ABSTRACT

Our objective was to investigate the association of early metritis (EMET, diagnosed at <5 d in milk (DIM)) and late metritis (LMET, diagnosed at ≥5 DIM) with circulating concentrations of energy metabolites, minerals, and haptoglobin (Hp) throughout the first 14 d postpartum. A total of 379 purebred Jersey cows were enrolled in a prospective cohort study from a single herd in west Texas. Cows were examined for metritis using the Metricheck device (Simcro Ltd.) at 4, 7, and 10 DIM. Cows identified by farm employees as possible metritis cases were also evaluated for metritis. Blood samples were collected for analysis of concentrations of Ca, Mg, and glucose at DIM 1 through 5, 7, 10, and 14. Albumin, urea, fructosamine, free fatty acids (FFA), creatinine, and β-hydroxybutyrate (BHB) were analyzed at DIM 3, 5, 7, 10, and 14, and Hp at DIM 1 through 5 and 7. Data were analyzed using the MIXED and PHREG procedures of SAS (SAS Institute Inc.). A series of mixed general linear models accounting for repeated measures were fitted to the data. The independent variables metritis [no metritis (NMET), EMET, and LMET], DIM of analyte assessment, and parity were forced in all models. Multivariable Cox proportional hazard models were built to assess the risk of pregnancy and culling within 150 DIM. The overall metritis incidence was 26.9% (EMET = 49; LMET = 53; NMET = 277). Average concentrations of glucose, Mg, and urea were not associated with metritis. The associations of Ca, creatinine, BHB, and fructosamine with metritis were dependent on the DIM of analyte assessment. Cows categorized as EMET and LMET had, on average, lower albumin and fructosamine compared with NMET cows. Both EMET and LMET cows had, on average, greater BHB than NMET cows. A greater FFA concentration was only observed in cows diagnosed with EMET compared with NMET cows (EMET = 0.58, LMET = 0.52, NMET = 0.48 mmol/L). Additionally, circulating Hp concentration was greater for LMET and EMET compared with NMET cows, and EMET cows had greater Hp compared with LMET cows (EMET = 1.15; LMET = 1.00; NMET = 0.84). In conclusion, several blood biomarkers were temporally associated with early versus late metritis diagnosis in postpartum Jersey cows. No meaningful differences were observed in production, reproduction, or culling between EMET and LMET cows. These results suggest that cows with EMET undergo a more severe degree of inflammation and negative energy balance compared with NMET cows.

Key words: Jersey, haptoglobin, metabolites, metritis

INTRODUCTION

It is known that around 80 to 100% of dairy cows have some degree of bacterial contamination of their reproductive tract after calving (Sheldon et al., 2008). A timely and robust immune response is needed to defend the postpartum uterus against pathogens and to mediate uterine involution (LeBlanc, 2012). Failure to successfully achieve this scenario leads to a more abrupt inflammation and infection of the uterus, causing a disease known as metritis. Clinical metritis is characterized by an abnormally enlarged uterus and a fetid, watery, red-brownish uterine discharge present within 21 d after parturition (Sheldon et al., 2006). The incidence of metritis has been reported to be between 20 and 40% in the United States (Sheldon et al., 2009; Dubuc et al., 2010). The effect of metritis on lactation performance is striking. Metritis is associated with impaired reproduction, increased risk of death and culling, and reduced milk production, especially within the first 2 mo of lactation (Wittrock et al., 2011; de Oliveira et al., 2020; Machado et al., 2020). The economic losses caused by each metritis case have been reported to range between $267 and $410 (Lima et al., 2019).

Antimicrobial use in food animal production and the potential risk for selection of antimicrobial resistant
bacteria has raised public health concerns in recent years (Tragesser et al., 2006; Liu et al., 2016). Metritis is a disease that is widely treated with antimicrobials like ceftiofur (a third-generation cephalosporin); therefore, many researchers are seeking innovative alternative therapy strategies, including a targeted treatment approach. Recent research showed that DIM at metritis diagnosis is associated with disease severity; cows that were diagnosed with metritis cases that occurred within the first 5 DIM (early metritis, EMET) were less likely to cure and experienced more pronounced milk production and reproductive losses compared with cows that developed metritis after 5 DIM (late metritis, LMET), despite all cases being treated with systemic ceftiofur (Machado et al., 2020; de Oliveira et al., 2021).

The beginning of lactation is marked by decreased DMI, which can lead to a lack of nutrient support to the activated immune system (Vazquez-Aion et al., 1994). An impaired immune function associated with negative energy balance is known to be associated with the development of metritis (Galvão et al., 2010). Thus, the potential use of blood markers measured during the periparturient period has been extensively evaluated to better understand the dynamics of energy and mineral metabolism and their relationship with metritis incidence. Metabolic markers such as free fatty acids (FFA), BHB, and glucose have been associated with the risk of metritis (Chapinal et al., 2011; Bicalho et al., 2017; Menta et al., 2021). The imbalance of these markers is documented to impair immunity by reducing neutrophil function (Kimura et al., 1999; Hammon et al., 2006). Likewise, mineral imbalances such as hypocalcemia have been associated with metritis in Holstein cows (Martinez et al., 2012; Bicalho et al., 2014; Neves et al., 2018), as Ca plays a key role in the activation and function of neutrophils (Bréchard and Tschirhart, 2008). In addition to energy and mineral biomarkers, haptoglobin (Hp), an acute-phase protein, has been extensively reported to be associated with metritis incidence, with some evidence that it is also associated with the severity of the disease (Huzzey et al., 2009; Ceciliani et al., 2012; Machado et al., 2020).

Extensive work characterizing the association between metritis incidence and biomarkers of metabolism and inflammation has been done in Holstein cows, as demonstrated above. However, no studies have yet characterized the association of those biomarkers with metritis diagnosed either earlier or later in lactation. Evaluating the association of these biomarkers with metritis incidence based on DIM of diagnosis may aid in the understanding of how an earlier metritis event associates with greater production losses. Therefore, the objective of this study was to evaluate the association of EMET and LMET with circulating concentrations of metabolites, minerals, and Hp. In addition, we evaluated effects of EMET and LMET on reproductive performance, culling, and milk yield compared with nonmetritic (NMET) multiparous Jersey cows.

**MATERIALS AND METHODS**

**Study Animals, Farm Management, and Eligibility Criteria**

Data from 379 multiparous purebred Jersey cows from a prospective cohort study characterizing hypocalcemia in Jersey cows were used for the present study (Menta et al., 2021). Cows belonged to a commercial dairy farm in west Texas milking 3,800 Jersey and Jersey-Holstein crosses twice a day. The herd’s average mature-equivalent 305-d milk production was 7,058 kg. Cows were housed in dry lots with shades in the central area of the pen. Feed was offered as a TMR twice daily for both lactating and dry cows; the diet for lactating cows was calculated to exceed their nutritional requirements. Close-up cows received a negative DCAD TMR (Menta et al., 2021).

Reproductive management was designed by the herd veterinarian and had a voluntary waiting period of 35 d. A presynchronization protocol consisting of 2 PGF2α injections 14 d apart started at 53 ± 3 DIM, followed by Cosynch-48 (GnRH injection followed by PGF2α, 7 d later and GnRH and AI 48 h after). Pregnancy was diagnosed at 42 ± 3 d after breeding by the herd veterinarian via manual transrectal palpation. Cows that remained open were administered PGF2α at the time of pregnancy check and re-enrolled in a Cosynch-48 program the week after. Cows demonstrating estrus signs (aided by tail chalk application) were allowed to be bred by AI throughout the entire reproductive program.

As stated in the parent study, due to the farm’s historic use of oral Ca supplementation at the time of parturition, only half of all eligible cows were enrolled in this data set. Maternity employees were provided with randomized enrollment sheets for purebred Jersey cows blocked by parity and oral Ca treatment. Cow eligibility requirements were as follows: (1) be a multiparous purebred Jersey, (2) not receive an oral Ca supplement at calving, (3) not have a twin birth, and (4) have a gestation length >260 d.

All activities performed in this study were reviewed and approved by the Texas Tech University Institutional Animal Care and Use Committee (#18035-04).

**Data Collection and Time to Metritis Definition.** Metritis was diagnosed by the research team at 4, 7, and 10 DIM using a Metricheck device (Simcro Ltd.). Vaginal discharge was scored based on a modified scale of 1 to 5, where 1 = clear mucoid discharge with no
Menta et al.: TIME TO METRITIS DIAGNOSIS

smell; 2 = cloudy and mucoid discharge with no smell; 3 = mucopurulent discharge with <50% pus and no smell; 4 = mucopurulent discharge from white to yellowish to red-brownish color with ≥50% pus and not fetid; and 5 = fetid, watery, red-brownish discharge with or without pieces of necrotic tissues present (de Oliveira et al., 2020). Cows identified by farm employees as possible metritis cases were also evaluated for metritis by the research team and enrolled in the study regardless of DIM. Cows diagnosed with metritis at <5 and ≥5 DIM were classified as EMET and LMET, respectively. Metritic cows were treated by farm personnel and received 6.6 mg/kg of ceftiofur crystalline-free acid (Excede, Zoetis), on the day of diagnosis and 3 d later. A single researcher scored cows at 4 DIM for BCS using a 5-point scale, considering score 1 as emaciated and 5 as obese (Ferguson et al., 1994).

Milk yield was recorded weekly by the research group by manually inputting the cow’s production (directly obtained from the parlor’s milk flow system) into a handheld data entry device (Pocket CowCard, Valley Agricultural Software), which was then automatically downloaded into DairyComp 305 (Valley Agricultural Software) for the duration of the data collection period. Cow-level data related to health events not assessed by the research crew; reproduction, milk production, and culling data were collected from the farm’s database software (DairyComp 305).

Blood Collection and Analysis. Blood samples were collected by coccygeal venipuncture at 1, 2, 3, 4, 5, 7, 10, and 14 DIM when cows were restrained in headlocks after the morning milking while having access to fresh TMR using a 20-gauge × 2.54-cm needle into 9-mL vacuum tubes containing spray-dried lithium heparin (Greiner Bio-One). Tubes were gently inverted to allow proper anticoagulant mixing and immediately placed in a thermo-conductive passive temperature-regulating module (Coolrack; BioCision), which was kept inside a cooler containing ice water and transported to the laboratory within 2 h after collection for processing. Samples were then centrifuged at 1,200 × g for 15 min, and plasma was stored at −80°C until further analysis. Samples collected at 1, 2, 3, 4, 5, 7, 10, and 14 DIM were analyzed for Ca, Mg, and glucose; samples at 3, 5, 7, 10, and 14 DIM were analyzed for FFA, BHB, albumin, urea, fructosamine, and creatinine; Hp was measured at 1, 2, 3, 4, 5, and 7 DIM. All energy and mineral markers were measured using an automated clinical chemistry analyzer (RX Daytona, Randox Laboratories Ltd.) using reagents supplied by the manufacturer. Intra- and interassay coefficients of variation were as follows: 2.4 and 3.3% for total Ca; 2.8 and 2.6% for total Mg; 1.8 and 3.9% for glucose; 0.7 and 1.8% for FFA; 1.0 and 1.4% for BHB; 1.3 and 2.4% for albumin; 1.5 and 2.4% for fructosamine; 1.7 and 2.0% for urea; and 2.8 and 4.0% for creatinine, respectively. Serum Hp concentration was determined using a colorimetric assay via quantification of the haptoglobin/hemoglobin complex by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982). Assays were performed in 16- × 100-mm borosilicate tubes; briefly, 5 µL of sample, deionized water (blank), or standard was added to 7.5 mL of a solution containing 0.6 g/L of o-dianisidine, 13.8 g/L sodium phosphate monobasic, and 0.5 g/L EDTA (pH = 4.1). Immediately, 25 µL of a solution containing 0.3 g/L bovine hemoglobin was added to each tube, followed by incubation in a water bath at 37°C for 45 min. Then, 100 µL of a freshly prepared 156 mM hydrogen peroxidase solution was added to each tube, and samples were incubated at room temperature for 60 min. Finally, 200 µL from each tube was transferred to a 96-well plate and the optical density read at 450 nm in an Epoch2 Microplate Spectrophotometer (BioTek Instruments Inc.). Haptoglobin concentration was calculated using standard curves generated by serial dilutions of a sample of known concentration determined by a commercially available ELISA kit as previously described (Cooke and Arthington, 2013). Intra- and interassay coefficients of variation were 5.2 and 7.3%, respectively.

Statistical Analysis

As previously mentioned, the data set used in the present study came from another prospective cohort study originally designed to characterize hypocalcemia in Jersey cows (Menta et al., 2021). Therefore, for the current study, no formal sample size calculation was performed. However, before data analysis, we conducted a sample size calculation based on findings from another previous study evaluating the association of biomarkers with metritis cure risk (Machado et al., 2020). In that study, the difference in circulating Hp concentration between cured and uncured cows was 0.4 mg/mL, with a standard deviation of approximately 0.6 mg/mL for both groups. Using those numbers and assuming 80% power and α = 0.05, we determined that we needed at least 37 cows per group (EMET vs. LMET) to detect a statistical difference in Hp concentration. Sample size calculation was performed using MedCalc version 18.11.6 (MedCalc Software). Because we had approximately 50 cows per group, we decided to proceed with analysis.

Descriptive statistics analysis for the number of animals enrolled per group, parity, dystocia, stillborn calves, retained placenta (RP), average days submitted to negative DCAD during the dry period, and previous...
gestation length at metritis diagnosis was performed using the chi-squared and ANOVA functions of JMP 14 (SAS Institute Inc.). Univariable analyses were performed to screen variables associated with the outcomes of interest at $P \leq 0.20$ before inclusion in the multivariable models.

Generalized linear models were fitted to the data using the MIXED procedure of SAS (SAS Institute Inc.) to assess the association of metritis based on DIM at diagnosis and blood markers and milk production. The data comprised a series of repeated measures of each dependent variable throughout all blood collection days and weeks of lactation. The independent variables metritis (NMET, EMET, and LMET), DIM of analyte assessment, parity, and previous gestation length were forced in all models. Parity was dichotomized to represent second versus third or greater parities. To account appropriately for within-cow correlation, the error term was modeled by imposing a Toeplitz covariance structure for all models; the selection of Toeplitz was based on the lowest Akaike’s information criterion. The normality of the residuals was evaluated using the Shapiro-Wilk statistic and normal probability plots residuals using the Univariate procedure of SAS. If the normality of residuals criteria was not met, the dependent variable was square root–transformed. Only Hp did not meet normality and was square root–transformed. The SLICE option using a Tukey-Kramer multiple comparison adjustment was used to explore interactions between metritis and time.

To assess the effect of metritis on time to pregnancy and time to culling or death, multivariable Cox proportional hazard models were fitted using the PHREG procedure in SAS. For the reproduction outcome, cows were right-censored if not diagnosed as being pregnant before culling, death, or until 150 DIM. For time to culling or death, cows were right-censored if they were alive at the end of the data collection period, which was also fixed at 150 DIM. To illustrate the median calving to pregnancy interval and the median time to culling or death for each independent variable, Kaplan-Meier survival analysis was performed using MedCalc version 18.11.6 software (MedCalc Software).

A manual backward stepwise selection procedure was used in all models, and variables at $P \leq 0.10$ were retained as main effects. Dystocia, stillborn calves, RP, mastitis up to 14 DIM, average days submitted to negative DCAD during dry period, BCS, and previous gestation length were offered in all models and retained if $0.05 < P < 0.1$. The independent variables metritis (NMET, EMET, and LMET), DIM of analyte assessment, and parity were forced in all models. For all models, statistical significance was declared if $P \leq 0.05$, and a tendency was considered if $0.05 < P < 0.1$. Data are presented as least squares means ± standard error of the mean and as hazard ratios with estimated 95% CI.

### RESULTS

#### Descriptive Statistics

The descriptive statistics regarding the number of animals enrolled per group, parity, dystocia, stillborn calves, RP, mastitis up to 14 DIM, average days submitted to negative DCAD during dry period, previous gestation length, and RT at metritis diagnosis are presented in Table 1. The incidence of metritis in the study was 26.9% (EMET, n = 49; LMET, n = 53; NMET, n = 277).

#### Association of Metritis Based on DIM at Diagnosis with Blood Metabolites and Mineral Concentrations

The association of metritis based on DIM at diagnosis with several blood biomarkers of metabolism is

<table>
<thead>
<tr>
<th>Item</th>
<th>Metritis group$^1$</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>NMET</td>
<td>EMET</td>
<td>LMET</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals enrolled, no. (%)</td>
<td>277 (73.1)</td>
<td>49 (12.9)</td>
<td>53 (14.0)</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-parity animals enrolled, no. (%)</td>
<td>107 (72.8)</td>
<td>17 (11.6)</td>
<td>23 (15.6)</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third and greater parity animals enrolled, no. (%)</td>
<td>170 (73.3)</td>
<td>32 (13.8)</td>
<td>30 (7.9)</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystocia, no. (%)</td>
<td>7 (63.6)</td>
<td>3 (27.8)</td>
<td>1 (9.09)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillborn, no. (%)</td>
<td>5 (38.5)</td>
<td>5 (38.5)</td>
<td>3 (23.0)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained placenta, no. (%)</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastitis incidence up to 14 DIM, no. (%)</td>
<td>14 (5.0)</td>
<td>4 (8.2)</td>
<td>0 (0)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average days submitted to negative DCAD (SE)</td>
<td>23.0 (0.80)</td>
<td>24.3 (1.80)</td>
<td>20.9 (1.87)</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous gestation length, d (SE)</td>
<td>279.7 (0.33)</td>
<td>278.5 (0.79)</td>
<td>279.5 (0.76)</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average RT$^2$ at metritis diagnosis, °C (SE)</td>
<td>—</td>
<td>38.7 (0.05)</td>
<td>38.6 (0.05)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$NMET = nonmetritic cows; EMET = cows diagnosed with metritis at <5 DIM (early); LMET = cows diagnosed with metritis at ≥5 DIM (late).

$^2$Rectal temperature.
presented in Table 2. The concentration of albumin throughout the blood sampling period was lower for cows diagnosed with metritis compared with NMET \( (P < 0.01) \). Additionally, we observed a difference in albumin when comparing NMET to EMET \( (P < 0.01) \) and LMET \( (P < 0.01) \) cows. Similarly, FFA concentration throughout the blood sampling period was greater for cows diagnosed with metritis compared with NMET cows \( (P < 0.01) \). However, only EMET cows \( (P < 0.01) \) had higher FFA concentrations than NMET cows. The associations of metritis based on DIM at diagnosis with several variables were dependent on day of sampling \( (P \leq 0.04; \text{Table 2}) \). The concentration of BHB was 0.29 and 0.40 mmol/L greater for EMET and LMET, respectively, compared with NMET counterparts at 10 DIM \( (P < 0.01; \text{Figure 1B}) \). Also, EMET \( (P = 0.03) \) and LMET \( (P = 0.02) \) had, on average, 0.14 and 0.15 mmol/L greater BHB concentration when compared individually with NMET cows, respectively (Table 2). The concentration of Ca at 2 \( (P < 0.01) \) and 10 DIM \( (P = 0.06) \) was 0.11 and 0.10 mmol/L lower for EMET cows, respectively, compared with NMET (Figure 1C). Also, EMET cows tended to have, on average, 0.04 mmol/L lower Ca concentration when compared individually to NMET cows \( (P = 0.08; \text{Table 2}) \). Although the interaction term between metritis and time of sampling was significant for linear models assessing the association of blood concentration of creatinine \( (P = 0.01, \text{Figure 1D}) \), statistically significant differences between EMET, LMET, and NMET were not observed on any specific sampling day. The concentration of fructosamine tended to be lower across all time points \( (P \leq 0.06) \) when comparing EMET and LMET with NMET (Figure 2E). Moreover, EMET and LMET cows had on average 16.4 and 13.5 mmol/L, respectively, lower fructosamine concentrations when compared individually to NMET cows \( (P < 0.01; \text{Table 2}) \).

The time of metritis diagnosis, the main effect of metritis, and the interaction term metritis \( \times \) sampling time were not associated with serum concentrations of glucose, magnesium, or urea \( (P > 0.10) \).

### Association Between Metritis Based on DIM at Diagnosis and Haptoglobin

The associations between metritis based on DIM at diagnosis and Hp are presented in Table 2 and Figure 3. The serum concentration of Hp averaged 0.84, 1.15, and 1.00 sqrt g/L (square root–transformed) for NMET, EMET, and LMET, respectively. Cows categorized as EMET and LMET had, on average, 0.31 and 0.16 sqrt g/L higher Hp compared with those characterized as NMET \( (P < 0.01) \). Also, EMET cows had lower Hp concentration compared with LMET \( (P = 0.04) \), and a greater concentration of Hp than NMET counterparts \( (P < 0.01) \). Additionally, EMET cows had higher concentrations of Hp at 2, 3, 4, 5, and 7 DIM \( (P \leq 0.05) \) compared with NMET, and LMET cows had higher concentrations of Hp at 3, 4, 5, and 7 DIM \( (P \leq 0.05) \).

### Reproductive Performance

A multivariable Cox proportional hazard model was performed to evaluate the effect of metritis based on DIM at diagnosis on the hazard of pregnancy. The only variables retained in this model were parity and metritis based on DIM at diagnosis. Cows classified as LMET had decreased hazard of pregnancy compared with NMET counterparts \( (P = 0.04; \text{hazard ratio} = 0.60; \text{Table 3}) \). The proportion of pregnant cows for

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**Table 2.** Association between NMET, EMET, and LMET cows and variables related to energy metabolism, minerals, and haptoglobin measured in plasma of Jersey cows

<table>
<thead>
<tr>
<th>Variable</th>
<th>NMET</th>
<th>EMET</th>
<th>LMET</th>
<th>Metritis (M)</th>
<th>Time (T)</th>
<th>M × T</th>
<th>NMET vs. EMET</th>
<th>NMET vs. LMET</th>
<th>EMET vs. LMET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin, mmol/L</td>
<td>3.10</td>
<td>2.96</td>
<td>2.97</td>
<td>&lt;0.01</td>
<td>0.33</td>
<td>0.12</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BHB, mmol/L</td>
<td>0.77</td>
<td>0.91</td>
<td>0.92</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.06</td>
<td>2.01</td>
<td>2.05</td>
<td>0.20</td>
<td>&lt;0.01</td>
<td>0.04</td>
<td>0.08</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>54.44</td>
<td>53.76</td>
<td>54.56</td>
<td>0.92</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>0.70</td>
<td>0.94</td>
<td>0.72</td>
</tr>
<tr>
<td>Fructosamine, mmol/L</td>
<td>182.76</td>
<td>166.38</td>
<td>169.31</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.52</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>3.08</td>
<td>3.16</td>
<td>3.07</td>
<td>0.26</td>
<td>&lt;0.01</td>
<td>0.12</td>
<td>0.11</td>
<td>0.89</td>
<td>0.18</td>
</tr>
<tr>
<td>Magnesium, mmol/L</td>
<td>0.83</td>
<td>0.82</td>
<td>0.83</td>
<td>0.97</td>
<td>&lt;0.01</td>
<td>0.66</td>
<td>0.85</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>Free fatty acids, mmol/L</td>
<td>0.48</td>
<td>0.58</td>
<td>0.52</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.35</td>
<td>&lt;0.01</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>3.65</td>
<td>3.77</td>
<td>3.83</td>
<td>0.33</td>
<td>&lt;0.01</td>
<td>0.23</td>
<td>0.41</td>
<td>0.18</td>
<td>0.71</td>
</tr>
<tr>
<td>Haptoglobin, sqrt/g/L</td>
<td>0.84</td>
<td>1.15</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.12</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1NMET = nonmetritic cows; EMET = cows diagnosed with metritis at <5 DIM (early); LMET = cows diagnosed with metritis at ≥5 DIM (late).

2Square root-transformed haptoglobin.
Figure 1. The association of cows diagnosed with metritis at <5 DIM (EMET, red line; n = 49) and ≥5 DIM (LMET, black line; n = 53) and nonmetritic cows (NMET, gray line; n = 277) with (A) albumin, (B) BHB, (C) calcium, (D) creatinine, (E) fructosamine, (F) glucose, (G) magnesium, (H) FFA (free fatty acids), and (I) urea. Error bars represent SEM. Symbols indicate a difference at *P < 0.05: * = EMET vs. NMET; † = LMET vs. NMET; ¥ = EMET vs. LMET.
EMET, LMET, and NMET cows was 40.8 (20/49), 35.8 (19/53), and 52.7% (146/277), respectively (Figure 3A).

A multivariable Cox proportional hazard model was performed to evaluate the effect of metritis (without subcategorization into early or late) on the hazard of pregnancy. The only variables retained in this model were parity and metritis. Cows that were diagnosed with metritis up to 10 DIM had decreased hazard of pregnancy compared with NMET counterparts ($P = 0.01$; hazard ratio = 0.64; Table 3). The proportion of pregnant cows for metritic (MET) and NMET cows was 38.2 (39/102) and 52.7% (146/277), respectively (Figure 3B).

**Culling and Death**

A multivariable Cox proportional hazard model was performed to evaluate the effect of time to metritis diagnosis on culling up to 150 DIM (Table 4). Metritis based on DIM at diagnosis was not associated with culling or death likelihood ($P > 0.11$). The proportion of culled cows for EMET, LMET, and NMET cows was 22.4 (11/49), 20.7 (11/53), and 17.0% (47/277), respectively (Figure 4A).

A multivariable Cox proportional hazard model was performed to evaluate the effect of metritis (without subcategorization into early or late) on culling up to 150 DIM (Table 4). Metritis incidence was not associated with the hazard of removal from the herd ($P = 0.10$). The proportion of culled cows for MET and NMET cows was 21.6 (22/102) and 17.0% (47/277), respectively (Figure 4B).

**Milk Production**

The least squares means of milk production for NMET cows (34.0 kg/d), EMET cows (32.5 kg/d), and LMET cows (34.0 kg/d) by week of lactation are presented in Figure 5A. The variables retained in this model were parity, week of lactation, previous gestation length, time to metritis, and the interaction between time to metritis and week of lactation. No differences in average milk production were observed between EMET, LMET, and NMET cows in the first 9 wk of lactation. Cows categorized as EMET had or tended to have lesser production in the second ($P = 0.03$) and fifth ($P = 0.09$) weeks of lactation compared with NMET counterparts. In addition, each additional unit of previous gestation length ($P < 0.01$) increased production by 0.24 kg/d.

The least squares means of milk production for NMET cows (34.0 kg/d) and MET cows (33.3 kg/d) by week of lactation are presented in Figure 5B. The variables retained in this model were parity, week of lactation, previous gestation length, metritis, and the interaction between metritis and week of lactation. No differences in average milk production were observed between MET and NMET cows in the first 9 wk of lactation; MET cows had lower production only in the second ($P = 0.01$) week of lactation compared with NMET counterparts.
DISCUSSION

Our study used a convenient data set from a prospective cohort study designed to evaluate the association of Ca and energy markers with disease and performance in lactating Jersey cows (Menta et al., 2021). To better understand the metabolic and mineral imbalances associated with EMET and LMET, and to evaluate the effects of metritis on Jersey cows, we performed a prospective cohort study reclassifying metritic cows based on DIM at diagnosis. The underlying principle of classifying cows based on DIM of metritis diagnosis was based on recent findings on factors associated with metritis cure (Machado et al., 2020; Figueiredo et al., 2021). It has been demonstrated that DIM at diagnosis was associated with risk of cure and influenced lactation performance of Holstein cows. Ceftiofur-treated cows that were diagnosed with metritis after 5 DIM

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Kaplan-Meier survival analysis of calving to conception interval for Jersey cows (A) diagnosed with metritis at <5 DIM (EMET; n = 49) and ≥5 DIM (LMET; n = 53) and nonmetritic cows (NMET; n = 277); and (B) diagnosed with metritis up to 10 DIM (MET; n = 102) and nonmetritic cows (NMET; n = 277). The median calving to conception interval was 127, 120, and 109 d for EMET, LMET, and NMET cows, respectively ($P = 0.06$); the median calving to conception interval for MET and NMET cows was 109 and 121, respectively ($P = 0.02$).
had increased odds of cure and produced more milk compared with cows diagnosed with metritis before 5 DIM. Likewise, nontreated cows diagnosed with metritis at ≥8 DIM had impaired milk production, decreased risk of pregnancy, and increased culling risk compared with NMET cows (Machado et al., 2020). Similarly, another study demonstrated that DIM at metritis diagnosis is associated with the odds of cure (de Oliveira et al., 2021). In that study, cows that were diagnosed at ≥8 DIM had 1.17 higher odds of attaining clinical cure compared with cows diagnosed sooner. Another study established that 5 DIM was the threshold that maximized the sensitivity and specificity of models predicting metritis cure in treated metritic cows (Figueiredo et al., 2015). Because all of our cows diagnosed with metritis were also treated with ceftiofur, we used 5 DIM as our threshold to characterize EMET and LMET cows.

Table 3. Cox proportional hazard analyses evaluating the effect of DIM at metritis diagnosis and metritis on the hazard of pregnancy up to 150 d after parturition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Effect of DIM at metritis diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second parity Referent</td>
<td>1.36 (1.01–1.81)</td>
<td>0.04</td>
</tr>
<tr>
<td>≥Third parity</td>
<td>Referent</td>
<td>0.88 (0.47–1.66)</td>
</tr>
<tr>
<td>LMET vs. EMET</td>
<td>0.68 (0.43–1.08)</td>
<td>0.11</td>
</tr>
<tr>
<td>EMET vs. NMET</td>
<td>0.60 (0.37–0.97)</td>
<td>0.04</td>
</tr>
<tr>
<td>LMET vs. NMET</td>
<td>0.60 (0.37–0.97)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2: Effect of metritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second parity Referent</td>
<td>1.35 (1.01–1.80)</td>
<td>0.04</td>
</tr>
<tr>
<td>≥Third parity</td>
<td>Referent</td>
<td>0.64 (0.45–0.91)</td>
</tr>
<tr>
<td>MET vs. NMET</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1NMET = nonmetritic cows; EMET = cows diagnosed with metritis at ≥5 DIM (late); MET = metritic cows.

Haptoglobin is an acute-phase protein that is commonly used as an indicator of systemic inflammation (Ceciliani et al., 2012). Albumin is a negative acute-phase protein that is inversely correlated with Hp; a lower concentration of albumin in the blood suggests a reduction of liver functionality due to systemic inflammation that reduces albumin synthesis (Trevisi et al., 2016). Many studies have demonstrated an association between inflammation status around parturition and lactational performance of dairy cows. For instance, it has been shown that Hp is associated with postpartum disease and impaired lactation performance (Huzzey et al., 2009; Dervishi et al., 2016; Machado et al., 2020). Moreover, reduced concentrations of albumin have been associated with decreased reproductive performance in dairy cows (Krause et al., 2014). Also, Hp concentrations measured at 3 DIM have been associated with metritis severity (Huzzey et al., 2009). Although we observed an association between albumin and metritis, no differences were found when comparing EMET and LMET. However, our results demonstrated that EMET had greater Hp concentrations than LMET and NMET cows, suggesting that EMET may represent a more severe metritis case than LMET. This could help explain why EMET affected milk production and reproductive performance in a more pronounced manner than LMET (Machado et al., 2020), although it is important to note that major differences in milk production between EMET and LMET were not observed in our study. Normally, milk yield losses associated with metritis are seen until 90 DIM (Wittrock et al., 2011) in Holsteins. We speculate that the lack of association herein is due to the difference milk production magnitude between breeds, as Jerseys produce a lower volume of milk than Holsteins (Olson et al., 2010). Also, future research evaluating difference in milk components in Jersey metritic cows is needed, because fat and protein have a high economic importance to the dairy industry, and Jerseys are known for their greater milk total ash content compared with Holsteins (Cerbulis and Farrell, 1976). Elevated Hp concentration in the postpartum period has been associated with milk production losses, regardless of whether the cow has metritis (Machado et al., 2020; Martins et al., 2021). Additionally, others have observed that increased concentrations of Hp are associated with impaired resumption of ovarian activity and lower risk of pregnancy (Krause et al., 2014; Huzzey et al., 2015). In contrast, high concentrations of Hp in clinically healthy cows was associated with greater neutrophil response and improved reproductive performance (Nightingale et al., 2015). Hence, we believe that the differences in Hp concentration between EMET and LMET cows provide more evidence to support the rationale that EMET cases are more severe than LMET.

Table 4. Cox proportional hazard analyses evaluating the effect of DIM at metritis diagnosis and metritis on the hazard of culling or being sold or dying up to 150 d after parturition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Effect of DIM at metritis diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second parity Referent</td>
<td>5.68 (1.71–18.83)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥Third parity</td>
<td>Referent</td>
<td>1.24 (0.38–4.084)</td>
</tr>
<tr>
<td>LMET vs. EMET</td>
<td>1.69 (0.62–4.59)</td>
<td>0.30</td>
</tr>
<tr>
<td>EMET vs. NMET</td>
<td>2.11 (0.83–5.36)</td>
<td>0.11</td>
</tr>
<tr>
<td>LMET vs. NMET</td>
<td>5.63 (1.70–18.65)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥Third parity</td>
<td>Referent</td>
<td>1.90 (0.89–4.05)</td>
</tr>
</tbody>
</table>

1NMET = nonmetritic cows; EMET = cows diagnosed with metritis at <5 DIM (early); LMET = cows diagnosed with metritis at ≥5 DIM (late); MET = metritic cows.
It is important to highlight that Hp concentration was only assessed until 7 DIM, and that some cows in the LMET group had increased circulating concentrations of Hp. However, most cows in the LMET group had developed metritis by 7 DIM.

In our study, FFA and BHB were associated with the main effect of metritis. The association between metritis and increased concentrations of BHB and FFA has been consistently reported in the literature (Galvão et al., 2010; Chapinal et al., 2011; Menta et al., 2021). Increased concentrations of FFA and BHB are known to exist in periparturient cows due to the excess energy demands (Chapinal et al., 2012). A study evaluating the association of metritis on behavioral parameters showed that cows diagnosed with metritis have decreased feeding time (Merenda et al., 2021), which leads to greater lipolysis. Also, greater concentrations of FFA and BHB have been associated with impaired

Figure 4. Kaplan-Meier survival analysis of culling or death for Jersey cows (A) diagnosed with metritis at <5 DIM (EMET; n = 49) and ≥5 DIM (LMET; n = 53) and nonmetritic cows (NMET; n = 277); and (B) diagnosed with metritis up to 10 DIM (MET; n = 102) and nonmetritic cows (NMET; n = 277; P = 0.51).
neutrophil function (Hammon et al., 2006), and neutrophils are recognized as the main defense cell responsible for bacterial clearance after uterine infection (Hussain, 1989). Herein, we also observed that EMET and LMET cows had, on average, greater BHB than NMET cows, whereas greater FFA concentration was only observed in cows diagnosed with EMET compared with NMET cows. Although no association between metritis and glucose concentration was observed in this data set, we showed a time-dependent association between fructosamine and metritis incidence: NMET cows had increased levels of fructosamine compared with EMET and LMET cows. Fructosamine is a marker of retrospective glucose concentration (Voziyan et al., 2003; Caré et al., 2018); therefore, it is not correlated with the actual glucose concentration, which partly explains the lack of association with glucose. Also, fructosamine has been associated with postpartum inflammation and reduced DMI (Caré et al., 2018; Mammi et al., 2021; Martins et al., 2021), which could partly explain its higher concentration in cows diagnosed with metritis.

Although most studies report that metritis has detrimental effects on milk yield, reproductive performance, and culling of dairy cows, in our study, metritis only caused reproductive losses and some marginal losses in milk yield (de Oliveira et al., 2020; Machado et al.,

**Figure 5.** Least squares means of milk production by week of lactation for Jersey cows (A) diagnosed with metritis at <5 DIM (EMET; n = 49) and ≥5 DIM (LMET; n = 53) and nonmetritic cows (NMET; n = 277); and (B) metritis diagnosed up to 10 DIM (MET; n = 102) and nonmetritic cows (NMET; n = 277). Error bars represent SEM. Symbols indicate a difference at $P < 0.05$: * = EMET vs. NMET (panel A) or MET vs. NMET (panel B); ¥ = EMET vs. LMET.
However, it is important to acknowledge that most of the studies on this topic evaluated Holstein cows, whereas the present data set only evaluated purebred Jersey cows. Reproductive losses caused by metritis were more pronounced in LMET than in EMET cows, in contrast to previous findings (Machado et al., 2020) and to our rationale that EMET cases were more severe than LMET cases. However, the milk yield losses caused by metritis in the second week of lactation were mainly associated with EMET. Further, we did not observe any differences in milk production when comparing EMET and LMET cows, as previously reported (Machado et al., 2020). Additionally, we did not observe any differences in culling when comparing metritic and NMET cows, or when comparing EMET and LMET cows. It is important to highlight that we used a convenient sample size, which may have impaired study power and our ability to appropriately determine some of these associations that have been consistently established in the literature (Dubuc et al., 2010; Galvão et al., 2020).

Although we did not report strong effects of metritis based on DIM at diagnosis on lactational performance, we speculate that the findings reported herein potentially explain the association of DIM of metritis diagnosis with disease severity and production losses reported in the past (Machado et al., 2020; Figueiredo et al., 2021). With EMET cows having greater Hp and FFA concentrations compared with LMET and NMET cows, respectively, we speculate that EMET cows undergo a greater degree of inflammation and lipolysis than LMET counterparts. However, we caution readers to carefully interpret our results, considering that we used a convenient data set and multiparous Jersey cows and, as reported in the literature, greater metritis incidence is seen in primiparous cows (Giuliodori et al., 2013). Furthermore, our data came from a single commercial farm in west Texas. More research is needed to elucidate whether EMET cases are more severe and require different treatment strategies than LMET cases in both primiparous and multiparous cows.

CONCLUSIONS

We observed that Hp concentrations were greater for EMET cows than for LMET and NMET counterparts, and that EMET cows had greater FFA concentrations than NMET cows, suggesting that EMET cows represent more severe metritis cases than LMET cows. However, this is in contrast with our findings related to lactational performance, as impaired reproductive performance compared with NMET cows was only observed for LMET cows; no significant differences in milk production, reproduction, or culling were observed between EMET and LMET cows. Also, EMET and LMET had lower albumin, BHB, and fructosamine compared with NMET cows.

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REFERENCES


Menta et al.: TIME TO METRITIS DIAGNOSIS


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