The objective of this study was to evaluate the effects of the use of intravaginal probiotics prepartum on the incidence risk of metritis postpartum and conception risk after first artificial insemination (AI). A total of 606 Holstein cows were enrolled 3 wk before their expected calving date from 2 farms. Cows were randomly assigned to either receive a 2-mL dose of a combination of 3 lactic acid bacteria (probiotic treatment) washed with approximately 2 mL of a sterile saline solution, into the vaginal canal twice weekly until parturition, or no intervention (control). Metritis diagnoses were carried out on 6 and 12 d postpartum. Vaginal discharge and rectal temperature were assessed, and vaginal discharge was scored on a scale from 1 to 4, where 1 = clear and 4 = fetid, purulent discharge. Metritis was defined as cows having a vaginal discharge score of 4 with or without fever (rectal temperature ≥39.5°C) on either 6 or 12 d postpartum, or both. Cows were bred after a 60-d voluntary waiting period primarily via the detection of estrus using automated activity monitors; cows not found in estrus were enrolled onto timed AI protocols to receive first breeding before 100 DIM. Pregnancy diagnosis was carried out at d 35 ± 7 post-AI on both farms. Data were analyzed via ANOVA using linear mixed regression models and survival analysis using a Cox proportional hazard model. Total incidence risk of metritis was 23.7% and 34.4% on farm A and farm B, respectively. Overall, the incidence of metritis was not different between treatment groups (control: 41.6 ± 3.8%; probiotic: 38.6 ± 4.0%); however, an interaction by farm was detected, where the probiotic treatment reduced metritis on one farm but not on the other. Conception risk after first AI was not affected by treatment. However, we detected an interaction between parity and treatment, where multiparous cows receiving the probiotic treatment were more likely to become pregnant compared with multiparous cows within the control group (hazard ratio: 1.33; 95% confidence interval: 1.10–1.60); no effect of probiotic treatment was found on the hazard of pregnancy for primiparous cows. In addition, the probiotic treatment was associated with an increased proportion of cows being detected in estrus for the first AI postpartum. In conclusion, vaginal probiotic treatment applied during the 3 wk prepartum was associated with a decreased incidence of metritis on one farm but not the other, suggesting that farm management may be a key player influencing treatment efficacy. Overall, probiotic treatment was found to have only limited effects on fertility in the current study.

Key words: lactic acid bacteria, uterine health, fertility

INTRODUCTION

Postpartum uterine disease presents a major economic challenge to the commercial dairy industry (Bartlett et al., 1986; Esslemont and Kossaibati, 2002). Metritis and endometritis are more prevalent in dairy cattle than other domestic species and are often associated with compromised welfare, reduced fertility, and milk production losses (Fourichon et al., 2000; Sheldon et al., 2008). Metritis and endometritis are distinguished by the depth of inflammation in the uterine wall (LeBlanc, 2008). Metritis is an acute inflammatory infection of the deeper layers of the uterus that may become systemic; it occurs within 21 d of parturition but is most common within 10 d (Sheldon et al., 2006, 2008, 2009). Endometritis is an infection of the uterine lining that occurs in cows more than 21 d postpartum and often reflects the proportion of cows that have failed to resolve bacterial infection in the uterus in the weeks following parturition (Sheldon et al., 2006, 2008). Positive diagnoses of metritis and endometritis have been correlated with lower conception rates and longer calv-
Both metritis and endometritis are the result of postpartum microbial contamination within the uterine lumen and endometrial tissue (Turner et al., 2014). The pathogens associated with uterine diseases at the time of infection are mostly anaerobic bacteria (e.g., Bacteroides spp., Treponella pyogenes, Fusobacterium necrophorum; Williams et al., 2005; Sheldon et al., 2008). Although such bacteria are found within the uterus of most cattle after calving, the bovine reproductive tract is naturally prepared to eliminate pathogens during the weeks following parturition (Sheldon et al., 2008). After parturition, bacterial contamination of the uterine lumen compromises uterine function in cattle, and, when pathogenic bacteria remain, it may result in uterine disease (Sheldon et al., 2006). At calving many factors can influence the occurrence of uterine diseases, such as retained fetal membranes, dystocia, size of the calf, and immune dysregulation (Sheldon et al., 2008, 2009; Esposito et al., 2014). It has been reported that 20 to 40% of dairy cows develop clinical metritis by 10 DIM, 20% develop clinical endometritis after 21 DIM, and 35 to 50% develop subclinical endometritis between 40 and 60 DIM (Kasimanickam et al., 2004; Gilbert et al., 2005; LeBlanc, 2008).

Antibiotics are the primary method of treatment for uterine infections; however, antibiotics have failed to address the suboptimal fertility associated with uterine diseases (Haimerl and Heuwieser, 2014; Figueiredo et al., 2021). Furthermore, concern is growing regarding the large-scale use of antibiotics in agriculture and the emergence of antibiotic-resistant microbes (Haimerl and Heuwieser, 2014; Machado et al., 2014). The dairy industry needs new alternatives to prevent uterine diseases, reduce the use of antibiotics, and address the reduced reproductive performance commonly associated with metritis.

The use of different probiotic strains has been proposed to prevent postpartum uterine infections and inflammation (Ametaj et al., 2014; Genís et al., 2016). Probiotics have been used in agriculture to improve gastrointestinal health, to treat and prevent bacterial infections, and to improve productivity and immunity (Uyeno et al., 2015). Moreover, evidence suggests that probiotics may confer health benefits beyond their application for reducing the incidence of metritis and endometritis. Previous research has indicated that the development of metritis is associated with the presence of Escherichia coli and certain E. coli virulence factors in the uterus within the first few days postpartum (Bi-calho et al., 2012; Kassé et al., 2016). Previous studies have shown the efficacy of a combination of lactic acid bacteria to reduce uterine inflammation and infection from E. coli, using both ex vivo and in vitro methods (Genís et al., 2016, 2017b). Lactobacilli are well known for their contribution to maintaining the balance of natural microbiota by competing with pathogens for tissue colonization and have been demonstrated to exhibit effective antimicrobial potency against bacterial pathogens (Lin et al., 2020) in both the gut and the mammary gland (Bouchard et al., 2015). Studies have examined the effects of lactobacilli species on reproductive health and performance in dairy cows and have found that cows that receive intravaginal lactobacilli have better uterine health and increased fertility compared with cows that did not receive lactobacilli (Ametaj et al., 2014; Deng et al., 2015; Genís et al., 2016). However, field studies showing the applicability of using intravaginal probiotics within a commercial setting are lacking, as are studies with appropriate sample sizes to associate the use of intravaginal probiotics with reproductive outcomes.

The objective of this study was to determine the effects of prepartum intravaginal application of probiotics on the incidence risk of metritis and subclinical endometritis, as well as conception risk after first AI and days to conception. We hypothesized that cows receiving prepartum intravaginal probiotic treatment would be less likely to develop metritis and subclinical endometritis and more likely to become pregnant at first postpartum insemination, as well as to have a shorter interval from calving to conception.

**MATERIALS AND METHODS**

This randomized controlled study was conducted at 2 locations. Farm A was at the University of British Columbia’s Dairy Education and Research Centre in Agassiz, Canada (49°13′59″ N, 121°46′01″ W), and farm B was a commercial farm in Agassiz, Canada. The study was approved by the University of British Columbia’s Animal Care Committee (A15-0089), and cows were cared for according to the guidelines outlined by the Canadian Council of Animal Care (2009).

**Animals and Housing**

A total of 606 Holstein dairy cows (farm A = 321; farm B = 285) were enrolled in this study. Lactating cows were dried off at approximately 45 and 60 d before their expected calving date on farms A and B, respectively, and moved to a dry pen on both farms thereafter. Cows were then moved into the prepartum pen approximately 21 d before their expected calving date and moved into
an adjacent maternity pen when showing physical signs of imminent calving. The maternity pens were bedded with straw (farm A) or sawdust (farm B). Before calving, fresh TMR was delivered once daily and pushed up an additional 4 times daily for both farms. After calving, cows were housed in a freestall barn system at both locations. On farm A, cows were housed in deep-bedded sand freestalls, and manure management was carried out by an automated scraper (approximately 6 times per day). On farm B, stalls were bedded with sawdust, and manure management was carried out using a flush system twice daily. Milking was performed twice daily (at 0500 h and 1500 h) in a conventional milking parlor on both farms. Fresh TMR was delivered to lactating cows twice daily on farm A (0700 h and 1600 h) and once daily at farm B (0700 h). The lactating TMR on each farm was formulated following the Nutrient Requirements of Dairy Cattle guidelines (NRC, 2001) to meet or exceed the requirements of a 620-kg Holstein cow producing 40 kg/d of 3.5% FCM. Water and TMR were available for ad libitum intake.

Body condition was assessed at enrollment (at 21 ± 3 d before the expected calving date) and at 6 and 12 d postpartum. Body condition was scored on a 5-point scale from thin (1) to obese (5) by increments of 0.25 as outlined by Edmonson et al. (1989). Gait scores were assessed on a 5-point scale from sound (1) to severely lame (5) in increments of 1 as outlined by Flower and Weary (2009) at enrollment and at 6 d postpartum. The lactation number into which the cow was entering was recorded at the time of calving. Milk production was recorded using 305-d mature-equivalent milk yields for 15 min at 5ºC and resuspended with sterile NaCl 0.9%. Each dose had a ratio of 25/25/2 of L. rhamnosus, P. acidilactici, and in 20 mL of de Man, Rogosa, and Sharpe medium (Oxoid Ltd.) for L. rhamnosus and P. acidilactici, and in 20 mL of de Man, Rogosa, and Sharpe medium for L. reuteri, and then incubated at 37°C in static conditions for 48 h. Bacteria were then centrifuged at 6,000 × g for 15 min at 5°C and resuspended with sterile NaCl 0.9%. Each dose had a ratio of 25/25/2 of L. rhamnosus, P. acidilactici, and L. reuteri, respectively, as previously described by Genís et al. (2017b). Lactobacillus reuteri was chosen as a probiotic within this combination due to its low adherent capacity in the endometrium, whereas P. acidilactici and L. rhamnosus were selected as 2 probiotics with moderate adherent capacity (++) following the methods described by Genís et al. (2016).

**Disease Diagnosis**

Vaginal discharge and rectal temperature were evaluated for each cow at 6 and 12 d postpartum for the diagnosis of metritis. Before vaginal examination, the vulva was thoroughly cleaned with a soft-bristled brush soaked in an iodine solution (Prepodyne Scrub, Kane Veterinary Supplies) diluted in warm water, to remove feces that could introduce bacteria into the vaginal canal. Discharge was evaluated using a Metrichew check device (Simcrotech), which was inserted into the vaginal canal. The device was cleaned with the same diluted
iodine solution as noted above between uses. Vaginal discharge was classified as follows: 1 = no discharge or clear mucus; 2 = cloudy mucus with <50% purulent; 3 = purulent ≥50%; and 4 = putrid (red/brown color, watery, foul smelling), following a scale adapted from Williams et al. (2005). Cows were classified as having metritis if they had at least one vaginal discharge score of 4 with or without fever at 6 or 12 d postpartum, as described by Sheldon et al. (2006).

At 42 ± 3 d postpartum, a cytological examination was performed via an endometrial cytoprobe (Cooper-Surgical Inc.) for cows on farm A (n = 205). Briefly, the vulva of the cow was cleaned, and the cytoprobe was inserted through the cervix into the uterus, protected by a metal sheath covered with a plastic sanitary sheath. Once in the cervix, the sanitary sheath was removed. The cytobrush was then exposed and rolled on the uterine wall once the instrument had entered into the uterine body. Once complete, the cytobrush was retracted back into the metal sheath, and the entire instrument was removed from the cow. Slides for cytological examination were prepared on farm by rolling the cytoprobe on a clean glass microscope slide (Fisherbrand, Microscope Slides, Fisher Scientific) and were stained using a Romanowsky stain (Diff-Quick, Fisher Diagnostics). Cover slips (Fisherbrand, Microscope Cover Glass, Fisher Scientific) were applied using slide glue once slides were dry (Fisher Chemical, Permoun, Mounting Medium, Fisher Scientific). Each slide was examined by a single trained observer at 400× magnification using a light microscope (Nikon Eclipse E200) to perform a differential cell count of 300 cells (PMN and endometrial cells), in 3 counts of 100 cells throughout the cytobrush slide, and then averaged. Cytological endometritis (CE) was defined as the presence of ≥5% PMN, as defined by Dubuc et al. (2010).

Breeding Program and Pregnancy Diagnosis

On both farms, cows were AI upon estrus, as detected by activity monitors. Cows were continuously monitored by a leg-mounted pedometer (farm A: Afim PedoPlus, Afikim; farm B: Rescounter II, GEA Farm Technologies), fitted immediately following calving. On farm A, cows were AI upon estrus, detected by the activity monitor using the a.m./p.m. rule. On farm B, cows were AI once daily between 10 a.m. and noon. Cows not found in estrus were examined by the herd’s veterinarian and were enrolled into one of the following protocols to receive first breeding before 100 DIM: (1) an i.m. dose of prostaglandin (PGF, 2 mL, 250 µg/mL cloprostenol; Estrumate, Schering-Plough Animal Health) and activity monitors observed daily for estrus detection; or (2) Ovsynch protocol: 100 µg i.m. of gonadorelin acetate (GnRH, 2 mL; Factrel, Zoetis), 7 d post-GnRH i.m. 2 mL of PGF, 56 h post-PGF i.m. 2 mL of GnRH, and insemination 12 h later.

Pregnancy diagnosis was carried out via transrectal ultrasonography at 35 ± 7 d after AI on both farms. A cow was considered pregnant when a viable embryo (with a heartbeat) was present. Conception risk at first AI was calculated by dividing the number of cows that were pregnant at 35 ± 7 d after AI by the number of inseminated cows.

Statistical Analyses

Sample size calculations were carried out to determine a minimum sample size (n = 90 per group) required to identify a difference of 18% (13% vs. 31%; Genís et al., 2018) in the incidence risk of metritis, and a minimum sample size (n = 178 per group) to identify a difference of 15% (45% vs. 30%; Peter et al., 2018) in the conception risk at first service between cows receiving the probiotic and control treatments, with 95% confidence and 80% power. Both sample sizes included adjustment for confounders (adjustment factor = 1.1) and the intraclass correlation coefficient between individuals in the cluster.

Distributions and normality tests were obtained using the Univariate procedure of SAS University Edition (SAS Institute Inc.). Parity was divided into primiparous (cows in first lactation) and multiparous (cows in second lactation or greater). Body condition score in second lactation or greater). Body condition score at enrollment, and at 6 and 12 d postpartum), calving ease, calf size, stillbirth, and milk production, and the interactions of treatment with farm and parity, with cow nested within farm as a random effect, using linear mixed regression models (GLIMMIX). Conception risk after first AI and whether cows were inseminated after a spontaneous estrus (vs. hormonal treatment) were used as binomial dependent variables and tested for the effects of treatment (control vs. probiotic), farm, preceding dry period length, parity, BCS (at enrollment and at 6 and 12 d postpartum), calving ease, calf size, stillbirth, and milk production, and the interactions of treatment with farm and parity, with cow nested within farm as a random effect, using linear mixed regression models (GLIMMIX).
had 2 lactations within the data set (11.6%, 70/606). Treatment was applied at random for the second lactation. Data were insufficient to test the effect of CE on conception risk after first AI. Separate models were built for the effect of treatment on metritis and CE, due to uterine diseases being intervening variables. All animals (n = 606) enrolled in the study were used within the analysis. However, 7.7% of enrolled cows were culled before first AI or before pregnancy diagnosis of first AI and thus not included for the assessment of conception risk after first AI. Effects of treatment (control vs. probiotic), metritis, and CE on hazard of pregnancy by 300 DIM was analyzed using a Cox proportional hazards regression model (PHREG procedure). For these models, treatment, farm, parity, and milk production were tested as explanatory variables, as well as the interactions of treatment with farm and parity. For hazard of pregnancy, observations were right-censored at culling or at 300 DIM if pregnancy had not been previously confirmed.

Unadjusted survival curves were drawn from the proportion of nonpregnant cows at each time point given, using the LIFETEST procedure. For all analyses, separate models were used for collinear variables, such that the effects of metritis and CE, the effects of BCS on 6 and 12 d postpartum, as well as the effects of 305-d equivalent milk yield, and accumulative milk yield at 45 and 60 d were all modeled separately from the collinear counterpart. For all models, only variables with a P-value <0.15 were kept in final models, using manual backward elimination. Differences between groups with $P \leq 0.05$ were considered significant, and those between $0.05 > P \leq 0.10$ were designated as a tendency.

**RESULTS**

**Animals and Farm**

A total of 606 Holstein dairy cows, 176 primiparous and 430 multiparous, were enrolled in this study. The average (±SD) number of doses of probiotics that the cows belonging to the probiotic treatment received was 5.7 ± 1.3, and the median was 6 (ranging from 1 to 9 doses of probiotic). The majority of cows received 5 to 7 doses (77%), and only a small proportion received either 1 to 4 doses (13%) or 8 to 9 doses (10%). Descriptive data of each farm are described in Table 1. Descriptive data regarding treatments are described in Table 2.

### Factors Affecting the Incidence Risk of Metritis

Total incidence risk of metritis was 23.7% and 34.4% on farm A and farm B, respectively. Calf size was not associated with the incidence risk of metritis [large calf (41.3 ± 6.7%) vs. medium calf (39.0 ± 5.1%) vs. small calf (33.9 ± 6.4%); $P = 0.6$]. Cows that were classified as having unassisted parturition had a lower incidence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Farm A</th>
<th>Farm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals enrolled</td>
<td>321</td>
<td>285</td>
</tr>
<tr>
<td>Treatment1 (%), [n/n]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51.7 [166/321]</td>
<td>48.7 [139/285]</td>
</tr>
<tr>
<td>Probiotic</td>
<td>48.3 [155/321]</td>
<td>51.3 [146/285]</td>
</tr>
<tr>
<td>Doses of probiotic2</td>
<td>5.8 ± 1.1</td>
<td>5.6 ± 1.6</td>
</tr>
<tr>
<td>Length of preceding dry period3 (d)</td>
<td>57.5 ± 13.1</td>
<td>32.6 ± 15.9</td>
</tr>
<tr>
<td>Milk production4</td>
<td>24.3 ± 6.2</td>
<td>32.1 ± 7.6</td>
</tr>
<tr>
<td>45 DIM (kg/d)</td>
<td>41.7 ± 6.4</td>
<td>39.7 ± 9.4</td>
</tr>
<tr>
<td>60 DIM (kg/d)</td>
<td>12,717 ± 1,936</td>
<td>11,567 ± 2,117</td>
</tr>
<tr>
<td>305 ME (kg)</td>
<td>75.4 ± 15.1</td>
<td>77.2 ± 22.1</td>
</tr>
<tr>
<td>DIM at first AI5 (d)</td>
<td>27 ± 2.2</td>
<td>2.9 ± 2.4</td>
</tr>
<tr>
<td>Days to conception7 (d)</td>
<td>126.0 ± 55.4</td>
<td>159.0 ± 98.7</td>
</tr>
<tr>
<td>Stillborn calves8 (%) [n/n]</td>
<td>8.7 [28/321]</td>
<td>7.7 [22/285]</td>
</tr>
</tbody>
</table>

1Treatment = percentage of cows that received either control or probiotic treatment.
2Doses of probiotic = number of doses of probiotic that was administered prior to calving.
3Length of preceding dry period = total days dry from previous lactation of the enrollment.
4Milk production = total milk production cumulative in 45 and 60 d postpartum in kg/d; 305 ME (mature-equivalent) total production (kg) during the entire lactation.
5DIM at first AI = days in milk when the first artificial insemination occurred.
6Total times bred = average total times that each cow was bred until achieving a positive pregnancy result.
7Days to conception = total days in milk to became pregnant.
8Stillborn calves = calves classified as alive or dead within 12 h of parturition.
risk of metritis compared with cows classified as having an assisted parturition (27.9 ± 2.0% vs. 48.3 ± 1.6%; P = 0.02). Cows that had stillborn calves had a higher incidence risk of metritis (44.3 ± 6.9%) compared with cows that had live calves (24.1 ± 2.3%; P < 0.01). Primiparous cows had a lower incidence risk of metritis compared with multiparous cows (28.2 ± 5.2% vs. 40.2 ± 4.0%, respectively; P = 0.03). Body condition scores at enrollment (P = 0.76) and 6 DIM (P = 0.68) were not associated with the incidence risk of metritis postpartum; however, cows with thin BCS measured at 12 DIM were more likely to have metritis compared with average and moderate BCS (respectively, 38.9 ± 3.6% vs. 25.2 ± 4.1% vs. 21.0 ± 5.2%; P < 0.01). The length of the preceding dry period was associated with metritis diagnosis (P = 0.02), as cows with a normal duration had a lower incidence risk of metritis compared with cows that had short and long durations of the dry period (36.4 ± 5.4% vs. 51.1 ± 6.0% vs. 42.1 ± 5.9%, respectively).

**Factors Affecting the Incidence Risk of Cytological Endometritis**

Total prevalence of CE on farm A was 32.2%. Calving difficulty (P = 0.38), calf size (P = 0.13), prepartum BCS (P = 0.58), and BCS at 6 d (P = 0.49) and at 12 d (P = 0.68) were not associated with the incidence risk of CE. Multiparous cows were more likely to have CE (42.5 ± 4.8%; P = 0.01) compared with primiparous cows (19.9 ± 5.3%). Cows that did not have metritis early postpartum were less likely to have CE compared with cows that were diagnosed with metritis (20.3 ± 4.8%, vs. 54.4 ± 7.0%; P < 0.001). The length of the preceding dry period was associated with CE, as cows with a short duration were more likely to have CE compared with cows that had a normal or longer-duration dry period (50.0 ± 14.0% vs. 27.3 ± 4.5% vs. 25.8 ± 7.9%, respectively; P = 0.05).

**Effect of Treatment on the Incidence Risk of Metritis and Cytological Endometritis**

We detected no effect of treatment on metritis (control = 41.6 ± 3.8%, vs. probiotic = 38.6 ± 4.0%; P = 0.42); however, we found a farm interaction (P = 0.005), as shown in Figure 1, where cows treated with probiotics were less likely to have metritis than control cows on farm A, but no effect of treatment was found on farm B. Cows receiving the probiotic treatment on farm A were also less likely to have CE (23.3 ± 5.5%) compared with animals assigned to the control treatment (39.1 ± 5.6%; P = 0.02); no CE data were collected on farm B. There was no effect of number of doses administered before calving on either metritis (P = 0.13) or CE (P = 0.24). No interaction was found between treatment and parity on the incidence risk of metritis (P = 0.41).

**Factors Associated with Fertility Outcomes**

We found no effect of treatment on conception risk after first AI (control = 27.4 ± 2.6%, vs. probiotic = 26.3 ± 2.6%; P = 0.72), but cows that were diagnosed with metritis had lower conception risk after first AI compared with cows that were not metritic (metritis = 16.7 ± 3.6%, vs. nonmetritic = 25.3 ± 2.5%; P = 0.04). Additionally, cows diagnosed with CE at 42 ± 3 d had lower conception risk after first AI compared with cows that had ≤5% PMN (7.1 ± 5.7% vs. 28.1 ± 3.7%, respectively; P < 0.001). Days in milk at culling (median ± SD) for control vs. probiotic cows were 188.5 ± 133.9 d vs. 205.0 ± 129.6, respectively (P = 0.71). From the 606 cows enrolled, 69.6% were bred by spontaneous estrus, 22.7% were bred following hormonal intervention, and 7.7% were not inseminated before culling. Cows that were bred by spontaneous estrus, detected by an automated activity monitor, had greater conception risk after first AI compared with cows that...
were bred following hormonal intervention (28.4 ± 2.3% vs. 13.7 ± 3.9%, respectively; \( P = 0.04 \)). Calving difficulty (\( P = 0.79 \)), cumulative milk production at 45 d (\( P = 0.99 \)) and 60 d (\( P = 0.57 \)), parity (\( P = 0.99 \)), and farm (\( P = 0.24 \)) were not associated with conception risk after first AI. We detected effects of treatment, metritis, and farm on the proportion of animals bred by spontaneous estrus (as detected by automated activity monitor). Cows in the probiotic treatment were more likely to be inseminated by spontaneous estrus compared with cows in the control treatment (87.5% ± 3.0 vs. 79.6% ± 2.9, respectively; \( P = 0.05 \)). A lower proportion of cows diagnosed with metritis were inseminated after spontaneous estrus compared with cows without metritis (72.9% ± 4.1 vs. 82.9% ± 2.6, respectively; \( P < 0.01 \)). Farm A inseminated fewer cows by spontaneous estrus compared with farm B (72.5% ± 4.2 vs. 84.4% ± 2.5, respectively; \( P < 0.009 \)).

The hazard of pregnancy in the first 300 DIM was associated with metritis, CE, and parity, as well as an interaction between treatment and parity. Multiparous cows receiving the probiotic treatment were more likely to become pregnant compared with multiparous cows in the control group [hazard ratio (HR) = 1.33; 95% CI = 1.10–1.60; \( P = 0.003 \)], but no effect of probiotic treatment was found on the hazard of pregnancy for primiparous cows, as shown in Figure 2. Primiparous cows were more likely to become pregnant compared with multiparous cows (HR = 1.26; 95% CI = 0.71–0.91; \( P = 0.05 \)). Cows that did not have metritis early postpartum were more likely to become pregnant compared with cows that had metritis (HR = 1.43; 95% CI = 1.17–1.75; \( P < 0.001 \); Figure 3). We detected no interaction between metritis (metritic vs. nonmetritic) and parity on the hazard of pregnancy (\( P = 0.85 \)). Cows without CE were more likely to become pregnant than cows diagnosed with CE (HR = 1.71; 95% CI = 1.21–2.41; \( P = 0.002 \); Figure 4). We found an interaction between CE and parity on the hazard of pregnancy, as multiparous cows that were not diagnosed as CE were more likely to become pregnant compared with multiparous cows that were diagnosed as CE (HR = 1.74; 95% CI = 1.16–2.60; \( P < 0.001 \)); no effect of CE was found on the hazard of pregnancy for primiparous cows, as shown in Figure 5.

**DISCUSSION**

Our study demonstrated the benefit of using intravaginal probiotics in reducing the incidence risk of metritis and CE, although farm differences were observed. Irrespective of farm, cows receiving probiotic treatment were found to have a decreased need of hormone intervention to synchronize cows for AI, due to increased detection of probiotic cows in estrus using automated activity monitors. No direct associations of probiotic treatment were found on fertility outcomes (conception risk at first AI or days to conception), although cows not diagnosed with metritis or CE had increased fertility. We found no effect of the number of doses administered before calving on metritis. However, the range in doses administered in this study did not have a large variation, as the majority of cows received 5 to 7 doses (77%) and only a small proportion received either 1 to
4 doses (13%) or 8 to 9 doses (10%). Future studies are needed to determine whether there is a minimum number of doses required before calving to be beneficial.

During the transition period, cows are subjected to dramatic changes in their metabolism, immune system, and endocrine function, resulting in increased susceptibility to diseases (Esposito et al., 2014; Zebeli et al., 2015). The objective of this study was to evaluate the effects of intravaginal probiotic use prepartum on the incidence risk of metritis and CE postpartum and overall fertility outcomes. The combination of the probiotic strains used in this study has previously been shown to reduce infection in vitro and in vivo (Genís et al., 2016). Genís et al. (2018) reported that the same strains of lactobacilli could regulate inflammation in the endometrium, which has the potential to alleviate the negative effects of uterine infections on fertility. For defense against uterine infections, including metritis and endometritis, species of lactobacilli have become frontrunners in the search for probiotic candidates to improve uterine health (Ametaj et al., 2014; Genís et al., 2016). In our study, the intravaginal application of probiotics twice per week during the 3-wk period before calving was able to reduce the incidence risk of metritis and CE; however, there was a farm interaction, where the use of probiotics only reduced the incidence of metritis on 1 of the 2 farms enrolled. It must be noted that this study did not have a negative control (sterile saline only), and thus this study cannot separate the effects of the application of intravaginal probiotics and the application of sterile saline on the development of uterine disease.

The development of uterine diseases is dependent on several factors, such as immunity, health, genetics, the uterine environment, and the number, species, and pathogenicity of bacteria (Williams et al., 2005;
Sheldon et al., 2008, 2009). The presence of pathogenic microorganisms alone is not usually enough to induce illness, and clinical disease is frequently the result of the interaction of these numerous factors, which could explain the difference in the incidence of uterine diseases reported between farms. Generally accepted risk factors for uterine disease, which also vary by farm, many of which were also described in this study, include the calving environment, twins, size of the calf, difficulty calving (dystocia), and retained placenta (LeBlanc, 2008; Sheldon et al., 2008). In the current study, we did not find an association between calf size and uterine disease; this may be due to using a visual assessment of calf size, which is more subjective than a quantitative assessment, such as measuring calf weight. Unfortunately, environmental factors were not measured objectively during the study and thus could not be assessed for their influence on the efficacy of the probiotics between the different farms. However, anecdotally, the farm in which the probiotics were more effective housed prepartum and parturient cows under housing conditions that were more regularly maintained (i.e., maternity pen cleaned more often, cows visibly cleaner). The findings reported in this study are in agreement with other studies that have shown that the incidence risk of uterine disease (i.e., metritis and subclinical endometritis) varies by farm and can range from 8 to 40% for metritis (Zwald et al., 2004; Galvão et al., 2011) and from 5 to 30% for subclinical endometritis (LeBlanc et al., 2002; Galvão et al., 2011). Future research is needed to understand the interaction between prepartum intravaginal probiotics and housing conditions.

Probiotics have the potential to enhance animal health and immunity. By increasing the density and diversity of commensal bacteria, competitive effects can
reduce a pathogen’s ability to adhere to and colonize in the epithelial layers (Guillot, 2001). Furthermore, some beneficial microbes produce antimicrobial substances and interfere with production of bacterial toxins (Vandenbergh, 1993; Brandão et al., 1998). Probiotics can also stimulate the immune response to eliminate pathogens more effectively compared with antimicrobial treatment (Isolauri et al., 2001). Thus, the modulatory effects of probiotics on the composition of the resident microbial community could have offered protection from invading pathogens. Evidence also suggests that application of lactic acid probiotics within the vagina of dairy cows can increase the immune response at the local level (Kassé et al., 2016).

Lactobacilli have been associated with beneficial characteristics including their ability to adhere to epithelial tissue and production of antimicrobial substances (Mu et al., 2018). Lactobacilli can inhibit growth of bacterial pathogens by producing acetic acid, lactic acid, hydrogen peroxide, and bacteriocins (Arotutcheva et al., 2001). A study by Genís et al. (2017a), administering intravaginal probiotics containing *L. rhamnosus*, *P. acidi-dilactici*, and *L. reuteri*, did not find that the probiotics were able to reach the endometrium; however, they did observe a downregulation of genes involved in mounting an immune response to bacterial infection (β-defensins and *MUC1*), suggesting that the probiotics present in the vagina were able to reduce the number of pathogens reaching the endometrium.

Polymorphonuclear neutrophils are the primary phagocytic cells responsible for eliminating bacteria from the uterine environment (Sheldon et al., 2009). Immunity in the female genital tract is faced with unique challenges. The uterine immune response must be able to selectively detect and eliminate foreign invaders but must not protect against spermatozoa and a developing fetus (Beagley and Gockel, 2003; Wira and Fahey, 2004). Additionally, excessive inflammation can

---

*Figure 4.* Survival curve of the effect of cytological endometritis diagnosis on the hazard of pregnancy in the first 300 DIM.
impair fertilization, ovarian function, and embryonic development (Gilbert, 2011). Cows that were diagnosed with metritis and CE had lower fertility compared with cows that did not have uterine disease. The dairy industry relies on the establishment of pregnancy, and it is commonly acknowledged that uterine infections reduce reproductive outcomes. A meta-analysis reported that metritis caused subfertility in cattle: time to first pregnancy was increased, conception rate was reduced by 20%, and the interval between calving and conception was increased by 18.6 d (Fourichon et al., 2000). Cows with subclinical endometritis had a 27% reduction in conception rate and had a 32-d increase in time to pregnancy (LeBlanc et al., 2002). Infertility caused by uterine infection is influenced by the modulatory effects of bacteria and inflammation along the reproductive tract. Importantly, it has been discovered that the effects of uterine infection on fertility persist even after successful treatment (Borsberry and Dobson, 1989). The mechanisms by which uterine infections affect fertility are multimodal and involve uterine function, ovarian function, the hypothalamus, and the pituitary gland (Sheldon et al., 2008; Bromfield et al., 2015).

Cows that were diagnosed with metritis and CE had more days to conception and were more likely to require hormonal intervention (e.g., prostaglandin, Ovsynch) for the first AI postpartum. A study conducted by Ametaj et al. (2014) did not find that intravaginal administration of lactic acid bacteria improved conception risk at first insemination, nor were there any effects on the cumulative insemination rate, but significant inferences cannot be drawn due to the low sample size of the study. Peter et al. (2018) did find that reproductive performance was improved after administration of Lactobacillus buchneri in cows with subclinical endometritis. Overall conception at first service in that study was found to be 45.7% for the probiotic treatment, compared with 30% for their placebo group. Interestingly, Peter et al. (2018) also found a protective effect of probiotic use in cows with uterine disease, where cows diagnosed with subclinical endometritis had a first-service conception rate of 60% if they received the probiotic treatment but only 13.3% if they were in the placebo group; however, again it is noteworthy that the sample size of that study was limited in terms of fertility outcomes. Furthermore, Peter et al. (2018) found a median number of days to conception of 74 d for cows receiving the probiotic and 164 d for cows receiving a placebo. This same study found lower levels of proinflammatory factors for cows receiving the
probiotic, suggesting that the improved pregnancy rate may be a result of reduced inflammation in the uterus.

Ovarian function is affected by changes in cellular pathways induced by bacterial infection. Both follicular growth and oocyte development are inhibited in the presence of pathogens (Bromfield et al., 2015). In general, cows with uterine infection have lower chances of resumption of cyclicity postpartum, produce smaller ovarian follicles, and have lower circulating concentrations of estradiol (Sheldon et al., 2008), which is essential for induction of peak LH required for ovulation (Roche, 1996; Forde et al., 2011). These factors could potentially be the link between probiotic use and the reduction in hormonal intervention needed for first insemination postpartum. It should be noted that this study was carried out on 2 farms, where management for first insemination was based on the use of estrus detection using automated monitors, such that the enrollment to a hormonal fertility treatment most likely reflects this group of cows having some ailment that reduced their ability to demonstrate estrus appropriately, such as those related to health, as described above. Furthermore, the low conception risk found for inseminations after hormonal intervention may also be linked to that subpopulation of cows being ill or having some sort of reproductive disfunction.

**CONCLUSIONS**

Overall, this study demonstrated the efficacy of administering probiotics intravaginally to prepartum cows at reducing the incidence risk of metritis and cytological endometritis, although this association was influenced by farm effects. Although further research is required to determine the most effective strains, dose, timing and frequency of administration, and important factors associated with individual farms (e.g., management, housing environment), this study demonstrates potential for preventative probiotics to become a powerful tool to reduce the need of antibiotics for the treatment of uterine infections. Moreover, probiotics could serve as a more effective tool due to their ability to prevent uterine infections and decrease the need of hormone intervention at insemination, due to increased detection of cows in estrus for first postpartum AI.

**ACKNOWLEDGMENTS**

This study was supported by a contribution from the Natural Sciences and Engineering Research Council (NSERC; Ottawa, Canada), the Dairy Industry Research and Education Committee (DIREC, British Columbia Dairy Association; Burnaby, Canada), and the Mitacs Globalink Program (Ottawa, Canada). We are grateful to Sandra Genís (Institut de Recerca i Tecnologia Agroalimentàries, Barcelona, Spain) for all her help and expertise needed for the concepts of this study. We are also grateful to the University of British Columbia’s Dairy Education and Research Centre (Agassiz, Canada) as well as to their farm personnel for contributing to this research project. The authors have not stated any conflicts of interest.

**REFERENCES**


ORCIDs
A. M. L. Madureira https://orcid.org/0000-0001-9696-8478
T. A. Burnett https://orcid.org/0000-0003-2417-4016
C. T. Boyd https://orcid.org/0000-0001-5184-4228
R. L. A. Cerri https://orcid.org/0000-0002-8169-8900