Association between 4-day vaginal temperature assessment during the dry period and performance in the subsequent lactation of dairy cows during the warm season^{1,2}

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ABSTRACT: The objective of the study was to investigate the relationships between vaginal temperature during the dry-period and health, milk production, and reproduction in the subsequent lactation of cows during the warm season. A total of 105 nonlactating Holstein cows from 2 dairies were enrolled in the study during summer. At enrollment, cows were between 250 and 260 d of gestation. Vaginal temperature (VT) and corral ambient temperature and humidity were recorded every 5 min for 4 consecutive days starting at enrollment. Cows were categorized as presenting high (HT) or low temperature (LT) based on the median values of average VT and were followed until 300 d in milk (DIM) of the subsequent lactation to evaluate health disorders, culling rate, milk yield, and reproductive efficiency. Cows that became pregnant were followed until subsequent calving. Cows were monitored for uterine diseases (UTD) and mastitis (MAST) by farm personnel. Individual milk yield was recorded monthly until 300 DIM. Cows classified as HT had shorter ($P < 0.01$) gestation length (273.9 ± 0.9 vs. 278.7 ± 1.0 d) and spent fewer ($P < 0.01$) days in the close-up pen (14.3 ± 0.8 vs. 19.4 ± 1.0 d) than LT cows. Hazard to UTD or MAST in the first 60 DIM was

greater for HT than LT cows (adjusted hazard ratio [AHR] = 5.15, 95% CI = 1.91 to 13.86). Cows classified as HT had greater hazard to MAST in the first 300 DIM compared with LT cows (AHR = 2.39; 1.03 to 5.56). Vaginal temperature was not associated with milk yield. In contrast, the interaction between VT category and month of lactation tended to influence milk yield. This interaction was observed because cows categorized as LT had greater ($P < 0.01$) milk yield in the first month of lactation compared with HT cows (39.2 ± 1.6 vs. 33.7 ± 1.5 kg), whereas milk yield tended ($P = 0.07$) and was greater ($P = 0.05$) for HT cows in the ninth (32.7 ± 1.6 vs. 28.5 ± 1.9 kg) and tenth (29.9 ± 1.7 vs. 25.0 ± 2.0 kg) month of lactation, respectively. Pregnancy per AI at first service, interval from calving to pregnancy, and percentage of cows calving in the subsequent lactation did not differ between HT and LT cows. In conclusion, VT assessed between 20 and 30 d before expected calving is associated with health outcomes and milk production in the subsequent lactation. In addition, cows susceptible to be affected by postpartum disorders after calving may be identified during the summer by evaluating VT temperature at 250 to 260 d of gestation.

Key words: dairy cow, health disorders, summer heat stress, vaginal temperature.

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INTRODUCTION

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Heat stress has profound effects on productive performance of dairy cows (West, 2003). Lactating dairy cows exposed to heat stress have reduced DMI, which results in significant reduction in milk yield during periods of elevated temperatures. Besides the negative effects of hyperthermia on immediate milk loss of lactating cows, exposure to heat stress during the dry period also impacts milk production in the subsequent lactation (Collier et al., 2006). Compromised mammary cell

proliferation is a potential mechanism by which heat-stressed dry cows have reduced milk production in the next lactation (Tao et al., 2011). The impact of heat stress in late gestation on milk production might also be related to increased incidence of postpartum disorders. There is evidence that cows exposed to heat stress during the dry period have greater incidence of health disorders after calving (Thompson and Dahl, 2012).

Even though several studies evaluated the effects of heat stress during the dry period on performance in the subsequent lactation, most experiments did not investigate whether subpopulations of cows are more susceptible to be affected by heat stress. It is possible that dry cows less resilient to heat stress may be more prone to have postpartum health disorders and decreased milk production and fertility in the next lactation. It is unknown whether body core temperature in late gestation is a useful predictor to identify this subpopulation of cows.

We hypothesized that during the warm season, dry cows with greater vaginal temperature (VT) than their counterparts would have greater incidence of postpartum health disorders and reduced productive and reproductive performance in the subsequent lactation. The objective of this study was to evaluate overall performance of Holstein cows categorized by VT assessed in the dry period.

MATERIALS AND METHODS

This study, including all the procedures performed, was approved by the Kansas State University's Institutional Animal Care and Use Committee (IACUC).

Animals, Housing, and Feeding

The experiment was conducted in 2 commercial dairies located in southwest Kansas. Thirty-seven primiparous and 68 multiparous (second lactation = 37; and third lactation = 31) dry Holstein cows with 250 to 260 d of gestation were enrolled in the study from June to July 2014. Mean ($\pm$ SEM) and median DIM at dry-off were 325.6 (4.7) and 317, respectively. Cows eligible to be enrolled in the study had a locomotion score < 3 (Sprecher et al., 1997) and 4 functioning quarters at dry-off. Enrollment of cows in the study was based on weather forecast to ensure cows were exposed to similar weather conditions in the first 4 d after enrollment. The criterion for enrollment was forecast of maximum temperature $> 32^{\circ}\text{C}$ during the week of enrollment. Cows were enrolled weekly in cohorts (replicates). In Dairy A, a total of 90 cows were enrolled in 4 replicates. First, second, third, and fourth replicates consisted of 18, 18, 24, and 30 cows, respectively. In Dairy B, a total of 15 cows were enrolled in 1 replicate. Body condition score

was assessed at enrollment using a scale from 1 to 5 (Ferguson et al., 1994) and categorized into low BCS (**LBCS**; ≤ 3.5) or high BCS (**HBCS**; ≥ 3.75). Cows were followed until 300 DIM of the next lactation to evaluate health disorders, culling rate, milk yield, and reproductive efficiency. Cows that became pregnant were followed until subsequent calving. In Dairy A, dry cows were housed in dry-lot pens with shaded areas. Lactating cows were housed in free-stall barns equipped with fans and sprinklers, with access to a dirt exercise lot, and milked thrice daily. In Dairy B, lactating and dry cows were housed in dry-lot corrals with shade. Cows were milked twice daily. Cows from both dairies were fed once daily with a total mixed ration formulated to meet or exceed nutrient requirements (NRC, 2001). After 260 d of gestation, cows were moved from the far-off pen to the close-up pen and were fed acidogenic salts. Days spent in the close-up pen for each individual cow was recorded in the on-farm management software (DairyComp 305; ValeyAg Software, Tulare, CA).

Vaginal Temperature, Ambient Temperature and Humidity, and Milk Yield Measurements

At enrollment, 250 to 260 d of gestation, a calibrated temperature logger (iButton DS1922L; Embedded Data Systems, Lawrenceburg, KY) fitted in a blank controlled internal drug release (CIDR) was inserted into the vagina to collect VT measurements. Vaginal temperature was assessed while cows were in the far-off pen. Loggers were calibrated for accuracy of $\pm 0.13^{\circ}\text{C}$ and were fixed in the CIDR device using a moisture-resistant flexible plastic (Parafilm; Bemis Company, Inc., Oshkosh, WI) and silicone aquarium sealant (Loctite; Henkel Corporation, Rocky Hill, CT). Vaginal temperature was collected every 5 min for 4 consecutive days while cows were in the far-off pen. Time of CIDR insertion and removal were documented. During the time that VT was being monitored, ambient temperature and humidity were recorded every 5 min from the pens where cows were housed by placing temperature loggers (HOBO U23 Pro v2; Onset Computer Corp., Pocasset, MA) approximately 3 m above ground level. The following equation was used to calculate temperature-humidity index (**THI**): $\text{THI} = T - (0.55 - 0.55 \text{ RH}/100) \times (T - 58)$, where T and RH are dry bulb temperature ($^{\circ}\text{F}$) and relative humidity, respectively (NOAA, 1976).

After CIDR removal, measurements recorded by loggers were downloaded to a computer. Vaginal temperature measurements collected within 1 h after CIDR insertion were excluded to ensure accuracy of the temperature readings. For statistical analysis, average VT of all readings was calculated for each cow, and median values of average VT were calculated within

each parity group. Cows with average VT less than the median value were classified as low temperature (**LT**), and cows with average VT greater than or equal to the median value were classified as high temperature (**HT**).

Milk yield was recorded for individual cows using a parlor management software (DairyPlan C21; GEA Farm Technologies, Naperville, IL). In the first day of the month, average milk yield of the last 7 d was recorded in the on-farm management software (DairyComp 305). This information was extracted from calving to 300 DIM to obtain milk yield for each month of lactation (1 to 10). Milk yield information was limited according to DIM. The range of the minimum DIM for first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, and tenth months were from 5 to 34, 35 to 64, 65 to 94, 95 to 124, 125 to 154, 155 to 184, 185 to 214, 215 to 244, 245 to 274, and 275 to 304, respectively.

Definitions of Health Disorders

During the duration of the study, the standard operating procedures for diagnosis and treatment of diseases of each dairy was maintained according to the veterinarian protocol. Diagnosis and treatments were recorded in the on-farm management software on a daily basis by trained farm personnel. Health parameters of interest in the current study were: uterine diseases (**UTD**; retained placenta and metritis) and mastitis (**MAST**). Cows that did not release fetal membranes within 24 h after calving were considered to have retained placenta. Metritis was characterized by the presence of fetid uterine discharge between 4 and 21 DIM. Lactating cows with abnormal milk (i.e., presence of clots or distinct coloration) with or without udder swelling or systemic symptoms were considered a case of mastitis. For statistical analyses, cows were classified by the occurrence of mastitis in the first 60 (**MAST60**) or 300 (**MAST300**) DIM.

Reproductive Management

Estrus detection was performed by a trained technician in the morning. Cows were considered to be in estrus based on tail paint removal and were eligible to be inseminated after 53 DIM. Cows were presynchronized with 2 injections of PGF_{2α} (cloprostenol; 500 µg of Estrumate; Merck Animal Health, Madison, NJ) given 14 d apart, starting at 56 ± 3 DIM. Cows not inseminated in estrus 14 d after PGF_{2α} treatments were enrolled in the Cosynch-72 timed AI protocol, which consisted of a GnRH injection followed 7 d later by PGF_{2α} injection and 3 d later a GnRH injection and AI. Pregnancy diagnosis was conducted by transrectal ultrasonography at 36 ± 3 d after AI. Pregnancy was characterized by the presence

of fluid and an embryo with a heartbeat. Nonpregnant cows received a PGF_{2α} injection to induce estrus. Cows not re-inseminated after 7 d of nonpregnancy diagnosis were enrolled in the GGPG protocol (Dewey et al., 2010), which consisted of treating cows with GnRH 7 d before initiating the Cosynch-72 timed AI protocol.

Pregnancy per AI was calculated by dividing the number of cows diagnosed pregnant by the number of cows that received AI. Days open (**DOPN**) was defined as the interval between the date of calving and the date a cow received an insemination that resulted in pregnancy.

Statistical Analyses

Binary variables were analyzed by logistic regression using the LOGISTIC procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Complete case analyses were conducted to assess adjusted odds ratio (**AOR**) of health disorders. The rate at which cows were culled, diagnosed with health disorders, inseminated at first service, and became pregnant were analyzed by the Cox proportional hazard using the PHREG procedure of SAS. The interval from calving to occurrence of an event were evaluated by the Kaplan-Meier survival analysis using the LIFETEST procedure of SAS. Continuous variables were analyzed by ANOVA using the GLM procedure of SAS or by ANOVA for repeated measures using the MIXED procedure of SAS. All models included parity (primiparous vs. multiparous), BCS category at enrollment (high vs. low), VT category (high vs. low), dairy, average daily maximum THI of each replicate, and the interaction between parity and VT category as fixed effects. Models for repeated measures (e.g., milk yield analyses) also included interactions between month of lactation and parity, month of lactation and BCS category, month of lactation and VT category, VT category and parity, and month of lactation and VT category and parity. Additional analyses were performed to further investigate the association between VT category and milk yield. Models for additional analyses consisted of including health disorders in the first 60 DIM as an independent variable or excluding cows that were removed from the herd from the analysis. Unstructured covariance structure was used for the repeated measure analysis based on the Akaike's information criterion (**AIC**). Other covariance structures evaluated were: compound symmetry and autoregressive (1). Dairy and average daily maximum THI of replicates were forced to remain in all the models. Variables and interactions with a *P* value > 0.10 were removed from the model by stepwise backward elimination. Statistical significance was defined as *P* < 0.05 and statistical tendencies as 0.05 ≤ *P* ≤ 0.10.

Table 1. Percentage of cows classified as having high or low vaginal temperature per replicate and ambient temperature, relative humidity, and temperature-humidity index (THI) during vaginal temperature assessment

Item	Replicate number ¹				
	1 (Dairy A)	2 (Dairy A)	3 (Dairy A)	4 (Dairy A)	1 (Dairy B)
Cows classified HT ² , %	52.9 (9/17)	33.3 (6/18)	54.2 (13/24)	43.3 (13/30)	80.0 (12/15)
Cows classified LT ² , %	47.1 (8/17)	66.7 (12/18)	45.8 (11/24)	56.7 (17/30)	20.0 (3/15)
			Mean		
Daily maximum temperature, °C	35.8	37.0	37.4	34.7	33.6
Daily minimum temperature, °C	16.9	16.6	19.8	19.8	21.7
Daily maximum relative humidity, %	84.7	92.2	82.1	77.8	94.5
Daily minimum relative humidity, %	18.7	25.1	23.6	29.7	48.5
Daily maximum THI	79.2	82.2	83.4	81.4	80.6
Daily minimum THI	61.4	61.8	66.7	66.4	68.3

¹Weekly cohorts of cows enrolled in the study.

²Vaginal temperature category was determined using median values of VT of cows from all replicates calculated separately for each parity.

RESULTS

Temperature-Humidity Index, Vaginal Temperature, and Parturition Descriptive Data

One primiparous cow from dairy A lost the CIDR insert during the first replicate and her records were excluded from the study.

Average daily THI during the period of enrollment was 73.4 for dairy A (4 replicates) and 74.2 for dairy B (1 replicate). In addition, average daily maximum THI was 81.5 for dairy A and 80.6 for dairy B, and average daily minimum THI was 63.9 and 65.8 for dairies A and B, respectively. Average daily maximum and minimum temperature were 36.2°C and 18.2°C for dairy A, and 33.6°C and 19.2°C for dairy B, respectively. Average daily maximum and minimum relative humidity were 84.5% and 24.3% for dairy A, and 94.5% and 32.5% for dairy B, respectively. Average daily maximum and minimum ambient temperature, relative humidity, and THI for each replicate are described in Table 1.

Cows classified as HT had greater ($P < 0.01$) VT compared with LT cows (Table 2). In addition, primiparous cows had greater ($P < 0.01$) VT than multiparous cows (38.91 ± 0.03 vs. $38.82 \pm 0.03^\circ\text{C}$). Vaginal temperature was not associated with the interaction between parity and VT category (Table 2). In addition, VT was not ($P = 0.52$) associated with BCS category.

Projected 305-d mature equivalent milk yield, BCS, days after calving at dry-off and at enrollment, and dry period length did not differ between HT and LT cows (Table 2). In contrast, HT cows had decreased ($P < 0.01$) gestation length and lesser ($P < 0.01$) days spent in the close-up pen than LT cows (Table 2). Primiparous cows had shorter ($P \leq 0.03$) gestation length (275.0 ± 1.0 vs. 277.6 ± 0.8 d), dry period length (51.7 ± 2.2 vs. 56.8 ± 1.8 d), and days spent in the

close-up pen (15.5 ± 1.0 vs. 18.2 ± 0.8 d) than multiparous cows. The interaction between parity and VT category affected ($P = 0.02$) gestation length and tended ($P = 0.10$) to affect days spent in the close-up pen.

Health Disorders

Cows classified as HT were more likely ($P < 0.01$) to have UTD, UTD or MAST60, and UTD or MAST300 and tended ($P = 0.08$) to be less likely to be culled by 300 DIM compared with LT cows (Table 3). No difference was detected in culling by 30 DIM. Vaginal temperature category was not associated with incidence of MAST60 or calving in the subsequent lactation, but there was a tendency ($P = 0.09$) for the interaction between VT category and parity to be associated with MAST300 (Table 3). The interaction between VT category and parity was not associated with other health disorders, culled by 300 DIM, or calving in the subsequent lactation. Dairy was not ($P \geq 0.21$) associated with health disorders, culling, or calving in the subsequent lactation. Average maximum THI of replicate was ($P = 0.01$) and tended ($P = 0.07$) to be associated with UTD or MAST300 and MAST300, respectively.

Category of VT was not associated with hazard to MAST60 (Table 4). In contrast, hazard to UTD or MAST60, UTD or MAST300, and MAST300 were greater ($P \leq 0.04$) for HT compared with LT cows (Table 4). Dairy, BCS category, and the interaction between parity and VT category did not ($P \geq 0.12$) affect hazard of occurrence of health disorders. Average maximum THI of replicate was ($P = 0.04$) associated only with hazard to UTD or MAST300. In the other models that evaluated the hazard to disease, average maximum THI was not ($P > 0.14$) associated with health disorders. Interval from calving to UTD or MAST60, UTD or MAST300, or MAST300 differed ($P < 0.01$)

Table 2. Prepartum descriptive data (mean $\pm$ SEM) of cows classified as low or high vaginal temperature before calving

Item	Vaginal temperature (VT) category		P-value		
	Low temperature	High temperature	VT category ¹	Parity	Interaction
Number of cows	51	53			
Average vaginal temperature, °C	38.72 (0.03)	39.01 (0.03)	< 0.01	< 0.01	0.43
Previous projected 305-d mature equivalent milk yield, kg	12,599 (340)	12,340 (280)	0.48	0.22	0.55
Body condition score ²	3.7 (0.06)	3.7 (0.05)	0.49	0.41	0.63
Days after calving at dry-off	317.7 (9.7)	318.4 (8.0)	0.95	0.91	0.22
Days after calving at enrollment	348.1 (9.7)	351.8 (8.0)	0.73	0.74	0.19
Days of gestation at enrollment	252.9 (0.5)	254.1 (0.4)	0.03	0.44	0.93
Gestation length, d	278.7 (1.0)	273.9 (0.9)	< 0.01	0.02	0.02
Dry period length, d	56.2 (2.3)	53.3 (1.9)	0.24	0.03	0.15
Days spent in close-up pen	19.4 (1.0)	14.3 (0.8)	< 0.01	0.01	0.10

¹Vaginal temperature category was determined using median values of VT calculated separately for each parity.

²Body condition score is on a 1 (thin) to 5 (obese) scale.

between VT category. In addition, the interval from calving to MAST60 tended ($P = 0.08$) to differ between VT category. Mean days to UTD or MAST60 ($\pm$ SEM) for LT and HT cows were 37.8 ± 3.5 and 42.2 ± 1.3 , respectively. Kaplan-Meier survival curve representing the interval from calving to UTD or MAST300 is depicted in Fig. 1. Mean days to MAST300 for LT and HT cows were 132.0 ± 9.1 and 137.6 ± 5.5 , respectively.

Cows classified as HT tended ($P = 0.09$) to have lesser hazard of being culled than LT cows (Table 4). In addition, multiparous cows tended ($P = 0.06$) to be culled at a faster rate compared with primiparous cows (adjusted hazard ratio [AHR] = 0.44, 95% CI = 0.19 to 1.02), however, the interaction between parity and VT category was not associated with hazard of being culled. Body condition score affected ($P = 0.05$) hazard of culling because HBCS was culled at a faster rate than LBCS cows (AHR = 1.97, CI = 1.00 to 3.90). Dairy and average maximum THI were not ($P \geq 0.53$) associated with hazard of culling. According to the Kaplan-Meier survival curve, the interval from calving to culling did not differ between HT and LT cows. Mean days to culling were 240.6 ± 12.2 d and 222.9 ± 14.3 d for HT and LT cows, respectively.

Milk Yield

Vaginal temperature category was not ($P = 0.99$) associated with milk yield; however, the interaction between VT category and month of lactation tended ($P = 0.10$) to affect milk yield (Fig. 2). Cows classified as LT had greater ($P < 0.01$) milk yield in the first month of lactation (39.2 ± 1.6 vs. 33.7 ± 1.5 kg) compared with HT cows, whereas HT cows tended ($P = 0.07$) to have and had ($P = 0.05$) greater milk yield in the ninth (32.7 ± 1.6 vs. 28.5 ± 1.9 kg) and tenth (29.9 ± 1.7 vs. 25.0 ± 2.0 kg) months of lactation, re-

spectively. Overall milk yield did not ($P = 0.13$) differ between primiparous and multiparous cows, but a tendency ($P = 0.09$) for the interaction between parity and month of lactation was observed because multiparous cows had greater milk yield in the first, fourth, and fifth months of lactation than primiparous cows. The interaction between VT category, parity, and month of lactation was not ($P = 0.15$) detected. Cows categorized as HBCS tended ($P = 0.08$) to have reduced milk yield compared with LBCS cows (36.6 ± 1.3 vs. 39.0 ± 1.1 kg), and no interaction was detected ($P = 0.37$) between BCS category and month of lactation.

Inclusion of health disorder within 60 DIM as a covariate in the model resulted in similar findings. Tendencies for interactions between parity and month of lactation ($P = 0.10$), and VT category and month of lactation ($P = 0.08$) were observed. In addition, BCS category and health disorder were associated ($P \leq 0.01$) with milk yield, and a tendency ($P = 0.10$) for an association between VT category and milk yield was detected. Cows that were not diagnosed with health disorders had greater milk yield than cows diagnosed with mastitis or uterine diseases (38.5 ± 1.1 vs. 31.3 ± 1.6 kg).

In the analysis that excluded cows that were removed from the herd within 300 DIM, VT category and the interaction between VT category and parity were not ($P \geq 0.22$) associated with milk yield. In contrast, an interaction between VT category, parity, and month of lactation was detected ($P < 0.01$). Among primiparous cows (Fig. 3A), LT cows had ($P < 0.01$) and tended to have ($P = 0.07$) greater milk yield in the first and fourth months of lactation compared with HT cows, respectively. Among multiparous cows (Fig. 3B), milk yield differed ($P < 0.05$) in the second and third months of lactation between VT categories. Dairy and average daily maximum THI were not ($P \geq 0.34$) associated with milk yield in any of the models.

Table 3. Incidence and adjusted odds ratio (AOR) of health disorders, culling until 300 DIM, pregnancy per AI at first insemination, and calving in the subsequent lactation of cows classified as low or high vaginal temperature (VT) before calving

Item	Incidence		AOR (95% CI)	P-value		
	Low temperature	High temperature		VT Category ¹	Parity	Interaction
UTD ² , %	2.1	22.0	11.71 (2.48–115.10)	< 0.01	0.08	0.26
MAST60 ³ , %	8.7	20.4	2.47 (0.76–9.19)	0.15	0.09	0.39
UTD or MAST60, %	10.9	40.8	6.94 (2.23–21.57)	< 0.01	0.52	0.53
MAST300 ⁴ , %	—	—	—	0.28	0.51	0.09
Primiparous	28.6	22.2	0.72 (0.14–3.63)	—	—	—
Multiparous	20.0	50.0	4.47 (1.14–17.56)	—	—	—
UTD or MAST300, %	26.5	56.5	5.60 (1.87–16.79)	< 0.01	0.96	0.65
Pregnancy per AI at first AI, %	20.0	26.1	1.73 (0.59–5.13)	0.32	0.66	0.35
Culled until 30 DIM, %	9.8	7.6	0.80 (0.22–2.94)	0.73	0.18	0.55
Culled until 300 DIM, %	43.1	30.2	0.43 (0.17–1.09)	0.08	0.05	0.35
Calving subsequent lactation, %	47.1	54.7	1.65 (0.71–3.83)	0.24	0.65	0.91

¹VT category: low temperature = cows with vaginal temperature below the median value; high temperature = cows with vaginal temperature above or equal to the median value. Median values were calculated separately for each parity.

²UTD: cows diagnosed with uterine disorders (retained placenta or metritis).

³MAST60: cows diagnosed with mastitis in the first 60 d in milk.

⁴MAST300: cows diagnosed with mastitis in the first 300 d in milk.

Reproductive Performance

Pregnancy per AI at first service (23.2%) did not differ between VT category (AOR = 1.73, CI = 0.59 to 5.13). Hazard to first insemination or hazard to pregnancy in the first 300 DIM were not different between HT and LT cows (Table 4). In addition, BCS category ($P \geq 0.41$), parity ($P \geq 0.55$), or the interaction between parity and VT category ($P \geq 0.49$) did not affect hazard to first AI or hazard to pregnancy. Furthermore, the interval from calving to first AI or from calving to pregnancy in the first 300 DIM did not ($P \geq 0.54$) differ between VT category. Mean ($\pm$ SEM) and median days to first AI were 67.3 ± 1.3 and 62, respectively. Mean ($\pm$ SEM) and median days to pregnancy were 147.8 ± 7.7 and 129, respectively. Percentage of cows that became pregnant and calved in the subsequent lactation was similar between LT and HT cows (Table 3).

DISCUSSION

It is well accepted that providing shade and evaporative cooling to dry cows is an effective manner for minimizing the effects of heat stress on production of the subsequent lactation. Economic implications of investing in these types of heat abatement strategies for dry cows has been recently assessed. The report indicated that cooling dry cows is profitable for 89% of cows in the U.S. (Ferreira et al., 2016). Profit is achieved in spite of situations where building a new barn is required, regardless of the greater investment needed compared with facilities in which a barn is already present. To the authors' knowledge, there is a limited number of exper-

iments evaluating the benefits on production by providing shade and evaporative cooling only during the dry period (no heat abatement after calving). Acclimation to thermal stress may play a role in adaption to heat stress conditions by altering cellular signaling (Collier et al., 2008). Acclimation may assist cows in coping with heat stress after calving and it should be considered in production systems where no heat abatement is present for postpartum cows (e.g., pasture-based farms and dry-lot corral systems). Other alternatives to minimize the effects of heat stress on profitability of dairy herds should be considered besides investing on facilities. Selection of heat-tolerant dairy cattle may be a better strategy to reduce economic losses caused by heat stress because it may impact the entire herd regardless of the stage of production. Promising results that used genomic selection to identify heat-tolerant and susceptible dairy cows have been recently reported (Garner et al., 2016). Findings from the current study provide evidence that on-farm data may also be used to identify dairy cows susceptible to heat stress.

The authors hypothesized that during the warm season, dry cows with elevated VT would have greater incidence of postpartum health disorders. Findings reported herein support our hypothesis because HT cows had an increased hazard of having uterine diseases or mastitis in the first 60 d after calving compared with LT cows. We focused on evaluating the association between vaginal temperature and mastitis and postpartum uterine diseases because both are the most common health problems in dairy cows after calving (Santos et al., 2010). In addition, occurrence of these disorders rep-

Table 4. Adjusted hazard ratio (AHR) to uterine disease (UTD) or mastitis in the first 60 DIM (MAST60) or 300 DIM (MAST300), first AI, and pregnancy of cows classified as low (LT) or high (HT) vaginal temperature before calving

Item	AHR	95% CI	P-value		
			VT category ¹	Parity	Interaction
MAST60					
LT	Referent		0.12	0.09	0.43
HT	2.59	0.79– 8.45			
UTD or MAST60					
LT	Referent		< 0.01	0.87	0.44
HT	5.22	1.94– 14.06			
MAST300					
LT	Referent		0.04	0.35	0.12
HT	2.42	1.04– 5.62			
UTD or MAST300					
LT	Referent		< 0.01	0.80	0.99
HT	4.02	1.86– 8.67			
First AI					
LT	Referent		0.53	0.55	0.84
HT	1.16	0.73– 1.85			
Pregnancy					
LT	Referent		0.90	0.79	0.49
HT	0.97	0.59– 1.60			

¹VT category: LT = cows with vaginal temperature below the median value; HT = cows with vaginal temperature above or equal to the median value. Median values were calculated separately for each parity.

resents significant economic losses to dairy producers because of costs associated with treatment (Heikkilä et al., 2012; Liang et al., 2017) and decreased production (Bell and Roberts, 2007; Schukken et al., 2009) and reproductive efficiency during lactation (Ahmadzadeh et al., 2009; Giuliadori et al., 2013). Cows that have mastitis or uterine disease are less likely to be cyclic after calving and become pregnant at first AI compared with healthy cows (Santos et al., 2004; Huszenicza et al., 2005; Ribeiro et al., 2016). Although cows classified as HT had increased hazard to be treated for major postpartum disorders, no differences were detected in reproductive performance between VT categories. In fact, differences in postpartum health disorders were mainly detected because of the greater likelihood of HT cows being diagnosed with uterine disease. No differences were detected in the analysis that evaluated the occurrence of mastitis within 60 DIM.

To the authors' knowledge, this is the first study that identified dry cows susceptible to being treated for postpartum disorders by only using information of prepartum VT during conditions conducive to heat stress. Although periparturient immune responses and DMI were not evaluated in this study, the authors speculate that these traits may have differed between HT and LT cows. Other researchers (do Amaral et al., 2011) reported that dry

Figure 1. Kaplan-Meier survival analysis for days to occurrence of uterine diseases or mastitis in the first 300 DIM for cows classified as low (LT, $n = 51$) or high (HT, $n = 53$) vaginal temperature before calving. Solid line represents cows with vaginal temperature less than the median value. Dotted line represents cows with vaginal temperature greater than or equal to the median value. Mean days to occurrence of health disorders were 134.7 ± 6.2 and 99.9 ± 10.9 d for LT and HT cows, respectively ($P < 0.01$).

cows provided no heat stress abatement had suppressed immunity after calving (indicated by neutrophil phagocytosis and oxidative burst responses) compared with cows that were cooled (do Amaral et al., 2011). These researchers (do Amaral et al., 2011) reported that rectal temperature assessed twice daily during the prepartum period differed in approximately 0.4°C between cooled and not cooled cows. In the current study, the average vaginal temperature differed in approximately 0.3°C between HT and LT cows. Because of similar differences detected in body core temperature across experiments, we speculate that physiological processes not investigated in this study (e.g., immune responses and regulation of feed intake) differed between HT and LT cows, and were likely associated with the observed differences in postpartum health. Differences in gestation length and days spent in the close-up pen between HT and LT cows represent further evidence that we were able to identify cows susceptible to being treated for postpartum disorders during summer by assessing VT. Dry cows without heat stress abatement had a reduced dry period length compared with cooled cows because of shorter days of gestation (do Amaral et al., 2011; Tao et al., 2012). Considering that prepartum diets influence metabolism and postpartum health (Janovick et al., 2011), reduced days spent in the close-up pen of HT cows may also be associated with differences observed in postpartum disorders between HT and LT cows.

It is important to consider that other factors may have influenced VT of cows during the dry period. Differences in gestation length at enrollment were detected between HT and LT cows. Cows classified as HT had 1.2 d greater gestation length at enrollment compared with LT cows. It is possible that VT is higher near calving, which potentially could explain temperature differences between HT and LT cows. Nevertheless, we believe that the small difference detected in gestation

Figure 2. Milk yield of cows classified as low (LT, $n = 51$) or high (HT, $n = 53$) vaginal temperature before calving. Solid line represents cows with vaginal temperature less than the median value, and the dotted line represents cows with vaginal temperature greater than or equal to the median value before calving. Vaginal temperature was not ($P = 0.99$) associated with milk yield, but the interaction between vaginal temperature and month of lactation tended ($P = 0.10$) to affect milk yield. Within a month, pairwise differences and tendencies are represented as follows: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

length at enrollment was random and did not affect VT nor interfere with our research question. Furthermore, differences in VT between LT and HT cows could be related to subclinical infections present at enrollment (i.e., subclinical mastitis during the dry period). Although cows with clinical disorders were not eligible to be enrolled in the study, there is the possibility that HT cows had an established inflammatory process while VT was assessed resulting in higher body core temperature. Indeed, it has been shown that locomotion disorders before calving are associated with postpartum health events in the first 30 DIM (Oetzel and Miller, 2012). Nonetheless, by assessing VT of dry cows under conditions of heat stress, it was possible to identify cows susceptible to being affected by postpartum disorders after calving. Although herds used in this study had similar management and facilities for dry cows, it is important to consider that farm may influence VT of cows. To control for a potential herd effect, dairy was included in all statistical analyses. Dairy was not associated with VT, production, health, or reproductive outcomes.

An experiment conducted by Tao et al. (2011) demonstrated that compromised mammary cell proliferation is a potential culprit for reduced milk production observed in the subsequent lactation of heat-stressed dry cows. The previous study, however, did not evaluate whether differences in postpartum health disorders influence milk production of cows cooled and not cooled during the dry period. Because postpartum health disorder in the first 60 DIM impact milk production (Dubuc et al., 2011; Wittrock et al., 2011) and may be a potential confounder in the effect of heat stress during the dry period on production, findings from the current study are relevant besides expanding on the current knowledge of the impact of heat stress on dry cows. Based on the analysis that included

Figure 3. Milk yield of cows classified as low (LT) or high (HT) vaginal temperature before calving that were not removed from the herd within 300 DIM. Solid line represents cows with vaginal temperature less than the median value. Dotted line represents cows with vaginal temperature greater than or equal to the median value. (A) Among second-lactation cows (primiparous status at enrollment), LT had ($P < 0.01$) and tended ($P = 0.07$) to have greater milk yield in the first (41.5 ± 2.4 vs. 30.9 ± 2.1 kg) and fourth month (44.4 ± 2.0 vs. 39.8 ± 1.8 kg) of lactation compared with HT cows. (B) Among $\geq$ third-lactation cows (multiparous status at enrollment), LT had ($P < 0.05$) greater milk yield in the second- (52.6 ± 1.7 vs. 47.2 ± 1.4 kg) and third month (52.5 ± 1.9 vs. 44.9 ± 1.6 kg) of lactation compared with HT cows. Within a month, pairwise differences and tendencies are represented as follows: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

occurrence of health disorders within 60 DIM in the model, it seems that postpartum health does not confound the association between VT category and milk yield. These results augment conclusions from previously discussed earlier findings (Tao et al., 2011), in which heat stress during the dry period alters mammary cell proliferation, thus impacting milk production in the subsequent lactation. In addition, findings from the current study agrees with previous work in which cows with a greater temperature before calving had decreased milk yield in early lactation. Our study, however, is novel in detecting those differences without actively cooling cows, but by identifying cows more susceptible to being affected by heat stress by evaluating VT before calving. Furthermore, it is possible that the reduced milk yield of HT cows is associated with impaired mammary gland development due to reduced gestation length compared with LT cows. Calving earlier than expected may have impacted galactopoiesis influencing early milk yield.

In the analyses that evaluated the association between VT and milk yield, interactions between VT category and month of lactation tended to be associated

with milk yield. Such interactions were detected mainly because of differences in milk yield between HT and LT cows in early lactation. Differences in milk yield were also observed in late lactation. It is important to note that differences observed in late lactation may not reflect physiological responses to heat stress during the dry period. It is possible that differences in hazard to culling between HT and LT cows biased these results, which limits inferences that heat stress impacts milk yield in late lactation. To conduct an analysis with reduced bias, the authors of the current experiment excluded cows that were removed from the herd within 300 DIM, in which no differences in milk yield in late lactation were observed. Therefore, these results indicate that the impact of heat stress on dry cows in milk yield is predominantly in early lactation. The observed difference in hazard of culling within 300 DIM of HT and LT cows is intriguing, especially because LT cows were less likely to have postpartum disorders. It is possible that LT cows tended to be removed from the herd at a faster rate than HT cows because of differences in heat stress in early lactation. Greater milk yield of LT cows was likely accompanied by greater heat load compared with HT cows, in which may have resulted in greater extent of heat stress, possibly impacting cull rate. This speculation, however, requires further investigation because LT cows likely had an increased ability to regulate body core temperature than HT cows despite greater heat load. Moreover, only a tendency was detected in the Cox proportional hazard and there was no difference in the survival analysis or percentage of cows that calved in the subsequent lactation. Furthermore, no differences were detected in culling up to 30 DIM.

Body core temperatures and ambient THI indicate that cows in the current study were in moderate heat stress conditions during the dry period. Although LT cows had a lower temperature than HT cows, LT cows were also affected by heat stress. Lack of differences in hazard to insemination and pregnancy between VT categories suggest that heat stress during the dry period had a carryover effect on reproductive efficiency of LT and HT cows. Unfortunately, the absence of a control group (cows under thermoneutral conditions) limits our inference. Heat stress affects fertility of cows in a multifactorial manner. Researchers demonstrated that in vitro heat shock of oocytes impacts nuclear maturation by impairing resumption of meiosis and inducing cell apoptosis, and consequently reducing fertilization rate (Roth and Hansen, 2005). In addition, exposure to heat stress has carryover effects on concentrations of steroid hormones in follicular fluid (Roth et al., 2001). Considering that development from primordial to preovulatory follicle stage requires approximately 4 mo in cattle (Webb and Campbell, 2007), cows in the current study likely had

compromised oocytes after the end of the voluntary waiting period. Indeed, reduced pregnancy outcomes at first AI observed in this study suggest that heat stress during the prepartum and periparturient periods affected reproductive efficiency of cows in early lactation.

In conclusion, results from this observational study demonstrate that cows susceptible to being treated for postpartum disorders may be identified at the farm level during the summer by evaluating body core temperature at 250 to 260 d of gestation. Differences detected in postpartum health disorders suggest that cows with higher VT before calving are more susceptible to the negative effects of heat stress than cows with lower VT. In addition, cows with higher VT before calving have decreased milk yield in early lactation compared to LT cows. Nonetheless, reproductive performance is similar between HT and LT cows. Further experiments should be conducted to investigate effectiveness of cooling strategies in subpopulations of cows (e.g., cows with high or low VT during the dry period) and its impact on subsequent performance.

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