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Associations among milk production and rectal temperature on pregnancy maintenance in lactating recipient dairy cows

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ABSTRACT

The objective of this study was to evaluate the associations among milk production, rectal temperature, and pregnancy maintenance in lactating recipient dairy cows. Data were collected during an 11-mo period from 463 Holstein cows (203 primiparous and 260 multiparous) assigned to a fixed-time embryo transfer (ET) protocol. Only cows detected with a visible corpus luteum immediately prior to ET were used. Rectal temperatures were collected from all cows on the same day of ET. Milk production at ET was calculated by averaging individual daily milk production during the 7 d preceding ET. Pregnancy diagnosis was performed by transrectal ultrasonography 21 d after ET. Cows were ranked and assigned to groups according to median milk production (median = 35 kg/d; HPROD = above median; LPROD = below median) and rectal temperature ($\leq 39.0^\circ\text{C}$ = LTEMP; $> 39.0^\circ\text{C}$ = HTEMP). A milk production \times temperature group interaction was detected ($P = 0.04$) for pregnancy analysis because HTEMP cows ranked as LPROD were 3.1 time more likely to maintain pregnancy compared with HTEMP cows ranked as HPROD ($P = 0.03$). Milk production did not affect ($P = 0.55$) odds of pregnancy maintenance within LTEMP cows, however, and no differences in odds of pregnancy maintenance were detected between HTEMP and LTEMP within milk production groups ($P > 0.11$). Within HTEMP cows, increased milk production decreased the probability of pregnancy maintenance linearly, whereas within LTEMP cows, increased milk production increased the probability of pregnancy maintenance linearly. Within HPROD, increased rectal temperature decreased the probability of pregnancy maintenance linearly, whereas within LPROD cows, no associations between rectal temperatures and probability of cows to maintain pregnancy were detected. In summary, high-producing dairy cows with rectal temperatures below 39.0°C did not experience reduced pregnancy maintenance to ET compared to cohorts with reduced milk production.

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1. Introduction

In the U.S. dairy industry, milk production per cow increased whereas reproductive efficiency decreased over

the last few decades (Lucy, 2001). Most of these reproductive losses can be attributed to increased embryonic mortality (Zavy, 1994). More than 50% of dairy cows that conceive lose their pregnancy during the initial 6 weeks of gestation (Santos et al., 2004). Several physiological consequences of increased milk production can be associated with early pregnancy losses, such as increased incidence of metabolic and reproductive postpartum

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diseases, intensified postpartum negative energy balance, and reduced circulating concentrations of steroids (Opsomer et al., 2000; Lucy, 2001; Vasconcelos et al., 2003). More specifically, high producing cows have lesser plasma progesterone (P4) concentrations (Vasconcelos et al., 1999) compared to less-productive cohorts, mainly due to their greater DMI (Harrison et al., 1990), increased hepatic blood flow, and consequent hepatic catabolism of progesterone (P4) (Sangsrivavong et al., 2002; Vasconcelos et al., 2003). P4 affects the uterine environment (Thatcher et al., 2001; Green et al., 2005) and early embryonic development (Mann and Lamming, 2001), whereas reduced P4 concentrations after artificial insemination (AI) is detrimental to subsequent pregnancy rates (Stronge et al., 2005; Mann et al., 2006; Demetrio et al., 2007).

Many studies also reported that increased body temperature is detrimental to reproductive function in dairy cattle, particularly early embryo development and survival (Hansen and Arechiga, 1999; Wolfenson et al., 2000; Hansen et al., 2001). These outcomes become of greater concern during the summer, when increased environmental temperatures contribute to greater increases in body temperature and substantial decreases in reproductive efficiency of dairy cows (Badinga et al., 1985; Sartori et al., 2002). However, this decrease in reproductive performance appears to be influenced by milk production (Badinga et al., 1985; Al-Katanani et al., 1999; Sartori et al., 2002; López-Gatius, 2003) because high-producing dairy cows have a greater average body temperature compared to less-productive cohorts due to their hastened metabolism and increased heat production (Berman et al., 1985; Kadzere et al., 2002).

However, Ravagnolo and Misztal (2000) reported that the correlation between milk production and heat tolerance is weak in dairy cows, indicating that selection for heat tolerant and high-producing dairy cows is possible. Umphrey et al. (2001) also reported weak partial correlation between milk yield and rectal temperature. Based on these observations, it was hypothesized that higher-producing dairy cows with normal body temperature do not experience reduced pregnancy maintenance compared to lesser producing cohorts. The objectives of this study were to evaluate the relationships among rectal temperature, milk production, and pregnancy maintenance of lactating recipient dairy cows within one year.

1.1. Materials and methods

This experiment was conducted from February to December 2007 at a commercial dairy farm located in Araras, Brazil. The latitude, longitude, and altitude of this location are, respectively, 22°21' south, 47°23' west, and 614 m. The animals utilized were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

1.2. Animals and diets

Data were collected during an 11-mo period from 463 lactating recipient Holstein cows (203 primiparous and 260

multiparous). Cows were housed according to parity into 12 free stall barns with access to an adjoining sod-based area. Barns were cooled by intermittent sprinkling and forced ventilation to minimize the effects of heat stress. Cows were fed *ad libitum* with a TMR based on corn silage, bermudagrass (*Cynodon dactylon* cv. coast-cross), ground corn, cottonseed, soybean meal, and a mineral and vitamin mix, which was balanced to meet the nutritional requirements of lactating dairy cows (NRC, 2001). Cows were milked three times daily in a side-by-side milking system. Daily milk yield for each cow was recorded automatically.

1.3. Reproductive management

During the experiment, all non-pregnant recipient cows which were more than 55 d in milk (DIM) were evaluated monthly by transrectal ultrasonography examinations (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer; Tokyo, Japan) to determine estrous cyclicity by presence of a corpus luteum (CL). Cows determined as estrous cycling were assigned to a monthly ovulation synchronization + fixed-time embryo transfer (ET) protocol. Prior to ET, all cows received a health evaluation which included clinical and subclinical mastitis exam (Smith et al., 1984; Dohoo and Leslie, 1991; Maunsell et al., 1999), endometritis (LeBlanc et al., 2002; Kasimanickam et al., 2004) and lameness evaluation (Sprecher et al., 1997). Only cows diagnosed as healthy were assigned to ET to prevent confounding effects between health issues and environmental heat on rectal temperatures and pregnancy maintenance. At ET, mean milk production was 35.2 ± 0.40 kg/d, with mean DIM of 204 ± 6.3 d and BCS of 3.0 ± 0.02 (Wildman et al., 1982). For the estrous synchronization protocol, cows received a 100 µg injection of GnRH (Fertagyl®; Schering-Plough Co., São Paulo, Brazil) and received an intravaginal P4 releasing device (CIDR®, containing 1.9 g of P4; Pfizer Animal Health, São Paulo, Brazil) on d 0, a 25 mg injection of prostaglandin F_{2α} (Lutalyse®; Pfizer Animal Health) and CIDR removal on d 7, and a 1 mg injection of estradiol cypionate (ECP®; Pfizer Animal Health) on d 8. Transrectal ultrasonography examinations (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer) were performed in all recipient cows immediately before ET (d 17) to verify presence of a CL. Only cows detected with a visible CL were assigned to ET, which was performed with fresh and frozen Holstein embryos (34.5% frozen and 65.5% fresh; Table 1) which originated from *in vivo* procedures and obtained from a private company (Policlinica Pioneiros; Paraná, Brazil). Embryo collection and ET procedures were similar to those described by Vasconcelos et al. (2011), and embryos were originated from a combination of 75 donors (nonlactating heifers and cows) and 7 sires. Embryos were assigned randomly to recipient cows (Table 1). Pregnancy diagnosis was performed by detecting a viable conceptus with transrectal ultrasonography (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer) 21 d after ET (d 38 of the study). Cows that did not become pregnant to ET were assigned to different breeding procedures and consequently removed from the study.

Table 1

Distribution of cows and embryo type (in parenthesis; frozen or fresh, respectively), according to season, parity, milk production, and rectal temperature at the time of embryo transfer.^{a,b}

Season	Mild	Warm
Primiparous (n = 203)		
LPROD-LTEMP	32 (3/29)	37 (9/28)
LPROD-HTEMP	11 (2/9)	21 (15/6)
HPROD-LTEMP	25 (2/23)	54 (10/44)
HPROD-HTEMP	11 (2/9)	12 (3/9)
Multiparous (n = 260)		
LPROD-LTEMP	41 (20/21)	41 (16/25)
LPROD-HTEMP	31 (18/13)	14 (12/2)
HPROD-LTEMP	38 (12/26)	62 (16/46)
HPROD-HTEMP	17 (12/5)	16 (8/8)

^a Milk production; median = 35 kg/d of milk; HPROD = above the median; LPROD = below the median; Rectal temperature; $\leq 39.0^\circ\text{C}$ = LTEMP; $> 39.0^\circ\text{C}$ = HTEMP

^b Mild season (fall and winter) = April to September; Warm season (spring and summer) = January to March, and October to December.

1.4. Sample collection

Dry bulb temperature and relative humidity were obtained every 10 min from a meteorological station located 40 km from the dairy farm (Brazilian Air Force Academy, Meteorological Station; Pirassununga, Brazil), and summarized on a daily basis. Dates of ET were divided into two seasons due to the tropical climate characteristics of the region: warm season (spring and summer; September, October, November, December, January, and February), and mild season (fall and winter; March, April, May, June, July, and August). Milk production at ET was calculated by averaging individual daily milk production during the 7 d preceding ET. Rectal temperature was measured using a digital thermometer (G-Tech; São Paulo, Brazil); in the morning of the day of ET (06:00–10:00 h) after milking. Rectal body temperature was evaluated instead of temperature–humidity index to account for individual heat tolerance (Srikandakumar and Johnson, 2004). Blood samples were collected, concurrently with rectal temperature assessment, via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately, maintained at 4°C for 12 h, and centrifuged at $1500 \times g$ for 15 min at room temperature for serum collection. Serum was stored at -20°C until analyzed for P4 concentrations using Coat-A-Count solid phase ^{125}I RIA kit (DPC Diagnostic Products Inc., Los Angeles, CA). The intra- and interassay CV were, respectively, 5.0% and 6.9%. The assay sensitivity was 0.01 ng/mL.

1.5. Statistical analysis

The UNIVARIATE procedure of SAS (SAS Inst., Inc., Cary, NC) was utilized to determine the median milk production at ET. Cows were ranked and assigned to groups according to median milk production at ET (median = 35 kg/d; HPROD = above median; LPROD = below median) to divide the experimental herd into high-producing and less-producing cows. Cows were also ranked and assigned to groups according to rectal temperature at ET ($\leq 39.0^\circ\text{C}$ = LTEMP; $> 39.0^\circ\text{C}$ = HTEMP), given that 39.0°C

is considered the threshold for heat stress in dairy cattle (Berman et al., 1985; West, 2003). Distribution of cows into groups, according to parity and season, is described in Table 1.

Physiological data were analyzed using the MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for milk production and temperature effects on P4 concentrations contained the effects of production group (HPROD or LPROD), temperature group (HTEMP or LTEMP), season, parity, and all resultant interactions, whereas DIM and CL volume were included as covariates. Data were analyzed using barn as a random variable. The model statement used to compare P4 concentrations at ET in cows diagnosed as pregnant or non-pregnant contained the effects pregnancy status, season, parity, and all resultant interactions, whereas DIM and CL volume were included as covariates. The model statement used for milk production effects on rectal temperatures contained the effects of production group, season, parity, and the resultant interactions, whereas DIM was included as a covariate. Results are reported as least squares means and were separated using LSD.

Pregnancy data were analyzed as using the GLIMMIX procedure of SAS with a binomial distribution and logit link function. The model statement used for production and temperature comparison contained the effects of production group, temperature group, season, parity, embryo type, and all resultant interactions, whereas DIM, P4 concentrations at ET, and BCS were included as covariates. Embryo donor and sire were initially included in the model. Given that no significant interactions among these variables with production and temperature groups were detected ($P > 0.23$), embryo donor and sire were removed from the model to prevent a substantial reduction in degrees of freedom. Data were analyzed using barn as a random variable. Results are reported as least squares means and as adjusted odds ratios based on a 95% confidence interval, and were separated by LSD.

The probability of cows to maintain pregnancy was evaluated according to P4, milk production within temperature groups, and rectal temperature within production groups. The GLM procedure of SAS was initially used to determine if each individual measurement influenced pregnancy maintenance linearly, quadratically, or cubically, and also to determine effects of season and parity on the statistical model. No effects or interactions were detected; therefore probability of pregnancy maintenance was evaluated across season and parities. The LOGISTIC procedure was used to generate the regression model, determine the intercept and slope(s) values according to maximum likelihood estimates from each significant continuous order effect, and the probability of pregnancy maintenance was determined according to the following equation: Probability = $(e^{\text{logistic equation}})/(1 + e^{\text{logistic equation}})$. Logistic curves were constructed according to the minimum and maximum values detected for rectal temperature and milk production. Pearson correlations were calculated among rectal temperatures, milk production, and serum P4 concentrations. The GLM procedure was utilized to determine

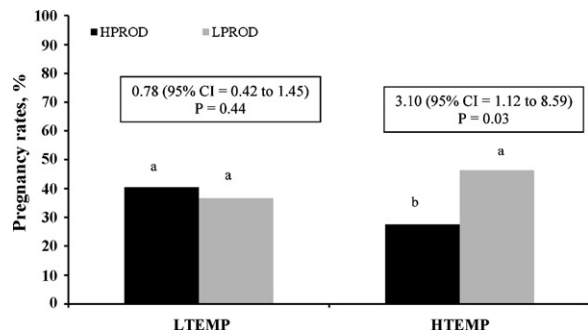


Fig. 1. Pregnancy maintenance of lactating recipient Holstein cows ranked according to milk production (median = 35 kg/d of milk; HPROD = above the median; LPROD = below the median) and rectal temperature ($\leq 39.0^\circ\text{C}$ = LTEMP; $> 39.0^\circ\text{C}$ = HTEMP) at the time of embryo transfer. Results are reported as least square means. Information reported within boxes represents the likelihood of LPROD cows maintaining pregnancy compared to HPROD cows, based on calculated odds ratio and 95% confidence interval (CI). A production \times temperature rank interaction was detected ($P = 0.01$). Within HTEMP cows, those ranked as LPROD had greater mean pregnancy maintenance ($P = 0.05$) and were 3.1 time more likely to maintain pregnancy ($P = 0.03$) compared to HPROD cohorts. Milk production did not affect pregnancy maintenance, however, either as least square means ($P = 0.58$) or odds ratio ($P = 0.44$), within LTEMP cows. Variables within temperature group not bearing a common letter differ ($P < 0.05$).

effects of season and parity on correlation coefficients. No effects or interactions were detected; therefore correlation coefficients reported herein were determined across seasons and parities.

For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant, or according to the highest order interaction detected.

2. Results

Associations between season and climate conditions are described in Table 2. Dry bulb temperatures (mean, maximum, and minimum) and relative humidity were increased during the warm season compared with the mild season.

A production group \times temperature group interaction was detected ($P = 0.01$; Fig. 1) for the analysis of pregnancy maintenance. Within HTEMP cows, those ranked as HPROD had lesser ($P = 0.05$) pregnancy maintenance compared to LPROD cohorts. Further, based on the calculated odds ratio, HTEMP cows ranked as LPROD were 3.1 time more likely (95% confidence interval = 1.12–8.59) to maintain pregnancy compared with HTEMP cows ranked as HPROD ($P = 0.03$). However, production group did not affect pregnancy maintenance, either as least square means ($P = 0.58$) or odds ratio ($P = 0.44$), within LTEMP cows. No differences in pregnancy maintenance were detected between HTEMP and LTEMP cows when data were stratified by production group ($P > 0.11$; data not shown). In addition, the probability of cows to maintain pregnancy was evaluated according to production and temperature groups. Within HTEMP cows, increased milk production decreased the probability of pregnancy maintenance linearly ($P = 0.05$; Fig. 2), whereas within LTEMP cows, milk

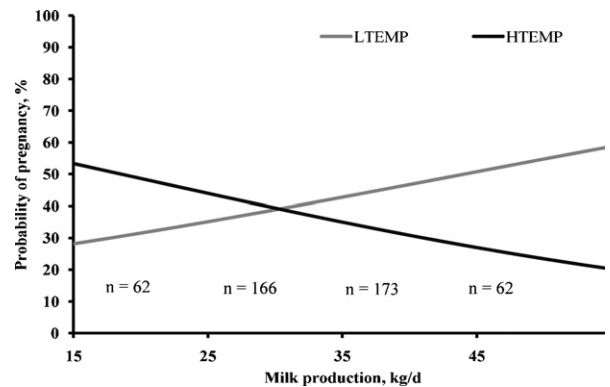


Fig. 2. Relationship between milk production at the time of embryo transfer, according to rectal temperature ($\leq 39.0^\circ\text{C}$ = LTEMP, $n = 330$; $> 39.0^\circ\text{C}$ = HTEMP, $n = 133$), and the probability of lactating recipient dairy cows to maintain pregnancy. Values above the x-axis indicate the number of cows within each milk yield section. A positive linear effect was detected for LTEMP cows ($P = 0.02$), whereas a negative linear effect was detected for HTEMP cows ($P = 0.05$).

production increased the probability of pregnancy maintenance linearly ($P = 0.01$; Fig. 2). Within HPROD, elevated rectal temperature decreased the probability of pregnancy maintenance linearly ($P = 0.02$; Fig. 3), whereas within LPROD cows, rectal temperature and probability of cows to maintain pregnancy were not associated ($P = 0.96$; Fig. 3).

Serum P4 concentrations were similar ($P = 0.24$) among cows that became pregnant to ET compared to those that remained non-pregnant (3.08 ng/mL vs. 3.20 ng/mL; SEM = 0.090). Further, serum P4 concentrations did not affect the probability of cows to maintain pregnancy, even when production and temperature groups were accounted into the analysis (data not shown).

Serum P4 concentrations were correlated with milk production ($r = -0.10$; $P = 0.03$) and rectal temperature ($r = 0.15$; $P < 0.01$). At the time of ET, P4 concentrations covariately adjusted to CL volume were reduced ($P = 0.03$)

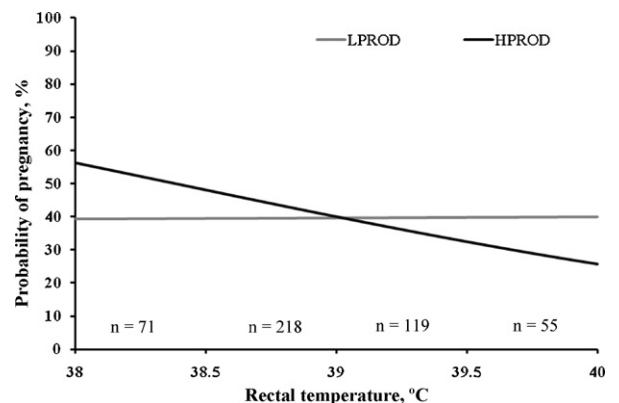


Fig. 3. Relationship between rectal temperature at the time of embryo transfer, according to milk production (median = 35 kg/d of milk; HPROD = above the median, $n = 235$; LPROD = below the median, $n = 228$), and the probability of lactating recipient dairy cows to maintain pregnancy. Values above the x-axis indicate the number of cows within each rectal temperature section. A linear effect was detected for HPROD cows ($P = 0.02$), but not for LPROD cows ($P = 0.96$).

Table 2Dry bulb temperature and humidity (\pm SD) according to monthly meteorological data and season.^{a,b}

	Dry bulb temperature, °C		Relative humidity, %	
	Mean	Maximum	Minimum	Mean
Monthly averages ^b				
January	23.9 \pm 1.6	33.1 \pm 2.1	17.5 \pm 0.6	73.2 \pm 8.3
February	23.5 \pm 0.9	33.1 \pm 0.9	18.4 \pm 0.4	79.9 \pm 3.8
March	21.0 \pm 1.1	30.2 \pm 0.8	12.4 \pm 2.2	74.5 \pm 2.7
April	18.1 \pm 1.4	30.0 \pm 1.2	7.4 \pm 0.7	61.3 \pm 7.0
May	17.3 \pm 0.6	27.7 \pm 1.0	8.1 \pm 1.2	68.4 \pm 1.6
June	13.7 \pm 0.3	21.2 \pm 0.6	7.7 \pm 0.9	81.8 \pm 1.9
July	13.8 \pm 1.8	22.1 \pm 2.7	7.8 \pm 1.4	77.77 \pm 7.0
August	19.7 \pm 2.0	31.5 \pm 2.0	9.4 \pm 2.9	59.5 \pm 12.2
September	16.1 \pm 2.0	24.3 \pm 3.0	9.7 \pm 3.2	73.0 \pm 11.0
October	20.6 \pm 2.4	31.9 \pm 2.9	11.6 \pm 3.4	60.7 \pm 11.2
November	17.6 \pm 2.2	25.9 \pm 2.8	10.8 \pm 3.2	72.2 \pm 8.9
December	22.6 \pm 0.6	32.0 \pm 1.7	14.9 \pm 1.3	71.2 \pm 7.0
Season ^c				
Mild	18.9 \pm 2.1	29.8 \pm 2.3	9.4 \pm 2.9	65.9 \pm 9.3
Warm	15.8 \pm 2.5	23.3 \pm 2.7	9.3 \pm 2.8	78.5 \pm 7.6
	23.4 \pm 1.0	32.3 \pm 1.6	16.9 \pm 2.0	74.6 \pm 6.7
	22.0 \pm 1.2	27.7 \pm 1.5	17.3 \pm 1.9	80.2 \pm 6.1

^a Obtained every 10 min from a meteorological station located 40 km from the research site (Brazilian Air Force Academy, Meteorological service; Pirassununga, Brazil), and summarized on a daily basis

^b Within monthly or season values, upper row = daily averages; lower row = 06:00–10:00 h averages

^c Mild season (fall and winter) = April to September; Warm season (spring and summer) = January to March, and October to December.

in HPROD cows compared to LPROD cows (3.08 compared to 3.33 ng/mL, respectively; SEM=0.085), and greater ($P<0.01$) in HTEMP cows compared with LTEMP cohorts (3.38 compared to 3.03 ng/ml, respectively; SEM=0.083). Milk production and rectal temperature were negatively correlated ($r=-0.19$; $P<0.01$), whereas HPROD cows had lesser ($P<0.01$) mean rectal temperatures compared to LPROD cows (38.9 compared to 39.0 °C, respectively; SEM=0.05).

3. Discussion

Meteorological data described in Table 2 suggest that cows were exposed to heat stress conditions throughout the year, given that maximum dry bulb temperatures were greater than 25 °C during every month of the study, which is considered the upper limit temperature at which Holstein cows can maintain a stable body temperature (Berman et al., 1985). In addition, Dikmen and Hansen (2009) recently reported that upper limit temperature for Holstein cows in subtropical environment and housed in conditions similar to those reported herein was 28.4 °C, whereas in the present study maximum dry bulb temperatures were greater than 28.4 °C during both mild and warm seasons (Table 2). However, the frequency at which dry bulb temperatures were above both heat stress thresholds

were likely decreased during the mild season compared with the warm season because of the differences detected for mean dry bulb temperature between seasons. During the period at which cows were evaluated for rectal temperatures (06:00–10:00 h; Table 2), maximum dry bulb temperatures during the warm season were above the threshold suggested by Berman et al. (1985), but below the threshold suggested by Dikmen and Hansen (2009). One can speculate that the time of rectal temperature evaluation was not adequate to evaluate environmental effects, whereas increased rectal temperature (>39.0 °C) could have been caused by health challenges rather than heat stress. Nevertheless, only apparently healthy cows were assigned to ET in the present study, and all cows were evaluated for rectal temperature during the same period of the day. Consequently, increased rectal temperatures was likely due to dry bulb temperatures observed during the evaluation period within the warm season, and inefficiency of cows to dissipate heat absorbed during the previous day and resume proper body temperature, given that maximum dry bulb temperatures were greater than 28.4 °C (Dikmen and Hansen, 2009) during both mild and warm seasons (Table 2). It is also important to note that meteorological data was obtained from a meteorological station located 40 km from the research site, and may not precisely represent the environmental (temperature and

humidity) conditions that cows were exposed to throughout the year.

Results detected for pregnancy analysis according to production and temperature groups (Figs. 1–3) could help in explaining some of the inconsistent perspectives about the effects of milk production on reproduction of dairy cattle. Although decreased reproductive performance of dairy cows has been attributed, at least partially, to increased milk yield (Royal et al., 2000; Lucy, 2001), research studies reported both negative (Dematawewa and Berger, 1998; Vasconcelos et al., 2006) and positive effects (Nebel and McGilliard, 1993; Stevenson, 1999) of milk production on reproductive parameters of dairy herds. Perhaps these differences in outcomes can be attributed to body temperature of the evaluated cows which can modulate, as reported herein, how milk production influences pregnancy establishment and maintenance in dairy cattle. Supporting this rationale, López-Gatius (2003) indicated that detrimental effects of increased milk yield on pregnancy rates are more evident during warm periods compared to cool periods. Further, the majority of reproductive losses in dairy cattle can be attributed to increased embryonic mortality (Zavy, 1994), whereas Santos et al. (2004) reported in a review of the literature that milk production alone does not affect late embryonic and fetal losses in dairy cows, and concluded that there is little or no indication that milk production is a risk factor for reduced pregnancy establishment and maintenance in dairy cattle.

The deleterious effects of increased body temperatures due to heat stress on pregnancy establishment and maintenance have been well characterized. Previous data indicate rectal temperature was negatively associated with the probability of lactating recipient dairy cows becoming pregnant to both AI and ET (Vasconcelos et al., 2006). Biggers et al. (1987) reported that heat stress was detrimental to embryonic development when applied from d 8 to 16 of pregnancy by decreasing conceptus weight. In addition, Ryan et al. (1993) reported that heat stress may retard embryonic development or cause failure of embryonic development during the initial 14 d of the pregnancy. In his review, Lucy (2001) suggested that there is an additive effect between heat stress and milk production in regards to decreased reproductive performance of dairy cattle. This rationale supports results of the present study, which indicated that increased milk production only impaired pregnancy maintenance in recipient dairy cows when associated with rectal temperatures above the heat stress threshold (Figs. 1 and 2). Similarly, increased rectal temperatures were detrimental to pregnancy maintenance only in high-producing cows, suggesting that rectal temperatures are not critical to pregnancy maintenance in lesser producing cohorts (Fig. 3). This latter outcome deserves further investigation; given that it can lead to development of novel hypothesis for how heat stress and hyperthermia influences reproductive efficiency of dairy cattle.

Circulating concentrations of P4 after breeding have been positively associated with pregnancy per AI in dairy and beef cows (Robinson et al., 1989; Stronge et al., 2005; Demetrio et al., 2007). Progesterone is required for proper establishment and maintenance of pregnancy (Spencer and

Bazer, 2002) by preparing the uterine environment for conceptus growth and development, modulating the release of hormones that may regress the CL and disrupt gestation (Bazer et al., 1998) and by regulating endometrial secretions and structural changes that are essential for proper embryo development (Gray et al., 2001; Wang et al., 2007). High-producing dairy cows have lesser plasma concentrations of P4 (Vasconcelos et al., 1999) compared to lesser productive cohorts mainly due to their greater DMI (Harrison et al., 1990), increased hepatic blood flow, and consequent hepatic catabolism of P4 (Sangsritavong et al., 2002; Vasconcelos et al., 2003). Because of this relationship, reduced P4 concentrations have been considered one of the mechanisms by which increased milk production can decrease reproductive performance in dairy cows (Lucy, 2001). Further, P4 concentrations can be altered by environmental and rectal temperatures because in cows under heat stress conditions, blood flow might shift to peripheral tissues for cooling purposes (West, 2003), and consequently reduce hepatic catabolism of P4. In the present study, serum P4 concentrations were weakly correlated with milk production and rectal temperature. Still, P4 concentrations at the time of ET were reduced in HPROD cows compared to LPROD cows, and greater in HTEMP cows compared with LTEMP cohorts. Conversely, serum P4 concentrations were not associated with pregnancy maintenance or the probability of cows to maintain pregnancy. These outcomes are supported by previous efforts from our research group indicating that serum P4 concentrations at the time of ET was not associated with the probability of recipient cows to maintain pregnancy, although a positive association was detected between P4 concentrations and pregnancy rates per AI (Demetrio et al., 2007). Therefore, P4 concentrations at the time of ET may not be a critical factor for maintenance of pregnancy in recipient dairy cows because embryos are typically well developed when transferred (morula or blastocyst stage), and consequently do not require increased amounts of P4 for proper establishment and growth (Demetrio et al., 2007).

During the present study, all cows were diagnosed as clinically healthy, offered similar diets, and housed in similar installations throughout the year. Therefore, differences in rectal temperatures, particularly within cows with similar milk production, can be attributed to their ability of dissipating heat. Pszczola et al. (2009) suggested that decreased heat tolerance may be one of the reasons for the lesser reproductive performance of high-producing dairy cows. Ravagnolo and Misztal (2000) concluded that genetic variation for heat tolerance is important and that selection for milk production and heat tolerance is possible because of the low negative correlation between them. Surprisingly, in the present study, milk production and rectal temperature were weakly negatively correlated, whereas HPROD cows had reduced, although marginally, rectal temperatures compared to LPROD cows. Dikmen and Hansen (2009) reported that milk yield does not influence rectal temperatures in high-producing Holstein cows maintained in subtropical environments. Therefore, these results indicate that increased milk production does not necessarily mean increased body temperatures, and selection for high-producing dairy cows with adequate heat tolerance and

consequent body temperatures is possible, and desired (Ravagnolo and Misztal, 2000).

4. Conclusions

Results from this study indicate that high-producing dairy cows with rectal temperatures below 39.0 °C do not experience reduced pregnancy maintenance compared to cohorts with lesser milk production. Correspondingly, increased rectal temperatures were detrimental to pregnancy maintenance in high-producing cows, but not in lesser productive cohorts. Milk production and rectal temperatures were not positively associated; therefore, dairy cows can be selected for increased milk production and optimal reproductive performance if heat tolerance is included into the selection criteria.

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