Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows

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ABSTRACT

The primary objective of this study was to identify relationships between endometritis and metabolic state during the calving transition and early lactation periods. A subset of mixed age and breed dairy cows ($n = 78$) from a seasonal, pasture-grazed herd of 389 cows was examined. The selected cows were grouped as having endometritis at d 42 postpartum or being unaffected by endometritis. Endometritis was defined as $>6\%$ (upper quartile) of uterine nucleated cells being polymorphonuclear cells (H-PMN; $n = 38$); unaffected by endometritis was defined as $\leq 1\%$ of nucleated cells being polymorphonuclear (L-PMN; $n = 40$). Milk yield was determined at each milking, and milk composition (fat and protein) was determined at 2-wk intervals. Blood samples collected on d -14 , 0 (d of calving), 4, 7, 14, 28, and 42 were analyzed for indicators of energy status (nonesterified fatty acids, glucose, and urea), liver function (albumin, globulin, glutamate dehydrogenase, and aspartate aminotransferase), inflammation (haptoglobin), and mineral status (Ca and Mg). Samples collected weekly from d 21 to 63 or 70 were analyzed for progesterone content. The postpartum anovulatory interval was defined to end on the first day postpartum that plasma progesterone concentration was ≥ 1 ng/mL. A greater percentage of H-PMN cows failed to ovulate before d 63 or 70 (34%) compared with L-PMN cows (10%), although the proportions of cows ovulating within either polymorphonuclear group was similar through d 56 postpartum. Plasma concentrations of albumin and the albumin:globulin ratio were consistently lower in H-PMN cows. Plasma Mg was lower, whereas glutamate dehydrogenase and aspartate aminotransferase were higher, in H-PMN cows during early lactation compared with L-PMN cows. Circulating metabolites indicative of energy status (nonesterified fatty acids, glucose, and urea) were not different

between polymorphonuclear groups. Among 3- to 5-yr-old cows, daily milk yield for the first 42 d after calving was lower for H-PMN cows than for L-PMN cows. Among cows >5 yr old, protein percentage was lower in H-PMN cows compared with L-PMN cows. In summary, endometritis at 42 d postpartum in the herd studied was associated with an increased likelihood of remaining anovulatory. These cows had lower albumin concentrations throughout the calving transition period, perhaps indicating impaired liver function, with lower plasma Mg and evidence of hepatocellular damage in early lactation. Similar profiles of nonesterified fatty acids and glucose indicated that energy status was not a risk factor for endometritis.

Key words: dairy cow, endometritis, metabolic, transition

INTRODUCTION

A uterine bacterial infection develops in many cows soon after calving (Sheldon et al., 2009). Approximately 35% of cows may be clinically infected within the first 21 d postpartum (metritis), and about 10 to 20% will retain, or contract, endometritis beyond this period (Borsberry and Dobson, 1989; McDougall et al., 2007; Sheldon et al., 2009). Fertility is reduced in dairy cows with endometritis (McDougall et al., 2007; Pleticha et al., 2009). The prevalence of endometritis diminishes with time postpartum (McDougall et al., 2007; Gautam et al., 2009) because most infected cows recover spontaneously. Although some physiological differences during calving transition were evident between genetic strains of Holstein-Friesian cows selected for production within either pasture-based or TMR-based systems, the risk of uterine infection was the same for both strains (Olmos et al., 2009). A constraint within a seasonal, pasture-based dairy system is that breeding begins on a calendar date, irrespective of previous calving date for individual cows. A high level of reproductive performance is required to maintain a 365-d calving interval. Within-herd risk factors for endometritis in a pasture-based seasonal dairy system include late calving and

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periparturient disorders, although breed and age are not risk factors in this environment (McDougall et al., 2007).

Nutrition-related risk factors for uterine infections include lowered DMI (Marquardt et al., 1977; Zamet et al., 1979), possibly due to reduced hunger (Urton et al., 2005; Huzzey et al., 2007). Metabolic markers of increased mobilization of body tissues in early lactation, such as NEFA and BHBA concentrations, have also been associated with extended periods of postpartum anovulation (Canfield and Butler, 1990) and with an increased incidence of peripartum disease (Duffield, 2000). Lower DMI may further depress immune function during early lactation (Goff, 2006; Hammon et al., 2006). Increased concentrations of non-specific markers of inflammation such as acute-phase proteins have also been associated with uterine disease and poorer fertility (Sheldon et al., 2004).

Sheldon et al. (2009) estimated that the incidence of subclinical endometritis, defined as an elevated percentage of polymorphonuclear (PMN) cells in the uterus, is 3- to 4-fold higher than the prevalence of endometritis as measured using gross clinical procedures. Endometritis at this subclinical level has also been associated with poorer reproductive performance (Kasimanickam et al., 2004; Galvão et al., 2009; McDougall et al., 2009). In addition to an associated delay in establishing normal estrous cycles (McDougall et al., 2007; Senosy et al., 2009), embryonic losses are likely to contribute to reduced fertility in cows with uterine inflammation (Hill and Gilbert, 2008).

The objective of this study was to investigate the relationships between metabolic indicators of nutrient status and liver function during the periparturient period, and the occurrence of endometritis at 42 d postpartum. The working hypothesis was that naturally occurring endometritis in pasture-grazed dairy cows is associated with imbalances in energy, protein, and mineral metabolism during the transition and early lactation periods.

MATERIALS AND METHODS

Animal Management

Experimental cows enrolled in this study ($n = 78$) were a subset from a seasonal, pasture-based herd of 389 cows in Hawera, New Zealand ($39^{\circ}36'S$ $174^{\circ}18'E$). The herd was managed following recommended principles for pasture-based seasonal dairying (Macdonald and Penno, 1998). Although DMI was not quantified, prepartum feeding management was intended to provide 102 MJ of ME/500 kg of BW per day using pasture and hay supplement. Pasture allocations after calving

aimed to achieve an average daily intake of 206 MJ of ME/500 kg of BW. All procedures had prior approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Experimental Design and Animal Selection

Cows that calved between July 10 and August 23 ($n = 329$) were considered eligible for enrollment. The cows were categorized into quartiles based on the percentage of intrauterine nucleated cells that were PMN ($\leq 1\%$, 2–3%, 4–6%, and $> 6\%$) on d 42 (d of calving designated as d 0) postpartum. Cows diagnosed with one or more peripartum conditions ($n = 89$), including dystocia, retained fetal membranes, mastitis, lameness, hypocalcemia, or having delivered a dead calf, were excluded from further analysis. From the remaining cows, 38 were diagnosed with endometritis, as defined by a high ($> 6\%$) percentage of PMN (**H-PMN**). A random selection of 40 of the 48 cows with $\leq 1\%$ uterine PMN (**L-PMN**) were used for comparative examination of associations between PMN status and indicators of metabolic state during the transition period. As assessed using the Metrichick (Simcrotech, Hamilton, New Zealand; www.simcrotech.co.nz/products) examination procedure (McDougall et al., 2007), none of the L-PMN cows was positive for clinical endometritis (Metrichick score < 3), whereas 4 of the 38 H-PMN cows were diagnosed as having clinical endometritis (Metrichick score > 2). None of the experimental cows were treated for endometritis, and PMN results of individual cows were not provided to the herd manager.

The PMN groups had a similar breed mix (Holstein-Friesian, Jersey, and Holstein-Friesian $\times$ Jersey cross-breds), age or parity (2 yr old are first parity, 3 yr old are second parity, and so on), genetic merit, prepartum BCS (1–10 scale; Roche et al., 2004) and BW, with indifferent mean calving date and calf birth weights (Table 1). The age distribution of cows 2, 3, 4 to 6, and older than 6 yr among the H-PMN (11, 5, 13, and 9 cows) and L-PMN (14, 5, 16, and 8 cows) groups, respectively, was not different using χ^2 tests (Pearson χ^2 , $P = 0.34$; likelihood ratio, $P = 0.20$, and linear-by-linear association, $P = 0.15$).

The means and distribution profiles of PMN percentage for the PMN groups were markedly different by design (Table 1 and Figure 1).

Uterine Cytology

Cytological samples of the endometrium were collected 42 ± 2.6 d (mean $\pm$ SD) after calving. The vulva was cleaned and a modified, triple-guarded, AI pipette was passed through the cervix. A fine, sterile stylette

Table 1. Breed, age, genetic merit, prepartum BCS and BW, calving date, calf weight, and reproduction indices for cows classified as having a low (L-PMN, $n = 40$) or high (H-PMN, $n = 38$) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum¹

Item	L-PMN	H-PMN	P-value
Breed ratio, HF:HF×J:J ²	20:6:14	18:7:13	0.92
Age (yr)	4.0 ± 2.2	4.8 ± 2.8	0.44
Breeding worth ³	91.3 ± 43.2	90.7 ± 43.2	0.95
Prepartum BCS ⁴	4.5 ± 0.5	4.3 ± 0.5	0.28
Prepartum BW (kg)	479 ± 86	470 ± 94	0.66
Calving date	August 6 ± 13.8 d	August 4 ± 12.6 d	0.59
Calf weight (kg)	31.1 ± 8.0	31.9 ± 7.8	0.65
d 42 PMN (%)	0.7 ± 0.5	26.6 ± 23.3	<0.001
FSCR ⁵ (%)	54	31	0.05
Six-wk pregnancy rate ⁶ (%)	70	54	0.16
Final pregnancy rate ⁷ (%)	95	79	0.04

¹Values are the mean ± SD.

²Holstein Friesian (HF; ≥12/16 HF) and Jersey (J; ≥12/16 J).

³Genetic merit (New Zealand Animal Evaluation Ltd.; www.aeu.org.nz).

⁴Body condition score on a 1 to 10 scale; Roche et al. (2004).

⁵First-service conception rate.

⁶Percentage of cows pregnant by 6 wk after the start of the seasonal breeding period.

⁷Percentage of cows confirmed pregnant after the 93-d seasonal breeding period.

(length, 500 mm; internal diameter, 0.5 mm; external diameter, 0.7 mm) with an attached sterile cytology brush (Cyto-brush, Ebos Group Ltd., Christchurch, New Zealand) was inserted through the AI pipette into the uterus. The brush was gently rotated a quarter turn while in contact with the endometrium, immediately anterior to the cervix. The stylette was retracted into the AI pipette and all sampling equipment was removed from the cow. Recovered material was smeared onto

a microscope slide and air-dried. Slides were stained (Diff-Quik, Dade Behring, Newark, DE) within 1 h of sample collection and examined by a veterinary pathologist (IVABS, Massey University, Palmerston North, New Zealand). Areas of each slide that contained small clusters of epithelial cells (5–20 cells per cluster) were preferentially selected and all intact identifiable nucleated cells were counted in those fields. Approximately 200 nucleated cells per slide were counted, with PMN distinguished from non-PMN cells (Barlund et al., 2008).

Figure 1. Frequency distribution of polymorphonuclear (PMN) cells for cows classified as having a low (L-PMN, $n = 40$) and high (H-PMN, $n = 38$) percentage of PMN cells in cytological samples collected from the endometrium at d 42 postpartum.

Blood Sampling and Plasma Metabolite Measurements

A weekly blood sample was collected from every cow by coccygeal venipuncture from 28 d before due calving date to 63 (all cows) or 70 d (26 cows) after calving. Additional samples were collected on the day of calving (d 0) and 4 d after calving. Blood was collected in evacuated tubes containing heparin as an anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and immediately placed into iced water. After centrifugation at $1,500 \times g$ for 12 min, plasma were harvested and stored at -20°C .

Plasma samples collected on d -14 , 0 (d of calving), 4, 7, 14, 28, and 42 were analyzed for concentrations of NEFA (mmol/L; commercial kit using the acyl Co-A synthetase, acyl-CoA oxidase method, Wako, Osaka, Japan), glucose (mmol/L; hexokinase method based on formation of NADPH_2), urea (mmol/L; urease hydrolysis method based on formation of glutamate and NAD), albumin (g/L; bromocresol green reaction at

pH 4.1), total protein (g/L; Biuret reaction method), haptoglobin (g/L; commercial kit based on binding to hemoglobin to preserve peroxidase activity at low pH; Phase kit, Tridelta Development Ltd., Co., Kildare, Ireland), glutamate dehydrogenase (**GDH**, IU/L; catalyzing activity of NADH-dependent conversion of α -ketoglutarate to glutamate), aspartate aminotransferase (**ASAT**, IU/L; catalyzing activity of transamination of L-aspartate to oxaloacetate), Ca (mmol/L; α -cresolphthalein complexone method), and Mg (mmol/L; xlidyl blue reaction). All assays were colorimetric and performed at 37°C with a Roche Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN) by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand). The interassay and intraassay CV for all assays were ≤ 11 and $\leq 2\%$, respectively.

In addition, levels of Cu ($\mu\text{mol/L}$), Zn ($\mu\text{mol/L}$), and Se (nmol/L) were determined by Gribbles Veterinary Pathology Ltd. in the d -14 plasma samples of 10 randomly selected cows (5 of either PMN group) as a measure of herd-level status for these minerals.

Determination of Plasma Progesterone and Postpartum Anovulatory Intervals

Progesterone was measured in plasma collected weekly from d 21 to 63 or 70 postpartum using a commercial kit (Progesterone Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). The average intraassay CV was 6.4% and the minimum detectable level was 0.03 ng/mL. Progesterone data were used to determine the postpartum anovulatory interval (**PPAI**), which was defined as the interval from calving to the first sample day that plasma progesterone concentration was ≥ 1 ng/mL. The PPAI could not be determined for 13 of 38 H-PMN and 4 of 40 L-PMN cows, for which concentrations remained < 1 ng/mL throughout the sampling period.

Milk Production

Individual milk yields (kg/cow per d) were measured daily (GEA Farm Technology, Oelde, Germany), and fat and CP concentrations were determined every 2 wk by the infrared technique (FT120, Foss Electric, Hillerød, Denmark) from a composite of samples collected during a consecutive a.m. and p.m. milking. Milk SCC was also recorded for individual cows at 2-wk intervals.

Statistical Analysis

Means comparisons for descriptive variables presented in Table 1 were conducted using one-way ANOVA.

Plasma data were analyzed in a generalized linear model, with cow included as a random effect, and a first-order autoregressive covariance structure to account for repeated measures within cow over time. Fixed effects in the model were PMN group and sample day (as a categorical variable). A group $\times$ time interaction term was included initially, but removed from the final model if not significant ($P > 0.05$). Globulin concentrations were assumed by subtracting albumin from total protein, and the albumin:globulin ratio derived. Data for NEFA, ASAT, GDH, and haptoglobin were not normally distributed, so they were log-transformed for statistical analysis. The untransformed raw means and SEM are presented, unless stated otherwise.

To assess the relationship between PMN status and PPAI, the frequency of cows that ovulated by week was cross-tabulated by PMN group, and differences were investigated by a χ^2 test using pairwise comparisons of the proportions. Additionally, Kaplan-Meier survival curves were plotted against PPAI for cows in each PMN group, and differences between groups were tested by log-rank test.

Milk yields over the first 42 d of lactation and $\log_{10}$ SCC were analyzed in a generalized linear model using a first-order autoregressive covariance structure, with DIM nested within cow, and cow as a random effect. Fixed effects included PMN group, breed, age (coded as 2, 3–5, and > 5 yr old), and DIM. Data are presented as estimated marginal means of milk yield (kg/cow per d; SEM) over the first 42 d of lactation. Associations between milk composition and $\log_{10}$ SCC variables, and PMN group, age group, week in milk, and breed were performed. Those with an association at $P < 0.2$, as well as PMN group, were included in a final model.

Statistical analyses were performed using 'R' version 2.9.2 (R Development Core Team, 2009), with the addition 'Zelig' package (Imai et al., 2008, 2009), or PASW Statistics 18 (SPSS, Chicago, IL).

RESULTS

Breed composition, age, prepartum BCS and BW, calving date, and calf weights were similar between the PMN groups (Table 1). Selected reproduction indices are included in Table 1 as descriptive statistics.

Associations Between PMN and PPAI

Proportionately more ($P = 0.02$) cows in the L-PMN (90%) group had ovulated by 63 and 70 d postpartum compared with those in the H-PMN group (66%). This difference was significant only after d 56 and was due to fewer of the H-PMN cows ovulating after d 56 post-

Figure 2. Kaplan-Meier survival curves of interval from calving to first ovulation for cows classified as having a low (L-PMN, $n = 40$ with 4 censored) or high (H-PMN, $n = 38$ with 13 censored) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum.

partum (Figure 2). From survival analysis, the median PPAI tended to be greater in cows of the H-PMN than the L-PMN group (56 vs. 49 d, $P = 0.07$).

Associations Between PMN and Indicators of Energy Status and Blood Proteins

No differences ($P > 0.1$) were detected in NEFA (Figure 3a), glucose (Figure 3b), urea (Figure 3c), or globulin (Figure 4a) concentrations between PMN groups, but albumin concentrations were greater ($P = 0.02$) in L-PMN compared with H-PMN cows (Figure 4b). Consequently, the ratio of albumin:globulin tended ($P = 0.06$) to be greater in the L-PMN group (Figure 4c). No PMN group $\times$ time interactions were detected for these variables.

Associations Between PMN and Indicators of Liver Function and Inflammation

Mean plasma concentration of ASAT tended ($P = 0.09$) to be greater in H-PMN cows (Figure 5a), but no group $\times$ time interaction was detected ($P > 0.1$). A group $\times$ time interaction ($P < 0.05$) was detected for plasma GDH, where concentrations in H-PMN cows (14.9 ± 1.3 IU/L) were greater ($P = 0.04$) than in L-PMN cows on d 28 (9.7 ± 1.0 IU/L; Figure 5b). A group $\times$ time interaction ($P < 0.05$) was also detected

for plasma haptoglobin (Figure 5c), where concentrations in H-PMN cows (0.071 ± 0.006 g/L) tended ($P = 0.06$) to be greater on d 42 as compared with L-PMN cows (0.064 ± 0.005 g/L).

Associations Between PMN and Mineral Status

Mean plasma Ca was not different between the PMN groups (Figure 6a). A group $\times$ time interaction for Mg concentrations was detected ($P < 0.05$), with plasma Mg on d 14 postpartum being lower ($P < 0.01$) in H-PMN (0.73 ± 0.03 mmol/L) than in L-PMN cows (0.86 ± 0.03 mmol/L; Figure 6b).

Among the 10 cows randomly selected to represent mineral status at herd-level, mean ($\pm$ SD) concentration of Cu was 13.2 ± 3.1 μ mol/L (range: 7–18), Se was 405 ± 80 nmol/L (range: 320–580), and Zn was 12.7 ± 2.4 μ mol/L (range: 10–16).

Associations Between PMN and Milk Production

Average milk yield during the first 42 DIM was lower ($P = 0.04$) in the H-PMN (16.6 ± 0.22 kg/cow per d) cows compared with L-PMN cows (17.2 ± 0.23 kg/cow per d). This difference was subject to an age $\times$ PMN group interaction ($P < 0.001$), where L-PMN cows in the 3- to 5-yr-old age group had a higher milk yield (19.7 ± 0.3 kg/cow per d; $n = 6$) compared with H-PMN cows of similar age (17.7 ± 0.35 kg/cow per d; $n = 13$). No significant differences were observed in average milk yield over the first 42 DIM between 2-yr-old cows ($n = 25$) or cows older than 5 yr ($n = 19$) within either PMN group. There was no interaction between PMN group and DIM (Figure 7; $P = 0.28$), or PMN group and breed ($P = 0.99$).

No differences in milk fat ($P = 0.65$) and protein ($P = 0.21$) percentages were observed between the H-PMN ($5.26 \pm 0.10\%$ and $4.05 \pm 0.05\%$, respectively) and L-PMN cows ($5.31 \pm 0.10\%$ and $4.14 \pm 0.06\%$, respectively). Likewise, no difference ($P = 0.78$) was observed in the fat:protein ratio between H-PMN (1.29 ± 0.02) and L-PMN cows (1.30 ± 0.02). For protein, however, a PMN $\times$ age group interaction was found, with milk of L-PMN cows greater than 5 yr old having a higher ($P = 0.04$) protein percentage ($4.28 \pm 0.12\%$) compared with H-PMN cows of similar age ($3.92 \pm 0.09\%$). No other interactions involving PMN group with age, breed, or DIM were detected.

The SCC did not differ ($P = 0.53$) between the H-PMN and L-PMN cows (80; 95% CI = 57–113 and 69; 95% CI = 50–97 $\times 10^3$ geometric mean SCC/mL, respectively). Breed, age group, and DIM were not significant factors ($P > 0.2$).

Figure 3. Plasma concentrations of (a) NEFA, (b) glucose, and (c) urea, during the transition period for cows classified as having a low (L-PMN, $n = 40$) or high (H-PMN, $n = 38$) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum. Raw means and SE (error bars) are presented.

Figure 4. Plasma concentrations of (a) globulin, (b) albumin, and (c) the albumin:globulin ratio for cows classified as having a low (L-PMN, $n = 40$) or high (H-PMN, $n = 38$) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum. Raw means and SE (error bars) are presented.

DISCUSSION

The primary objective of this study was to explore associations between the occurrence of endometritis and metabolic status during the transition period and in early lactation. Endometritis in the current study was defined on the basis of uterine PMN percentage. All the L-PMN cows had PMN <1% and a negative test for gross endometritis using the Metrichheck procedure, whereas 4 of 38 H-PMN cows (uterine PMN >6%) were also diagnosed with gross endometritis (i.e., Metrichheck score >2). The majority (90%) of cows in the H-PMN group, however, suffered what is commonly referred to as “subclinical” endometritis (Sheldon et al., 2009).

The working hypothesis, that a lower energy status throughout the transition period is a risk factor for endometritis, was not supported by the results of this study based on production data and plasma indicators of metabolic status. The results indicate that endometritis was associated with an inflammatory response and perhaps impaired liver function, coupled with low plasma Mg in early lactation. The current study also supports the existence of an association between endometritis, reduced milk yield, and anovulation beyond d 56 postpartum.

Association Between PPAI and PMN

These results indicate that ovulatory status before the diagnosis of endometritis (at 42 d postpartum) was not a risk factor for this condition, but that elevated PMN percentage at d 42 was associated with a greater likelihood of cows remaining anovulatory beyond d 63. This differs from reports of clinical metritis or endometritis, where “early cyclicity (by 21 DIM) was the main determinant of later uterine health” (Galvão et al., 2009), vaginal discharge was associated with delayed onset of postpartum cyclicity (Opsomer et al., 2000), and Metrichheck detection of endometritis at 6 wk postpartum was not associated with the prevalence of anestrus in seasonal herds (McDougall et al., 2007). The present findings indicate that cows with a uterine infection at 6 wk postpartum are no less likely to have resumed ovulating than those with a healthy uterus; cows with a prolonged PPAI (>56 d), however, are more likely to have endometritis as a preceding condition. A similar finding was recently reported (Senosy et al., 2009). Bacterial contamination of the endometrium was associated with reduced function of the dominant ovarian follicle (Sheldon et al., 2002; Williams et al., 2007). These previous studies limited their investigation to the first 4 wk postpartum; by comparison, the current findings and those of Senosy et al. (2009) demonstrate a link between uterine infection and ovarian function 5 to

Figure 5. Plasma concentrations of (a) aspartate aminotransferase (ASAT), (b) glutamate dehydrogenase (GDH), and (c) haptoglobin for cows classified as having a low (L-PMN, $n = 40$) or high (H-PMN, $n = 38$) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum. Raw means and SE (error bars) are presented.

6 wk postpartum, but not before that time. It is therefore possible that the physiological mechanisms that underlie reported associations between PPAI and the severity of uterine disease (metritis versus endometritis; Sheldon et al., 2009) are not the same.

Indicators of Energy Status and Liver Function

Albumin can be considered a negative acute-phase protein (Fleck, 1989), with subnormal concentrations indicating impaired liver function, following a diverted synthesis to positive acute-phase proteins (Bertoni et al., 2008). Accordingly, the lower concentrations of albumin (and albumin:globulin ratio) may indicate impaired liver function in this study's H-PMN cows. Notably, this condition was evident from at least 14 d before calving. Green et al. (2009) also reported lower albumin in pasture-grazed cows with endometritis compared with cows unaffected by endometritis. Albumin depression may be evidence of liver fat infiltration (Chamberlain and Wilkinson, 1996), a reported risk factor for uterine disease (Zerbe et al., 2000). This seems an unlikely reason within pasture-grazed systems where cows have lower milk yields than cows in intensive systems fed TMR diets.

Lower plasma albumin could also be a consequence of greater degree of AA scavenging for gluconeogenesis (Bell et al., 2000). Again, this is an unlikely explanation for lower plasma albumin among H-PMN cows in the current study. Profiles of plasma NEFA, glucose, and urea for both PMN groups that grazed the same forage diet indicate that DMI and energy status were similar (Bauman and Currie, 1980; Reist et al., 2002). Further studies are necessary to explain the preceding condition of lower albumin in H-PMN cows and whether any mechanistic link to endometritis exists. The influence of inflammatory and anti-inflammatory cytokines (Bertoni et al., 2009) should be included in such future studies.

Elevated plasma concentrations of ASAT and GDH in early lactation among H-PMN cows indicate liver dysfunction and, potentially, hepatic tissue damage (Bertoni et al., 2008). This is further evidence that the challenge to liver function among cows diagnosed with endometritis was greater. Postpartum disease may have a detrimental effect on liver function, and in some cases can result in hepatic failure (Sweeney et al., 1988). This previous report implied liver function was compromised because of systemic toxins from postpartum disease. The current study, however, provides evidence that liver function may have been compromised prepartum, and we found evidence of hepatocellular damage in early lactation among cows diagnosed with endometritis.

Several previous reports link increased haptoglobin, a positive acute-phase, inflammatory-response protein, to the presence of uterine disease, and haptoglobin has been suggested as an early predictor of clinical metritis (Drillich et al., 2007; Huzzey et al., 2009). Haptoglobin concentration is also increased in cows with retained fetal membranes (Mordank, 2009), a key risk factor

Figure 6. Plasma concentrations of (a) Ca and (b) Mg for cows classified as having a low (L-PMN, n = 40) or high (H-PMN, n = 38) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum. Raw means and SE (error bars) are presented.

Figure 7. Daily milk yield during the first 42 DIM for cows classified as having a low (L-PMN, $n = 40$) or high (H-PMN, $n = 38$) percentage of polymorphonuclear (PMN) cells in cytological samples collected from the endometrium at d 42 postpartum. Estimated marginal means and SE (error bars) are presented.

for uterine infection. In the current study, plasma haptoglobin was marginally higher in H-PMN at d 42 compared with L-PMN cows. This result is difficult to interpret, given the low concentrations and small difference involved at d 42. Perhaps more importantly, haptoglobin did not differ between PMN groups in the early postpartum period, particularly during the first 7 d postpartum when haptoglobin was reported to be markedly increased in cows that develop clinical metritis within the first 2 wk postpartum (Huzzey et al., 2009). In the current study, cows with calving disorders that predispose severe uterine infection were excluded from enrollment. Among those that were enrolled, the relatively low concentrations of haptoglobin indicated that neither PMN group exhibited an acute inflammatory response indicative of a severe, early uterine infection.

Mineral Status

Mineral tests using d -14 samples of 10 randomly selected cows indicated normal levels of Cu (Suttle,

1994), Se (Wichtel, 1998), and Zn (Underwood and Suttle, 1999) at the herd level. Further tests conducted by the herd veterinarian in 6 randomly selected animals at about d 42 (data not presented) also indicated normal levels of such trace elements considered important for long-term immune function (Goff, 2006). On the basis of this information, Zn and Se were not measured as a variable of interest in the current study.

The Ca profile through the transition period was consistent with previous reports of pasture-grazed cows (Burke and Roche, 2007) and did not differ between the PMN groups. By contrast, a group $\times$ time interaction was detected for Mg profiles, with H-PMN cows having lower circulating concentrations of Mg at d 14 postpartum. Bertoni et al. (2008) also reported plasma Mg to be depressed in cows with impaired liver function. This is an interesting result because, as with lower plasma albumin, lower plasma Mg in early lactation was reported to be associated with a delayed onset of cyclicity in pasture-grazed cows (Burke et al., 2006; Burke and Roche, 2007). The possibility of a common cause or underlying condition involving liver malfunction as a

predisposing factor for endometritis and anestrus in pasture-grazed dairy cows warrants further investigation.

Because of the association between dietary Mg and milk fever (Roche and Berry, 2006) and the very high potassium content of pasture (Roche et al., 2002), supplementary Mg was applied to pasture. As it is not stored in the body, Mg must be continuously sourced from diet. Lower plasma Mg may therefore indicate a lower pasture DMI among H-PMN cows. Similar concentrations of plasma urea and NEFA between the PMN groups, however, suggest that the difference in plasma Mg on d 14 was unlikely to be a consequence of lower DMI in H-PMN cows at this time. Further studies on the role of Mg during postpartum recovery of the reproductive axis are required.

CONCLUSIONS

Endometritis at 42 d postpartum in the herd studied was associated with lower concentrations of albumin and a lower albumin:globulin ratio throughout the transition period. Endometritis was also associated with lower Mg and evidence of hepatocellular damage in early lactation, as well as a greater incidence of anovulation at 9 to 10 wk postpartum. Milk yield for 3- to 5-yr-old cows and protein percentage for cows >5 yr old were lower in cows with endometritis. Energy status, as measured by selected metabolites, was not detected as a risk factor for this uterine condition.

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