pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 35(6) : 258-265 (2018)
http://dx.doi.org/10.17555/jvc.2018.12.35.6.258

Journal of Veterinary Clinics
www.ksvc.or.kr

Associations of Puerperal Metritis with Serum Metabolites, Uterine Health, Milk Yield, and Reproductive Performance in Dairy Cows

Jae-Kwan Jeong, Hyun-Gu Kang and Ill-Hwa Kim[1]

Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea

(Received: October 22, 2018 / Accepted: November 16, 2018)

Abstract : We aimed to identify the relationships between puerperal metritis (PM) and serum metabolites, uterine health, milk yield, and reproductive performance in dairy cows. Blood samples from 150 Holstein dairy cows were collected just after calving, and at 1, 2, and 4 weeks postpartum to measure serum concentrations of β-hydroxybutyrate (BHBA), urea nitrogen, total cholesterol (TCH), albumin, aspartate aminotransferase (AST), calcium, and magnesium. PM was diagnosed by the presence of fever (≥ 39.5°C) and a watery, fetid uterine discharge during the first 14 days after calving. Cows were divided into two groups on the basis of the presence or absence of the disease: a control group (n = 83) and a PM group (n = 67). The cows diagnosed with PM were subcutaneously administered with 2.2 mg/kg ceftiofur for 3-5 days. The serum concentrations of BHBA tended to be higher ($P = 0.06$) and AST was higher ($P < 0.05$) in the PM group than in the control group 1 week after calving, whereas serum concentrations of urea nitrogen, TCH, albumin, calcium, and magnesium were lower ($P < 0.05$-0.0001) after calving in the PM group than in the control group. The probability of clinical endometritis was higher (odds ratio = 5.40, $P < 0.001$) in the PM group than in the control group. Moreover, the proportion of neutrophils in the uterus was also higher in the PM group than in the control group 4, 6, and 8 weeks after calving ($P < 0.001$). The mean milk yield 1 and 2 months after calving was lower ($P = 0.05$) in the PM group than in the control group. The hazard of pregnancy by 180 days after calving tended to be lower (hazard ratio = 0.60, $P = 0.07$) in the PM group than in the control group, which led to an extended mean interval between calving and pregnancy (19 days) in the PM group ($P < 0.01$). In conclusion, PM is associated with higher postpartum concentrations of BHBA and AST, and lower concentrations of urea nitrogen, TCH, albumin, calcium, and magnesium. Moreover, PM is associated with subsequent poor uterine health, lower milk yield, and poorer reproductive performance in dairy cows.

***Key words* :** puerperal metritis, metabolite, uterine health, milk yield, reproduction, dairy cow.

Introduction

Puerperal metritis (PM) is caused by an acute bacterial uterine infection that commonly manifests within the first 21 days after calving, and is characterized by an abnormally enlarged uterus with a watery, fetid, red-brown uterine discharge and the presence of fever (rectal temperature ≥ 39.5°C) (34). The disease has been recognized as a serious postpartum disease, which is associated with lower milk production and poorer reproductive outcomes, and can be life-threatening in dairy cows (26,33,38). The incidence of PM ranges from 8% to 30% (1,9,14). In most cases, affected animals are promptly treated using antibiotics and supportive therapy, and the antibiotics that are commonly used are ceftiofur, ampicillin, oxytetracycline, and penicillin (9,12,25). Nevertheless, the economic losses associated with the disease are estimated at $380 per case, including the costs of treatment, lower milk yield, delayed pregnancy, and potentially culling or death (9). In addition, cows with PM have a higher risk of subsequent clinical and cytological endometritis (3,25).

Diverse factors contribute to the etiology, severity, and progression of the disease. It is reported that calving disorders (dystocia, twins, and stillbirth) and retained placenta are important risk factors for PM (10). In addition, negative energy balance (NEB) during the transition period, due to lower dry matter intake (DMI) and greater energy requirement for milk production (15), may impair immune function, leading to a greater incidence of postpartum metritis, as well as metabolic disorders. Thus, poor nutritional management of the herd may increase the incidence of PM, especially in high-yielding dairy herds. Likewise, cows that develop metritis show lower DMI, starting up to 2 weeks before calving, and continuing until 4-5 weeks after calving (18,21), leading to immunosuppression (6,23) and uterine health disorders (18).

Previous studies show that higher non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA), and lower calcium concentrations, during early lactation are associated with impaired neutrophil function, resulting in a higher incidence of PM (18,27). Thus, measurement of circulating concentrations of metabolites during the peripartum and early lactation periods can provide valuable information on the risk and progression of certain postpartum diseases, including metri-

[1]Corresponding author.
E-mail : illhwa@cbu.ac.kr

tis. However, studies of the relationships between blood metabolite concentrations and the incidence of PM have been few in number.

We hypothesized that determining the relationships between serum metabolite concentrations (especially those related to energy and mineral status) during the postpartum period in cows with PM might help to establish strategies for the prevention and treatment of the disease. Therefore, the first objective of our study was to determine the statistical relationships between serum metabolites (BHBA, urea nitrogen, total cholesterol [TCH], albumin, aspartate aminotransferase [AST], calcium, and magnesium) and PM in dairy cows.

The effects of PM on subsequent uterine health, milk yield, and reproductive performance have been published numerous times (1,11). However, the criteria for the diagnosis of PM, including the measurement of body temperature and evaluation of the vaginal discharge, were not consistent among these studies. Moreover, the degree of adverse impact of the disease differed among the studies, possibly due to differences in the characteristics of the animals, herd health management, treatment method, and environment (regional geography and climate). Therefore, the second objective of the study was to determine the effects of PM on subsequent uterine health, milk production, and reproductive performance in dairy cows in Chungcheong Province.

Materials and Methods

Animals

This study was performed on four dairy farms located in Chungcheong Province. Each farm contained 90-200 milking cows, which were maintained in loose housing systems. They were fed total mixed rations and milked twice daily. Milk yield was measured monthly by staff from the Korean Animal Improvement Association and the yield data were analyzed. At the beginning of the study, a total of 219 Holstein cows that had recently calved were included, but 69 cows were subsequently excluded because they developed multiple postpartum disorders. Thus, data from 150 cows, with 2.4 ± 1.4 lactations (mean ± standard deviation; 41 primiparous and 109 multiparous cows), were analyzed. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University.

Study design

Measurement of rectal temperature and evaluation of the uterine discharge of the cows were undertaken daily at approximately 10:00 h between calving and 14 days postpartum. PM was diagnosed by the presence of fever (≥ 39.5°C) and a watery, fetid uterine discharge during the period. The cows were divided into two groups on the basis of the presence or absence of the disease: a control group (n = 83) and a PM group (n = 67). All the cows diagnosed with PM were subcutaneously administered with ceftiofur sodium (2.2 mg/kg) for 3-5 days. No negative control group (with PM, but not treated with antibiotics) was established because this is a severe illness that carries a significant risk of death or culling if left untreated.

We compared the serum concentrations of metabolites (BHBA, urea nitrogen, TCH, albumin, AST, calcium, and magnesium) just after calving, and 1, 2, and 4 weeks postpartum between cows that did or did not have PM. In addition, the relationships between PM and uterine health (the incidence of clinical endometritis and the proportion of neutrophils in the uterus), milk yield (mean yield 1 and 2 months after calving), and reproductive performance (the hazard of pregnancy by 180 days after calving) were determined.

Case definition

All cows received reproductive health checks weekly by veterinarians on the research team, which included an examination of their ovarian structures (follicle and corpus luteum) and uterus by transrectal palpation and ultrasonography. The definitions of the postpartum disorders that were used in the present study were similar to those described in previous publications (8,29,34). Retained placenta was defined as the retention of the fetal membranes for longer than 24 h. PM was defined by the presence of fever (≥ 39.5°C) and a watery, fetid uterine discharge during the first 14 days after calving. Clinical endometritis was diagnosed 4 weeks after calving by examining any vaginal discharge using the Metricheck tool. Cows with a mucopurulent discharge (< 50% pus) were diagnosed with clinical endometritis. Ketosis was diagnosed by the presence of the following clinical signs: anorexia, depression, and an acetone odor on the breath. Milk fever was diagnosed by the presence of weakness, recumbency, and a favorable response to calcium therapy after calving. Abomasal displacement was diagnosed by a 'ping' sound during abdominal auscultation. Mastitis was diagnosed by the production of abnormal milk or signs of inflammation in one or more quarters of the udder. Lameness was diagnosed if an abnormal gait or lack of weight-bearing on a limb was observed, and this included diagnoses of interdigital or digital dermatitis. The incidence of postpartum disorders was finalized by 4 weeks postpartum, except for particular disorders for which the timing of diagnosis is given above. Cows diagnosed with metabolic disorders (ketosis, milk fever, or abomasal displacement), mastitis, or lameness were excluded from the study, but cows with both PM and retained placenta remained in the study.

Blood sampling and uterine cytology

Blood samples from 150 Holstein dairy cows were collected just after calving (range: 30 min-3 h), and 1, 2, and 4 weeks postpartum to measure serum concentrations of BHBA, urea nitrogen, TCH, albumin, AST, calcium, and magnesium. Ten milliliters of blood were placed into plain plastic centrifuge tubes, which were immediately placed in an ice bath. The samples were then centrifuged at 2,000 × g for 10 min at 4°C, and the serum was harvested and frozen at −80°C until assayed.

Samples for uterine cytology were collected from the cows 4, 6, and 8 weeks after calving as previously described (22). Briefly, after cleaning the vulva, a cytobrush and stainless steel rod (which was guarded by a stainless steel sheath and covered with a protective plastic sheath) were introduced into the vagina. The plastic sheath was pulled back at the external ostium of the cervix, and the stainless steel sheath, stainless steel rod, and cytobrush were passed into the body of the uterus. The sheath was then retracted to expose the cytobrush, which was rotated clockwise to obtain cellular material from the endometrium. After removal from the vagina, the brush was rolled onto a glass slide and the sample was air-dried. All slides were stained using Diff-Quick (Sysmex Inc., Kobe, Japan), according to the manufacturer's guidelines. Each slide was examined microscopically (200 × magnification) by the same researcher. The numbers of epithelial endometrial cells and neutrophils were counted (up to 200 cells per slide) and the percentage of neutrophils was calculated.

Health and reproductive management

The cows diagnosed with PM were subcutaneously administered with ceftiofur sodium (2.2 mg/kg) (Unixel, Uni Biotech Co., Ltd, Anyang, Korea) and supportive medication for 3-5 days. Cows with clinical endometritis were treated with an injection of cloprostenol (500 μg), a $PGF_{2\alpha}$ analogue (Estrumate, MSD Animal Health, Seoul, Korea) if they had a corpus luteum (CL), or one intrauterine infusion of 2% povidone-iodine solution (Betadine solution, Korea Pharma Co., Ltd., Hwasung, Korea) if they had no CL, and retreated if necessary.

The voluntary waiting period from calving to the first artificial insemination was 40 days. In addition to estrus detection, Ovsynch synchronization was performed using a combination of gonadorelin (100 μg), a GnRH analogue (Godorel, Uni Biotech) on Day 0, prostaglandin $F_{2\alpha}$ on Day 7, and gonadotropin releasing hormone (GnRH) on Day 9. Cows that exhibited estrus naturally were inseminated according to the am-pm rule, whereas cows treated with Ovsynch underwent timed artificial insemination. Pregnancy was diagnosed 35-40 days after insemination by transrectal ultrasonography. Data regarding reproductive performance were collected for a minimum of 180 days postpartum, or until pregnancy or culling.

Measurement of serum metabolite concentrations

The concentrations of BHBA, urea nitrogen, TCH, albumin, AST, calcium, and magnesium in serum samples were measured using a 7180 Biochemistry Automatic Analyzer 710 (Hitachi Ltd., Tokyo, Japan) and commercial enzyme assay kits (Wako Pure Chemical Ltd., Osaka, Japan), according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were < 5% for each assay.

Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM). For statistical analyses, cow parity was categorized as either 1 or ≥ 2 and calving season as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February). Statistical analyses were performed using SAS (version 9.4, SAS Inst., Cary, NC, USA).

The risk of clinical endometritis was analyzed by logistic regression using the LOGISTIC procedure. The logistic regression model included calving season, cow parity, retained placenta, and PM. Farm was included in the model as a random effect. Backward stepwise regression was used for all models, and elimination was performed based on the Wald statistic criterion when $P > 0.11$. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by logistic regression. Results are presented as percentages and ORs with their respective 95% CIs.

Statistical analysis of mean milk yield 1 and 2 months after calving was carried out using the general linear models (GLM) procedure. The model included calving season, cow parity, and group (control or PM). Farm was included in the model as a random effect.

Cox's proportional hazard model with the PHREG procedure was used to compare the hazard of pregnancy by 180 days after calving between groups. This estimated the hazard of a cow being pregnant at a given time. The time variable used in this model was the interval in days between calving and pregnancy. Cows that died, were sold, or were not pregnant by 180 days after calving were excluded. The cox model included calving season, cow parity, and group, and farm was included in the model as a random effect. The proportional hazard rate was determined on the basis of interactions between explanatory variables and time. The mean number of days to pregnancy was determined by survival analysis using the Kaplan-Meier model and the LIFETEST procedure within the SAS software. A survival plot was generated using the survival option within MedCalc software (12.7.1, MedCalc Software, Mariakerke, Belgium).

The effects of group (control or PM), cow parity (primiparous or multiparous), sampling time (weeks postpartum), and two-way interactions between group, cow parity, and sampling time, on serum metabolite concentrations and the proportion of neutrophils in the uterus were determined using a mixed model.

A P-value ≤ 0.05 was considered statistically significant and $0.05 < P < 0.1$ was considered to indicate a notable trend.

Results

Relationships between PM and serum metabolite concentrations

Fig 1 shows comparisons of serum metabolite concentrations between the control and PM groups just after calving, and 1, 2, and 4 weeks after calving. The serum concentrations of BHBA tended to be higher ($P = 0.06$) and AST was higher ($P < 0.05$) in the PM group 1 week after calving (Fig 1A and E, respectively). However, the serum concentrations of urea nitrogen, TCH, albumin, calcium, and magnesium were lower ($P < 0.05$-0.0001) in the PM group than in the control group during the postpartum period (Fig 1B, C, D, F, and G).

Relationship between PM and uterine health

Table 1 shows the variables included in the final logistic regression model to establish the risk factors for clinical endometritis. The probability of clinical endometritis was higher (OR = 5.40, $P < 0.001$) in cows with PM than in cows without PM. In addition, cows with a retained placenta had a

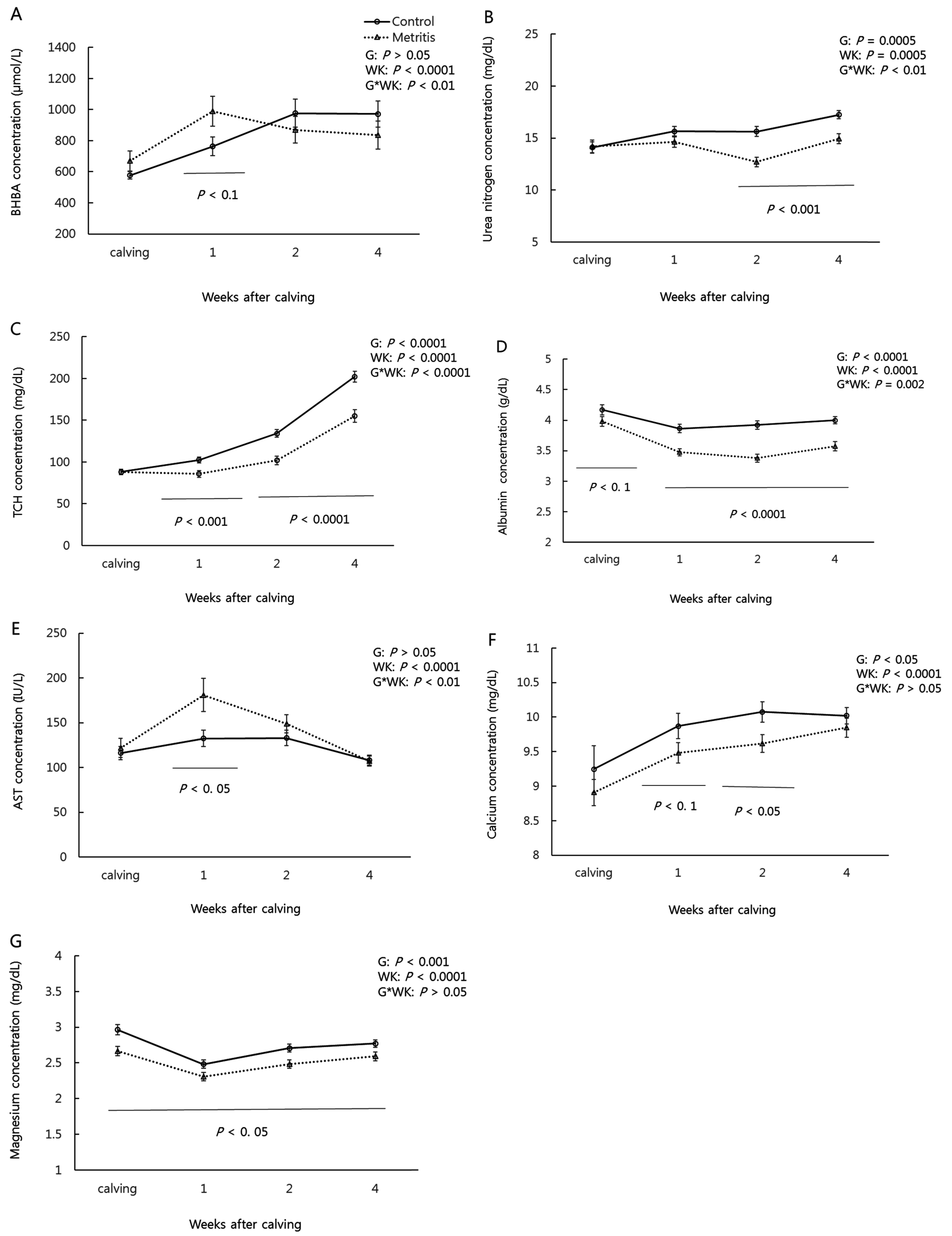

Fig 1. Serum β-hydroxybutyrate (BHBA; A), urea nitrogen (B), total cholesterol (TCH; C), albumin (D), aspartate aminotransferase (AST; E), calcium (F), and magnesium (G) concentrations in cows without puerperal metritis (PM) (n = 83) and those with PM (n = 67) just after calving, and at 1, 2 and 4 weeks after calving. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect.

higher risk of clinical endometritis (OR = 2.72, $P < 0.05$) than cows without.

Fig 2 shows a comparison of neutrophil levels in the uterus 4, 6, and 8 weeks after calving between the control and PM groups. The proportion of neutrophils in the uterus 4, 6, and 8 weeks after calving was higher in the PM group than in the control group ($P < 0.01$-0.001).

Relationship between PM and milk yield

The GLM procedure revealed that mean milk yield 1 and 2 months after calving in the PM group was lower ($P = 0.05$) than in the control group (Table 2). In addition, multiparous cows had higher mean milk yield 1 and 2 months after calving than primiparous cows ($P < 0.0001$), whereas there was no association with calving season (Table 2, $P > 0.1$).

Relationship between PM and reproductive performance

Table 3 shows the factors affecting the hazard of pregnancy by 180 days after calving, analyzed using the PHREG procedure. The hazard of pregnancy at this time point tended to be lower (hazard ratio = 0.60, $P = 0.07$) in the PM group than in the control group, which led to an extended mean interval between calving and pregnancy (a 19-day increase)

Table 1. Odds ratio (OR) and variables included in the final logistic regression model used to identify risk factors for clinical endometritis

Variable	Adjusted OR	95% CI[1]	*P*-value
Calving season			> 0.1
Cow parity			> 0.1
Retained placenta			
No	Reference		
Yes	2.72	1.153-6.438	< 0.05
PM[2]			
No	Reference		
Yes	5.40	2.210-13.182	< 0.001

[1]Confidence interval.
[2]Puerperal metritis, diagnosed by the presence of fever (rectal temperature ≥ 39.5°C) and a watery, fetid uterine discharge during the first 14 days after calving.

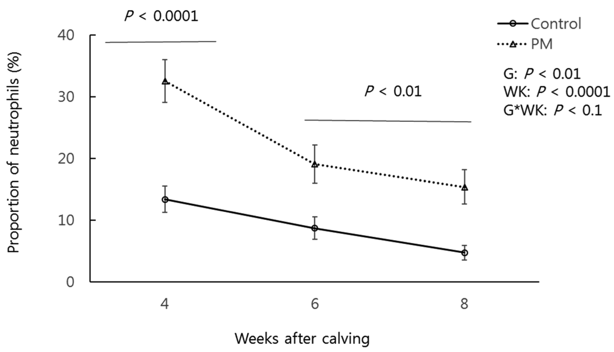

Fig 2. Comparison of neutrophil levels in the uterus between the control group (n = 83) and the PM group (n = 67) 4, 6, and 8 weeks after calving. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect.

Table 2. Least squares means of variables included in the statistical model for mean milk yield 1 and 2 months after calving

Variable	Milk yield (kg)	*P*-value
Calving season		> 0.1
Cow parity		
Primiparous	34.5 ± 1.2	
Multiparous	40.9 ± 0.7	< 0.0001
PM[1]		
No	38.9 ± 0.9	
Yes	36.5 ± 1.1	0.05

[1]Puerperal metritis, diagnosed by the presence of fever (rectal temperature ≥ 39.5°C) and a watery, fetid uterine discharge during the first 14 days after calving.

Table 3. Factors affecting the hazard of pregnancy by 180 days after calving, analyzed using the PHREG procedure

Variable	Hazard ratio	95% CI[1]	*P*-value
Calving season			> 0.1
Cow parity			> 0.1
PM[2]			
No	Reference		
Yes	0.60	0.343-1.046	0.07

[1]Confidence interval.
[2]Puerperal metritis, diagnosed by the presence of fever (rectal temperature ≥ 39.5°C) and a watery, fetid uterine discharge during the first 14 days after calving.

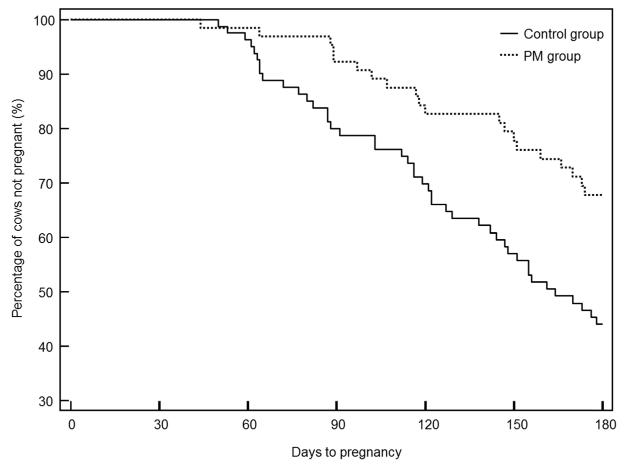

Fig 3. Survival curves for the interval to pregnancy in the control and PM groups. The hazard of pregnancy by 180 days after calving was lower (HR: 0.46, $P < 0.01$) in the PM group (n = 67) than in the control group (n = 83). The mean (± SEM) days to pregnancy was 142.8 ± 4.9 in the control group and 162.1 ± 4.2 in the PM group.

in the PM group ($P < 0.01$), as shown by the survival curves (Fig 3). However, calving season and cow parity did not affect the hazard ($P > 0.1$).

Discussion

We evaluated the relationships PM and serum metabolite concentrations, uterine health, milk yield, and reproductive performance in dairy cows. Our results indicate that PM is associated with higher BHBA and AST, and lower urea nitrogen, TCH, albumin, calcium, and magnesium concentrations during the postpartum period, indicating severe NEB and poor liver and immune function, along with lower uterine tone. Moreover, PM is associated with subsequent poor uterine health, and reductions in milk yield and reproductive performance.

The serum concentrations of BHBA and AST were higher in cows with PM than in those without 1 week after calving, whereas the concentrations of urea nitrogen, TCH, albumin, calcium, and magnesium were lower during the postpartum period. Consistent with our findings, a previous study showed that BHBA concentration is higher in cows with PM than in those without at the same time point (14). Additionally, cows developing metritis show lower DMI from 2 weeks before calving until 4-5 weeks after calving (18,21). The lower DMI before calving would result in greater serum NEFA concentrations before calving and subsequently greater BHBA concentrations after calving (36). Our finding that urea nitrogen concentration was lower in cows with PM than in those without 2 and 4 weeks after calving was also similar to that of a previous study in which urea nitrogen concentration was measured 3 weeks after calving (14). A low urea concentration in transitioning cows might be associated with NEB, reflecting a reduction in DMI or an inability of the liver to efficiently convert ammonia to urea (19). Thus, higher BHBA and lower urea nitrogen concentrations in the present study reflect more severe NEB during the postpartum period in cows with PM than in cows without the disease (35).

The liver is the main organ involved in adaptation to NEB during early lactation. Thus, several metabolites in the blood can reflect liver function during the postpartum period. Cholesterol is a constituent of several lipoproteins and a reduction in TCH could reflect changes in liver synthesis associated with fatty liver (20). Albumin also can be used as an indicator of liver dysfunction (37), and lower serum albumin concentration is associated with hepatocellular liver diseases and fatty liver (4). In addition, the activity of AST is often used as a biomarker of hepatocyte damage, and greater serum AST activity reflects active liver pathology (7). Therefore, cows suffering from hepatic lipidosis can be identified by their high levels of AST (16). Taken together, the lower TCH and albumin and higher AST concentrations identified in cows with PM in the present study are indicative of impaired liver function.

Our finding of a lower concentration of serum calcium in cows with PM is consistent with that of a previous study (27) in which cows that developed metritis during the first 12 days in milk (DIM) had lower serum calcium concentrations from the day of calving until 12 DIM than cows that did not develop metritis. The researchers also suggested that lower calcium concentration might result not only in the loss of nerve and muscle tone in the uterus but also a reduction in neutrophil function (impairment in phagocytosis) (27), resulting in the development of metritis, which is also consistent with our results. In addition, and similar to our finding that cows with PM had a lower serum magnesium concentration than those without PM, a previous study showed that magnesium concentration is lower in cows with endometritis than in those without (5). The lower magnesium concentration in the present study might result in hyperexcitability because of a reduction in nerve resting membrane potential (30), which is also involved in the development of PM.

Negative energy, mineral, and vitamin balance during the transition period leads to immunosuppression (6,23). Previous studies show that severe NEB and hypocalcemia are associated with impaired neutrophil function during the postpartum period (13,27). Neutrophil function (evaluated by the proportions of neutrophils demonstrating phagocytosis and an oxidative burst) is poorer in cows with subclinical hypocalcemia during the first 3 DIM (27). In addition, cows that develop uterine disease experience more severe NEB and impairment in neutrophil function (13), and these animals might be predisposed to uterine disease. Thus, our data suggest that severe NEB and reductions in liver and immune function, along with a loss of nerve and muscle tone in the uterus, reflected in changes in the concentrations of several serum metabolites, contribute to the morbidity associated with PM (18).

The probability of clinical endometritis and the proportion of neutrophils in the uterus 4-8 weeks after calving were higher in cows with PM than in those without, which indicates that cows with PM have delayed uterine involution and a persistent inflammatory response during the postpartum period. These findings are consistent with a previous study in which cows with PM had a greater incidence of clinical endometritis at 32 days, and cytological endometritis 39 days after calving (25). Another study also showed that cows with PM have a greater risk (2.2 times) of developing the disease than those without metritis (14).

Mean milk yield 1 and 2 months after calving in the PM group was lower than in the control group, which is consistent with previous findings (2,14). The lower milk yield might be the result of the DMI during early lactation and the consequent reduction in the energy available for milk synthesis in cows with metritis (2). One study found that multiparous cows with metritis produced less milk than healthy multiparous cows during early lactation (38), while in contrast, a previous study showed that milk production by primiparous cows is not affected by metritis and that the effect of metritis on milk production varied over time in multiparous cows (11). In this study, milk production was lower at first test-day, but was not different at the subsequent 3 tests (11).

Despite treatment with parental antibiotic (ceftiofur), cows with PM demonstrated poorer reproductive outcomes (lower hazard of pregnancy by 180 days after calving and an extended mean interval from calving to pregnancy) than control cows. These findings are consistent with those of a previous study in which cows with PM had a lower hazard of pregnancy by 150 DIM and a longer interval from calving to conception than cows without PM, despite the former being treated with ceftiofur (2.2 mg/kg for 3 days) (14). However, another study showed that reproductive outcomes (number of days at conception, conception rate at first insemination, and percentage of non-pregnant cows at >150 days after calving) in cows with metritis that are treated with intramuscular amoxicillin do not differ from those of healthy controls (1). On the other hand, it has been shown that treatment with ceftiofur in cows with metritis shortens by 22 days required to achieve the same percentage of pregnant animals compared to those not treated (31). Thus, the efficacy of antibiotics, including ceftiofur, for the subsequent improvement of reproductive outcomes requires further investigation (17).

The adverse effect of PM on reproductive performance might be associated with poor uterine health (a higher incidence of clinical endometritis and a higher proportion of neutrophils in the uterus), because uterine diseases may impair fertility (delayed resumption of cyclicity and impaired embryo development) (28,32). Also, it has been reported that the inflammation in the uterus is associated with the release of large amounts of $PGF_{2\alpha}$, because of tissue destruction, and this high $PGF_{2\alpha}$ concentration may inhibit ovulation and CL formation (24). Furthermore, poorer reproductive performance in cows with PM might be indirectly associated with impairment in immune and liver function, as well as energy deficiency, which are reflected in altered serum concentrations of metabolites during the postpartum period.

In summary, our results show that PM is associated with higher serum AST and BHBA, and lower urea nitrogen, TCH, albumin, calcium, and magnesium concentrations, during the postpartum period in dairy cows. Moreover, PM is associated with subsequent poor uterine health, and reductions in milk yield and reproductive performance in dairy cows. These data may indicate that the adverse effect of PM on the health, production, and reproduction of the cows affected might be exerted through severe NEB and impairment in liver and immune function, along with lower uterine tone, reflected in alterations in serum metabolite concentrations during the postpartum period. Therefore, the establishment of a nutritional management strategy to prevent NEB and mineral deficiency during transition should be emphasized to avoid PM in dairy herds. In addition, an effective regimen for the treatment of PM is also urgently required.

References

1. Armengol R, Fraile L. Comparison of two treatment strategies for cows with metritis in high-risk lactating dairy cows. Theriogenology 2015; 83: 1344-1351.
2. Bell MJ, Roberts DJ. The impact of uterine infection on a dairy cow's performance. Theriogenology 2007; 68: 1074-1079.
3. Benzaquen ME, Risco CA, Archbald LF, Melendez P, Thatcher M-J, Thatcher WW. Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J Dairy Sci 2007; 90: 2804-2814.
4. Bernabucci U, Ronchi B, Basiricò L, Pirazzi D, Rueca F, Lacetera N, Nardone A. Abundance of mRNA of apolipoprotein B_{100}, apolipoprotein E, and microsomal triglyceride transfer protein in liver from periparturient dairy cows. J Dairy Sci 2004; 87: 2881-2888.
5. Burke CR, Meier S, McDougall S, Compton C, Mitchell M, Roche JR. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J Dairy Sci 2010; 93: 5363-5373.
6. Cai TQ, Weston PG, Lund LA, Brodie B, McKenna DJ, Wagner WC. Association between neutrophil functions and periparturient disorders in cows. Am J Vet Res 1994; 55: 934-943.
7. Cebra CK, Garry FB, Getzy DM, Fettman MJ. Hepatic lipidosis in anoretic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. J Vet Intern Med 1997; 11: 231-237.
8. Cook N, Oetzel G, Nordlund K. Modern techniques for monitoring high-producing dairy cows. 1. Principles of herd-level diagnoses. In Pract 2006; 28: 510-515.
9. Drillich M, Beetz O, Pfützner A, Sabin M, Sabin H-J, Kutzer P, Nattermann H, Heuwieser W. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. J Dairy Sci 2001; 84: 2010-2017.
10. Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ. Risk factors for postpartum uterine diseases in dairy cows. J Dairy Sci 2010; 93: 5764-5771.
11. Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ. Effects of postpartum uterine diseases on milk production and culling in dairy cows. J Dairy Sci 2011; 94: 1339-1346.
12. Espadamala A, Pereira R, Pallarés P, Lago A, Silva-del-Río N. Metritis diagnosis and treatment practices in 45 dairy farms in California. J Dairy Sci 2018; 101: 9608-9616.
13. Galvão KN, Flaminio MJBF, Brittin SB, Sper R, Fraga M, Caixeta L, Ricci A, Guard CL, Butler WR, Gilbert RO. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. J Dairy Sci 2010; 93: 2926-2937.
14. Giuliodori MJ, Magnasco RP, Becu-Villalobos D, Lacau-Mengido IM, Risco CA, de la Sota RL. Metritis in dairy cows: Risk factors and reproductive performance. J Dairy Sci 2013; 96: 3621-3631.
15. Goff JP, Horst RL. Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci 1997; 80: 1260-1268.
16. González FD, Muiño R, Pereira V, Campos R, Benedito JL. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. J Vet Sci 2011; 12; 251-255.
17. Haimerl P, Heuwieser W. Invited review: Antibiotic treatment of metritis in dairy cows: A systematic approach. J Dairy Sci 2014; 97: 6649-6661.
18. Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol 2006; 113: 21-29.
19. Hayton A, Husband J, Vecqueray R. Nutritional management of herd health. In: Dairy Herd Health, Oxfordshire: CABI. 2012: 248.
20. Herdt TH, Liesman JS, Gerloff BJ, Emery RS. Reduction of serum triacylglycerol-rich lipoprotein concentrations in cows with hepatic lipidosis. Am J Vet Res 1983; 44: 293-296.
21. Huzzey JM, Veira DM, Weary DM, von Keyserlingk MAG. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy Sci 2007; 90: 3220-3233.
22. Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, Johnson WH. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 2004; 62: 9-23.
23. Kehrli ME Jr, Goff JP. Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. J Dairy Sci 1989; 72: 1188-1196.
24. Kindahl H, Odensvik K, Aiumlamai S, Fredriksson G. Utero-ovarian relationships during the bovine postpartum period. Anim Reprod Sci 1992; 28: 363-369.
25. Lima FS, Vieira-Neto A, Vasconcellos GSFM, Mingoti RD, Karakaya E, Solé E, Bisinotto RS, Martinez N, Risco CA, Galvão KN, Santos JEP. Efficacy of ampicillin trihydrate or caftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows. J Dairy Sci 2014; 97: 5401-5414.
26. Mahnani A, Sadeghi-Sefidmazgi A, Cabrera VE. Consequences and economics of metritis in Iranian Holstein dairy farms. J Dairy Sci 2015; 98: 6048-6057.
27. Martinez N, Risco CA, Lima FS, Bisinotto RS, Greco LF, Ribeiro ES, Maunsell F, Galvão K, Santos JEP. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J Dairy Sci 2012; 95: 7158-7172.
28. Mateus L, Lopes da Costa L, Bernardo F, Robalo Silva J. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod Dom Anim 2002; 37: 31-35.
29. McDougall S, Macaulay R, Compton C. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. Anim Reprod Sci

2007; 99: 9-23.
30. National Research Council. Mineral tolerance of animals, seventh ed. Washington, DC: National Academy Press. 2005.
31. Piccardi M, Romero G, Veneranda G, Castello E, Romero D, Balzarini M, Bó GA. Effect of puerperal metritis on reproductive and productive performance in dairy cows in Argentina. Theriogenology 2016; 85: 887-893.
32. Ribeiro ES, Gomes G, Greco LF, Cerri RLA, Vieira-Neto A, Monteiro Jr PLJ, Lima FS, Bisinotto RS, Thatcher WW, Santos JEP. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J Dairy Sci 2016; 99: 2201-2220.
33. Sheldon IM, Dobson H. Postpartum uterine health in cattle. Anim Reprod Sci 2004; 82-83; 295-306.
34. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining postpartum uterine disease in cattle. Theriogenology 2006; 65: 1516-1530.
35. Urton G, von Keyserlingk MAG, Weary DM. Feeding behavior identifies dairy cows at risk for metritis. J Dairy Sci 2005; 88: 2843-2849.
36. Vazquez-Añon M, Bertics S, Luck M, Grummer RR, Pinheiro J. Peripartum liver triglyceride and plasma metabolites in dairy cows. J Dairy Sci 1994; 77: 1521-1528.
37. West HJ. Effect of on liver function of acetonaemia and the fat cow syncrome in cattle. Res Vet Sci 1990; 48: 221-227.
38. Wittrock JM, Proudfoot KL, Weary DM, von Keyserlingk MAG. Short communication: Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. J Dairy Sci 2011; 94: 2408-2412.