Risk factors for postpartum uterine diseases in dairy cows

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ABSTRACT

The objective of this observational study was to investigate the risk factors for metritis, purulent vaginal discharge, and cytological endometritis. The hypothesis was that purulent vaginal discharge and cytological endometritis would have different risk factors because they represent distinct manifestations of uterine disease. Data generated from 1,363 Holstein cows (3 herds) enrolled in a randomized clinical trial were used. Calving history, periparturient disease incidence, and body condition score at calving and at 63 d in milk (DIM) were recorded. Serum nonesterified fatty acid concentration was measured once during the week before expected calving. Serum nonesterified fatty acid, β -hydroxybutyric acid, and haptoglobin (Hapto) concentrations were measured at 4 ± 3 , 11 ± 3 , and 18 ± 3 DIM. Serum progesterone concentration was measured at 21 ± 3 , 35 ± 3 , 49 ± 3 , and 63 ± 3 DIM. Metritis was diagnosed by farm managers within the first 20 DIM using a standardized definition. Cows were examined at 35 ± 3 DIM by a veterinarian for purulent vaginal discharge (mucopurulent or worse vaginal discharge; Metricheck device) and cytological endometritis ($\geq 6\%$ polymorphonuclear cells on endometrial cytology; cytobrush device). Statistical analyses were performed using multivariable logistic regression models for each disease, accounting for the random effect of herd. Risk factors for metritis included increased nonesterified fatty acid prepartum (≥ 0.6 mmol/L), dystocia, retained placenta, and increased Hapto in the first week postpartum (≥ 0.8 g/L). Risk factors for purulent vaginal discharge included twinning, dystocia, metritis, and increased Hapto (≥ 0.8 g/L) in the first week postpartum. Risk factors for cytological endometritis included low body condition score at parturition (≤ 2.75), hyperketonemia ($\geq 1,100$ μ mol/L), and increased Hapto (≥ 0.8 g/L) in the first week postpartum. These results support the hypothesis that some of the risk factors for purulent

vaginal discharge and cytological endometritis are different, which supports that they are distinct manifestations of uterine disease.

Key words: dairy cow, endometritis, risk factor, uterine disease

INTRODUCTION

Postpartum uterine diseases are frequently diagnosed in dairy cows and have been shown to have negative effects on reproductive performance (Kelton et al., 1998; Gilbert et al., 2005; Overton and Fetrow, 2008). Many large-scale epidemiological studies have investigated risk factors for uterine diseases (Gröhn et al., 1990; Correa et al., 1993; Kim and Kang, 2003). Unfortunately, a great deal of variation exists in the terminology used to describe uterine diseases and most studies have not standardized disease definitions, leading to wide variation in reported incidence (Kelton et al., 1998). Recently, metritis was defined as a condition causing systemic signs of illness (e.g., fever, anorexia, decreased milk production), characterized by a foul-smelling, brown-red, watery vaginal discharge occurring within the first 21 DIM (Sheldon et al., 2006). Endometritis was defined as chronic uterine inflammation occurring ≥ 20 DIM, diagnosed clinically or cytologically, based on a reduction of subsequent reproductive performance.

Previous studies have suggested that risk factors for metritis include dystocia, twins, retained placenta (**RP**), stillbirth, abortion, and prolapsed uterus (Gröhn et al., 1990; Correa et al., 1993; Kaneene and Miller, 1995). Reduced feeding time and DMI before parturition are predictors of metritis (Urton et al., 2005; Huzzey et al., 2007). Increased serum NEFA, increased serum BHBA, and decreased DMI are detrimental to neutrophil function, which is involved in the pathophysiology of metritis (Hammon et al., 2006). Increased haptoglobin (**Hapto**) concentration in serum is associated with an increased subsequent risk of metritis (Smith et al., 1998; Sheldon et al., 2001; Huzzey et al., 2009).

Reported risk factors for endometritis include RP, twins, dystocia, parity, season, and BCS (Markusfeld, 1987; Gröhn et al., 1990; LeBlanc et al., 2002). Little evidence exists of the potential link between metritis and endometritis in the literature because most studies

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consider these conditions separately. Increased Hapto concentration early in the postpartum period has been associated with an increased incidence of endometritis (Sheldon et al., 2001; Williams et al., 2005). Energy balance indicators, such as increased NEFA and decreased DMI prepartum, and increased BHBA early postpartum, have been associated with an increased risk of endometritis (Hammon et al., 2006). Metabolic disorders, such as abomasal displacement, hypocalcemia, and ketosis, increase the risk of endometritis (Markusfeld, 1987; Correa et al., 1993; Whiteford and Sheldon, 2005). Uterine disease has an unfavorable effect on time of return to ovarian cyclicity, but it is unclear if ovarian cyclicity has an effect on the incidence of endometritis (Sheldon, 2004).

A recent study (Dubuc et al., 2010) investigated the relationship between clinical and cytological diagnostic criteria for endometritis and found a weak agreement. Clinical and cytological endometritis each had detrimental effects on reproduction, and these effects were additive when both conditions were diagnosed simultaneously. It was proposed that clinical and cytological endometritis diagnostic criteria might reflect different manifestations of uterine disease. Further, it was suggested that purulent vaginal discharge terminology should be encouraged in place of clinical endometritis as purulent vaginal discharge is not necessarily indicative of endometrial inflammation. Information on the risk factors for purulent vaginal discharge and cytological endometritis may help to better understand and confirm the difference between these conditions.

The objective of the present study was to investigate the risk factors for metritis, purulent vaginal discharge, and cytological endometritis. We hypothesized that purulent vaginal discharge and cytological endometritis would have different risk factors because they represent distinct manifestations of uterine disease.

MATERIALS AND METHODS

A total of 1,363 Holstein cows from 3 commercial dairy herds, including 2 in southwestern Ontario, Canada, and 1 in western New York were enrolled in this prospective observational study. The study was part of a larger research project involving a randomized clinical trial on therapies for preventing and treating uterine diseases in 2,178 Holstein cows. Cows enrolled in the present study did not receive treatments that could potentially influence their uterine health status. Herd size ranged from 300 to 1,000 lactating cows. Cows were housed in freestall barns. Cows from 2 herds ($n = 1,090$) were milked 3 times daily and 1 herd ($n = 273$) was milked twice daily. Individual cow data management was performed on farm with DairyComp 305

(Valley Agricultural Software, Tulare, CA). Cow parity group (first, second or greater), calving date, season of calving (winter: December to February; spring: March to May; summer: June to August; fall: September to November), DHI test-day milk production, and peripartum events and diseases (twins, dystocia, RP, and metritis; diagnosed by farmers) were recorded. Definitions of RP (failure to pass fetal membranes by 24 h after parturition), metritis (systemic illness with fetid vaginal discharge), and dystocia (pull necessitating ≥ 2 people or a calf-jack, taking longer than 15 min, or veterinary assisted) were standardized at the beginning of the study. Hands-on training on disease diagnosis (RP and metritis) and dystocia were not provided to dairy personnel. Body condition was scored by the same person within herd, using a 5-point scale (Ferguson et al., 1994), at parturition and at 63 DIM. Nutritional information (e.g., diets) from the study herds was not collected.

Herds were visited weekly from September 2007 until November 2008 by a research technician and a veterinarian for data collection. Cow lists were generated weekly using DairyComp 305 commands. Cows were enrolled in the study 1 wk before expected calving date and were followed until 63 ± 3 DIM. At $d 4 \pm 3$ prepartum, and at $d 4 \pm 3$, 11 ± 3 , 18 ± 3 , 21 ± 3 , 35 ± 3 , 49 ± 3 , and 63 ± 3 postpartum, a blood sample (10 mL) from the coccygeal vein, artery, or both was collected into an evacuated sterile tube without anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Samples were kept chilled and allowed to clot. Within 5 h of blood collection, samples were centrifuged to harvest serum, which was frozen at -20°C . Prepartum samples were tested for NEFA. Postpartum samples collected on $d 4 \pm 3$, 11 ± 3 , and 18 ± 3 were tested for NEFA, BHBA, and Hapto, whereas samples collected on $d 21 \pm 3$, 35 ± 3 , 49 ± 3 , and 63 ± 3 were tested for progesterone (P4). Serum chemistry analyses were conducted at the Animal Health Laboratory of the University of Guelph. The NEFA, BHBA, and Hapto concentrations in serum were determined with an automated serum analyzer (Hitachi 911, Roche Diagnostics, Indianapolis, IN). The NEFA and BHBA concentrations were determined with Randox NEFA and Randox BHBA kits (Randox Laboratories Canada Ltd., Mississauga, ON, Canada). The analytical sensitivity of the NEFA and BHBA assays was 0.1 mmol/L and $15 \text{ } \mu\text{mol/L}$, respectively. The inter- and intraassay coefficients of variation were 3.9 and 4.1%, respectively, for NEFA, and 4.9 and 4.5%, respectively, for BHBA. The Hapto concentration was determined by measuring the hemoglobin binding capacity, using a methemoglobin reagent made on site according to a method described elsewhere (Skinner et al., 1991; Skinner and Roberts, 1994). The

analytical sensitivity of the Hapto assay was 0.03 g/L. The inter- and intraassay coefficients of variation were 1.9 and 2.0%, respectively. The P4 concentration was determined with a sequential competitive immunoassay (Immulite, Siemens, Mississauga, ON, Canada) validated for use in cattle (Martin et al., 2007). The inter- and intraassay coefficients of variation for P4 were 9.6 and 7.2%, respectively.

Cows were examined at 35 ± 3 DIM for diagnosis of uterine diseases using clinical (vaginal discharge and cervical diameter by transrectal palpation) and cytological (endometrial cytobrush) techniques (LeBlanc et al., 2002; Barlund et al., 2008). Prior to examination, the vulva was cleaned with paper towels. Then, a cytological sample of the endometrium from the dorsal aspect of the uterine body was collected using a cytobrush (VWR CanLab, Mississauga, ON, Canada) adapted for use in cattle (Kasimanickam et al., 2004, 2005; Barlund et al., 2008). Slides for cytological examination were prepared on farm by rolling the cytobrush on a clean glass microscope slide, and were fixed with cytofixative (Cytoprep, Fisher Scientific, Nepean, ON, Canada). Slides were maintained in a transport container at a temperature greater than 10°C to avoid freezing. Then, transrectal palpation for determination of cervical diameter was performed and results were classified as <5 cm, 5 to 7.4 cm, and ≥ 7.5 cm (LeBlanc et al., 2002). Last, vaginal discharge was visually examined with a Metrichick device (Simcrotech, Hamilton, New Zealand) technique and scoring: 0 = no discharge; 1 = clear mucus; 2 = mucus with flecks of pus; 3 = mucopurulent discharge; 4 = purulent discharge; 5 = foul-smelling discharge (McDougall et al., 2007).

A total of 3 veterinarians (1 per herd) were involved. All participating veterinarians received standard instructions and documentation to standardize the reporting of clinical procedures and findings at the start of the study. Hands-on training sessions were provided for the use and interpretation of Metrichick and cytobrush devices. Multiple validations of procedures and classification of findings were performed throughout the study, always using the same person (expert) as the referent for comparing result classifications. Minor adjustments to techniques and result interpretation were made if necessary.

All endometrial cytology slides were stained with modified Wright Giemsa stain (Protocol-Hema3, Biochemical Sciences, Swedesboro, NJ), and coverslips were applied once dry (Barlund et al., 2008). Each slide was examined at 400 $\times$ magnification to perform a differential cell count of 100 cells (polymorphonuclear neutrophils and endometrial cells) by a single observer. The observer was blinded to the on-farm examination

findings. The slide examination procedure was repeated twice, in 2 different locations on each slide, and the average of the results was calculated. If the 2 results had a difference >10% polymorphonuclear neutrophils, a third count was performed and the average was calculated from the 2 most similar results.

Uterine disease definition criteria were based on a significant reduction in subsequent reproductive performance (LeBlanc et al., 2002; Gilbert et al., 2005). These diagnostic criteria were established in a population of cows enrolled in the randomized clinical trial described previously, and are reported elsewhere (Dubuc et al., 2010). In summary, the diagnostic criteria for purulent vaginal discharge and cytological endometritis were based on the optimal thresholds for vaginal discharge and endometrial cytology for predicting pregnancy status at 120 DIM. Purulent vaginal discharge was defined as the presence of mucopurulent ($\geq 50\%$ pus) or worse (purulent or foul) vaginal discharge based on the Metrichick scoring chart (McDougall et al., 2007). Cytological endometritis was defined as the presence of $\geq 6\%$ polymorphonuclear neutrophils in endometrial cytology.

Statistical Analyses

Individual cow data were exported from DairyComp 305 to Microsoft Excel (Microsoft Corp., Redmond, WA). Statistical analyses were performed with SAS (Version 9.2, SAS Institute, Inc., Cary, NC) considering the cow as the experimental unit.

Serum NEFA concentrations during the last week prepartum (d -1 to -7) and postpartum serum concentrations of NEFA, BHBA, and Hapto sampled in wk 1 (d 1 to 7), 2 (d 8 to 14), and 3 (d 15 to 21) were analyzed as continuous variables. Dichotomization of these values was performed for each metabolite, within each sampling period, for each disease. Selection of the optimal threshold for dichotomization was based on the highest sum of sensitivity and specificity for predicting the occurrence of each disease. Progesterone was reported as a continuous variable and was classified using 1 ng/mL as a threshold value (Stevenson et al., 2006). Body condition scores, at parturition and at 63 DIM, were classified as fat (≥ 3.75), moderate (3.0 to 3.5), and thin (≤ 2.75).

Metritis, purulent vaginal discharge, and cytological endometritis were considered as outcomes. Predictor variables included NEFA, BHBA, Hapto, P4, BCS, season, parity group, twins, dystocia, RP, metritis (only for purulent vaginal discharge and cytological endometritis models), and milk production. Univariable associations of each predictor with each disease were tested (Pear-

Table 1. Optimal thresholds of serum metabolite concentrations, stratified by sampling period, for predicting metritis in 1,363 Holstein cows¹

Serum metabolite	Threshold	Se, ² %	Sp, ³ %	Cows above threshold, %	OR ⁴ (95% CI)	P-value ⁵
NEFA						
1 to 7 d before parturition	≥0.6 mmol/L	25.1	84.7	16.9	1.86 (1.31–2.63)	<0.01
1 to 7 DIM	≥1.1 mmol/L	31.3	70.2	30.0	1.07 (0.78–1.47)	0.67
8 to 14 DIM	≥0.8 mmol/L	45.5	54.0	45.9	0.98 (0.73–1.31)	0.89
BHBA						
1 to 7 DIM	≥1,500 μmol/L	12.8	91.2	9.4	1.53 (0.97–2.40)	0.06
8 to 14 DIM	≥1,300 μmol/L	16.6	83.9	16.7	1.04 (0.70–1.54)	0.64
Haptoglobin						
1 to 7 DIM	≥0.8 g/L	51.6	79.0	25.8	4.01 (2.95–5.44)	<0.01
8 to 14 DIM	≥0.4 g/L	48.8	75.9	27.9	3.01 (2.22–4.06)	<0.01

¹Serum samples were only considered if sampling occurred before the diagnosis of metritis.

²Sensitivity.

³Specificity.

⁴Odds ratio.

⁵P-value from univariable association of threshold with metritis.

son χ^2) using the FREQ procedure. Serum predictors were only considered for metritis if sample collection occurred before the disease diagnosis. Variables with $P < 0.15$ were retained and analyzed in a multivariable mixed logistic regression model for each disease, where herd was treated as a random effect and all biologically meaningful interaction terms were tested (GLIMMIX procedure). A manual backward elimination procedure was used until only significant variables or interactions were retained ($P < 0.05$). Least squares means of adjusted disease prevalence were obtained for variables retained in the final models.

RESULTS AND DISCUSSION

A total of 1,363 cows were enrolled. However, only 1,295 cows were examined at 35 DIM because 68 cows had been culled ($n = 40$) or died ($n = 28$). Reasons for culling included low milk production ($n = 16$), mastitis ($n = 13$), and locomotion problems ($n = 11$). The incidence of dystocia, twins, RP, metritis, purulent vaginal discharge, and cytological endometritis were 9.2, 8.9, 11.8, 17.6, 14.6, and 18.7%, respectively. Among cows with RP, 33.2% developed metritis; among cows with metritis, 32.5 and 31.8% developed purulent vaginal discharge and cytological endometritis, respectively. Of all cows with uterine diseases, 22.8% had both purulent vaginal discharge and cytological endometritis. Among cows with purulent vaginal discharge, only 41.7% had cytological endometritis at the same time, whereas among cows with cytological endometritis, only 32.1% had purulent vaginal discharge. The median time for diagnosis of metritis was 6 d postpartum. Only 1% of metritis cases were diagnosed on or after 13 DIM but before 17 DIM.

Optimal thresholds of metabolites for predicting metritis are presented in Table 1. Optimal thresholds for purulent vaginal discharge and cytological endometritis were identical. Thus, only values for purulent vaginal discharge are reported in Table 2. The final logistic regression model for metritis is reported in Table 3. The final logistic regression models for purulent vaginal discharge and cytological endometritis at 35 DIM are presented in Table 4.

The incidence of metritis and the prevalence of purulent vaginal discharge and cytological endometritis found in this study support results from other studies (LeBlanc et al., 2002; Barlund et al., 2008; Overton and Fetrow, 2008). The optimal thresholds of metabolites (NEFA, BHBA, and Hapto) for predicting metritis were consistent with those found elsewhere (Carson, 2008; Huzzey et al., 2009). Elevated prepartum NEFA was associated with increased risk of metritis, but postpartum NEFA and BHBA concentrations were not (Table 1). The optimal NEFA threshold for predicting metritis in the 7 d before parturition was 0.6 mmol/L. This is higher than that reported in a similar study of 55 herds ($n = 2,365$ cows) in Canada and the United States, where a threshold of 0.3 mmol/L was reported (Carson, 2008). In the present study, NEFA concentration ≥ 0.6 mmol/L in the 7 d before parturition was associated with 1.6 times the odds of developing metritis (Table 3). Elevated NEFA concentration is detrimental to neutrophil functions and uterine health (Hammon et al., 2006). The present results are consistent with an immunosuppressive effect on uterine health associated with increased lipid mobilization during the 7 d before parturition. Elevated NEFA concentration may reflect a decrease in DMI, which may lead to a reduction of antioxidant and calcium intake (Bell, 1995; Horst et al.,

Table 2. Optimal thresholds of serum metabolite concentrations, stratified by sampling period, for predicting purulent vaginal discharge in 1,295 Holstein cows

Serum metabolite	Threshold	Se, ¹ %	Sp, ² %	Cows above threshold, %	OR ³ (95% CI)	P-value ⁴
NEFA						
1 to 7 d before parturition	≥0.5 mmol/L	54.3	52.5	49.2	1.31 (1.02–1.68)	0.03
1 to 7 DIM	≥1.0 mmol/L	41.2	65.7	36.0	1.34 (1.04–1.74)	0.02
8 to 14 DIM	≥0.9 mmol/L	42.8	63.8	37.9	1.32 (1.02–1.70)	0.03
15 to 21 DIM	≥0.7 mmol/L	40.8	62.9	38.0	1.17 (0.91–1.51)	0.23
BHBA						
1 to 7 DIM	≥1,100 μmol/L	28.3	83.7	20.4	1.36 (1.01–1.83)	0.05
8 to 14 DIM	≥700 μmol/L	59.0	48.0	53.7	1.33 (1.04–1.71)	0.03
15 to 21 DIM	≥1,000 μmol/L	26.7	77.9	23.2	1.28 (0.96–1.71)	0.09
Haptoglobin						
1 to 7 DIM	≥0.8 g/L	39.1	79.6	25.0	2.51 (1.91–3.29)	<0.01
8 to 14 DIM	≥0.3 g/L	46.5	66.8	36.5	1.75 (1.36–2.26)	<0.01
15 to 21 DIM	≥0.4 g/L	16.0	84.6	15.5	1.05 (0.75–1.47)	0.79

¹Sensitivity.²Specificity.³Odds ratio.⁴P-value from univariable association of threshold with purulent vaginal discharge.

1997). Postpartum NEFA and BHBA concentrations were not associated with the risk of metritis, which supports the results of Carson (2008).

Haptoglobin ≥0.8 g/L in the first 7 DIM was associated with more than 2 times the odds of developing metritis (Table 3). This supports another study, which reported that cows with a Hapto concentration ≥1.0 g/L at 3 DIM were 7 times more likely to develop metritis (Huzzey et al., 2009). These results support other studies that found an association between elevated Hapto in the postpartum period and metritis (Smith et al., 1998; Sheldon et al., 2001). In the present study, only serum samples that were taken before the diagnosis of metritis were considered for this analysis. Even then, the effect of elevated Hapto remained in the final model (Table 3) when adjusted for other variables that could potentially

cause an increase in Hapto concentration, such as dystocia and RP. This may indicate that the association of elevated Hapto concentration preceding metritis was not a consequence of the disease itself or due to other active known inflammatory problems. Elevated Hapto concentration was a risk factor for purulent vaginal discharge and cytological endometritis at 35 DIM. This may suggest that increased inflammation in the early postpartum period, likely from the uterus, may be associated with greater odds of maintaining endometrial, cervical, or vaginal inflammation.

Other variables in this study associated with increased odds of developing metritis were dystocia and RP (Table 3). This supports previous study results (Gröhn et al., 1990; Correa et al., 1993; Kaneene and Miller, 1995). Dystocia is usually associated with human intervention

Table 3. Final logistic regression model, accounting for the random effect of herd, of variables (by class) associated with subsequent risk of metritis in 1,363 Holstein cows

Variable	OR ¹	95% CI	P-value
NEFA ² 1–7 d before parturition, mmol/L			
<0.6	Referent		
≥0.6	1.59	1.06–2.32	0.02
Hapto ³ 1–7 DIM, g/L			
<0.8	Referent		
≥0.8	2.17	1.51–3.12	<0.01
Dystocia			
No	Referent		
Yes	2.12	1.30–3.45	<0.01
Retained placenta			
No	Referent		
Yes	6.25	3.57–10.0	<0.01

¹Odds ratio.²Serum NEFA concentration.³Serum haptoglobin concentration.

Table 4. Final logistic regression models, accounting for the random effect of herd, of variables associated with purulent vaginal discharge (mucopurulent or worse vaginal discharge) or cytological endometritis ($\geq 6\%$ polymorphonuclear cells at endometrial cytology) in 1,295 Holstein cows

Uterine disease	Variable	Class	n	OR ¹	95% CI	P-value
Purulent vaginal discharge	Hapto, ² 1–7 DIM; g/L	<0.8	1,009	Referent		
		≥ 0.8	286	2.02	1.29–3.13	<0.01
	Twins	No	1,216	Referent		
		Yes	79	2.16	1.09–4.27	0.03
	Dystocia	No	971	Referent		
		Yes	324	2.13	1.43–3.98	<0.01
	Metritis	No	1,107	Referent		
		Yes	188	2.33	1.41–3.77	<0.01
Cytological endometritis	BHBA, ³ 1–7 DIM; $\mu\text{mol/L}$	<1,100	1,033	Referent		
		$\geq 1,100$	262	1.41	1.05–2.00	0.03
	Hapto, ² 1–7 DIM; g/L	<0.8	993	Referent		
		≥ 0.8	302	1.59	1.12–2.27	<0.01
	BCS at parturition	≤ 2.75	401	Referent		
		3.0–3.5	688	0.53	0.42–0.95	0.03
		≥ 3.75	206	0.62	0.46–1.35	0.12

¹Odds ratio.²Serum haptoglobin concentration.³Serum BHBA concentration.

and a potential for increased contamination of the uterus and vagina during manual extraction. The proposed contribution of RP in the pathogenesis of metritis is in providing an ideal environment for bacterial growth because of the large amount of necrotic tissue present, the delay of lochia expulsion, and potential lesions to the uterus from manual removal (Kaneene et al., 1986; Bolinder et al., 1988; Sheldon et al., 2004). The consistent association between RP and metritis could be partially explained by the impaired immune response that occurs in both circumstances, which presumably may be linked (Gunnink, 1984; Gilbert et al., 1993; Hammon et al., 2006).

Risk factors for cows with purulent vaginal discharge at 35 DIM were dystocia, twins, and metritis (Table 4). Dystocia and twins are likely associated with increased bacterial contamination of the uterus, trauma, and higher risk of developing RP and metritis (Sheldon, 2004). It is important to understand that not all cows with RP will experience metritis, and that not all cows with metritis will develop purulent vaginal discharge. It appears that these uterine diseases are linked, but one is not necessary for the development of the others.

Cows were more likely to have cytological endometritis if they experienced hyperketonemia ($\geq 1,100 \mu\text{mol/L}$) during the first 7 DIM (Table 4), or had a thin BCS (≤ 2.75) at parturition. Hyperketonemia increased the odds of developing cytological endometritis (Hammon et al., 2006), by having an immunosuppressive effect, or by reflecting a reduction of DMI linked with a reduced intake of antioxidants and calcium (Bell, 1995; Horst et al., 1997).

Cows that had $>1 \text{ ng}$ of P4/mL at 21 or 35 DIM, or both, had a prevalence of purulent vaginal discharge and cytological endometritis 10 to 12% lower ($P < 0.01$) than cows with $\leq 1 \text{ ng}$ of P4/mL when examined in univariable analyses. Interestingly, this predictor was not significant in the final multivariable models ($P > 0.20$) of purulent vaginal discharge and cytological endometritis. But the magnitude of this difference in prevalence was consistent at 49 and 63 DIM, suggesting a potential confounding effect, when considering other variables simultaneously in the models. It appears that cows with uterine diseases were less likely to have $>1 \text{ ng}$ of P4/mL. This may be directly or indirectly associated with some risk factors, such as hyperketonemia, dystocia, and twins, which were associated with greater incidence of anovulation (Walsh et al., 2007).

Some of the risk factors for purulent vaginal discharge and cytological endometritis were different. Risk factors for purulent vaginal discharge included factors that plausibly increase uterine trauma and bacterial contamination, such as dystocia, twins, and metritis. Risk factors for cytological endometritis, such as hyperketonemia and thin body condition may reflect the effect of immune suppression or metabolic imbalance in the peripartum period. This difference supports the weak agreement (42%) between purulent vaginal discharge and cytological diagnostic criteria. Although some cows will have both simultaneously, the presence of mucopurulent or worse vaginal discharge may not be reflective of endometrial inflammation. Cows with such vaginal discharge might have cervicitis or vaginitis, but no endometrial inflammation. These results support the

assertion that the terminology “clinical endometritis” may not be accurate, and that the use of the terms “purulent vaginal discharge” may be more appropriate. Purulent vaginal discharge and cytological endometritis (taken individually) at 35 DIM had detrimental effects on subsequent reproductive performance in another study (Dubuc et al., 2010). In that study, these effects were additive in cows with both conditions simultaneously, suggesting that the detrimental effects were of different origins. Overall, because both conditions have some different risk factors and additive effects on reproductive performance, these data support the assertion that they are different manifestations of reproductive tract disease.

CONCLUSIONS

Risk factors for metritis were associated with metabolic status in the prepartum period, as well as problems at or after parturition (dystocia and RP). Elevated Hapto concentration during the postpartum period was associated with metritis, cytological endometritis, and purulent vaginal discharge. Some risk factors for cytological endometritis and purulent vaginal discharge were different. Cows were at greater risk of experiencing cytological endometritis if they were hyperketonemic in early postpartum. Purulent vaginal discharge was more likely to occur in cows with dystocia, twins, and metritis (diagnosed by dairy farmers).

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