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# Failure of clinical cure in dairy cows treated for metritis is associated with reduced productive and reproductive performance

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## ABSTRACT

Objectives were to assess reproductive and productive outcomes associated with failure of clinical cure in dairy cows diagnosed with metritis following antimicrobial therapy. This retrospective cohort study included data from 3 experiments performed in 5 dairies. Metritis was characterized by the presence of watery, fetid, reddish-brownish vaginal discharge within 21 DIM (study d 0). Cows not diagnosed with metritis (i.e., cows may have had other diseases postpartum; NoMT; n = 1,194) were paired based on lactation number and calving date. All cows with metritis received antimicrobial therapy (ampicillin or ceftiofur). Clinical cure was evaluated on d 10 based on vaginal discharge score, and cows were categorized as cured (MTC; n = 1,111) or not cured (MTnoC; n = 299). Purulent vaginal discharge (28 ± 3 or 32 ± 3 DIM), cytological endometritis (35 ± 3 or 39 ± 3 DIM), and estrous cyclicity (50 ± 3 and 64 ± 3, 36 ± 3 and 50 ± 3, or 37 ± 5 and 51 ± 5 DIM) were evaluated in subgroups of cows. Proportions of cows with purulent vaginal discharge and cytological endometritis were greatest for MTnoC (91.7 and 91.4%), intermediate for MTC (74.0 and 73.3%), and smallest for NoMT (38.1 and 36.4%). Proportion of cyclic cows was smaller for MTnoC compared with MTC and NoMT (62.0, 71.0, and 71.0%). Pregnancy per artificial insemination following first service was smaller for cows with metritis compared with their counterparts with no metritis (NoMT = 28.1, MTC = 26.1, MTnoC = 22.0%). Pregnancy loss tended to be greater for MTnoC compared with MTC (NoMT = 11.5, MTC = 11.1, MTnoC = 18.4%). Hazard of pregnancy by 300 DIM was smallest for MTnoC, intermediate for MTC, and greatest for NoMT. Death by 60 DIM (3.9, 1.1, and 0.6%) and removal from herd by 300 DIM (26.3, 17.4, and 15.4%) were greatest for MTnoC compared with MTC and NoMT, respectively. Milk production among multiparous cows was smaller for MTnoC compared with MTC and NoMT in the first 10 mo postpartum, whereas MTC produced less milk compared with NoMT only during the first 2 mo postpartum (NoMT = 42.0 ± 0.22, MTC = 40.6 ± 0.28, MTnoC = 37.7 ± 0.54 kg/d). Failure of clinical cure was not associated with milk yield in primiparous cows (NoMT = 35.2 ± 0.31, MTC = 33.9 ± 0.31, MTnoC = 35.0 ± 0.52 kg/d). Cows diagnosed with metritis that do not undergo clinical cure by 10 d of onset of antimicrobial therapy have impaired reproductive performance, reduced milk production, and increased risk of leaving the herd.

**Key words:** uterine health, fertility, culling, milk yield

## INTRODUCTION

Metritis is a polymicrobial disease associated with inflammation of multiple layers of the uterine lining that affects 20 to 40% of dairy cows within the first 21 d postpartum (Sheldon et al., 2009; Dubuc et al., 2010a; Jeon et al., 2015). In addition to its effects on animal welfare (Stojkov et al., 2015), metritis is associated with increased odds of purulent vaginal discharge and cytological endometritis (Lima et al., 2014), delayed resumption of estrous cyclicity (Sheldon et al., 2009; Santos et al., 2010), impaired reproductive performance (Santos et al., 2010; Ribeiro et al., 2013; de Oliveira et al., 2020), increased risk of death and culling (Bartlett et al., 1986; Dubuc et al., 2011a; de Oliveira et al., 2020), and reduced milk production (Dubuc et al., 2011b; Wittrock et al., 2011). Altogether, the economic burden associated with each case of metritis ranges between US$267 and 410, considering losses in reproduction, production, costs incurring from treatment, potential discard of nonsellable milk, and replacement of dead and culled cows (Overton and Fetrow; 2008, Lima et al., 2019).

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Antimicrobial therapy is widely recommended for treatment of metritis, although nearly half of affected cows undergo spontaneous clinical cure within 2 wk of diagnosis if left untreated (Chenault et al., 2004; McLaughlin et al., 2012; de Oliveira et al., 2020). Clinical cure in cows treated with ceftiofur crystalline free acid or ceftiofur hydrochloride was 25 to 35% greater compared with untreated controls (Chenault et al., 2004; McLaughlin et al., 2012; de Oliveira et al., 2020). Antimicrobial therapy based on ceftiofur also improved milk production during the first 2 mo of lactation, decreased the proportion of cows classified by farm personnel as ineligible for reproduction, and increased the hazard of pregnancy by 300 DIM (de Oliveira et al., 2020). Despite improving several productive and reproductive outcomes in cows diagnosed with metritis, antimicrobial therapy did not restore milk production, culling, and hazard of pregnancy to those observed in counterparts without metritis (de Oliveira et al., 2020). Clinical signs of uterine disease, evaluated as the presence of reddish or brownish, watery, and fetid vaginal discharge, persisted in 15 to 20% of cows treated with antibiotics in the first 6 to 8 d after completion of treatments (Lima et al., 2014; de Oliveira et al., 2020). Furthermore, experiments that considered absence of fever together with evaluation of vaginal discharge to define clinical cure reported that 25 to 35% of cows treated for metritis failed to undergo clinical cure (Chenault et al., 2004; McLaughlin et al., 2012; Lima et al., 2014). It is important to highlight that similar failure of clinical cure has been reported following treatment of metritis with ceftiofur and ampicillin (Lima et al., 2014; Merenda et al., 2021).

It remains unclear whether the gap in productive and reproductive performance between cows diagnosed with metritis and treated with antimicrobials and cows that do not have metritis is associated with clinical cure of the former. It is possible that diminished uterine health, productivity, and fertility outcomes in the fraction of cows that does not respond to antimicrobial therapy alone explains the gap observed between cows treated for metritis and counterparts not diagnosed with the disease. Because previous experiments were designed to evaluate the effects of therapeutic strategies on health and fertility, the relative contribution of clinical cure remains unknown. We hypothesized that uterine health, fertility, and productive performance are reduced in cows that fail to undergo clinical cure for metritis after receiving antimicrobial therapy compared with herdmates that achieved clinical cure. Moreover, we hypothesized that the decline in milk production associated with failure of clinical cure following treatment for metritis is observed in both primiparous and multiparous cows. Objectives of this study were to assess risk factors associated with failure of clinical cure following antimicrobial therapy in cows diagnosed with metritis. Furthermore, we evaluated the association between clinical cure in cows treated for metritis and ensuing uterine health, resumption of estrous cyclicity, pregnancy per AI (**P/AI**) and pregnancy loss after first insemination postpartum, hazard of pregnancy, milk yield, and culling in lactating dairy cows.

## MATERIALS AND METHODS

### Study Population and Inclusion Criteria

Data from 3 experiments conducted between 2012 and 2018 in 5 dairy farms located in the state of Florida were included in this retrospective cohort study (herds 1 and 2: de Oliveira et al., 2020; herd 3: Lima et al., 2014; herds 4 and 5: Merenda et al., 2021). Study protocols are depicted in Figure 1. Number of lactating cows and herd rolling average for milk yield for each herd were 4,400 and 12,500 kg (herd 1), 1,800 and 10,500 kg (herd 2), 5,700 and 11,000 kg (herd 3), 2,300 and 10,333 kg (herd 4), and 2,500 and 12,049 kg (herd 5), respectively. Lactating Holstein cows were milked twice (herd 4) or thrice daily (herds 1, 2, 3, and 5). Cows were fed a TMR to meet or exceed the nutritional requirements for a 650- to 680-kg Holstein cow producing 40 to 45 kg/d of 3.5% fat-corrected milk (NRC, 2001). Lactating cows were housed in tunnel (herd 1) or naturally ventilated freestall barns (herds 2 to 5) equipped with sprinklers and fans over the feed bunk and fans over the stalls. Cows had ad libitum access to water and deep-bedded sand stalls. Cows were classified as primiparous (lactation = 1) or multiparous (lactation >1) for statistical analyses.

Calving was monitored by trained farm personnel, and cows were assisted as defined by each herd's standard operational procedures. Date and season of calving (spring = March to May; summer = June to August; fall = September to November; winter = December to February) were recorded for each cow. Calving difficulty was scored using a 4-point scale (1 = unassisted parturition; 2 = minimal assistance provided by 1 technician without use of mechanical traction; 3 = mechanical extraction of the calf; 4 = severe dystocia requiring cesarean section or fetotomy) validated for dairy cattle (Schuenemann et al., 2011). Calving difficulty score ≥2 characterized dystocic delivery. Stillbirth was defined as calves delivered dead or that died by 24 h after delivery. Calf sex and twinning were recorded by farm personnel. Retained fetal membranes (**RFM**) was characterized as membranes not detached by 24 h after parturition, based on visual inspection.

### Definition of Metritis and Failure of Clinical Cure

Metritis was diagnosed based on visual assessment of vaginal discharge retrieved using a Metricheck device (Simcro). Cows were evaluated systematically at 4, 6, and 8 DIM (Lima et al., 2014), 5, 7, and 9 DIM (de Oliveira et al., 2020), or 4, 6, 8, 10, and 12 DIM (Merenda et al., 2021). In addition to these pre-established intervals postpartum, rectal temperature was assessed in all cows, and those with fever (i.e., rectal temperature ≥39.5°C) were submitted to an unscheduled evaluation of vaginal discharge on the same day. Descriptive statistics for timing of metritis diagnosis are as follows: minimum = 2 DIM, maximum = 19 DIM, mean ± standard deviation (SD) = 6.4 ± 2.5 DIM, and median = 6 DIM. Vaginal discharge was scored using a 5-point scale (1 = no discharge, clear mucus, or clear lochia; 2 = clear mucus with flecks of pus; 3 = mucopurulent discharge with <50% pus; 4 = mucopurulent discharge with ≥50% pus or reddish mucous discharge, not fetid; 5 = watery, reddish or brownish, and fetid discharge) described for dairy cows (adapted from Chenault et al., 2004). Metritis was defined for cows with vaginal discharge score 5 (n = 1,410), and the day of diagnosis was considered study d 0. Rectal temperature was measured concurrently with evaluation of vaginal discharge (i.e., data available only for cows diagnosed with metritis), and cows with rectal temperature ≥39.5°C were considered febrile (metritis without fever, n = 878; metritis with fever, n = 532). For assessment of the association between metritis and outcomes of interest, cows with metritis were matched with herdmates not diagnosed with metritis (vaginal discharge score ≤4 between 4 and 12 DIM; n = 1,194) based on parity and calving date. Incidences of metritis reported in each study were 36.1% (Lima et al., 2014), 25.3% (de Oliveira et al., 2020), and 19.0% (Merenda et al., 2021). Body condition score was evaluated at d 0 using a scale of 1 (emaciated) to 5 (obese) with 0.25-unit increments (Edmonson et al., 1989), as depicted in the BCS chart by Elanco (Elanco Animal Health, 2009).

Cows diagnosed with metritis received antimicrobial therapy according to experimental protocols starting on d 0. The following treatments were applied across all 3 experiments: ampicillin trihydrate (Polyflex, Boehringer Ingelheim Vetmedica), 11 mg/kg of BW, i.m. injected once daily during 5 consecutive days (n = 566; Lima et al., 2014; Merenda et al., 2021); ceftiofur hydrochloride (Excenel RTU sterile suspension, Zoetis), 2.2 mg/kg of BW, i.m. injected once daily for 5 consecutive days (n = 263; Lima et al., 2014); and ceftiofur crystalline free acid (Excede sterile suspension, Zoetis), 6.6 mg/kg of BW, s.c. injected twice 72 h apart (n = 596; de Oliveira et al., 2020; Merenda et al., 2021). Vaginal discharge was re-evaluated in cows diagnosed with metritis on d 10 and scored as previously described. Failure of clinical cure in cows diagnosed with metritis was defined as vaginal discharge score 5 on d 10. Information regarding clinical cure following treatment for metritis was not disclosed to herd managers and staff. Cows were categorized according to diagnosis of metritis on d 0 and uterine health on d 10, as follows: cows not diagnosed with metritis (**NoMT**; n = 1,194), cows diagnosed with metritis that underwent clinical cure (**MTC**; n = 1,111), and cows diagnosed with metritis that failed to undergo clinical cure (**MTnoC**; n = 299).

### Evaluation of Purulent Vaginal Discharge, Cytological Endometritis, and Estrous Cyclicity

Presence of purulent vaginal discharge was assessed in a subgroup of cows (NoMT: n = 910; MTC: n = 894; MTnoC: n = 227). Vaginal discharge was evaluated at 32 ± 3 DIM (Lima et al., 2014) or 28 ± 3

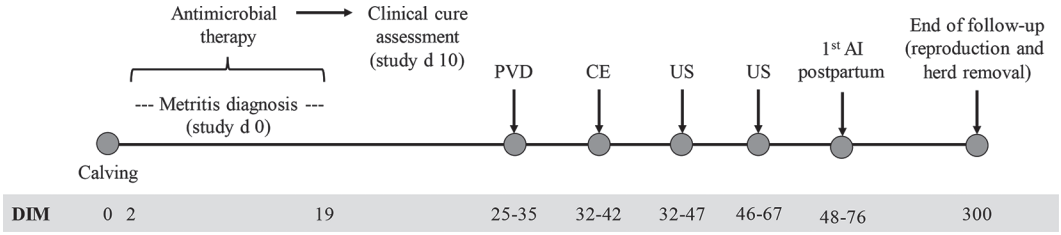


**Figure 1.** Diagram of study procedures. Range in DIM encompasses differences in protocol across studies. Metritis was diagnosed based on evaluation of vaginal discharge (i.e., vaginal discharge score 5; reddish-brownish watery and fetid discharge). All cows with metritis received antimicrobial therapy (ampicillin trihydrate, ceftiofur hydrochloride, or ceftiofur crystalline free acid), and clinical cure was evaluated 10 d after diagnosis based on inspection of vaginal discharge. Cows without metritis were paired based on lactation number and calving date. Study groups consisted of cows not diagnosed with metritis (NoMT; n = 1,194), cows diagnosed with metritis that underwent clinical cure (MTC; n = 1,111), and cows diagnosed with metritis that failed to undergo clinical cure (MTnoC; n = 299). CE = assessment of cytological endometritis; PVD = assessment of purulent vaginal discharge; US = ultrasonographic evaluation of ovarian structures for characterization of estrous cyclicity based on presence of a corpus luteum.

DIM (Merenda et al., 2021) using the Metricheck device and scored as described previously (Chenault et al., 2004). Cows with vaginal discharge score ≥3 were diagnosed with purulent vaginal discharge (Dubuc et al., 2010b). Endometrial cytology was performed in a subgroup of cows (NoMT: n = 239; MTC: n = 372; MTnoC: n = 88). Endometrial cytology was evaluated at 39 ± 3 DIM (Lima et al., 2014) or 35 ± 3 DIM (Merenda et al., 2021) using a cytobrush attached to a stainless-steel gun (Lima et al., 2013). In brief, cows had their perineal area cleaned and disinfected with ethanol (70% vol/vol) before insertion of the cytology gun protected by a plastic sheath. Once the cytology gun was manipulated through the cervix into the uterine body, the cytobrush was exposed and rolled against the endometrium. The cytobrush was recovered, rolled onto a glass slide after collection, air-dried, and stained using Diff-Quik (JorVet Dip Quick Stain Kit, Jorgensen Labs) for differential cell count under microscope. Cows with percentage of polymorphonuclear leukocytes ≥6% were diagnosed with cytological endometritis (Dubuc et al., 2010b; Denis-Robichaud and Dubuc, 2015). Resumption of estrous cyclicity postpartum was assessed in a subgroup of cows (NoMT: n = 486; MTC: n = 729; MTnoC: n = 174). Cows had their ovaries evaluated using transrectal ultrasonography at 50 ± 3 and 64 ± 3 DIM (Lima et al., 2014), 36 ± 3 and 50 ± 3 DIM (de Oliveira et al., 2020), and 37 ± 5 and 51 ± 5 DIM (Merenda et al., 2021). Cows with a visible corpus luteum with diameter >15 mm in at least 1 of the examinations were classified as estrous cyclic, whereas counterparts without visible corpus luteum in both exams were deemed anovular.

### Reproductive Management and Calculation of Reproductive Outcomes

In herd 1, after a 48-d voluntary wait period (**VWP**), cows were observed once daily for signs of estrus based on rubbing of a mount detection patch (Estrotect, Rockway). Cows not inseminated by 55 ± 3 DIM were treated with $PGF_{2\alpha}$, and those not inseminated by 72 ± 3 DIM were enrolled in a 7-d Ovsynch-56 protocol (GnRH, 7 d later $PGF_{2\alpha}$, 56 h later GnRH, 16 h later timed AI). Pregnancy was diagnosed 33 ± 3 d after AI via transrectal ultrasonography and 70 ± 3 d after AI via rectal palpation. Cows diagnosed as nonpregnant received an injection of GnRH and were enrolled in the 7-d Ovsynch-56 protocol 7 d later. In herd 2, after a 50-d VWP, cows were observed once daily for signs of estrus based on removal of tail chalk. Cows not inseminated by 70 ± 3 DIM were enrolled in a 7-d Ovsynch-56 protocol. Pregnancy was diagnosed 43 ± 3 and 70 ± 3 d after AI via rectal palpation, and nonpregnant cows were enrolled in a 7-d Ovsynch-56 protocol. In herd 3, after a 60-d VWP, cows were observed once daily for signs of estrus based on removal of tail chalk. Cows were treated with $PGF_{2\alpha}$ at 50 ± 3 and 64 ± 3 DIM, and those not inseminated by 76 ± 3 DIM were enrolled in a 5-d Cosynch-72 protocol (GnRH, 5 and 6 d later $PGF_{2\alpha}$, 2 d later GnRH and timed AI). Pregnancy was diagnosed 34 ± 3 and 62 ± 3 d after AI via transrectal ultrasonography, and nonpregnant cows were enrolled in the 5-d Cosynch-72 protocol. In herds 4 and 5, cows were treated with 2 injections of $PGF_{2\alpha}$ (herd 4: 35 ± 3 and 49 ± 3 DIM; herd 5: 39 ± 3 and 53 ± 3 DIM) and were enrolled in a 7-d Cosynch-72 protocol (GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH and timed AI) starting at 63 ± 3 DIM (i.e., no estrus detection before first AI, VWP = 63 d). Pregnancy was diagnosed 40 ± 3 d after AI via rectal palpation and 70 ± 3 d after AI via transrectal ultrasonography. Cows diagnosed as nonpregnant were enrolled in the 7-d Cosynch-72 protocol.

Loss to follow-up represents a potential bias for analyses of selected reproductive outcomes. For instance, cows more severely affected by metritis or failure of clinical cure might have been withheld from insemination (i.e., due to death, culling, and reproductive culling) more extensively compared with counterparts that were not diagnosed with metritis or those that underwent clinical cure. Therefore, the proportion of cows that received at least 1 AI during lactation was analyzed to complement interpretation of results regarding P/AI. Pregnancy was diagnosed 38 ± 8 (first exam) and 66 ± 7 d after AI (second exam) across all experiments. Therefore, first and second exams are referred to as 38 and 66 d after AI, respectively. Pregnancy per AI was calculated as the number of cows diagnosed pregnant 38 or 66 d after AI divided by the total number of cows that received AI. Pregnancy loss was calculated as the number of cows that lost a pregnancy between 38 and 66 d of gestation divided by the number of cows pregnant 38 d after AI. The follow-up period for reproductive outcomes was 300 DIM. Time to pregnancy was defined as DIM at the AI that resulted in a pregnancy confirmed at 66 d.

### Milk Production and Removal from Herd

Herds 1, 2, 4, and 5 participated in the monthly DHIA test, and individual production was recorded in the herd's management software system (PCDART, Dairy Records Management System). Test days were aligned with month postpartum, and milk production data from the first 10 mo postpartum were collected for NoMT, (n = 854), MTC (n = 640), and MTnoC cows

(n = 212). Date, DIM, and reason for removal from herd (i.e., died or culled) were recorded for cows that left the herd by 300 DIM.

### Experimental Design, Sample Size Calculations, and Statistical Analyses

This retrospective cohort study encompasses data from 3 previously published experiments that evaluated clinical cure in cows treated for metritis and recorded data related to subsequent fertility and production parameters (Lima et al., 2014; de Oliveira et al., 2020; Merenda et al., 2021). The final data set used for statistical analyses included 1,194 cows not diagnosed with metritis, 1,111 cows diagnosed with metritis that underwent clinical cure, and 299 cows diagnosed with metritis that failed to undergo clinical cure. Sample size calculation regarding time to pregnancy was performed using an online tool (Kohn and Senyak, 2020), whereas calculations for binary and continuous outcomes were performed using the POWER procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc.). Considering that 21.2% of cows treated for metritis in our study failed to achieve clinical cure, which agrees with values reported by others (Chenault et al., 2004; McLaughlin et al., 2012), 1,410 cows with metritis were deemed sufficient to detect a 20% decrease in the hazard of pregnancy [hazard ratio (**HR**) = 0.83] for MTnoC compared with MTC ($\alpha = 0.05$, $\beta = 0.20$). Conversely, using purulent vaginal discharge as a proxy for clinical cure failure after treatment for metritis, due to the prolonged exposure to clinical signs of uterine disease in the latter cohort, pointed toward a greater decrease in hazard of pregnancy (HR = 0.72; LeBlanc et al., 2002). For analyses of risk factors associated with failure of clinical cure after treatment for metritis, results from post hoc power calculations based on observed differences and number of cows available per condition were 0.08 (parity), 0.99 (calving season), 0.17 (dystocia), >0.99 (RFM), 0.51 (twinning), 0.38 (stillbirth), 0.05 (BCS on d 0), 0.97 (fever on d 0), and 0.09 (treatment) for $\alpha = 0.05$. Because of limited number of experimental units and restricted statistical power, interactions between risk factors were not included in the multivariable model. Considering an SD for milk production of 4.7 kg/d (Douglas et al., 2006; Smith et al., 2009; Yasui et al., 2014; Greco et al., 2015), numbers of cows available per group of metritis clinical cure were deemed sufficient to detect a difference in milk yield of 1.5 kg/d in primiparous and 1.4 kg/d in multiparous cows ($\alpha = 0.05$, $\beta = 0.20$).

For all statistical analyses, the association between independent variables and outcomes of interest was assessed initially using univariable models. All independent variables with $P \leq 0.10$ in univariable analyses were included in each multivariable model. Independent variables were removed from multivariable models using a backward stepwise elimination method if $P > 0.10$ (i.e., sequentially from the largest to smallest $P$-value), with the exception of study and group of metritis clinical cure that were forced into all final models. Type of AI was categorized as following estrus detection or timed AI protocol, and BCS was categorized as low (≤2.75), moderate (3.00 to 3.50), or high (≥3.75).

Binary responses were analyzed by logistic regression using the LOGISTIC, FREQ, and GLIMMIX procedures of SAS. Initial models for the analyses of risk factors associated with failure of clinical cure in cows diagnosed with metritis included the fixed effects of study, parity, calving season, dystocia, RFM, twinning, stillbirth, BCS category, antimicrobial therapy, and fever. Initial models for the analyses of purulent vaginal discharge, cytological endometritis, estrous cyclicity, and reproductive outcomes included the fixed effects of study, group of metritis clinical cure, parity, BCS category, and first AI type and season (i.e., analyses of P/AI and pregnancy loss only). Initial models for the analyses of death and removal from herd (i.e., died or culled) included the fixed effects of study, group of metritis clinical cure, calving season, and parity. Results were reported as odds ratios (univariable model) and adjusted odds ratios (multivariable models) followed by 95% confidence interval (**CI**). Receiver operator characteristic curves were built using JMP Pro version 15.0.0 (SAS Institute Inc.) to determine the DIM at diagnosis of metritis that resulted in greatest combined sensitivity and specificity to describe clinical cure failure following antimicrobial therapy. Time-dependent variables were analyzed via Cox's proportional hazard model using the PHREG procedure of SAS and Kaplan-Meier survival curves using the LIFETEST procedure of SAS. For analyses of time to removal from herd, cows were right-censored at completion of the follow-up period (60 or 300 DIM as indicated for each analysis). For analyses of time to pregnancy, cows were right-censored when they were deemed no longer eligible for reproduction, left the herd before 300 DIM, or reached 300 DIM as a nonpregnant cow. Initial models included the fixed effects of study, group of metritis clinical cure, and parity. Results were reported as adjusted HR (**AHR**) and 95% CI, unless indicated otherwise. Milk production for the first 10 mo postpartum was analyzed by ANOVA for repeated measures using the GLIMMIX procedure fitting a normal distribution and considering cow nested within study as a random variable. Initial models included the fixed effects of study, group of metritis clinical cure, parity, month postpartum, and relevant 2-way (group of metritis clinical cure and par-

ity) and 3-way interactions (group of metritis clinical cure, parity, and month postpartum). Heterogeneous first-order autoregressive was selected as structure of covariance because it resulted in the smallest Bayesian and Akaike's information criteria. Assumptions of normality of residuals and homogeneity of variance were evaluated using the UNIVARIATE procedure.

Data regarding rectal temperature and type of antimicrobial therapy were available only for cows diagnosed with metritis. Therefore, a separate set of analyses were conducted to assess the association between fever on d 0 (fever vs. no fever), the 2-way interaction between group of metritis clinical cure and fever, and the effect of antimicrobial therapy (ampicillin vs. ceftiofur) on outcomes of interest considering MTC and MTnoC only. These 3 independent variables were included as fixed effects in the final multivariable model for the analyses described earlier and were subsequently removed using a backward stepwise elimination method if $P > 0.10$. Orthogonal contrasts were built to evaluate the effects of metritis (NoMT vs. MTC + MTnoC) and failure of clinical cure (MTC vs. MTnoC). The *P*-values for all pairwise comparisons were adjusted using the method of Tukey. Differences with $P \leq 0.05$ were considered significant, and those with $P \leq 0.10$ were considered tendencies.

## RESULTS

### Risk Factors Associated with Failure of Clinical Cure in Cows Treated for Metritis

Among the 1,410 cows diagnosed with metritis that were enrolled in this study, 299 (21.2%) failed to achieve clinical cure by d 10. Among cows diagnosed with metritis that underwent clinical cure, 35.1% (n = 390) had fever on d 0. Of those cows diagnosed with metritis that failed to undergo clinical cure, 47.5% (n = 142) had fever on d 0. Failure of clinical cure in cows treated for metritis was greater ($P < 0.001$) among those with RFM or fever at diagnosis of metritis (Table 1). Furthermore, failure of clinical cure tended to be greater ($P = 0.08$) in cows that calved during winter, spring, and summer compared with herdmates that calved during fall. However, proportion of cows with failure of clinical cure did not differ ($P \geq 0.10$) among cows that calved during winter, spring, and summer. Failure of clinical cure in cows treated for metritis was not associated ($P \geq 0.14$) with parity, dystocia, BCS at diagnosis of metritis, or antimicrobial therapy. Moreover, the interactions between fever at diagnosis of metritis and parity, and between fever at diagnosis of metritis and RFM, were not associated ($P \geq 0.12$) with failure of clinical cure. Days postpartum at diagnosis of metritis influenced ($P < 0.001$) the risk of clinical cure failure, and 5 DIM was the threshold that resulted in the largest combined sensitivity and specificity to explain risk of clinical cure failure based on receiver operator characteristic curves (Table 2). Moreover, the same threshold was identified for cows with fever at diagnosis of metritis and counterparts without fever. Areas under the curve for cows with and without fever on d 0 were 0.64 and 0.69, respectively.

### Association Between Failure of Clinical Cure in Cows Treated for Metritis and Purulent Vaginal Discharge and Cytological Endometritis

Both metritis and failure of clinical cure were associated ($P < 0.001$) with the proportion of cows with purulent vaginal discharge, which was greatest for MTnoC, intermediate for MTC, and smallest for no NoMT (Table 3). Proportion of cows with purulent vaginal discharge was greater ($P < 0.0001$) in primiparous compared with multiparous cows (77.2 vs. 68.1%). Cows with metritis and fever on d 0 had greater ($P < 0.01$) odds of developing purulent vaginal discharge compared with cows diagnosed with metritis and without fever on d 0 (88.1 vs. 82.4%). Proportion of cows with purulent vaginal discharge was not associated ($P \geq 0.52$) with the interaction between fever on d 0 and failure of clinical cure and with the antimicrobial therapy used to treat cows diagnosed with metritis. Proportion of cows with cytological endometritis was associated ($P < 0.0001$) with metritis and failure of clinical cure in cows treated for metritis (Table 3). The proportion of cows diagnosed with cytological endometritis was not associated ($P \geq 0.19$) with parity or BCS. Moreover, fever on d 0, antimicrobial therapy, or the interaction between fever and failure of clinical cure were not associated ($P \geq 0.21$) with the odds of cytological endometritis in cows treated for metritis.

### Association Between Failure of Clinical Cure in Cows Treated for Metritis and Reproductive Performance

Resumption of estrous cyclicity postpartum tended to be associated ($P = 0.07$) with group of metritis clinical cure. Compared with MTC counterparts, MTnoC cows were less likely ($P = 0.03$) to resume estrous cyclicity, although metritis was not associated ($P = 0.14$) with the odds of resumption of estrous cyclicity (Table 3). Proportion of cows that resumed estrous cyclicity was greater ($P < 0.001$) for multiparous compared with primiparous cows (74.1 vs. 61.3%). Resumption of estrous cyclicity postpartum was not associated ($P = 0.31$) with BCS. Moreover, fever on d 0, antimicro-

bial therapy, and interaction between fever and failure of clinical cure were not associated ($P \geq 0.39$) with resumption of estrous cyclicity postpartum in cows treated for metritis.

The proportion of cows that received at least 1 AI during the lactation was associated ($P < 0.001$) with group of metritis clinical cure and was greatest for NoMT, intermediate for MTC, and lowest for MTnoC (Table 3). A greater ($P < 0.001$) proportion of primiparous cows received at least 1 AI compared with multiparous counterparts (92.2 vs. 85.2%). An interaction between category of metritis clinical cure and presence of fever on d 0 was observed ($P = 0.03$) for cows diagnosed with metritis. Among cows without fever, the proportion of cows that received at least 1 AI was greater ($P < 0.001$) for MTC compared with MTnoC (91.3 vs. 80.5%). Conversely, clinical cure was not associated ($P = 0.69$) with the risk of receiving at least 1 AI among cows with fever on d 0 (MTC = 88.6 vs. MTnoC = 87.3%). Body condition score at diagnosis of metritis was not associated ($P = 0.64$) with the proportion of cows that received at least 1 AI during the current lactation. Furthermore, antimicrobial therapy had no effect ($P = 0.13$) on the probability of receiving at least 1 AI.

Pregnancy per AI for cows diagnosed with metritis tended to be smaller ($P = 0.08$) on d 38 and was smaller ($P = 0.05$) on d 66 after first AI postpartum compared with that observed in nonaffected cows (Table 3). Nevertheless, P/AI on d 38 and 66 was not associated ($P \geq 0.19$) with clinical cure in cows treated for metritis. Cows that received timed AI for first insemination postpartum had greater ($P < 0.001$) P/AI on d 38 (36.6 vs. 28.1%) and 66 (31.5 vs. 24.4%) compared with counterparts inseminated following estrus detection. Pregnancy per AI was not associated ($P \geq 0.13$) with parity, BCS, fever on d 0, antimicrobial therapy, or interaction between fever and failure of clinical cure (i.e., the last 3 variables assessed among cows diagnosed with metritis only). Although no association between occurrence of metritis and pregnancy loss was observed ($P = 0.29$), risk of pregnancy loss between d 38 and 66 after first AI postpartum tended to be greater ($P = 0.10$) for MTnoC compared with MTC. Body condition score and fever

**Table 1.** Risk factors associated with failure of clinical cure in cows treated for metritis[1]

| | | Univariable analyses | | Multivariable analyses | |
|---|---|---|---|---|---|
| Risk factor | Cure failure, % (n) | OR (95% CI) | *P*-value | AOR (95% CI) | *P*-value |
| Parity | | | | | |
| Primiparous | 21.8 (653) | Referent | 0.65 | — | — |
| Multiparous | 20.7 (757) | 0.94 (0.73–1.22) | | — | |
| Calving season | | | | | |
| Fall | 13.4 (417) | Referent | <0.0001 | Referent | 0.08 |
| Winter | 22.5 (511) | 1.87 (1.32–2.66) | | 1.59 (1.05–2.42) | |
| Spring | 25.3 (316) | 2.19 (1.50–3.19) | | 1.79 (1.07–2.99) | |
| Summer | 28.9 (166) | 2.62 (1.69–4.06) | | 2.06 (1.16–3.63) | |
| Dystocia | | | | | |
| No | 20.7 (1,150) | Referent | 0.32 | — | — |
| Yes | 23.4 (260) | 1.18 (0.85–1.62) | | — | |
| RFM[2] | | | | | |
| No | 17.6 (1,068) | Referent | <0.0001 | Referent | <0.001 |
| Yes | 32.5 (342) | 2.25 (1.71–2.96) | | 2.14 (1.60–2.85) | |
| Twinning | | | | | |
| No | 20.7 (1,326) | Referent | 0.05 | Referent | 0.14 |
| Yes | 29.8 (84) | 1.63 (1.00–2.65) | | 1.47 (0.88–2.46) | |
| Stillbirth | | | | | |
| No | 20.7 (1,296) | Referent | 0.10 | Referent | 0.20 |
| Yes | 27.2 (114) | 1.43 (0.93–2.21) | | 1.35 (0.85–2.12) | |
| BCS on d 0[3] | | | | | |
| Low | 20.8 (379) | 0.97 (0.73–1.30) | 0.98 | — | — |
| Mod | 21.3 (924) | Referent | | — | |
| High | 21.5 (107) | 1.01 (0.62–1.65) | | — | |
| Fever on d 0[3] | | | | | |
| No | 17.9 (878) | Referent | <0.0001 | Referent | <0.001 |
| Yes | 26.7 (532) | 1.67 (1.29–2.17) | | 1.69 (1.29–2.20) | |
| Treatment | | | | | |
| Ampicillin | 21.9 (556) | Referent | 0.59 | — | — |
| Ceftiofur | 20.7 (854) | 0.93 (0.72–1.21) | | — | |

[1]OR = odds ratio from univariate analyses; AOR = adjusted odds ratio from multivariable analyses.
[2]RFM = retained fetal membranes
[3]d 0 = day of diagnosis of metritis.

**Table 2.** Sensitivity (Sn.), specificity (Sp.), and proportion of cows that failed to undergo clinical cure after antimicrobial treatment for metritis according to DIM at diagnosis of metritis

| | Cows without fever at diagnosis of metritis (d 0) | | | | Cows with fever at diagnosis of metritis (d 0) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cure failure after treatment,[1] % (n) | | | | Cure failure after treatment,[1] % (n) | |
| DIM | Sn. | Sp. | Below DIM | Above DIM | Sn. | Sp. | Below DIM | Above DIM |
| 2 | 0.04 | 1.00 | 85.7 (6/7) | 17.3 (151/871) | 0.01 | 0.98 | 25.0 (2/8) | 26.7 (140/524) |
| 3 | 0.13 | 0.98 | 58.8 (20/34) | 16.2 (137/844) | 0.13 | 0.95 | 47.5 (19/40) | 25.0 (123/492) |
| 4 | 0.44 | 0.79 | 31.1 (69/222) | 13.4 (88/656) | 0.46 | 0.71 | 36.7 (65/177) | 21.7 (77/355) |
| 5 | 0.55[2] | 0.72[2] | 30.0 (87/290) | 11.9 (70/588) | 0.60[2] | 0.61[2] | 35.9 (85/237) | 19.3 (57/295) |
| 6 | 0.76 | 0.49 | 24.5 (120/489) | 9.5 (37/389) | 0.77 | 0.42 | 32.5 (109/335) | 16.8 (33/197) |
| 7 | 0.85 | 0.41 | 23.8 (134/562) | 7.3 (23/316) | 0.88 | 0.25 | 29.9 (125/418) | 14.9 (17/114) |
| 8 | 0.93 | 0.22 | 20.7 (146/706) | 6.4 (11/172) | 0.92 | 0.15 | 28.2 (131/464) | 16.2 (11/68) |
| 9 | 0.94 | 0.15 | 19.5 (148/760) | 7.6 (9/118) | 0.96 | 0.06 | 27.0 (136/503) | 20.7 (6/29) |
| 10 | 0.97 | 0.10 | 19.1 (153/801) | 5.2 (4/77) | 0.99 | 0.04 | 27.2 (140/515) | 11.8 (2/17) |
| 11 | 0.98 | 0.08 | 18.9 (154/816) | 4.8 (3/62) | 0.99 | 0.03 | 27.1 (141/520) | 8.3 (1/12) |
| 12 | 0.98 | 0.02 | 17.9 (154/859) | 15.8 (3/19) | 0.99 | 0.01 | 26.8 (141/526) | 16.7 (1/6) |
| 13 | 0.99 | 0.02 | 17.9 (155/864) | 14.3 (2/14) | 0.99 | 0.01 | 26.7 (141/528) | 25.0 (1/4) |
| 14 | 0.99 | 0.01 | 18.0 (156/869) | 11.1 (1/9) | 1.00 | 0.01 | 26.8 (142/530) | 0.0 (0/2) |
| 15 | 0.99 | 0.01 | 17.9 (156/872) | 16.7 (1/6) | 1.00 | 0.00 | 26.7 (142/532) | — |
| 16 | 0.99 | 0.00 | 17.8 (156/875) | 33.3 (1/3) | — | — | — | — |
| 17 | 0.99 | 0.00 | 17.8 (156/877) | 100.0 (1/1) | — | — | — | — |
| 18 | 1.00 | 0.00 | 17.8 (156/877) | 100.0 (1/1) | — | — | — | — |
| 19 | 1.00 | 0.00 | 17.9 (157/878) | — | — | — | — | — |

[1]Proportion of clinical cure failure following treatment for cows diagnosed with metritis below (includes DIM threshold) or above DIM threshold.
[2]DIM threshold with the greatest combined sensitivity and specificity.

on d 0, antimicrobial therapy, and interaction between fever and failure of clinical cure were not associated ($P \geq 0.12$) with pregnancy loss after first AI postpartum (i.e., the last 3 variables assessed among cows diagnosed with metritis only).

Cows diagnosed with metritis had reduced ($P < 0.001$) hazard of pregnancy compared with nonaffected counterparts, independent of clinical cure (MTC: AHR = 0.80, 95% CI = 0.72–0.88; MTnoC: AHR = 0.63, 95% CI = 0.53–0.74; Figure 2). Furthermore, hazard

**Table 3.** Purulent vaginal discharge, cytological endometritis, estrous cyclicity, pregnancy outcomes following first AI postpartum, and removal from herd according to occurrence of metritis and failure of clinical cure in response to antimicrobial therapy

| | Group[1] | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|
| Item | NoMT | MTC | MTnoC | Group | C1 | C2 |
| Purulent vaginal discharge, % (n) | 38.1 (910)[a] | 74.0 (894)[b] | 91.7 (227)[c] | <0.001 | <0.001 | <0.001 |
| Cytological endometritis, % (n) | 36.4 (227)[a] | 73.3 (397)[b] | 91.4 (81)[c] | <0.001 | <0.001 | <0.001 |
| Estrous cyclicity, % (n) | 71.0 (486)[A] | 71.0 (729)[A] | 62.0 (174)[B] | 0.07 | 0.14 | 0.03 |
| Received at least 1 AI, % (n) | 92.6 (1,194)[a,A] | 90.1 (1,111)[a,B] | 83.2 (299)[b] | <0.001 | <0.001 | <0.001 |
| P/AI first insemination[3] | | | | | | |
| d 38, % (n) | 32.3 (1,067) | 29.8 (978) | 27.4 (238) | 0.22 | 0.08 | 0.49 |
| d 66, % (n) | 28.1 (1,065) | 26.1 (978) | 22.0 (238) | 0.13 | 0.05 | 0.19 |
| Pregnancy loss after first AI, % (n) | 11.5 (366) | 11.1 (307) | 18.4 (68) | 0.23 | 0.29 | 0.10 |
| Death by 60 DIM, % (n) | 0.6 (1,194)[a] | 1.0 (1,111)[a] | 3.9 (299)[b] | <0.001 | <0.001 | 0.001 |
| Removal from herd[4] | | | | | | |
| By 60 DIM, % (n) | 2.7 (1,194)[a,A] | 4.3 (1,111)[a,B] | 10.7 (299)[b] | <0.001 | <0.001 | <0.001 |
| By 300 DIM, % (n) | 15.4 (1,194)[a] | 17.4 (1,111)[a] | 26.3 (299)[b] | <0.001 | <0.001 | <0.001 |

[a,b]Means with different lowercase superscripts differ ($P \leq 0.05$).
[A,B]Means with different uppercase superscripts tended to differ ($P \leq 0.10$).
[1]NoMT = cows not diagnosed with metritis postpartum; MTC = cows diagnosed with metritis that underwent clinical cure following antimicrobial therapy; MTnoC = cows diagnosed with metritis that failed to undergo clinical cure following antimicrobial therapy. Results are presented as LSM from final multivariable models followed by the total number of cows considered for statistical analyses.
[2]Group = effect of group (NoMT vs. MTC vs. MTnoC); C1 = effect of metritis (NoMT vs. MTC + MTnoC); C2 = effect of failure of clinical cure (MTC vs. MTnoC).
[3]P/AI = pregnancy per AI.
[4]Includes cows that died and those that were culled by farm personnel.

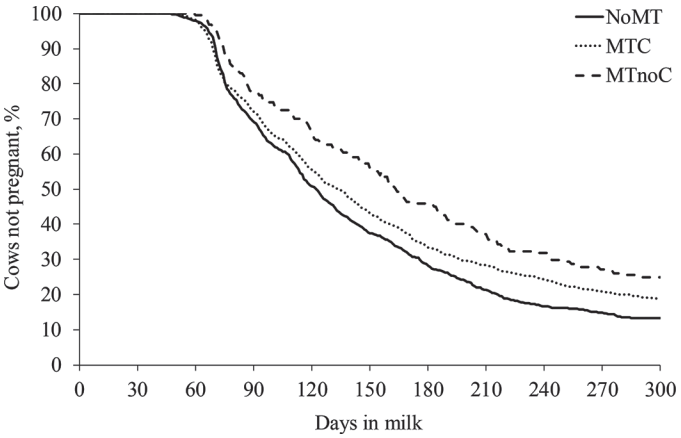


**Figure 2.** Survival curves for time to pregnancy by 300 DIM for cows not diagnosed with metritis (NoMT), cows diagnosed with metritis that underwent clinical cure after termination of treatment (MTC), and cows diagnosed with metritis that failed to undergo clinical cure after termination of treatment (MTnoC). Median days to pregnancy: NoMT = 123; MTC = 134; MTnoC = 163. Percentage of cows censored: NoMT = 25.4; MTC = 31.1; MTnoC = 43.5. Effect of metritis: $P < 0.001$; effect of clinical cure: $P < 0.001$.

of pregnancy was smaller ($P < 0.001$) for MTnoC compared with MTC (AHR = 0.79, 95% CI = 0.67–0.93). Primiparous cows had greater ($P < 0.001$) hazard of pregnancy compared with multiparous herdmates (AHR = 1.24, 95% CI = 1.13–1.37). Cows that calved during summer (AHR = 1.27, 95% CI = 1.09–1.47) or winter (AHR = 1.23, 95% CI = 1.08–1.41) had greater ($P < 0.05$) hazard of pregnancy compared with cows that calved during spring, whereas hazard of pregnancy did not differ between cows that calved in fall and spring (AHR = 1.17, 95% CI = 0.97–1.40). Fever on d 0, interaction between fever and group of metritis clinical cure, and antimicrobial therapy were not associated ($P \geq 0.15$) with hazard of pregnancy in cows treated for metritis.

### Association Between Failure of Clinical Cure in Cows Treated for Metritis and Death, Removal from Herd, and Milk Production

Both metritis and failure of clinical cure were associated ($P \leq 0.001$) with risk of death during the first 60 DIM, which was greatest for MTnoC, intermediate for MTC, and lowest for NoMT (Table 3). Parity, calving season, fever on d 0, interaction between fever and group of metritis clinical cure, and antimicrobial therapy were not associated ($P \geq 0.11$) with the proportion of cows that died by 60 DIM (i.e., the last 3 variables assessed among cows diagnosed with metritis only). Both metritis and failure of clinical cure were associated ($P < 0.001$) with risk of leaving the herd during the first 60 DIM, which was greatest for MTnoC, intermediate for MTC, and lowest for NoMT. Moreover, MTnoC cows had increased ($P < 0.001$) hazard of leaving the herd during the first 60 DIM compared with NoMT (AHR = 4.13, 95% CI = 2.58–6.61) and MTC cows (AHR = 2.53, 95% CI = 1.64–3.94). Hazard of leaving the herd was also greater ($P = 0.03$) for MTC compared with NoMT (AHR = 1.62, 95% CI = 1.05–2.50). Primiparous cows were less likely ($P = 0.05$) to leave the herd by 60 DIM (4.1 vs. 6.1%). Calving season, fever on d 0, interaction between fever and group of metritis clinical cure, and antimicrobial therapy were not associated ($P \geq 0.13$) with risk of leaving the herd during the first 60 DIM (i.e., the last 3 variables assessed among cows diagnosed with metritis only).

Metritis and failure of clinical cure were also associated ($P < 0.001$) with risk of leaving the herd by 300 DIM, which was greatest for MTnoC compared with MTC and NoMT. Cows treated for metritis that failed to undergo clinical cure had increased ($P < 0.001$) hazard of leaving the herd during the first 300 DIM, compared with NoMT (AHR = 1.90, 95% CI = 1.47–2.45) and MTC (AHR = 1.64, 95% CI = 1.27–2.12; Figure 3). In contrast, hazard of leaving the herd did not differ ($P = 0.13$) between MTC and NoMT (AHR = 1.16, 95% CI = 0.96–1.41). Primiparous cows were less likely ($P < 0.001$) to leave the herd by 300 DIM (14.3 vs. 25.6%). Calving season tended to be associated ($P = 0.07$) with risk of leaving the herd by 300 DIM. However, pairwise comparisons did not reveal differences in proportion of cows that left the herd according to calving season (fall = 21.0, winter = 16.5, spring = 21.4, summer = 18.7%). An interaction between group of metritis clinical cure and fever on d 0 was observed ($P < 0.01$) for removal from herd by 300 DIM. Among cows without fever on d 0, risk of being removed from the herd was greater ($P < 0.001$) for MTnoC compared with MTC (32.5 vs. 16.4%). However, percent of cows removed from the herd by 300 DIM did not differ ($P = 0.80$) between MTnoC and MTC cows with fever (17.6 vs. 18.6%, respectively). Hazard of being removed from the herd during the first 300 DIM was also affected ($P < 0.01$) by the interaction between group of metritis clinical cure and fever on d 0. Hazard of removal from the herd was greater for MTnoC compared with MTC among cows without fever on d 0 (AHR = 2.21, 95% CI = 1.62–3.02) but not among cows with fever (AHR = 0.97, 95% CI = 0.61–1.50). Antimicrobial therapy was not associated ($P \geq 0.95$) with risk of being removed from the herd in the first 300 DIM among cows treated for metritis.

An interaction among group cure, parity, and month postpartum was observed ($P < 0.001$) for milk production (Figure 4). For multiparous cows, milk yield for MTnoC was consistently smaller compared with NoMT

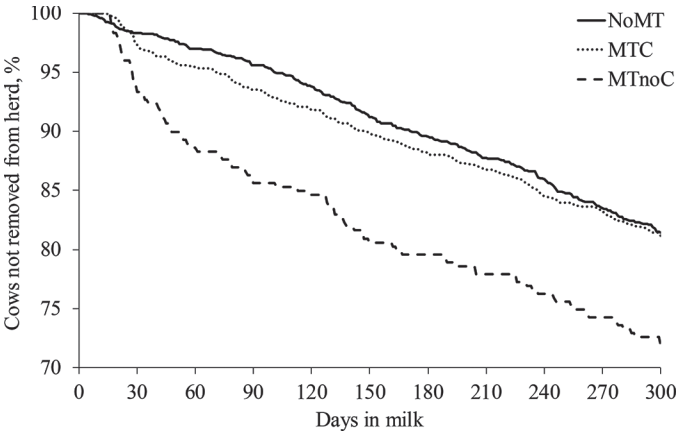


**Figure 3.** Survival curves for time to removal from herd by 300 DIM for cows not diagnosed with metritis (NoMT), cows diagnosed with metritis that underwent clinical cure after termination of treatment (MTC), and cows diagnosed with metritis that failed to undergo clinical cure after termination of treatment (MTnoC). Percentage of cows censored: NoMT = 81.4; MTC = 81.2; MtNoC = 71.9. Effect of metritis: $P < 0.001$; effect of clinical cure: $P < 0.001$.

and MTC counterparts, whereas milk production for MTC was smaller than that observed in NoMT only at the first 2 mo postpartum. By contrast, only marginal and transitory differences in milk production were observed among metritis cure groups for primiparous cows.

## DISCUSSION

Our results support the need to further advance preventative methods and therapy protocols, considering that approximately 20% of cows treated for metritis did not undergo clinical cure. Failure of clinical cure was assessed using a visual scale for evaluation of vaginal discharge adapted from Chenault et al. (2004) 10 d after diagnosis of metritis and onset of antimicrobial therapy. Besides being readily available to dairy herd managers to diagnose metritis and assess clinical cure after treatment, uterine health measured using this visual scale has been linked with measurable differences in reproductive performance and profitability (Dubuc et al., 2010b; Denis-Robichaud and Dubuc, 2015; Lima et al., 2019). Moreover, failure to undergo clinical cure was associated with increased risk of death by 60 DIM, increased proportion of cows with purulent vaginal discharge and cytological endometritis, delayed resumption of estrous cyclicity postpartum, increased risk of removal from herd, and decreased hazard of pregnancy by 300 DIM. Results from the present study also underscore the importance of preventative strategies, as reproductive losses were still observed in cows that underwent clinical cure, which supports that the damage to reproduction remained despite resolution of clinical signs. Of importance for interpretation of the present results, herd managers and staff were blinded to information about failure of clinical cure collected by study personnel. Therefore, knowledge of study outcomes by those responsible for breeding and culling decisions is not expected to be a source of bias.

The biological processes underlying failure of clinical cure in dairy cows treated for metritis have not been elucidated. Nevertheless, previous studies suggest that it is associated with specific changes in uterine microbiome that differ from those observed in cows that undergo clinical cure. For instance, cows that failed to

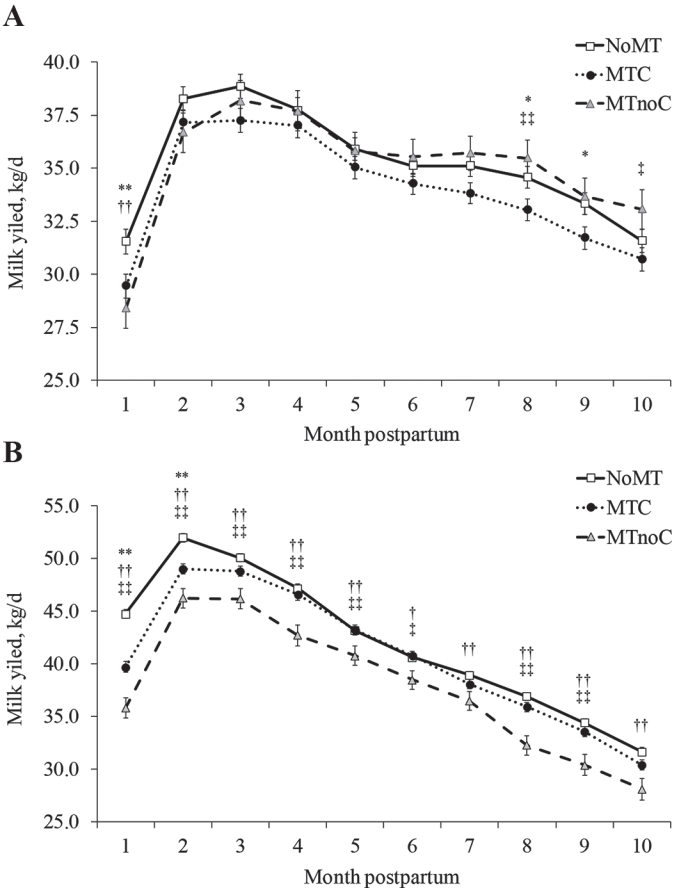


**Figure 4.** Milk production in the first 10 mo postpartum for primiparous (A) and multiparous cows (B). NoMT = cows not diagnosed with metritis postpartum; MTC = cows diagnosed with metritis that underwent clinical cure following antimicrobial therapy; MTnoC = cows diagnosed with metritis that failed to undergo clinical cure following antimicrobial therapy. LSM ± SEM for primiparous (NoMT = 35.2 ± 0.31; MTC = 33.9 ± 0.31; MTnoC = 35.0 ± 0.52 kg/d) and multiparous cows (NoMT = 42.0 ± 0.22; MTC = 40.6 ± 0.28; MTnoC = 37.7 ± 0.54 kg/d). Error bars represent SEM. Within month postpartum, different symbols indicate that LSM differed ($P \leq 0.05$; **NoMT vs. MTC, ††NoMT vs. MTnoC, ‡‡MTC vs. MTnoC) or tended to differ ($P \leq 0.10$; *NoMT vs. MTC, †NoMT vs. MTnoC, ‡MTC vs. MTnoC).

achieve clinical cure 2 d after completion of antimicrobial therapy had greater relative abundance of core uterine pathogens such as *Bacteroides*, *Fusobacterium*, and *Porphyromonas* compared with cows that achieved clinical cure (Jeon et al., 2018). Increased prevalence of these uterine pathogens in cows that fail to undergo clinical cure is of particular relevance, considering that a synergistic relationship among the 3 genera has been deemed pivotal during establishment of metritis postpartum (Jeon et al., 2015). The specific mechanisms deployed by bacteria to evade the immune system and to resist exposure to antimicrobials provide additional insights on the role of *Bacteroides*, *Fusobacterium*, and *Porphyromonas* for failure of clinical cure. Bacterial biofilm is an exopolysaccharide matrix produced by bacteria in all the aforementioned genera (Bradshaw et al., 1998; Lo et al., 2009; Reis et al., 2014), although its relevance during the development and resolution of metritis in dairy cows has not been established. Biofilm formation enhances bacterial survival by delaying penetration and diminishing exposure to antimicrobial molecules (Duguid et al., 1992; Hoyle et al., 1992; Suci et al., 1994; Bradshaw et al., 1998). Conversely, bacterial biofilm is equipped with channels that allow enclosed microorganisms assess to water and nutrient supplies (Lawrence et al., 1991). Biofilm also promotes formation of either single- or multispecies communities anchored to a target surface, thus facilitating synergistic interaction among microorganisms (Costerton et al., 1978). Other virulence factors associated with attachment or motility of bacteria within the uterus of dairy cows with metritis have been studied (Bicalho et al., 2012). However, the role of biofilm formation during establishment of uterine diseases and failure of clinical cure in response to antimicrobial therapy remains unexplored.

Retention of fetal membranes has been described as a risk factor for metritis in dairy cattle (Correa et al., 1993; Dubuc et al., 2010a; Vieira-Neto et al., 2016) and was identified as a risk factor for failure of clinical cure in the present study. Peripheral blood leukocytes collected from cows with RFM had reduced killing and chemotactic activities compared with those of cows without RFM from the week preceding parturition to 2 wk postpartum (Kimura et al., 2002). Cows with RFM had also reduced plasma concentrations of IL8, which has been characterized as a major chemoattractant in cotyledons (Kimura et al., 2002). Because resolution of clinical signs in a large portion of cows is mediated by the immune system, it is reasonable to speculate that impairment of innate immunity observed in cows with RFM is an important component underlying failure of clinical cure of metritis. In fact, experiments that included untreated control groups showed that 75 to 80% of cows that undergo clinical cure after receiving antimicrobial therapy would have experienced spontaneous cure if left untreated (Chenault et al., 2004; McLaughlin et al., 2012; de Oliveira et al., 2020). Moreover, RFM has been associated with changes in the vaginal microbiome, such as increases in bacterial load and relative abundance of *Fusobacteria* and *Bacteroidetes* on d 7 postpartum, compared with cows without RFM (Bicalho et al., 2017). In fact, increased abundance of bacteria from these 2 phyla in utero has been associated not only with establishment of metritis postpartum (Jeon et al., 2015) but also with failure of clinical cure (Jeon et al., 2018). Twinning and stillbirth were identified as risk factors for clinical cure failure in univariable models, but not in the final multivariable model. Path analyses of postpartum diseases in dairy cows indicate that twinning and stillbirth have a relationship with metritis, both directly and via increased incidence of RFM (Correa et al., 1993). Results from the present study support that a similar network underlies the odds of clinical cure failure in dairy cows treated for metritis. It is important to consider that the number of cows presented with either twinning or stillbirth is limiting compared with that of cows with RFM. Therefore, it is possible that the present study lacks statistical power to identify the association among twinning, stillbirth, and failure of clinical cure.

Fever has been associated with increased phagocytic activity of peripheral blood leukocytes in dairy cows with metritis (Jeon et al., 2016). Nevertheless, our results are in agreement with previously published studies that depicted a reduced probability of cure in cows with fever at diagnosis of metritis compared with herdmates without fever (Giuliodori et al., 2013). Moreover, cows diagnosed with metritis and fever had lower hazard of pregnancy compared with counterparts diagnosed with metritis without fever (Giuliodori et al., 2013). Occurrence of fever in cows with metritis suggests a systemic exposure to pyrogens such as LPS from gram-negative bacteria (Li et al., 2006). In cows diagnosed with metritis, uterine microbiome and concentration of bacteria based on quantification of the 16S gene did not differ between cows with fever and counterparts without fever (Jeon et al., 2016). Although the volume of uterine contents was not evaluated, these results suggest that development of fever in cows with metritis is not associated with greater exposure to gram-negative bacteria. Moreover, anatomical and histological evaluation of the uterine wall between cows with or without fever at diagnosis of metritis has not been conducted. It is plausible that more extensive damage to the endometrium and uterine stroma leads to greater escape of bacteria and bacterial components (e.g., LPS) from the uterus in cows with fever, which requires a prolonged interval

for tissue repair and remission of clinical signs. Vascular changes in cows with fever may also contribute to systemic exposure to bacterial pyrogens. Experiments with rats showed that exposure to ambient temperature of 40.5°C for 30 min led to hyperthermia followed by capillary dilation, vascular stasis, and extravasation of intravascular content into the interstitium in various organs (Vlad et al., 2010). It is also possible that fever in cows with metritis is mediated by damage-associated molecular pattern molecules in addition to pathogen-associated molecular pattern molecules, thus identifying a cohort of cows with more extensive damage to uterine tissues (Sheldon et al., 2019). Finally, increased body temperature has been associated with protein denaturation and DNA damage, possibly leading to cell death and delayed tissue healing (Roti Roti, 1982).

Previous studies have showed that cows diagnosed with metritis postpartum are at a greater risk of developing purulent vaginal discharge and cytological endometritis compared with counterparts not diagnosed with metritis (Cheong et al., 2011; Vieira-Neto et al., 2016). Although our results also depicted that the proportion of cows with purulent vaginal discharge and cytological endometritis was greater among those diagnosed with metritis compared with counterparts without metritis, incidences of both diseases were greatest for cows that fail to undergo clinical cure. In fact, over 90% of cows diagnosed with metritis that fail to resolve clinical signs by 6 to 8 d after completion of treatment were diagnosed with purulent vaginal discharge or cytological endometritis. It is likely that prolonged persistence of core pathogens associated with uterine disease such as *Bacteroides*, *Fusobacterium*, and *Porphyromonas* extend the interval to cure (Jeon et al., 2018), thus extending the time to re-establishment of normal vaginal discharge and population of resident immune cells in the endometrium. However, metritis has been associated with delayed resumption of estrous cyclicity postpartum (Santos et al., 2010; Vieira-Neto et al., 2016), and our results support that such a response is observed only in cows that fail to undergo clinical cure. Uterine infection has been associated with reduced rate of growth by ovarian follicles, decreased intrafollicular concentrations of estradiol, and impaired ovulatory capacity (Sheldon et al., 2002; Williams et al., 2007). Expression of LH receptors in granulosa cells was reduced in cows diagnosed with metritis compared with counterparts not diagnosed with the disease in the first 21 DIM (Piersanti et al., 2019). Because failure of clinical cure is likely associated with increased bacterial load and prolonged persistency of pathogens within the reproductive tract (Bicalho et al., 2017; Jeon et al., 2018), it is possible that more prolonged and more extensive damage to ovarian follicles impairs resumption of estrous cyclicity in cows that did not undergo clinical cure after treatment for metritis.

Reproductive performance in the first 300 DIM was impaired in cows diagnosed with metritis, particularly among those that failed to undergo clinical cure. Cows diagnosed with metritis were less likely to receive a first AI postpartum compared with counterparts not diagnosed with the disease, which paralleled the increased risk of death and removal from herd by 60 DIM. Cows diagnosed with metritis had reduced P/AI after first insemination postpartum, as reported previously (Santos et al., 2010; Ribeiro et al., 2013). The impact of metritis on establishment and maintenance of pregnancy after first AI encompasses changes in the uterus, ovarian follicles, and enclosed oocytes. Uterine infusion of pathogenic *Escherichia coli* and *Trueperella pyogenes* altered the transcriptome of reproductive tissues (i.e., oocytes, granulosa cells, endometrium, and oviduct) and reduced the developmental capacity of oocytes fertilized in vitro (Dickson et al., 2020; Horlock et al., 2020; Piersanti et al., 2020). Moreover, exposure to LPS in vitro increased occurrence of meiotic arrest and failure of germinal vesicle breakdown (Bromfield and Sheldon, 2011). Percentage of cleaved, live, and high-quality embryos in response to superstimulation with FSH was reduced in Holstein donors with retained placenta or metritis compared with donors without uterine diseases postpartum (Ribeiro et al., 2016). Reproductive performance between cows with and without metritis continues to diverge after first AI postpartum, suggesting that changes to ovarian and uterine function may persist beyond 2 mo after exposure to pathogens described previously (Dickson et al., 2020; Horlock et al., 2020; Piersanti et al., 2020). Pregnancy per AI after first insemination did not differ between MTC and MTnoC. However, pregnancy loss after d 38 of gestation tended to be greater and hazard of pregnancy by 300 DIM was reduced for cows that failed to achieve clinical cure following antimicrobial therapy for metritis compared with counterparts that underwent clinical cure. Further studies are necessary to elucidate the mechanisms underlying fertility loss in cows with failure of clinical cure. It is possible that increased bacterial load and prolonged persistency of pathogens in the reproductive tract of cows that did not undergo clinical cure (Bicalho et al., 2017; Jeon et al., 2018) lead to more severe detrimental impacts on oocyte quality and embryo development. Our data support that failure of clinical cure predisposed cows to develop a continued state of uterine inflammation, considering that over 90% of this cohort were subsequently diagnosed with purulent vaginal discharge or

cytological endometritis. Previous studies have associated chronic uterine inflammation with histological changes in uterine glands, including necrosis and infiltrations of lymphocytes and eosinophils (Cupps, 1973). Persistency of endometrial inflammation has also been linked to increased pregnancy loss in dairy cows (Lima et al., 2013).

Cows diagnosed with metritis are at greater risk of death and removal from the herd compared with counterparts without metritis (Bartlett et al., 1986; Dubuc et al., 2011b; Giuliodori et al., 2013). Our results showed that removal of cows with metritis from the herd was largely explained by those that did not achieve clinical cure after completion of antimicrobial therapy. In fact, the risk of death and removal from the herd were 3.9- and 2.5-fold greater for MTnoC compared with MTC, respectively. Results from the present study also support that reduced milk production in cows that fail to undergo clinical cure after treatment for metritis is a contributing factor for removal from herd. Milk production in the first month postpartum was reduced in the compared cows with metritis, particularly for multiparous cows that did not undergo clinical cure. Experiments that administered bacterial LPS intravenously into dairy cows reported reduction in milk production (Waldron et al., 2003; Al-Qaisi et al., 2020). However, differences in blood concentrations of LPS between cows that fail to undergo clinical cure after treatment for metritis and those that achieve clinical cure remain unexplored. Wittrock et al. (2011) reported that milk production in the first 12 wk postpartum for cows that left the herd was smaller compared with herdmates that were retained. The same authors also reported that among cows that were culled by 12 wk postpartum, those with metritis had reduced milk production compared with cows without metritis. In contrast, milk production did not differ between cows with and without metritis that were not culled (Wittrock et al., 2011). Considering that multiparous cows that did not undergo clinical cure sustained reduced milk yield during the first 10 mo postpartum, it is possible that diminished productive efficiency also contributed to greater hazard of removal from herd by 300 DIM. Interestingly, failure of clinical cure was not associated with decreased milk production among primiparous cows in the present study. Based on our experimental design, it is not possible to define whether first-lactation cows are refractory to the detrimental effects of clinical cure failure after treatment for metritis or whether removal from affected cows masked the decline in milk production (i.e., loss to follow-up). Our results warrant further investigation of the association between failure of clinical cure and milk production across different lactation groups.

## CONCLUSIONS

Failure of clinical cure of metritis was negatively associated with reproductive and productive performance. Cows with metritis that failed to undergo clinical cure after antimicrobial therapy had impaired uterine health and estrous cyclicity. Failure to achieve clinical cure was also associated with reduced proportion of cows that received at least one AI and with hazard of pregnancy in the first 300 d postpartum. In addition, cows treated for metritis that failed to achieve clinical cure were more likely to be removed from the herd than cows without metritis and those that underwent clinical cure. Metritis was associated with reduced milk production in multiparous cows, and such decrease was more pronounced among those that failed to undergo clinical cure. Considering the detrimental influences of metritis, results highlight the need for development of methods to reduce the incidence of metritis and increase the probability of cure in cows treated for metritis.

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