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Association of uterine health in the first lactation with transition cow health and reproductive performance in the second lactation of Holstein dairy cows

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ABSTRACT

The objective of this study was to evaluate the effects of puerperal metritis (PM) diagnosed and treated during the early postpartum period of the first lactation on transition cow health, milk production, reproduction, and culling of dairy cows in their second lactation. Diagnosis of PM was based on fetid watery red-brown uterine discharge and rectal temperature above 39.5°C. Two farms were enrolled in this retrospective observational cohort study (farms A and B). In both farms, the following diseases were recorded during the first 30 DIM in lactation 1 and 2: clinical hypocalcemia (CH), retained fetal membrane (RFM), PM, hyperketonemia (KET), left displaced abomasum (LDA), and clinical mastitis (MAST). Statistical analyses were performed using SPSS for Windows separately for each farm. Linear and logistic regression models were used for continuous (e.g., milk yield) and binary (e.g., disease, pregnancy per AI, pregnancy loss) outcomes, respectively. Cox proportional hazard regression models were calculated to model the time to event outcomes for culling or death during the first 60 DIM and for pregnancy within 250 d of the second lactation. The initial models contained the following variables: year of calving, month of calving, calving ease, stillbirth, twins, days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1, and PM in lactation 2 as explanatory variables. A total of 4,834 cows (farm A) and 4,238 cows (farm B) in the second lactation were considered for statistical analyses. On farm A, the incidence of PM in lactations 1 and 2 were 20.1% and 11.2%, respectively. On farm B, the incidence of PM in lactations 1 and 2 were 14.4% and 8.5%, respectively. On both farms, cows with PM in their first lactation had greater odds for RFM and PM in their second lactation, whereas there was no association of PM in the first lactation with any other nonuterine diseases (i.e., CH, KET, LDA, and MAST) in the second lactation. Cows with PM in lactation 2 had reduced milk yield. The reduction in milk yield in second lactation was greater for cows that already experienced PM in lactation 1. On farm A, cows with PM in their first lactation had a greater hazard for culling within 60 DIM of the second lactation; however, the same association was not present on farm B. Cows with PM in lactation 1 had reduced pregnancy per AI at first service in the second lactation only on farm B. Cows with PM in lactation 2 had reduced pregnancy per AI at first service in the second lactation on both farms. Pregnancy loss in lactation 2 was only associated with PM in lactation 2 but not with PM in lactation 1. On both farms, cows had a reduced hazard for pregnancy in their second lactation within 250 DIM when they experienced PM in either lactation. In conclusion, PM in the first lactation had long-lasting negative consequences (i.e., risk of uterine disease and lower reproductive performance) for cows in their next lactation.

Key words: puerperal metritis, pregnancy loss, pregnancy per artificial insemination

INTRODUCTION

The transition to lactation is a crucial phase characterized by numerous physiological changes, requiring proper preparation to ensure a successful lactation for dairy cows (Drackley, 1999). Shortly after calving, cows undergo enormous shifts in nutrient requirements and undergo metabolic adaptation to maintain homeostasis (Bell, 1995). During this period, cows typically reduce their DMI, leading to a state of negative energy balance due to insufficient energy consumption relative to the energy demands of milk production (Drackley, 1999).

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The immune system undergoes an imbalanced state, and cows encounter a decline in the immune function (i.e., impaired leukocyte and neutrophil activity) around parturition (Aleri et al., 2016), resulting in an increased susceptibility to infectious diseases early postpartum (Esposito et al., 2014). The uterine lumen is almost always contaminated with a wide range of pathogenic bacteria (Sheldon et al., 2009). Depending on the immune function of the cow, as well as the pathogenicity of the bacteria and the bacterial load, development of a uterine disease may occur. Postpartum uterine disease presents a major economic challenge to the dairy industry (Pérez-Báez et al., 2021). Diagnosis of puerperal metritis (**PM**) is based on reddish-brown fetid watery discharge and a rectal temperature >39.5°C within the first 21 DIM (Sheldon et al., 2006). The prevalence of metritis ranges between 14.8% and 40.9% (Sannmann et al., 2013; Lima et al., 2014; Pohl et al., 2016). Metritis is associated with short- and long-term consequences in dairy cattle. Short-term consequences of metritis include an inflammatory response with pyrexia and an increase in acute phase proteins (e.g., haptoglobin; Pohl et al., 2015), reduced DMI and milk yield early postpartum (Pérez-Báez et al., 2019), pain (Stojkov et al., 2015), and subclinical ketosis (Dohoo and Martin, 1984). Long-term consequences include reduced milk production, delayed resumption of ovarian cyclicity, reduced pregnancy per AI (**P/AI**) and an increased risk for pregnancy loss thus impairing reproductive performance, and an increased culling risk over the entire lactation (Santos et al., 2010; Giuliodori et al., 2013; Bruinjé et al., 2024). Although the majority of milk loss due to metritis occurs in early lactation, there is great variability across herds. Milk losses range from 325 to 2,203 kg/lactation (Pérez-Báez et al., 2021). The decrease in milk production cannot be solely attributed to the immediate losses observed during the clinical presentation of metritis. This suggests that the effects of clinical disease on lactation biology continues beyond the period of clinical recovery. Even treatment with ceftiofur could not completely reverse the negative effects of metritis on milk yield, culling, and fertility (Piccardi et al., 2016; de Oliveira et al., 2020).

Clinical disorders appear to have long-lasting effects on dairy cow physiology that impair performance even after clinical recovery from health issues, particularly for uterine diseases (Ribeiro and Carvalho, 2017). To the best of our knowledge, all studies that evaluated the consequences of metritis on milk yield, reproductive performance, and culling patterns only used the lactation in which the disease occurred. It remains unclear, however, how long the consequences of metritis last and whether the long-term consequences can be observed even in the next lactation.

It has been shown that certain foot lesions are highly repeatable across lactations and may have long-term effects on cow survivability and productivity (Oikonomou et al., 2013). The same has been shown for clinical mastitis (**MAST**; Hertl et al., 2018), where primiparous cows that experienced a case of MAST in the first 100 DIM had an increased risk of recurrent cases over their entire productive life. Understanding the comprehensive consequences of clinical diseases is of utmost importance when assessing their impact on the profitability and sustainability of dairy farms. This knowledge should also shape the perception of the issue among stakeholders in the dairy industry (Carvalho et al., 2019). Furthermore, determining the duration of the adverse effects resulting from clinical diseases offers valuable insights for making management decisions concerning culling and breeding practices.

Therefore, the objective of this study was to evaluate the association of PM diagnosed and treated during the early postpartum period of the first lactation on milk production, reproduction, and culling of dairy cows in their second lactation. We hypothesized that PM has long-lasting detrimental consequences on production and reproductive performance and, consequently, increases the rate of culling of lactating cows. A secondary objective was to evaluate the association of PM in first lactation with calving related disorders and transition cow health events in second lactation.

MATERIALS AND METHODS

Study Design

This observational retrospective cohort study used health and performance data from 2 farms of 6 calendar years between January 2015 and December 2021. Therefore, this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Farm A was located in western Slovakia and farm B was located in northern Germany. Both farms kept exclusively Holstein Friesian cows. Cows on both farms were milked twice daily with 305-d milk yield of 11,500 kg. During the study period, farm A and farm B had an average of 2,823 ±216 (SD) and 2,872 ±110 (SD) calvings per year, respectively. Cows were housed in a freestall barn with concrete flooring and had ad libitum access to feed and water. The TMR was formulated to meet or exceed the dietary requirements of a 650 kg Holstein cow producing 40 kg/d of 3.5% FCM (NRC, 2001). All cows were fed a TMR twice daily according to their transition status (far-off, close-up, and fresh-cow diets) and feed was pushed up 10 times per day. Close-up prepartum cows were fed a moderate negative DCAD diet.

Transition Cow Management

On farms A and B, nulliparous were moved once weekly to the close-up pen at 256.5 ± 5.2 (SD) and 255.7 ± 4.5 (SD) days of gestation, respectively. Parous cows were dried-off approximately 55 d before expected parturition and moved to the far-off group. Transfer of parous cows from the far-off to the close-up group was conducted on a weekly basis at 254.6 ± 5.2 (SD) and 257.0 ± 4.5 (SD) days of gestation on farms A and B, respectively. On farm A, nulliparous and parous cows were housed together in pens with deep-straw bedding. On farm B, nulliparous and parous cows were kept separately in sand-bedded freestall pens during the close-up period. Prepartum nulliparous and parous cows were monitored hourly to detect signs of imminent parturition (i.e., restlessness, vaginal discharge with bloody traces, lying lateral with abdominal contractions, a visible or broken amniotic sac, or feet of the emerging calf outside the vulva). If any of the aforementioned signs of parturition occurred animals were moved into a bedded pack area, farm B (8 × 8 m), or an individual maternity pen bedded with fresh straw on farm A (3.5 × 3.5 m). If the cow had not delivered the calf within 1 h, calving assistance was provided (Schuenemann et al., 2011). On both farms, all parous cows received prophylactic calcium supplementation (Bovikalc, Boehringer Ingelheim, Ingelheim am Rhein, Germany) at parturition and 12 to 24 h later. Parous cows on farm A also received a drench including a mineral mix (400 g of calcium propionate, 200 g of magnesium sulfate, 100 g of potassium chloride, 50 g of sodium chloride) and 60 L of lukewarm water. After parturition, cows on both farms were milked twice daily. Postpartum cows were examined daily by the farm personnel until 10 DIM following standard operating procedures created by the herd manager. These examinations have been described in detail previously by Venjakob et al. (2021). During examinations, animals were monitored visually to assess general appearance, attitude, presence of fetal membranes outside the vulva, vaginal discharge, udder health, lameness, and manure consistency. Rectal temperature was measured using a digital thermometer. Transrectal massage of the uterus was conducted at least twice within the first 10 DIM to obtain and evaluate uterine discharge. Blood ketone concentration was evaluated twice using a cow side BHB test (Precision Xtra, Abbott Laboratories, Abbott Park, IL; validated by Iwersen et al., 2013); on farm A cows were tested between 5 and 10 DIM, and on farm B cows were tested between 2 and 14 DIM. Daily milk production was assessed to identify cows with disease. A definition of each disease of interest was provided to the farm personnel. Cows between 11 and 30 DIM were transferred to the regular milking barn. They were screened for health disorders based on visual observations performed by the farm personnel every morning at 0800 h and on deviations in their daily milk production. Cows deviating in their visual appearance or daily milk production (day-to-day reduction of >4.5 kg; Guterbock, 2004) were separated and examined thoroughly as described above.

Disease Definitions

The following diseases were recorded during the first 30 DIM: clinical hypocalcemia (**CH**), retained fetal membrane (**RFM**), PM, hyperketonemia (**KET**), left displaced abomasum (**LDA**) and MAST. Because both farms had a long-term history in collaborating with the research team, disease definitions were consistent between both farms: when cows were recumbent during one of the first 5 d postpartum and responded to an intravenous Ca infusion, cows were considered as having CH; KET was defined as serum BHB concentrations ≥1.2 mmol/L. Ketotic cows received 250 mL of propylene glycol orally for 3 to 5 d. When percussion of the left flank resulted in tympanic resonance auscultated with a stethoscope, cows were considered to suffer from LDA; when fetal membranes were not expelled by 24 h after calving, cows were diagnosed with RFM. Vaginal discharge was evaluated at least twice using transrectal palpation between 1 and 10 DIM. If cows presented with fever on any other day, they were also examined transrectally. Signs of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with a cow with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C was considered as PM (Sheldon et al., 2006). Cows diagnosed with PM were treated with ceftiofur hydrochloride (2.2 mg/kg of BW) intramuscularly for 3 consecutive days. Clinical mastitis was defined as visible signs of inflammation in an affected mammary gland (i.e., redness, swelling, pain, or heat) and alterations such as clots, flakes, discoloration, or abnormal consistency of secretions (Vasquez et al., 2017). Udder health was evaluated twice daily by farm personnel during regular milking, on both farms.

Reproductive Management

Farm A. Cows were inseminated after estrus detection according to the alert of the automated activity monitoring system (i.e., 2015 to 2019: CowManager SensOor, Agis, Harmelen, the Netherlands; 2020 and 2021: Nedap Livestock Management, Groenlo, the Netherlands) that was confirmed by a technician via transrectal palpation of either a highly contractile uterus or visualization of clear, stringy vaginal discharge, or both, or received timed AI after hormonal intervention. Voluntary waiting period was 50 DIM (January 2015 to June 2020) and 60

DIM (July 2020 to December 2021), respectively. The sensor system generated a list of cows eligible for breeding, based on the activity value. If cows were confirmed in estrus, insemination was conducted on the same day. Cows that were not bred until 70 ± 3 DIM received timed AI using a modified 7-d Ovsynch protocol including a second $PGF_{2\alpha}$ treatment on d 8 (GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16–18 h later timed AI; Wiltbank et al., 2015). Cows received second and subsequent AI services after detected estrus any time after a previous insemination. Cows not re-inseminated at detected estrus underwent pregnancy diagnosis through transrectal ultrasound (Easi-Scan:GO, IMV Imaging, Bellshill, Scotland) 39 ± 3 d after AI. A positive pregnancy diagnosis was considered when a heartbeat was detected. At 32 ± 3 d after AI, all cows received a GnRH treatment. Open cows with at least one corpus luteum (**CL**) ≥15 mm received 2 $PGF_{2\alpha}$ treatments 24 h apart, GnRH 32 h after the second $PGF_{2\alpha}$, and timed AI 16 to 18 h later. Cows that did not meet the criteria to be included in the CL group received a modified Ovsynch protocol (i.e., GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16–18 h later timed AI). All pregnancy diagnoses were conducted by trained farm personnel. A second pregnancy diagnosis was performed 80 ± 3 d after AI. Pregnancy loss was defined as the proportion of pregnant cows on 39 ± 3 d after AI that were found nonpregnant on 80 ± 3 d after AI.

Farm B. Cows were inseminated for first service using only timed AI after submission to a Double-Ovsynch protocol as described by Souza et al. (2008) and modified by Brusveen et al. (2009) as follows: GnRH, 7 d later PGF, 3 d later GnRH, 7 d later GnRH, 7 d later PGF, 24 h later PGF, 32 h later GnRH, and 16 to 18 h later timed AI at 72 ± 3 DIM. At 25 d post-AI, all cows received a GnRH treatment. Seven days later at 32 d post-AI, cows underwent pregnancy diagnosis through transrectal ultrasound (Easi-Scan:GO, IMV Imaging, Bellshill, Scotland). A positive pregnancy diagnosis was considered when a heartbeat was detected. Nonpregnant cows with at least one CL ≥ 15 mm received a $PGF_{2\alpha}$ treatment, a second $PGF_{2\alpha}$ 24 h later, GnRH 32 h after the second $PGF_{2\alpha}$, and timed AI 16 to 18 h later. Cows that did not meet the criteria to be included in the CL group received a modified Ovsynch protocol (i.e., 2 PGF treatments) with progesterone (**P4**) supplementation through an intravaginal P4-releasing device (**CIDR**; 1.38 g of progesterone, Eazi-Breed CIDR, Zoetis, Florham Park, NJ) from the time of the first GnRH to the first $PGF_{2\alpha}$ treatment of the protocol (GnRH and CIDR inserted, 7 d later CIDR removal and $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16–18 h later timed AI; Pérez et al., 2020). All pregnancy diagnoses were conducted by trained farm personnel. A second pregnancy diagnosis was performed 60 d post-AI. Pregnancy loss was defined as the proportion of pregnant cows on 32 d post-AI that were found nonpregnant on 60 d post-AI.

Statistical Analyses

Individual cow data of cows that calved between January 2015 and December 2021 were transferred from Dairy Comp 305 (Valley Ag Software, Tulare, CA) to Microsoft Excel (Office 2013, Microsoft Deutschland Ltd., Munich, Germany). Statistical analyses were performed using SPSS for Windows (version 25.0, SPSS Inc., Chicago, IL).

Descriptive statistics (i.e., gestation length, dry period length, days in the pre-fresh group) for cows in lactation 1, whether they were affected by PM in lactation 1 or not, were calculated using ANOVA in SPSS. Binary data such as calving related disorders (i.e., stillbirth, twins, calving assistance) and transition cow health events (i.e., RFM, PM, CH, MAST, LDA, KET) were analyzed using logistic regression models in SPSS. We only included PM in lactation 1 (yes vs. no) as an explanatory variable. Separate models were built for farms A and B.

To analyze the association between PM in the first lactation and second test-day 305-d mature-equivalent milk projection (**2nd305ME;** mean ± SD DIM at second test was 56.5 ± 14.5 d; tests were carried by the country's equivalent DHI testing organization; farm A: Plemenarske sluzby Slovenskej republiky s.p., Trnava, Slovakia; farm B: Milchkontroll- und Rinderzuchtverband eG, Güstrow, Germany) in the second lactation, linear models were used in SPSS. This outcome was chosen as it allows to include cows that were culled after the second test day. Furthermore, waiting for a complete 305-d milk production record would bias the estimate due to missing data of cows that did not complete the period of 305 d (McCarthy and Overton, 2018). Cow was used as the experimental unit. Separate models were built for farms A and B. As described by Dohoo et al. (2009) each variable considered for the mixed model was analyzed in a univariate model, including the variable as a fixed factor (i.e., categorical or continuous variable). The final multivariable mixed model then included all variables resulting in $P \leq 0.10$ within the univariate models. The initial models contained the following variables: the year of calving (2015–2021), month of freshening, calving ease (0 = not observed, 1 = unassisted calving, 2 = calving assisted by 1 person, 3 = calving assisted by more than 1 person), stillbirth (yes vs. no), twins (yes vs. no), days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1 (yes vs. no), PM in lactation 2 (yes vs. no). The explanatory variables and the interaction term PM

in lactation 1 and PM in lactation 2 were only included when $P < 0.1$. For all models, the interaction between PM in lactation 1 and PM in lactation 2 was used to assess differences between the following groups: (1) no PM in the first lactation and no PM in second lactation (**NoPM+NoPM**), (2) no PM in the first lactation and PM in the second lactation (**NoPM+PM**), PM in the first lactation and no PM in the second lactation (**PM+NoPM**), and (4) PM in the first lactation and PM in the second lactation (**PM+PM**).

During the model building process, 2nd305ME was tested for normality and homoscedasticity of residuals using graphical methods (histograms, Q-Q plots, and scatterplots) and Kolmogorov-Smirnov statistic, respectively.

Binary data, such as pregnancy at first service (yes vs. no) and pregnancy loss at first service (yes vs. no) within the second lactation, were analyzed using logistic regression models using the GENLINMIXED procedure of SPSS. Model building was as described above. Separate models were built for farms A and B. The initial models contained the following variables: year of calving (2015–2021), month of freshening, calving ease (0 = not observed, 1 = unassisted calving, 2 = calving assisted by 1 person, 3 = calving assisted by more than 1 person), stillbirth (yes vs. no), twins (yes vs. no), days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1 (yes vs. no), and PM in lactation 2 (yes vs. no) as explanatory variables. The explanatory variables and the interaction term PM in lactation 1 and PM in lactation 2 were only included when $P < 0.1$.

Two different Cox proportional hazard regression models were calculated, using the SURVIVAL procedure of SPSS, to model the time to event outcomes for culling or death during the first 60 DIM and for pregnancy within 250 d of the second lactation for farms A and B separately. The initial models contained the following variables: year of calving (2015–2021), month of freshening, calving ease (0 = not observed, 1 = unassisted calving, 2 = calving assisted by 1 person, 3 = calving assisted by more than 1 person), stillbirth (yes vs. no), twins (yes vs. no), days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1 (yes vs. no), and PM in lactation 2 (yes vs. no) were tested as risk factors. The explanatory variables and the interaction term PM in lactation 1 and PM in lactation 2 were only considered if P-value was below 0.1. The proportional hazard assumption was checked using Schoenfeld residuals. Median DIM at pregnancy were derived from Kaplan-Meier analysis.

To account for multiple comparisons, the reported P-value was adjusted using a Bonferroni correction. Variables were declared significant when $P < 0.05$.

RESULTS

Descriptive Statistics

On farm A, a total of 6,266 cows in the first lactation were eligible for enrollment in this retrospective analysis. From these cows, 22.8% (1,432/6,266) were culled during their first lactation. The incidence of PM in the first lactation was 20.1% (1,255/6,266). The proportion of cows leaving the herd in lactation 1 was greater for cows affected by PM in the first lactation (29.9%; 375/1,255) compared with cows without PM (21.1%; 1,057/5,011). A total of 4,834 cows in second lactation from farm A were considered for statistical analyses. Descriptive statistics from these cows can be found in Table 1. There were 3,503 cows (72.4%) with NoPM+NoPM, 454 cows (9.4%) with NoPM+PM, 705 cows (14.6%) with PM+NoPM, and 175 cows (3.6%) with PM+PM. Compared with cows without PM, cows with PM in their first lactation spent 1 d longer in the pre-fresh pen (22.7 vs. 23.8 d; $P = 0.001$) and had a greater stillbirth rate (2.2% vs. 3.6%; $P = 0.015$) in the second lactation.

On farm A, the incidence of PM in the second lactation was 11.2% (542/4,834). Compared with cows without PM, cows with PM in their first lactation had greater odds for RFM (odds ratio [**OR**] = 1.50; 95% CI 1.28–1.76; $P = 0.001$) and PM in their second lactation (OR = 1.91; 95% CI 1.58–2.32; $P = 0.001$). There was no association of PM in the first lactation with any other nonuterine diseases (i.e., CH, KET, LDA, MAST) in the second lactation (Table 1).

On farm B, a total of 5,407 cows in the first lactation were eligible for enrollment in this retrospective analysis. From these cows, 21.6% (1,169/5,407) were culled during their first lactation. The incidence of PM in the first lactation was 14.4% (778/5,407). The proportion of cows leaving the herd in lactation 1 was greater for cows affected by PM in the first lactation (28.3%; 220/778) compared with cows without PM (20.5%; 949/4,629). A total of 4,238 cows in second lactation from farm B were considered for statistical analyses. Descriptive statistics from these cows can be found in Table 1. There were 3,428 cows (70.6%) with NoPM+NoPM, 252 cows (5.2%) with NoPM+PM, 450 cows (9.3%) with PM+NoPM, and 108 cows (2.2%) with PM+PM. Cows with PM in their first lactation spent 1 d longer in the pre-fresh pen (20.3 vs. 21.2 d; $P < 0.01$), had a greater stillbirth rate (1.8% vs. 3.7%; $P < 0.01$), and a greater likelihood for calving assistance (31.3% vs. 46.8%; $P < 0.01$) in their second lactation. There tended to be more twin births in cows with PM (3.2% vs. 1.8%; $P = 0.06$) compared with cows with no PM.

On farm B, the incidence of PM in the second lactation was 8.5% (360/4,238). Cows with PM in their first

Table 1. Descriptive statistics[1] of cows in the second lactation for farms A and B that were either affected by puerperal metritis (PM)[2] or not in their first lactation

Variable	Farm A			Farm B		
	Cows with no PM (n = 3,954)	Cows with PM (n = 880)	*P*-value	Cows with no PM (n = 3,680)	Cows with PM (n = 558)	*P*-value
First lactation						
Gestation length, d	277	277	0.698	276	277	0.002
Dry period length, d	56.6	56.7	0.792	49.3	49.3	0.921
Days in the pre-fresh group	22.7	23.8	0.001	20.3	21.2	0.003
Second lactation						
Stillbirth, %	2.2	3.6	0.015	1.8	3.7	0.009
Twins, %	3.3	3.8	0.515	1.8	3.2	0.060
Calving assistance, %	21.0	20.2	0.578	31.1	46.8	0.001
Retained fetal membranes,[3] %	9.0	12.9	0.001	4.9	8.5	0.001
Puerperal metritis, %	12.2	19.6	0.001	5.6	11.0	0.001
Clinical hypocalcemia,[4] %	2.5	2.8	0.498	0.4	0.7	0.312
Hyperketonemia,[5] %	30.1	29.3	0.472	16.4	18.8	0.220
Left displaced abomasum,[6] %	1.6	1.4	0.510	1.4	1.2	0.732
Clinical mastitis,[7] %	8.2	8.6	0.613	3.8	4.9	0.311

[1]Results were derived from univariable linear or logistic regression models.

[2]PM was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge, and fever >39.5°C.

[3]Retained fetal membrane = fetal membranes were not expelled by 24 h after calving.

[4]Clinical hypocalcemia = when cows were recumbent during the first 5 DIM and rose in response to an intravenous Ca infusion.

[5]Hyperketonemia = blood BHB concentrations ≥1.2 mmol/L using a cow side BHB test.

[6]Left displaced abomasum = percussion of the left flank resulted in tympanic resonance auscultated with a stethoscope.

[7]Clinical mastitis = visible signs of inflammation in an affected mammary gland (i.e., redness, swelling, pain, or heat) and alterations such as clots, flakes, discoloration, or abnormal consistency of secretions.

lactation had greater odds for RFM (OR = 1.35; 95% CI 1.10–1.64; $P = 0.001$) and PM in their second lactation (OR = 2.06; 95% CI 1.47–2.91; $P = 0.001$). There was no association of PM in the first lactation with any other nonuterine diseases (i.e., CH, KET, LDA, MAST) in the second lactation (Table 1).

305-d Milk Yield

On farm A, there tended to be an interaction ($P = 0.08$) of PM in lactation 1 and PM in lactation 2 regarding 305-d milk yield projection based on the second milk test in lactation 2 from 4,335 cows based on multivariable models (Table 2). For cows affected by PM in lactation 1, the difference in 305-d milk yield projection based on the second milk test in lactation 2 between cows with PM in lactation 2 compared with cows unaffected by PM in lactation 2 was 605 ± 100 kg ($P < 0.01$). For cows unaffected by PM in lactation 1, the difference in 305-d milk yield projection based on the second milk test in lactation 2 between cows with PM in lactation 2 compared with cows unaffected by PM in lactation 2 was 403 ± 59 kg ($P < 0.01$). In addition to that, 305-d milk yield projection based on the second milk test in lactation 2 was affected by year of freshening ($P < 0.01$), month of freshening ($P < 0.01$), stillbirth ($P < 0.01$), twins ($P < 0.01$), 305-d milk yield in lactation 1 ($P < 0.01$), and days open in lactation 1 ($P < 0.01$).

On farm B, there was an interaction ($P = 0.04$) of PM in lactation 1 and PM in lactation 2 regarding 305-d milk yield projection based on the second milk test in lactation 2 from 3,994 cows based on multivariable models (Table 2). For cows affected by PM in lactation 1, the difference in 305-d milk yield projection based on the second milk test in lactation 2 between cows with PM in lactation 2 compared with cows unaffected by PM in lactation 2 was 1,232 ± 161 kg ($P < 0.01$). For cows unaffected by PM in lactation 1, the difference in 305-d milk yield projection based on the second milk test in lactation 2 between cows with PM in lactation 2 compared with cows unaffected by PM in lactation 2 was 840 ± 97 kg ($P < 0.01$). In addition, 305-d milk yield projection based on the second milk test in lactation 2 was affected by year of freshening ($P < 0.01$), month of freshening ($P < 0.01$), and 305-d milk yield in lactation 1 ($P < 0.01$).

Culling in Early Lactation

On farm A, cows with PM in their first lactation had a greater hazard for culling in their second lactation within the first 60 DIM (hazard risk [**HR**] = 1.27; 95% CI 1.01–1.60; $P = 0.04$). There was no association of PM in the second lactation ($P = 0.18$) with culling in their second lactation within the first 60 DIM. The interaction of PM in the first lactation and PM in the second lactation was not associated with culling in their second

Table 2. Association of puerperal metritis (PM)[1] in the first and second lactation[2] with 305-d milk yield projection based on the second milk test in lactation 2 for farms A (n = 4,335) and B (n = 3,994)

Variable[3]	NoPM+NoPM	NoPM+PM	PM+NoPM	PM+PM	*P*-value		
					PM in lactation 1	PM in lactation 2	Interaction term
Farm A							
305-milk yield, kg ± SEM	10,871 ± 83	10,467 ± 88	11,403 ± 91	10,438 ± 113	0.22	0.01	0.08
Farm B							
305-milk yield, kg ± SEM	12,200 ± 61	11,360 ± 107	12,319 ± 89	11,086 ± 159	0.42	0.01	0.04

[1]PM was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C.

[2]Based on the diagnosis of PM in the first and second lactation, cows were classified into 4 groups: (1) No PM in the first lactation and no PM in second lactation (NoPM+NoPM), (2) no PM in the first lactation and PM in the second lactation (NoPM+PM), (3) PM in the first lactation and no PM in the second lactation (PM+NoPM), and (4) PM in the first lactation and PM in the second lactation (PM+PM).

[3]Outcomes were derived from multivariable models accounting for covariates such as year of calving, month of freshening, calving ease, stillbirth, twins, days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1, and PM in lactation 2.

lactation within the first 60 DIM ($P = 0.23$). Culling in early lactation was associated with year of freshening ($P = 0.01$), twins ($P < 0.01$), dystocia ($P < 0.01$), days open in lactation 1 ($P < 0.01$), and stillbirth ($P < 0.01$). There tended to be an association of month of freshening ($P = 0.09$) with culling in their second lactation within the first 60 DIM.

On farm B, there was no association of PM in the first lactation ($P = 0.59$) and PM in the second lactation ($P = 0.30$) with culling in their second lactation within the first 60 DIM. The interaction of PM in the first lactation and PM in the second lactation was also not associated with culling in their second lactation within the first 60 DIM ($P = 0.53$). Culling in early lactation was associated with year of freshening ($P = 0.01$), dystocia ($P < 0.01$), and stillbirth ($P < 0.01$).

P/AI at First Service

On farm A, there was an interaction of PM in lactation 1 and PM in lactation 2 ($P < 0.01$; Table 3) regarding the association with P/AI at first service. Pregnancy per AI at first service was 41.7 ± 1.7%, 30.0 ± 2.6%, 38.2 ± 2.5%, and 40.3 ± 4.3% for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively. Considering only cows with PM in lactation 1, there was no difference in P/AI at first service in lactation 2 ($P = 0.66$) between cows with or without PM in lactation 2. For cows unaffected by PM in lactation 1, P/AI at first service in lactation 2 differed by 11.7 percentage units (SEM: 2.6%; $P < 0.01$) between cows with PM in lactation 2 compared with cows without PM in lactation 2. In addition, P/AI at first service in lactation 2 was associated with year of freshening ($P = 0.01$), month of freshening ($P = 0.01$), dystocia ($P < 0.01$), 305-d milk yield in lactation 1 ($P < 0.01$), and days open in lactation 1 ($P = 0.04$).

On farm B, there tended to be an interaction of PM in lactation 1 and PM in lactation 2 ($P < 0.10$; Table 3) regarding the association with P/AI at first service. Pregnancy per AI at first service was 53.2 ± 3.0%, 38.1 ± 4.3%, 49.3 ± 3.8%, and 23.8 ± 5.4% for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively. For cows affected by PM in lactation 1, P/AI at first service in lactation 2 ($P < 0.01$) differed by 25.5 percentage units (SEM: 5.5%) between cows with PM in lactation 2 compared with cows without PM in lactation 2. For cows unaffected by PM in lactation 1, P/AI at first service in lactation 2 differed by 15.5 percentage units (SEM: 3.6%; $P < 0.01$) between cows with PM in lactation 2 compared with cows without PM in lactation 2. In addition, P/AI at first service in lactation 2 was associated with year of freshening ($P = 0.01$), month of freshening ($P = 0.01$), 305-d milk yield in lactation 1 ($P = 0.02$), and days open in lactation 1 ($P < 0.01$)

Pregnancy Loss at First Service

On farm A, PM in lactation 1 was not associated with pregnancy loss at first service in lactation 2 ($P = 0.43$; Table 3). Puerperal metritis in lactation 2 was associated with pregnancy loss at first service in lactation 2 (OR = 1.95; 95% CI = 1.01–3.77; $P = 0.04$). There was no interaction of PM in lactation 1 and PM in lactation 2 regarding pregnancy loss at first service in lactation 2 ($P = 0.95$). Stillbirth in second lactation tended to be associated with pregnancy loss at first service in lactation 2 ($P = 0.08$).

On farm B, PM in lactation 1 was not associated with pregnancy loss at first service in lactation 2 ($P = 0.15$; Table 3). Puerperal metritis in lactation 2 was associated with pregnancy loss at first service in lactation 2 (OR = 2.66; 95% CI = 1.36–5.17; $P = 0.01$). There was

Table 3. Association of puerperal metritis (PM)[1] in the first and second lactation with reproductive performance in the second lactation for farms A and B[2]

Variable[3]	No. of cows	NoPM+NoPM	NoPM+PM	PM+NoPM	PM+PM	*P*-value		
						PM in lactation 1	PM in lactation 2	Interaction term
Farm A								
Pregnancy per AI, % ± SEM	4,096	41.7 ± 1.7	30.0 ± 2.6	38.2 ± 2.5	40.3 ± 4.3	0.12	0.01	0.01
Pregnancy loss, % ± SEM	1,842	6.9 ± 0.7	12.7 ± 3.0	9.3 ± 1.8	18.8 ± 4.9	0.43	0.04	0.95
Median days open, d	4,440	105	145	122	127	0.01	0.01	0.07
95% CI, d		101–108	131–154	113–130	115–142			
Farm B								
Pregnancy per AI, % ± SEM	3,848	53.2 ± 3.0	38.1 ± 4.3	49.3 ± 3.8	23.8 ± 5.4	0.04	0.01	0.09
Pregnancy loss, % ± SEM	1,823	8.6 ± 1.5	19.6 ± 5.3	11.8 ± 3.0	26.9 ± 11.6	0.15	0.01	0.90
Median days open, d	4,078	110	147	117	185	0.03	0.01	0.54
95% CI, d		109–111	138–155	102–131	162–207			

[1]PM was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C.

[2]Outcomes were derived from multivariable models accounting for covariates such as the year of calving, month of freshening, calving ease, stillbirth, twins, days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1, and PM in lactation 2.

[3]Based on the diagnosis of PM in the first and second lactation, cows were classified into 4 groups: (1) no PM in the first lactation and no PM in second lactation (NoPM+NoPM), (2) nNo PM in the first lactation and PM in the second lactation (NoPM+PM), (3) PM in the first lactation and no PM in the second lactation (PM+NoPM), and (4) PM in the first lactation and PM in the second lactation (PM+PM).

no interaction of PM in lactation 1 and PM in lactation 2 regarding pregnancy loss at first service in lactation 2 ($P = 0.90$). Stillbirth was associated with pregnancy loss at first service in lactation 2 ($P = 0.05$).

Time to Pregnancy

On farm A, there tended to be an interaction ($P = 0.07$) of PM in lactation 1 and PM in lactation 2 regarding the risk for pregnancy in second lactation within 250 DIM. Median DIM at pregnancy were 105 (95% CI 101–108 d), 145 (95% CI 131–154), 122 (95% CI 113–130), and 127 (95% CI 115–142) days for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM groups, respectively (Table 3; Figure 1). In addition, the hazard for pregnancy within 250 DIM was affected by year of freshening ($P = 0.02$), month of freshening ($P < 0.01$), days open in lactation 1 ($P < 0.01$), dystocia ($P < 0.01$), and 305-d milk yield in lactation 1 ($P < 0.01$).

On farm B, cows with PM in lactation 1 had a reduced hazard for pregnancy in their second lactation within 250 DIM compared with cows without PM in lactation 1 (HR = 0.88; 95% CI 0.79–0.98; $P = 0.03$). Cows with PM in lactation 2 had a reduced hazard for pregnancy in their second lactation within 250 DIM compared with cows without PM in lactation 2 (HR = 0.67; 95% CI 0.58–0.77; $P < 0.01$). There was no interaction ($P = 0.54$) of PM in lactation 1 and PM in lactation 2 regarding the risk for pregnancy in second lactation within 250 DIM. Median DIM at pregnancy were 110 (95% CI 109–111), 147 (95% CI 138–155), 117 (95% CI 102–131), and 185 (95% CI 162–207) days for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively (Table 3; Figure 1). In addition, the hazard for pregnancy within 250 DIM was affected by month of freshening ($P < 0.01$), days open in lactation 1 ($P = 0.04$), dystocia ($P < 0.01$), and stillbirth ($P < 0.01$).

DISCUSSION

Transition cow health events can have long-lasting effects on dairy cow physiology that impair performance even after clinical recovery from these issues. However, it has not been tested whether the long-term consequences of PM in first lactation still could be observed in the next lactation. Cows with PM in their first lactation had an increased risk for uterine disease (i.e., RFM and PM) in their second lactation. Puerperal metritis in the first lactation was not associated with any other nonuterine health–related events in the transition period in the second lactation; however, there was an increased risk for culling in early lactation on one farm. Interestingly, milk loss due to PM in the second lactation was greater for cows affected by PM in the first lactation at least ono ne farm. However, reproductive performance in the second lactation was compromised in cows with PM in the first lactation. It can be hypothesized that a greater susceptibility to PM in the second lactation for cows affected by PM in first lactation is a major risk factor for reduced reproductive performance.

The incidence of PM was greater in primiparous cows compared with secundiparous cows on both farms in our study. This agrees with previous studies (Pohl et al., 2016; Venjakob et al., 2019).

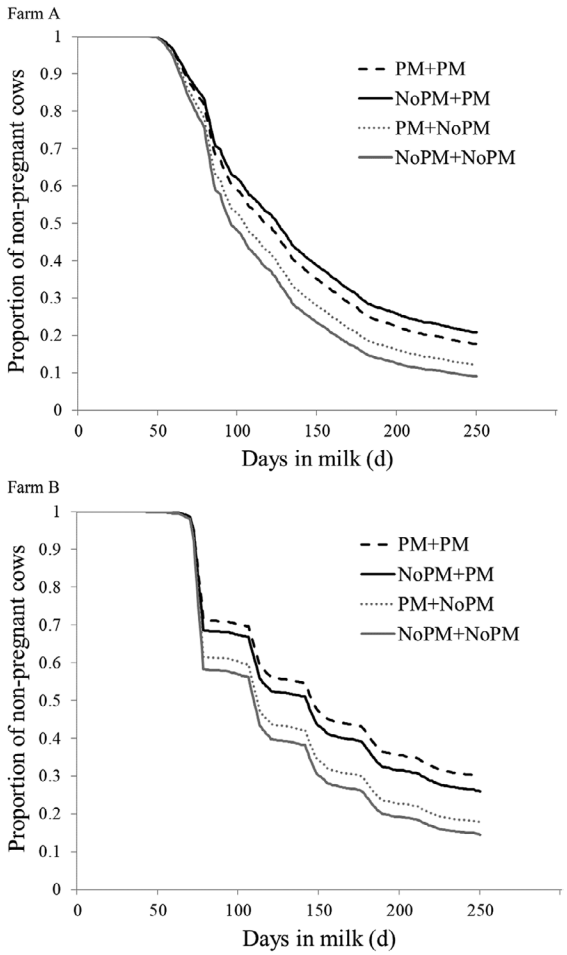

Figure 1. Kaplan-Meier survival analysis illustrating the association of puerperal metritis (PM) in the first and second lactations with time to pregnancy in the second lactation (farm A, 4,440 cows; farm B, 4,078 cows). Puerperal metritis was defined as sign of systemic illness (e.g., decreased milk production, dullness, or other signs of toxemia) in combination with an animal with an abnormally enlarged uterus, fetid watery red-brown uterine discharge and fever >39.5°C. Based on the diagnosis of PM in the first and second lactation, cows were classified into 4 groups: (1) no PM in the first lactation and no PM in the second lactation (NoPM+NoPM; gray solid line), (2) No PM in the first lactation and PM in the second lactation (NoPM+PM; black solid line), (3) PM in the first lactation and no PM in the second lactation (PM+NoPM; gray dotted line), and (4) PM in the first lactation and PM in the second lactation (PM+PM; black dashed line). Results were obtained from multivariable Cox proportional hazard regression model accounting for covariates such as the year of calving, month of freshening, calving ease, stillbirth, twins, days open in lactation 1, 305-d milk yield in lactation 1, PM in lactation 1, and PM in lactation 2. For farm A, the following variables were included: PM in lactation 1 ($P = 0.01$), PM in lactation 2 ($P < 0.01$), PM in lactation 1 × PM in lactation 2 ($P = 0.07$), year of freshening ($P = 0.02$), month of freshening ($P < 0.01$), days open in lactation 1 ($P < 0.01$), dystocia ($P < 0.01$), and 305-d milk yield in lactation 1 ($P < 0.01$). On farm A, median DIM at pregnancy were 105, 145, 122, and 127 d for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively. For farm B, the following variables were included: PM in lactation 1 ($P = 0.03$), PM in lactation 2 ($P < 0.01$), PM in lactation 1 × PM in lactation 2 ($P = 0.07$), month of freshening ($P < 0.01$), days open in lactation 1 ($P = 0.04$), dystocia ($P < 0.01$), and stillbirth ($P < 0.01$). On farm B, median DIM at pregnancy were 110, 147, 117, and 185 d for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively.

This is the first study that evaluated the long-term effects of PM across 2 lactations. Based on our results there seems to be an increased risk for uterine diseases in the second lactation in cows that were affected by PM in their first lactation. This was observed on both farms (farm A: OR = 1.91; farm B: OR = 2.06). Interestingly, we also observed a greater likelihood for RFM (farm A: OR = 1.50; farm B: OR = 1.35) in cows affected by PM in their previous lactation. The exact reason for the increased risk of PM and RFM in the second lactation remains speculative; however, it suggests long-term negative effects of PM for uterine health across consecutive lactations. The greater susceptibility of cows to PM and RFM in their second lactation in cows previously affected by PM could be influenced by several factors (e.g., long-lasting uterine damage, disturbed uterine function, or dysbiosis).

Long-lasting damage to the uterine wall associated with PM in the first lactation might be one reason for greater susceptibility for uterine disease in the second lactation. One concept is that intact epithelial barriers allow for commensal colonization, but that breach of this barrier with the invasion of epithelial or deeper cells triggers a substantial inflammatory response (LeBlanc, 2023). It has also been shown that cows affected by metritis had less glandular development and less epithelial proliferation within the deep endometrium up to 80 DIM (Sellmer Ramos et al., 2023), suggesting long-term changes in the tissue architecture in cows with PM. Tissue damage (determined by histology) across lactations seems to be unlikely as the uterine involution process itself involves damage, sloughing, and replacement of the luminal epithelium. Nevertheless, it would be interesting to study the consequences of PM for certain epithelial functions which are important for uterine health in future studies. It has been proposed that failure in endometrial tolerance to pathogenic bacteria and the innate immune response shape postpartum uterine disease (Sheldon et al., 2019). Tolerance against pathogens include tissue related strategies that allow an organism to maintain its health without directly reducing the pathogen load (Lopez, 2022). Therefore, by focusing research efforts on understanding these tolerance mechanisms, we can potentially develop new strategies for improving disease resilience.

The development of metritis was associated with dysbiosis of the uterine microbiota, characterized by a reduced richness and diversity, and an increased abundance of *Bacteroidetes*, *Porphyromonas*, and *Fusobacterium* (Galvão et al., 2019). Because the microbiome itself plays an important role for tolerance and resistance against pathogens (Sheldon et al., 2019), our results highlight the need for more comprehensive studies on long-term dysbiosis in animals with uterine diseases.

Cows with metritis in their previous lactation had compromised reproductive performance in their next lactation. This could be associated with the increased risk for RFM and metritis in their second lactation in particular for cows in the PM+PM group. Retained fetal membranes and metritis delay uterine involution, cause inflammation of the endometrium, affect follicle growth, which decreases estradiol secretion from dominant follicles, thus delaying the interval to first ovulation, and reduces pregnancy to first insemination (LeBlanc, 2008). The alterations in follicle growth and ovulation associated with uterine diseases implicate potential roles mediated by either direct effects of diseases within follicles reducing embryo quality (Ribeiro et al., 2016), or hypothalamic-pituitary changes that result in reduced luteinizing hormone secretion and failure of the gonadotrophin surge (Peter et al., 1989; Sheldon and Dobson, 2004). Ribeiro et al. (2016) demonstrated that inflammatory diseases before breeding reduced fertilization of oocytes and development to morula, and impaired elongation of early conceptus and secretion of IFN-τ in the uterine lumen. The authors postulated that reduced oocyte competence is a likely reason for carryover effects of diseases on developmental biology, but an impaired intrauterine environment was also shown to be involved. All of the studies mentioned above evaluated the consequences of metritis on reproductive performance using the same lactation in which the disease occurred. Although these studies might explain the negative effect on reproductive performance for cows with PM in their current lactation, they provide no clear arguments for the observed effects in cows with PM in their previous lactation. The group of cows with PM+NoPM is of particular interest, as they show a long-lasting effect of PM in the first lactation independent of PM in their second lactation.

In the present study, we observed a greater likelihood for RFM in cows affected by PM in their previous lactation. Genetic correlation between RFM and metritis is strong ranging from 0.56 to 0.79 (Heringstad, 2010: r = 0.64; Neuenschwander et al., 2012: r = 0.79; Parker Gaddis et al., 2014: r = 0.56), but has not been described for 2 consecutive parturitions.

Despite the low heritability of metritis, a wellness trait in US Holstein cows has been validated recently using genomic predictions (McNeel et al., 2017). The findings of this study suggested that health trait predictions were related to differences in metritis incidence among different quartiles. There was a difference in metritis incidence of 10.7% for cows in the first quartile (23.6%) compared with cows in the fourth quartile (12.9%). Unfortunately, we didn't have genomic data for cows in this study. For lameness, it has been shown that the repeatability of disease across lactations had a genetic component (Oikonomou et al., 2013). Future studies should evaluate the genetic proportion of the higher susceptibility for metritis in cows.

Another explanation might be a genetic component of susceptibility to uterine diseases. Heritability for metritis, however, has been described previously in several studies as low, ranging from 0.03 to 0.09 (Heringstad, 2010: $h^2 = 0.03$; Neuenschwander et al., 2012: $h^2 = 0.03$; Parker Gaddis et al., 2014: $h^2 = 0.04$; Vukasinovic et al., 2017: $h^2 = 0.09$). Genome-wide association studies have identified some potential candidate genes for uterine diseases (Guarini et al., 2019). Some of these genes are involved in immune signaling, such as toll-like receptor 4 (**TLR4**). The TLR4 is a pathogen recognition receptor that binds to pathogen associated molecular patterns. This activates a molecular cascade that will lead to the production of IL-1β, IL-6, and TNFα, CXCL1, CCL20, and IL-8 that are part of an effective immune response (Lima, 2020).

External validity of our study is limited because it was conducted on 2 commercial dairy farms. Nevertheless, these 2 farms represent a large proportion of herds with freestall housing, TMR feeding, and excellent reproductive management (i.e., greater conception rate, shorter days open, and so on). It is interesting to note here, that, although the reproductive management differed among these 2 farms (farm A: combination of estrus detection and timed AI and farm B: 100% timed AI) the detrimental effect of metritis in the first lactation on the reproductive performance in the second lactation was quite similar using the hazard risk for pregnancy within 250 DIM (farm A: HR = 0.88; 95% CI 0.79–0.97; farm B: HR = 0.88; 95% CI 0.79–0.98). There were, however, unexpected results regarding P/AI at first service on farm A for cows with PM+PM. Pregnancy per AI at first service in this group of cows on farm A was acceptable (i.e., 40.3% ± 4.3%). This subgroup of cows was rather small with 154 cows. Therefore, results have to interpreted with caution.

Both farms had a long-term history of collaboration with the research team and standard written operating procedures were provided by the herd manager in coordination with the researchers. Therefore, we are confident that the risk of misclassification is low and that diseases have been recorded consistently over the course of this study. Nevertheless, there is always a risk for misdiagnosis of metritis even among experienced observers (Sannmann and Heuwieser, 2015).

Another potential limitation of this study is to underestimate the consequence of PM across 2 consecutive lactations because of selection bias. Cows with PM in lactation 1 had a greater risk for culling in their first lactation compared with cows unaffected with PM on both farms (farm A: 21.1 % vs. 29.9%; farm B: 20.5% vs. 28.3%). It has been shown recently, that cows with more severe cases of metritis have a greater risk for dif-

ferences in production, reproductive performance and culling (Figueiredo et al., 2024). Therefore, more severe cases of PM in our study might have been culled in the first lactation limiting the negative effect of the disease in the next lactation.

CONCLUSIONS

The present study provides evidence that metritis in the first lactation has long-lasting negative consequences, particularly for the risk of uterine diseases and reproductive performance, even in their next lactation. Results from this study can help to improve our understanding of the development of the disease. Future studies should evaluate whether there are long-lasting differences in the microbiome of cows affected by the disease, structural changes in the uterus across consecutive lactations, or individual differences in tolerance and resistance, which might explain the differences leading to greater susceptibility for the development of metritis.

NOTES

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Nonstandard abbreviations used: 2nd305ME = second test-day 305-d mature-equivalent milk projection; CH = clinical hypocalcemia; CIDR = controlled intravaginal P4-releasing device; CL = corpus luteum; HR = hazard ratio; KET = hyperketonemia; LDA = left displaced abomasum; MAST = clinical mastitis; NoPM+NoPM = no PM in the first lactation and no PM in second lactation; NoPM+PM = no PM in the first lactation and PM in the second lactation; OR = odds ratio; P4 = progesterone; P/AI = pregnancy per AI; PM = puerperal metritis; PM+NoPM = PM in the first lactation and no PM in the second lactation; PM+PM = PM in the first lactation and PM in the second lactation; RFM = retained fetal membrane; TLR4 = toll-like receptor 4.

REFERENCES

Aleri, J. W., B. C. Hine, M. F. Pyman, P. D. Mansell, W. J. Wales, B. Mallard, and A. D. Fisher. 2016. Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period. Res. Vet. Sci. 108:8–17. https://doi.org/10.1016/j.rvsc.2016.07.007.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819. https://doi.org/10.2527/1995.7392804x.

Bruinjé, T. C., E. I. Morrison, E. S. Ribeiro, D. L. Renaud, and S. J. LeBlanc. 2024. Associations of inflammatory and reproductive tract disorders postpartum with pregnancy and early pregnancy loss in dairy cows. J. Dairy Sci. 107:1630–1644. https://doi.org/10.3168/jds.2023-23976.

Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin $F_{2\alpha}$ and estradiol-17β during Ovsynch in lactating dairy cows. J. Dairy Sci. 92:1412–1422. https://doi.org/10.3168/jds.2008-1289.

Carvalho, M. R., F. Peñagaricano, J. E. P. Santos, T. J. DeVries, B. W. McBride, and E. S. Ribeiro. 2019. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. J. Dairy Sci. 102:11701–11717. https://doi.org/10.3168/jds.2019-17025.

de Lima, F. S. 2020. Recent advances and future directions for uterine diseases diagnosis, pathogenesis, and management in dairy cows. Anim. Reprod. 17:e20200063. https://doi.org/10.1590/1984-3143-ar2020-0063.

de Oliveira, E. B., F. Cunha, R. Daetz, C. C. Figueiredo, R. C. Chebel, J. E. Santos, C. A. Risco, K. C. Jeong, V. S. Machado, and K. N. Galvão. 2020. Using chitosan microparticles to treat metritis in lactating dairy cows. J. Dairy Sci. 103:7377–7391. https://doi.org/10.3168/jds.2019-18028.

Dohoo, I. R., and S. W. Martin. 1984. Subclinical ketosis: Prevalence and associations with production and disease. Can. J. Comp. Med. 48:1–5.

Dohoo, P. J., S. W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. University of Prince Edward Island.

Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3.

Esposito, G., P. C. Irons, E. C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health, and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60–71. https://doi.org/10.1016/j.anireprosci.2013.11.007.

Figueiredo, C. C., S. Casaro, F. Cunha, V. R. Merenda, E. B. de Oliveira, P. Pinedo, J. E. P. Santos, R. C. Chebel, G. M. Schuenemann, R. C. Bicalho, R. O. Gilbert, S. R. Zas, C. M. Seabury, G. Rosa, W. W. Thatcher, R. S. Bisinotto, and K. N. Galvão. 2024. Evaluating differences in milk production, reproductive performance, and survival associated with vaginal discharge characteristics and fever in postpartum dairy cows. J. Dairy Sci. 107:6079–6089. https://doi.org/10.3168/jds.2023-23905.

Galvão, K. N., R. C. Bicalho, and S. J. Jeon. 2019. Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows. J. Dairy Sci. 102:11786–11797. https://doi.org/10.3168/jds.2019-17106.

Giuliodori, M. J., R. P. Magnasco, D. Becu-Villalobos, I. M. Lacau-Mengido, C. A. Risco, and R. L. de La Sota. 2013. Metritis in dairy cows: Risk factors and reproductive performance. J. Dairy Sci. 96:3621–3631. https://doi.org/10.3168/jds.2012-5922.

Guarini, A. R., D. A. L. Lourenco, L. F. Brito, M. Sargolzaei, C. F. Baes, F. Miglior, I. Misztal, and F. S. Schenkel. 2019. Genetics and genomics of reproductive disorders in Canadian Holstein cattle. J. Dairy Sci. 102:1341–1353. https://doi.org/10.3168/jds.2018-15038.

Guterbock, W. M. 2004. Diagnosis and treatment programs for fresh cows. Vet. Clin. North Am. Food Anim. Pract. 20:605–626. https://doi.org/10.1016/j.cvfa.2004.06.002.

Heringstad, B. 2010. Genetic analysis of fertility-related diseases and disorders in Norwegian Red cows. J. Dairy Sci. 93:2751–2756. https://doi.org/10.3168/jds.2009-2879.

Hertl, J. A., Y. H. Schukken, L. W. Tauer, F. L. Welcome, and Y. T. Gröhn. 2018. Does clinical mastitis in the first 100 days of lactation 1 predict increased mastitis occurrence and shorter herd life in dairy cows? J. Dairy Sci. 101:2309–2323. https://doi.org/10.3168/jds.2017-12615.

Iwersen, M., D. Klein-Jöbstl, M. Pichler, L. Roland, B. Fidlschuster, I. Schwendenwein, and M. Drillich. 2013. Comparison of 2 electronic cowside tests to detect subclinical ketosis in dairy cows and the influence of the temperature and type of blood sample on the test results. J. Dairy Sci. 96:7719–7730. https://doi.org/10.3168/jds.2013-7121.

LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. Vet. J. 176:102–114. https://doi.org/10.1016/j.tvjl.2007.12.019.

LeBlanc, S. J. 2023. Review: Postpartum reproductive disease and fertility in dairy cows. Animal 17(Suppl. 1):100781. https://doi.org/10.1016/j.animal.2023.100781.

Lima, F. S., A. Vieira-Neto, G. S. F. M. Vasconcellos, R. D. Mingoti, E. Karakaya, E. Solé, R. S. Bisinotto, N. Martinez, C. A. Risco, K. N. Galvão, and J. E. P. Santos. 2014. Efficacy of ampicillin trihydrate or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows. J. Dairy Sci. 97:5401–5414. https://doi.org/10.3168/jds.2013-7569.

Lopez, B. S. 2022. Can infectious disease control be achieved without antibiotics by exploiting mechanisms of disease tolerance? Immunohorizons 6:730–740. https://doi.org/10.4049/immunohorizons.2200043.

McCarthy, M. M., and M. W. Overton. 2018. Short communication: Model for metritis severity predicts that disease misclassification underestimates projected milk production losses. J. Dairy Sci. 101:5434–5438. https://doi.org/10.3168/jds.2017-14164.

McNeel, A. K., B. C. Reiter, D. Weigel, J. Osterstock, and F. A. Di Croce. 2017. Validation of genomic predictions for wellness traits in US Holstein cows. J. Dairy Sci. 100:9115–9124. https://doi.org/10.3168/jds.2016-12323.

Neuenschwander, T. F.-O., F. Miglior, J. Jamrozik, O. Berke, D. F. Kelton, and L. R. Schaeffer. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. Animal 6:571–578. https://doi.org/10.1017/S1751731111002059.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press.

Oikonomou, G., N. B. Cook, and R. C. Bicalho. 2013. Sire predicted transmitting ability for conformation and yield traits and previous lactation incidence of foot lesions as risk factors for the incidence of foot lesions in Holstein cows. J. Dairy Sci. 96:3713–3722. https://doi.org/10.3168/jds.2012-6308.

Parker Gaddis, K. L., J. B. Cole, J. S. Clay, and C. Maltecca. 2014. Genomic selection for producer-recorded health event data in US dairy cattle. J. Dairy Sci. 97:3190–3199. https://doi.org/10.3168/jds.2013-7543.

Pérez, M. M., R. Wijma, M. Scarbolo, E. Cabrera, F. Sosa, E. M. Sitko, and J. O. Giordano. 2020. Lactating dairy cows managed for second and greater artificial insemination services with the Short-Resynch or Day 25 Resynch program had similar reproductive performance. J. Dairy Sci. 103:10769–10783. https://doi.org/10.3168/jds.2020-18607.

Pérez-Báez, J., C. A. Risco, R. C. Chebel, G. C. Gomes, L. F. Greco, S. Tao, I. M. Thompson, B. C. do Amaral, M. G. Zenobi, N. Martinez, C. R. Staples, G. E. Dahl, J. A. Hernández, J. E. P. Santos, and K. N. Galvão. 2019. Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part I. Calving disorders and metritis. J. Dairy Sci. 102:9138–9150. https://doi.org/10.3168/jds.2018-15878.

Pérez-Báez, J., T. V. Silva, C. A. Risco, R. C. Chebel, F. Cunha, A. de Vries, J. E. P. Santos, F. S. Lima, P. Pinedo, G. M. Schuenemann, R. C. Bicalho, R. O. Gilbert, S. Rodrigez-Zas, C. M. Seabury, G. Rosa, W. W. Thatcher, and K. N. Galvão. 2021. The economic cost of metritis in dairy herds. J. Dairy Sci. 104:3158–3168. https://doi.org/10.3168/jds.2020-19125.

Peter, A. T., W. T. Bosu, and R. J. DeDecker. 1989. Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of *Escherichia coli* endotoxin. Am. J. Vet. Res. 50:368–373.

Piccardi, M., G. Romero, G. Veneranda, E. Castello, D. Romero, M. Balzarini, and G. A. Bo. 2016. Effect of puerperal metritis on reproductive and productive performance in dairy cows in Argentina. Theriogenology 85:887–893. https://doi.org/10.1016/j.theriogenology.2015.10.038.

Pohl, A., S. Bertulat, S. Borchardt, O. Burfeind, and W. Heuwieser. 2016. Randomized, controlled clinical trial on the efficacy of nonsteroidal antiinflammatory drugs for the treatment of acute puerperal metritis in dairy cows. J. Dairy Sci. 99:8241–8249. https://doi.org/10.3168/jds.2015-10775.

Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. J. Dairy Sci. 98:4544–4551. https://doi.org/10.3168/jds.2014-9181.

Ribeiro, E. S., and M. R. Carvalho. 2017. Impact and mechanisms of inflammatory diseases on embryonic development and fertility in cattle. Anim. Reprod. 14:589–600. https://doi.org/10.21451/1984-3143-AR1002.

Ribeiro, E. S., G. Gomes, L. F. Greco, R. L. A. Cerri, A. Vieira-Neto, P. L. J. Monteiro Jr., F. S. Lima, R. S. Bisinotto, W. W. Thatcher, and J. E. P. Santos. 2016. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J. Dairy Sci. 99:2201–2220. https://doi.org/10.3168/jds.2015-10337.

Sannmann, I., O. Burfeind, R. Voigtsberger, and W. Heuwieser. 2013. Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis. Theriogenology 79:961–969. https://doi.org/10.1016/j.theriogenology.2013.01.016.

Sannmann, I., and W. Heuwieser. 2015. Technical note: Intraobserver, interobserver, and test-retest reliabilities of an assessment of vaginal discharge from cows with and without acute puerperal metritis. J. Dairy Sci. 98:5460–5466. https://doi.org/10.3168/jds.2014-9169.

Santos, J. E. P., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Rep. Fert. Suppl. 67:387–403. https://doi.org/10.5661/RDR-VII-387.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvão, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. J. Dairy Sci. 94:5494–5501. https://doi.org/10.3168/jds.2011-4436.

Sellmer Ramos, I., J. Moraes, M. O. Caldeira, S. E. Poock, T. E. Spencer, and M. C. Lucy. 2023. Impact of postpartum metritis on the regeneration of endometrial glands in dairy cows. JDS Commun. 4:400–405. https://doi.org/10.3168/jdsc.2022-0338.

Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H.-J. Schuberth. 2009. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol. Reprod. 81:1025–1032. https://doi.org/10.1095/biolreprod.109.077370.

Sheldon, I. M., J. G. Cronin, and J. J. Bromfield. 2019. Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. Annu. Rev. Anim. Biosci. 7:361–384. https://doi.org/10.1146/annurev-animal-020518-115227.

Sheldon, I. M., and H. Dobson. 2004. Postpartum uterine health in cattle. Anim. Reprod. Sci. 82–83:295–306. https://doi.org/10.1016/j.anireprosci.2004.04.006.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530. https://doi.org/10.1016/j.theriogenology.2005.08.021.

Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208–215. https://doi.org/10.1016/j.theriogenology.2008.03.014.

Stojkov, J., M. A. G. von Keyserlingk, J. N. Marchant, and D. M. Weary. 2015. Assessment of visceral pain associated with metritis in dairy cows. J. Dairy Sci. 98:5352–5361. https://doi.org/10.3168/jds.2014-9296.

Vasquez, A. K., D. V. Nydam, M. B. Capel, S. Eicker, and P. D. Virkler. 2017. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. J. Dairy Sci. 100:2992–3003. https://doi.org/10.3168/jds.2016-11614.

Venjakob, P. L., R. Staufenbiel, W. Heuwieser, and S. Borchardt. 2019. Serum calcium dynamics within the first 3 days in milk and the associated risk of acute puerperal metritis. J. Dairy Sci. 102:11428–11438. https://doi.org/10.3168/jds.2019-16721.

Venjakob, P. L., R. Staufenbiel, W. Heuwieser, and S. Borchardt. 2021. Association between serum calcium dynamics around parturition and common postpartum diseases in dairy cows. J. Dairy Sci. 104:2243–2253. https://doi.org/10.3168/jds.2019-17821.

Vukasinovic, N., N. Bacciu, C. A. Przybyla, P. Boddhireddy, and S. K. DeNise. 2017. Development of genetic and genomic evaluation for wellness traits in US Holstein cows. J. Dairy Sci. 100:428–438. https://doi.org/10.3168/jds.2016-11520.

Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglandin $F_{2\alpha}$ during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. J. Dairy Sci. 98:8644–8654. https://doi.org/10.3168/jds.2015-9353.