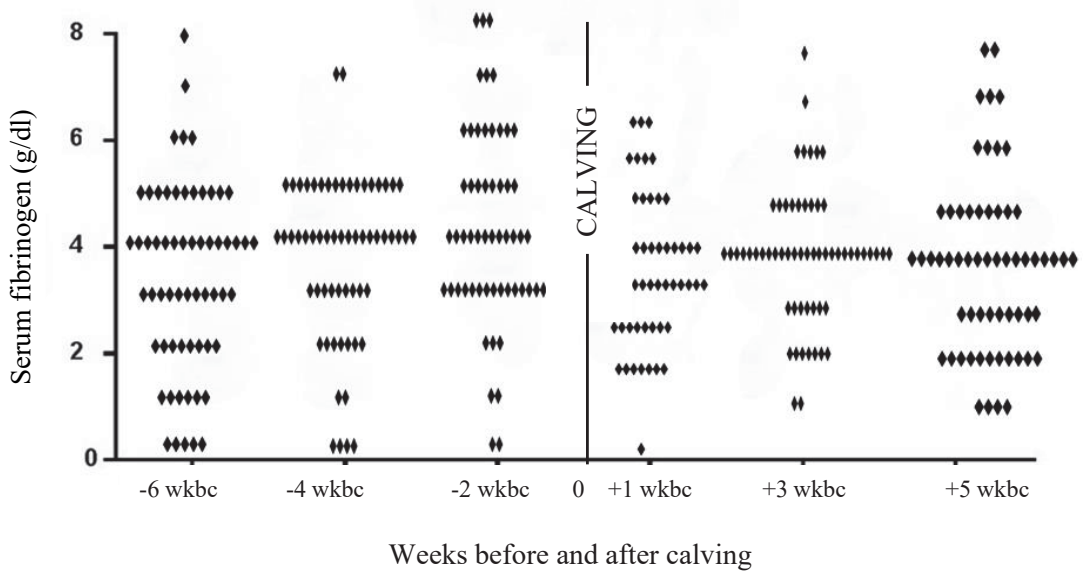

Figure 2. Serum fibrinogen (g/dl) in cows (n = 59) over time of examination; note the lack of difference at examinations.

endometritis (score ≤ 1); subclinical endometritis (< 5% PMNs on endometrial cytology) at EXAM 5; and cervicitis (score ≤ 1) at EXAM 6. In diseased cows, 100% (n = 11) had *T. pyogenes* (score ≥ 2) on EXAM 5, whereas 65% (n = 7) had clinical endometritis (score ≥ 2 of PVD), 36% (n = 4) had subclinical endometritis (≥ 18% PMNs on endometrial cytology), and 55% (n = 6) had cervicitis (score of 2) at EXAM 6. Diseased cows met at least 3 of 4 criteria. Prevalence of *T. pyogenes* at EXAM 5 (n = 15) was 25%.

In control cows, the distribution of PVD scores shifted to lower values (p = 0.04) from EXAM 4 to EXAM 6. However, this effect was not observed in diseased cows. By contrast, there was more vaginal discharge in diseased cows at EXAM 5 and EXAM 6 compared to EXAM 4 (p < 0.04 and p < 0.002, respectively). At EXAM 6, diseased cows had more (p = 0.008) PVD than the control cows. Mean PMN counts on endometrial cytology were not different (p > 0.05) from EXAM 4 to EXAM 6 in control cows. In diseased cows, mean PMN counts on the endometrial cytology at EXAM 4 were lower (p < 0.0005) than at EXAMs 5 and 6. Although mean PMN count at EXAM 4 was

different ($p > 0.05$) between control and diseased cows, it was higher at EXAM 5 and EXAM 6 for diseased cows compared to the control group ($p < 0.0001$ and $p < 0.02$, respectively). Cows with PVD score ≥ 2 had a higher ($p = 0.0001$) mean PMN count on endometrial cytology than those with lower scores. Cows with *T. pyogenes* had a higher mean PMN count on endometrial cytology at both EXAMs 5 and 6 compared to cows without this bacterial species ($p = 0.0001$ and $p = 0.02$, respectively). Similarly, cows with *T. pyogenes* were more likely to have clinical endometritis (n = 11; 73%) and subclinical endometritis (n = 10; 67%) at EXAM 5 (Table). Cervicitis scores were higher in diseased group compared to control group at EXAMs 5 and 6 ($p = 0.04$ and $p = 0.002$, respectively). Of the 22 diseased and control cows, 8 of 11 control cows and 4 of 11 diseased cows were pregnant after the first artificial insemination.

Concentrations of IgG, WBC components (PMNs, monocytes, lymphocytes, and eosinophils), and fibrinogen did not change ($p > 0.05$) over period or vary among control and diseased cows at any examination (Figures 3a and 3b, Figures 4a and 4b, and Figures 5a and 5b). Farm did not explain any variation ($p = 0.23$) in the data. A lack of variation ($p > 0.05$) over period (transition) for diseased and control groups and between groups at various examinations for all 3 inflammation indicators was also observed when the postpartum diseases (clinical and subclinical endometritis, presence of *T. pyogenes*, and cervicitis) were analyzed individually.

Discussion

Postpartum endometritis may reflect a generalized systemic inflammatory environment that can be attributed to a systemic metabolic condition that is common in the transition period.[23] Alternatively, it may be the direct result of impaired immune defences. To distinguish between these 2 possibilities and cast more light on the pathogenesis of postpartum endometritis, we sampled at 6 time points during the transition period. This study was also unique in defining the diseased animal based on several conditions. These are, in decreasing order of importance: 1) *T. pyogenes* (≥ 2 grade); 2) clinical endometritis (≥ 2 purulent vaginal discharge); 3) subclinical endometritis (≥ 18% PMNs), endometritis (≥ 18% PMNs), and 4) cervicitis (score of 2). *T. pyogenes* is associated with inflammation and infection of the endometrium in vivo and in vitro. It is the most prevalent uterine bacterial species at 3 weeks postpartum and is associated with a substantial reduction in pregnancy rate.[24] Disease definition used in our study was based on combining various chronic postpartum diseases, assuming that 2 conditions (clinical and

Table. Temporal changes in polymorphonuclear cells (PMN), purulent vaginal discharge (PVD), and cervicitis in control and diseased cows

	EXAM 4	EXAM 5	EXAM 6
PMN ≥ 18% Control	11 (24%)	14 (30%)	6 (13%)
PMN ≥ 18% Diseased	2 (13%)	10 (67%)	4 (27%)
PVD ≥ 2 Control	20 (43%)	21 (46%)	7 (15%)
PVD ≥ 2 Diseased	4 (27%)	11 (73%)	7 (47%)
Cervicitis (score = 2) Control	14 (30%)	14 (30%)	12 (26%)
Cervicitis (score = 2) Diseased	8 (53%)	11 (73%)	7 (47%)

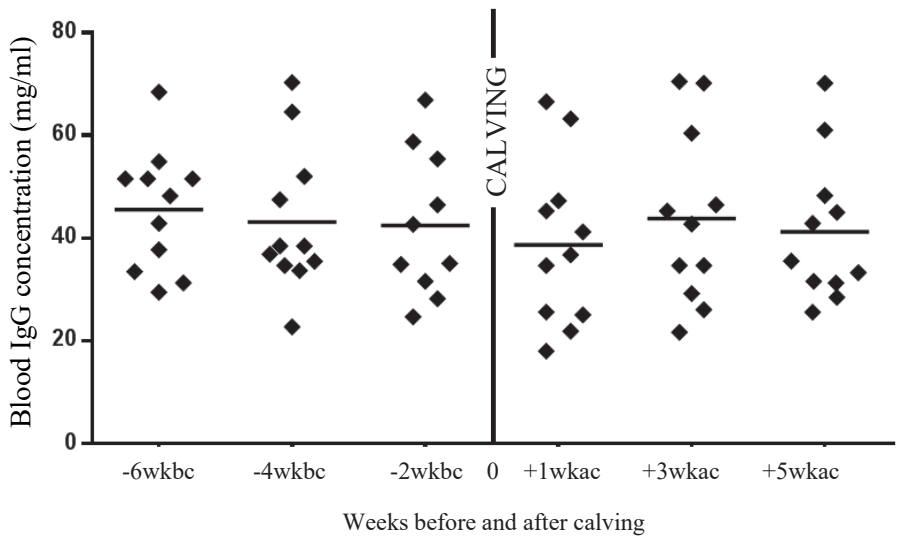

Figure 3a. Blood IgG concentrations in control (n = 11) cows; note the lack of difference at examinations

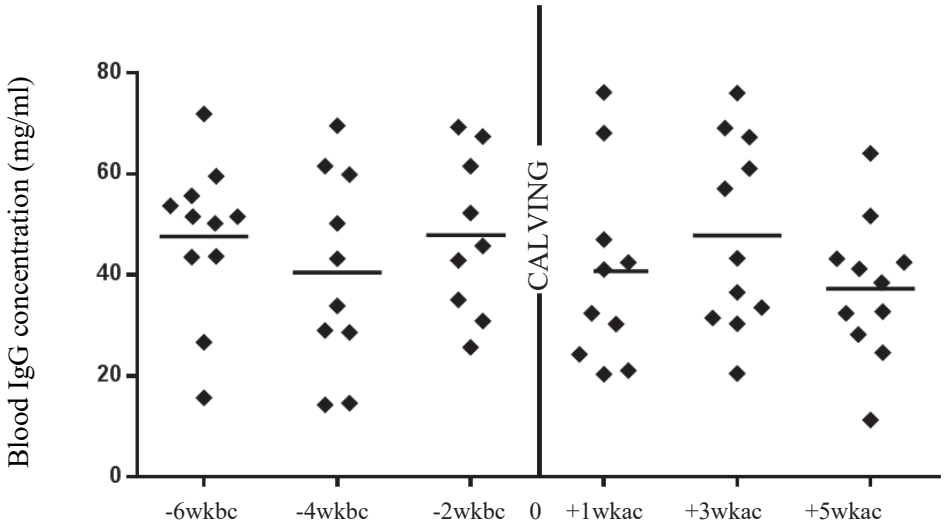

Figure 3b. Blood IgG concentrations in diseased cows (n = 11); note the lack of difference at examinations

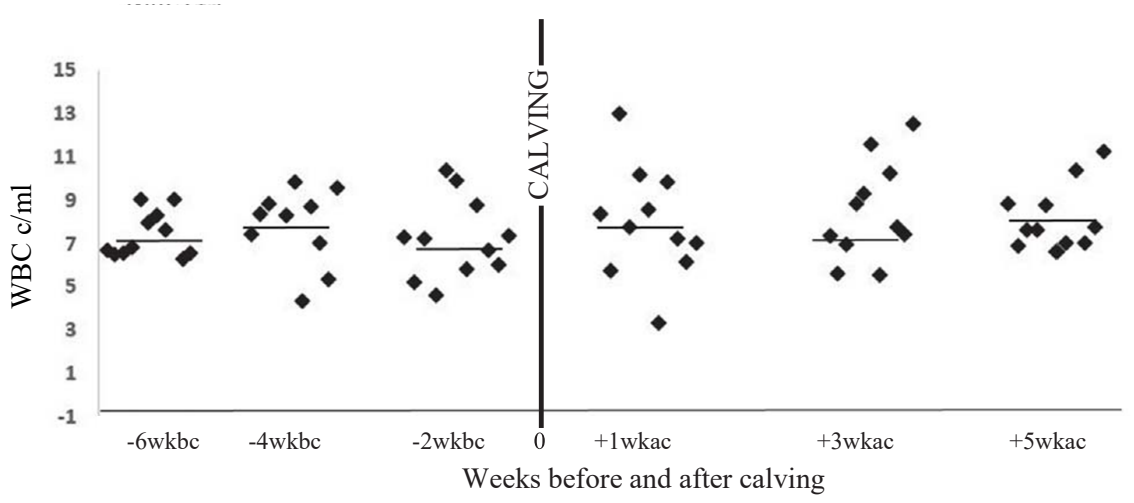

Figure 4a. WBC in control (n =11) cows; note the lack of difference at examinations

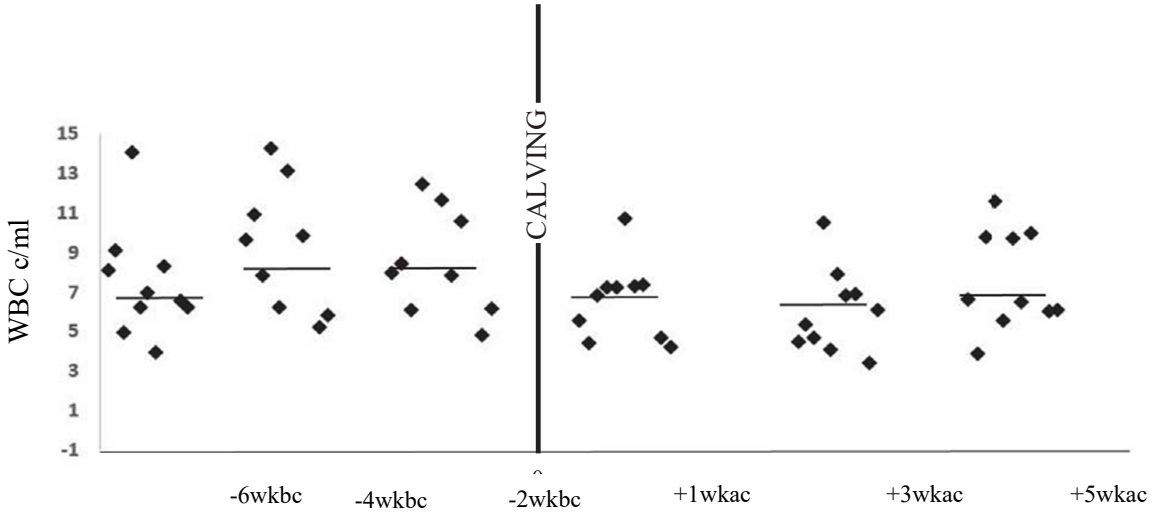

Figure 4b. WBC in diseased cows (n = 11); note the lack of difference at examinations

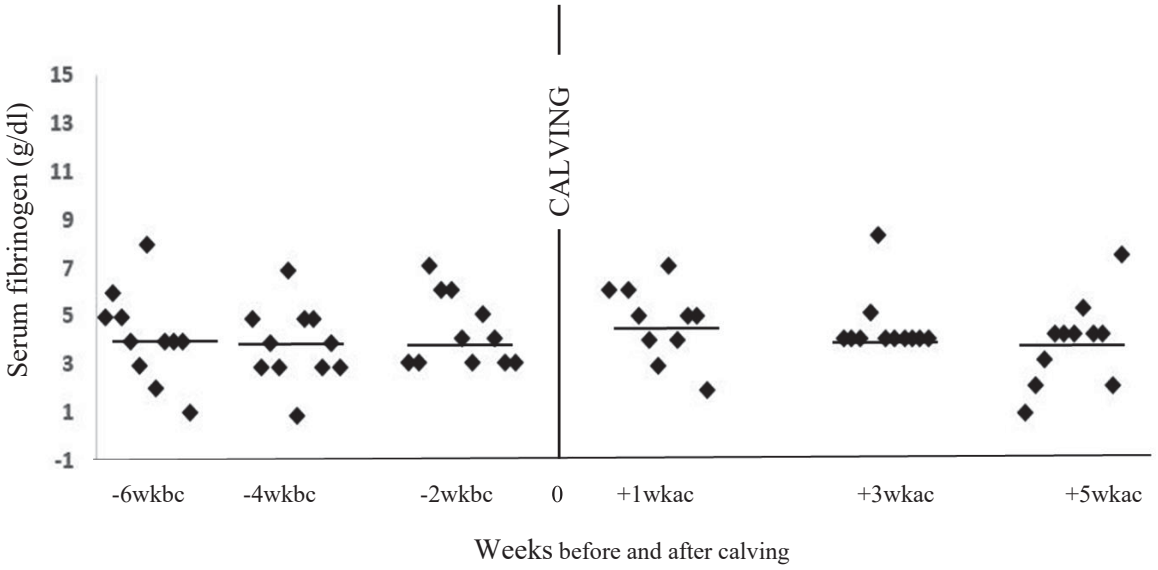

Figure 5a. Fibrinogen concentration in control cows (n = 11); note the lack of difference at examinations

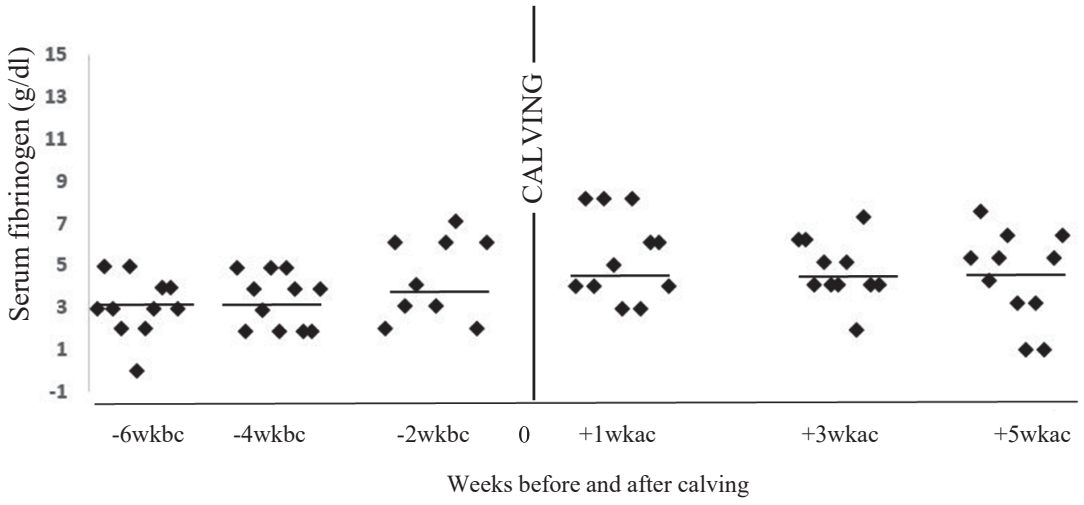

Figure 5b. Fibrinogen concentration in diseased cows (n = 11); note the lack of difference at examinations

subclinical endometritis) are more detrimental to fertility than is a single disease.[25] Proportion of PMNs at 5 and 7 weeks postpartum has been associated with concomitant bacterial infection[24] and with impaired reproductive performance.[18,27]

Since the most suitable threshold for the diagnosis of subclinical endometritis is still being debated, we used a threshold of 18% PMNs on endometrial cytology[28] for samples obtained between 20 and 33 DIM. Definition of disease chosen was meant to increase the likelihood of finding differences between control and diseased groups. Sample size calculation and using a systematic and consecutive enrollment design strengthened the experimental design. As less is known about the pathogenesis of postpartum endometritis, especially the role of systemic inflammatory markers, our study provided new insights into the systemic immune response of dairy cows to postpartum endometritis throughout the entire transition period.

Prevalence of clinical and subclinical endometritis and of cervicitis at EXAM 5 (n = 59) were similar to reported values for a similar time point during the postpartum period.[7,18,20] We observed a decrease in prevalence from EXAM 5 to EXAM 6, similar to the prevalence of clinical and subclinical endometritis that declined from 25.4% at 3 weeks to 14.7% at 8 weeks postpartum.[29] Similarly, prevalence of cervicitis throughout the early part of the postpartum period decreased. Like endometritis, this was most likely due to spontaneous cure that is expected to decrease with number of days postpartum.

Contrary to previous studies, the prevalence of *T. pyogenes* at EXAM 5 was 4 times greater than the prevalence reported at 28

DIM (6.3%),[30] and 1.2 times less than the prevalence reported at 21 days postpartum (30.2%).[31] However, the sample size in our study limited this comparison. On day 21 postpartum, < 40% of cows had *T. pyogenes* in uterine samples obtained using low-volume uterine lavage.[31] As prevalence of *T. pyogenes* was not greater than studies that had only 1 sampling, multiple sampling over the whole transition period probably did not affect results in our study. Definitively associated with endometrial lesions and fertility, *T. pyogenes* is the most recognized etiological agent of postpartum endometritis.[32] However, prevalence of *T. pyogenes* varies with herd, type of facility, management, and location. Cows with *T. pyogenes* (score ≥ 2) exhibited a greater probability of having clinical and/or subclinical endometritis ($p = 0.008$ and $p = 0.02$, respectively), but not cervicitis ($p > 0.05$).

We are the first group to assess systemic immune indicators of inflammation over the entire transition period (day – 40 to day + 35) in dairy cows with and without endometritis. There was no difference in IgG concentrations between EXAM 1 and EXAM 6, and among diseased and control cows at any examination throughout the entire transition period. Immunoglobulins have an important role in immune defence by serving as opsonin that enhance phagocytosis, and by stimulating the classical complement pathway.[14] As IgG is produced in part from the endometrium (mainly IgG1), with the balance of IgG1 derived from peripheral circulation and all IgG2 derived from peripheral circulation,[33,34] one would expect variations in IgG concentrations in the blood stream of cows during the transition period.[35] Decreased IgG concentrations in the peripheral circulation around calving could be explained by the immunosuppressant status of pregnancy-associated physiological phenomena, the effect of cortisol at calving, and the stress associated with high-producing cows in the postpartum period.[36] Some researchers have suggested that postpartum uterine diseases are related to an immunosuppressant status during the postpartum period, but they have provided no details about what specific features of this status could be involved. In our study, control and diseased cows did not have any locomotor, mammary gland or metabolic diseases that could have been potential sources of inflammation causing the changes in inflammatory indicators. Therefore, our chances of observing differences among control and diseased cows due to uterine anomalies alone was improved. Based on the results, and assuming that a large portion of total IgG in the uterine lumen is synthesized systemically, estimation of IgG serum concentrations is not a good inflammatory indicator to predict or monitor postpartum endometritis in dairy cows.[37] In addition to measuring variation in IgG concentrations, several researchers also reported a decrease in peripheral blood lymphocyte counts.[21,38] By contrast, as was the case for IgG, we did not observe variation in WBC numbers. Peripheral blood concentrations of total leukocytes or individual types (PMNs, monocytes, lymphocytes, and eosinophils) in the 2 groups of cows did not vary throughout the transition period, or differ between control and diseased cows at examinations. In normal cows, the prepartum period is usually accompanied by peripheral leukocytosis,[39] followed by leucopenia during the first week after calving.[40] Cows with subclinical endometritis had higher blood leukocyte counts and elevated PMN concentrations compared to control postpartum cows between days 45 and 55.[41]

Fibrinogen concentrations were not different between control and diseased cows at examinations during the transition period. Fibrinogen is a secreted plasma protein that is the precursor of fibrin and is used to form secondary hemostatic plugs at sites of vascular injury, in several inflammatory and traumatic conditions in cattle.[42] Analyses of metabolic blood parameters, serum inflammatory mediators, antibodies, and the cellular composition of cow blood for concentrations of elements like beta-hydroxybutyrate, haptoglobin and sialic acid around calving have been proposed, but none was satisfactory for disease prediction.[43] Increased fibrinogen concentrations (hyperfibrinogenemia) may be associated with inflammation or with dehydration. Although the ratio of plasma proteins/fibrinogen may help to distinguish dehydration from inflammation, dehydration was not evident in our study.

Conclusion

Concentrations of systemic inflammatory indicators (IgG, white blood cells, and fibrinogen) did not change over time nor did they differ between control and diseased postpartum cows at examinations. Therefore, we suggest that systemic inflammatory markers are not good indicators for diagnosing or monitoring endometritis in postpartum dairy cows.

Conflict of interest

None to declare.

References

1. Goff JP, Horst RL: Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci 1997;80:1260-1268.
2. Drillich M: An update on uterine infections in dairy cattle. Slov Vet Res 2006;43:11-15.
3. Overton TR, Yasui T: Practical applications of trace minerals for dairy cattle. J Anim Sci 2014;92:416-426.
4. Sheldon IM, Lewis GS, LeBlanc SJ: Defining postpartum uterine disease in cattle. Theriogenology 2006;65:1516-1530.
5. LeBlanc SJ: Postpartum uterine disease and dairy herd reproductive performance: a review. Vet J 2008;176:102-114.
6. Sheldon IM, Williams EJ, Miller AN, et al: Uterine diseases in cattle after parturition. Vet J 2008;176:115-121.
7. Williams EJ, Fischer DP, Pfeiffer DU, et al: Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 2005;63:102-117.
8. Sheldon IM, Dobson H: Postpartum uterine health in cattle. Anim Reprod Sci 2004;82:295-306.
9. Kasimanickam R, Duffield T, Foster R, et al: A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Can Vet J 2005;46:255-259.
10. Herath S, Dobson H, Sheldon IM: Use of the cow as a large animal model of uterine infection and immunity. J Reprod Immunol 2006;69:13-22.
11. Galvão KN, Flaminio MJBF, Brittin SB, et al: Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. J Dairy Sci 2010;93:2926-2937.

12. Herath S, Lilly ST, Santos NR, et al: Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. Reprod Biol Endocrinol 2009;7:55, https://doi.org/10.1186/1477-7827-7-55.
13. Sheldon IM, Cronin J, Schuberth HJ: Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 2009;81:1025-1032.
14. Singh J, Murray RD, Mshelia G, et al: The immune status of the bovine uterus during theperipartum period. Vet J 2008;175:301-309.
15. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.
16. Dhaliwal GS, Murray RD, Woldehiwet Z: Some aspects of immunology of the bovine uterus related to treatments for endometritis. Anim Reprod Sci 2001;67:135-152.
17. National Academies of Sciences, Engineering, and Medicine. 0. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. Washington, DC: The National Academies Press.
18. Tison N, Bouchard E, DesCoteaux L, et al: Effectiveness of intrauterine treatment withcephapirin in dairy cows with purulent vaginal discharge. Theriogenology 2017;89:305-317.
19. Gilbert RO: Management of reproductive disease in dairy cows. Vet Clin North Am Food Anim Pract 2016;32:387-410
20. Hartmann D, Rohkohl J, Merbach S, Heilkenbrinker, et al: Prevalence of cervicitis in dairy cows and its effect on reproduction. Theriogenology 2016;85:247-253.
21. Herr M, Bostedt H, Failing K: IgG and IgM levels in dairy cows during the periparturient period. Theriogenology 2011;75:377-385.
22. Faul F, Erdfelder E, Buchner A: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-191.
23. Sordillo LM, Contreras GA, Aitken SL: Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Health Res Rev 2009;10:53-63.
24. Foldi J, Kulcsar M, Pecsi A, et al: Bacterial complications of postpartum uterine involution in cattle. Anim Reprod Sci 2006;96:265-281.
25. Dubuc J, Duffield TF, Walton JS: Risk factors for postpartum uterine diseases in dairy cows. J Dairy Sci 2010;93:5764-5771.
26. Gilbert RO, Shin ST, Guard CL, et al: Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 2005;64:1879-1888.
27. Islam R, Kumar H, Nandi S: Determination of anti-inflammatory cytokine in periparturient cows for prediction of postpartum reproductive diseases. Theriogenology 2013;79:974-979.
28. Kasimanickam R, Duffield TF, Foster RA, et al: Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 2004;62:9-23.
29. Gautam G, Nakao T, Koike K, et al: Spontaneous recovery or persistence of postpartumendometritis and risk factors for its persistence in Holstein cows. Theriogenology 2010;73:168-179.
30. LeBlanc SJ, Duffield TF, Leslie KE, et al: The effect of treatment of clinical endometritis on reproductive performance in dairy cows. J Dairy Sci 2002;85:2237-2249.
31. Prunner I, Pothmann H, Wagener K, et al: Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows. Theriogenology 2014;82:1316-1322.
32. Barlund CS, Carruthers TD, Palmer CW: A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 2008;69:714-723.
33. Corbeil LB, Schurig GD, Duncan JR, et al: Immunoglobulin classes and biological functions of Campylobacter (Vibrio) fetus antibodies in serum and cervicovaginal mucus. Infect Immun 1974;10:422-429.
34. Beutler B, Ulevitch RJ: How we detect microbes and respond to them: the Toll-like receptors and their transducers. J Leukoc Biol 2003;74:479-485.
35. Fischer C, Drillich M, Heuwieser W, et al: Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the oestrous cycle and elevated in case of subclinical or clinical endometritis. Reprod Fertil Dev 2010;22:818-829.
36. Lucy MC: Stress, strain, and pregnancy outcome in postpartum cows. Anim Reprod 2019;16:455-464. Published online 2019 Oct 23. doi:10.21451/1984-3143-AR2019-0063.
37. Hazlett L, Wu M: Defensins in innate immunity. Cell Tissue Res 2011;343:175-188.
38. Kim IH, Na KJ, Yang MP: Immune responses during the peripartum period in dairy cows with postpartum endometritis. J Reprod Dev 2005;51:757-764.
39. Mateus L, da Costa LL, Bernardo F, et al: Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod Domest Anim 2002;37:31-35.
40. Guidry AJ, Paape MJ, Pearson RE: Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. Am J Vet Res 1976;37:1195-1200.
41. Raboisson D, Mounié M, Maigné E: Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review. J Dairy Sci 2014;97:7547-7563.
42. Hussain AM, Daniel RCW: Phagocytosis by uterine fluid and blood neutrophils and hematological-changes in postpartum cows following normal and abnormal parturition. Theriogenology 1992;37:1253-1267.
43. Kasimanickam R, Kasimanickam V, Olsen J, et al: Associations among serum pro- and anti inflammatory cytokines, metabolic mediators, body condition, and uterine disease in postpartum dairy cows. Reprod Biol Endocrinol 2013;11:103.