Impact of heat stress on health, production, and reproductive performance of dairy cows

by

Alexandre Lelandy Alves Scanavez

D.V.M., Universidade Federal de Uberlandia, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
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Abstract

Heat stress poses major challenges to the dairy industry, disrupting the well-being and productivity of cows. Besides affecting lactating cows, exposure to heat stress during the dry period increases core body temperature (CBT) and alters hormonal profile and mammary gland development, ultimately affecting milk yield in the subsequent lactation. Reproductive performance is severely reduced in dairy cows exposed to heat stress. Even though it is well accepted that estrus expression is reduced during periods of heat stress, it is not clear whether herd-level indicators of estrus-detection efficiency, such as insemination risk, are impacted during periods of heat stress. This dissertation focused on exploring the use of CBT during the dry period as a predictor of postpartum health, production, and reproductive performance during the subsequent lactation. Furthermore, potential implications of heat stress and other seasonal stressors on insemination risk were evaluated. Study 1 investigated the relationships between CBT during the dry period and health, milk production, and reproduction during the subsequent lactation. Dry cows with increased CBT were more susceptible to health disorders and had reduced milk yield early in the subsequent lactation. No association was observed between CBT during the dry period and reproductive performance after parturition. Study 2 explored factors associated with CBT in dry dairy cows and focused on determining the ideal time of the day to assess CBT of heat-stressed dry cows. Core body temperature was increased in cows pregnant with twins and was associated negatively with gestation length. Furthermore, results indicated that 2215 h is the most appropriate time of the day to assess CBT of dry cows exposed to heat stress. Study 3 aimed to compare physiologic and metabolic characteristics of cooled cows classified as having high or low CBT during the dry period. In addition, this study investigated the association between CBT during the dry period and health, milk yield, and reproductive performance after parturition. Cows with high CBT during the dry period had distinct concentrations of pregnancy-associated glycoprotein and indicators of energy balance during the transition period and had reduced milk yield compared with low-CBT cows. Furthermore, CBT during the dry period was a useful predictor of postpartum health disorders. Reproductive performance, however, did not differ between cows that had high or low CBT during the dry period. Study 4 investigated temporal patterns of insemination risk in large dairy herds and explored associations between insemination risk and herd-level traits. Seasonal variation of insemination risk was minimal, with increased insemination risk observed during autumn. Greater values of insemination risk were observed in dry-lot herds, with low mortality of cows, and longer voluntary waiting period for primiparous cows. In summary, assessment of CBT in dry cows may be a useful tool to identify groups of cows more likely to present health disorders and impaired productive performance after parturition. In addition, insemination risk is not reduced during the summer, but it is severely affected by herd-level traits such as housing system, mortality of cows, and voluntary waiting period for primiparous cows.

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Dedication

I dedicate this work to the three most important people in my life: my father, my wife, and my son. Father, you are the wisest person I know and my main inspiration of what a man should be like. Your selflessness, dedication, and integrity are key characteristics that helped to shape who I am and who I want to be. In the many times I failed, you were always there to tell me to stand up and try again. You were also there to celebrate every little victory with me. I really hope to be able to make you proud of me. I will be a successful man if I can ever be as good of a father as you are.

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Chapter 1 - Literature Review

Introduction

Cow health, reproduction, and productive performance are vital traits to achieve economic efficiency in dairy production systems. In order to optimize these traits, various stressors that disrupt homeostasis, and consequently reduce animal performance, must be avoided. Because the majority of world's domestic livestock population is located in areas affected by seasonal stressors (Collier et al., 2006), better understanding of the mechanisms by which animal productivity is impacted under adverse environmental conditions may aid advancements in livestock production. Heat stress is a condition that occurs when an animal in unable to dissipate adequate quantities of heat in order to maintain thermal balance (Bernabucci et al., 2014). It has been suggested that the dairy industry is the most affected industry by heat stress among all livestock species in the U.S. (St-Pierre et al., 2003), with annual economic losses estimated to range from $900 million to $1.5 billion.

Core body temperature (**CBT**) of dairy cows is increased during periods of heat stress (Dikmen and Hansen, 2009; Kaufman et al., 2018). Under conditions of hyperthermia, several physiologic processes are altered (Wheelock et al., 2010; Legrand et al., 2011), ultimately disrupting animal welfare (Polsky and von Keyserlingk, 2017) and productivity (Jordan, 2003; West, 2003). Furthermore, because milk yield is positively correlated with CBT (Berman et al., 1985), high-producing dairy cows are severely affected by sustained conditions of heat stress. Spiers et al. (2004) reported an association between CBT and magnitude of dry matter intake (DMI) reduction in lactating cows exposed to heat stress conditions. Because milk yield is largely driven by DMI (Veerkamp, 1998), productive performance is expected to be subpar under conditions of hyperthermia. It has been demonstrated, however, that reduction in DMI only accounts for 35 to 50% of the decrease in milk yield of heat-stressed dairy cows (Rhoads et al., 2009; Wheelock et al., 2010), suggesting that other factors are involved. Because activation of endogenous thermoregulatory mechanisms requires energy, maintenance requirements of heat-stressed dairy cows can be 7 to 25% greater than for cows in thermoneutral conditions (NRC, 2001).

In order to reduce CBT to more normal physiological values during exposure to heat stress, dairy cows adopt four distinctive thermoregulatory mechanisms (Collier et al., 2006). Efficacy of radiation, conduction, and convection is exclusively dependent on thermal gradient, and therefore, present limited potential to reduce CBT in conditions of elevated ambient temperature (Collier et al., 2006). Conversely, evaporation is highly effective under increased environmental temperatures, but its efficiency is reduced when ambient humidity also is increased.

Use of cooling systems to alleviate heat stress in dairy cows has been studied since 1948 (Seath and Miller, 1948). Commercial use of these systems, however, has only been widely adopted more recently. Heat abatement systems are usually comprised of fans or a combination of fans and soakers activated in short-duration cycles, resulting in water evaporation from the skin (Flamenbaum et al., 1986). When properly operated, these systems are effective in reducing CBT of heat-stressed dairy cows (Honig et al., 2012) and can ameliorate some of the deleterious effects of hyperthermia on their productivity (Collier et al., 2006). Unfortunately, most evaporative cooling systems require large quantities of water to operate, which can be an important limitation for its adoption in many areas where water is scarce. Therefore, studies focused on development of management practices or novelty technologies that will allow heat abatement with restricted use of natural resources (e.g., water) are necessary. This literature review will focus on the effects of heat stress on health, production, and reproductive performance of dairy cows.

Temperature-Humidity Index

Temperature-humidity index (THI) is a single value that represents the combined effects of ambient temperature and relative humidity associated with the magnitude of thermal stress (Bohmanova et al., 2007). Since THI was first described (Thom, 1959) several formulas have been proposed for its calculation. Dikmen and Hansen (2009) studied the association of THI calculated using various formulas with CBT of dairy cows under heat stress conditions. Their findings indicate that most equations used to calculate THI result in similar predictivity of CBT of cows. The following THI equation is commonly used in studies focused on heat-stressed dairy cows: $THI = T – (0.55 - 0.55 RH/100) \times (T – 58)$, where T and RH are dry bulb temperature (ºF) and relative humidity, respectively (NOAA, 1976).

Values of THI for lactating dairy cows were categorized arbitrarily into absent, mild, moderate, and severe by Armstrong (1990) based on results of studies conducted in the 1950s and 1960s (Bernabucci et al., 2010). Igono et al. (1992) suggested that milk yield is affected negatively when THI reaches 72. More recent data, however, demonstrated that milk yield is reduced in high-producing dairy cows when THI reaches 68 (Zimbelman et al., 2009). Furthermore, it has been reported that the proportion of time cows spend standing compared with other activities increases when THI is greater than 68 (Allen et al., 2015), confirming that this value is an appropriate threshold to determine heat stress conditions. Nonetheless, because high-producing dairy cows produce more metabolic heat, the THI at which moderate heat stress occurs in this population is likely lower than for cows that are not high-producing (Berman, 2005). Moreover, despite compelling evidence that heat stress has important deleterious effects on dry cows (Tao and Dahl, 2013), association of THI thresholds during the dry period and subsequent health, reproductive or productive performance has not been defined. Nevertheless, established THI thresholds for lactating cows (e.g., 68 or 72) are commonly used as references for dry cows (Ferreira et al., 2016; Fabris et al., 2017). Moreover, because THI thresholds were developed based on milk yield or DMI reduction, it is not clear whether similar thresholds are useful predictors of reduction in health or reproductive performance of heat-stressed dairy cows. It has been suggested that conception risk can be reduced when mean THI on the day of AI is between 51 and 56 (Schüller et al., 2014; 2017). Further studies are necessary to clarify the THI limit in which health, reproduction, and milk yield of dairy cows are unaffected.

Physiologic Alterations Induced by Heat Stress

Heat stress induces activation of several adaptative mechanisms in an attempt to maintain CBT within normal values. One of the primary autonomic mechanisms adopted by dairy cows to reduce heat load when exposed to heat stress are increases in respiration rate and sweating (Gebremedhin et al., 2008; Chen et al., 2015). Respiration rate and ambient temperature are highly correlated (Legates et al., 1991). Zimbelman et al. (2009) demonstrated that each 1-unit increase in THI results in an increase in respiration rate by approximately 2 breaths/min. In addition, it has been suggested that cows can be considered under heat stress when respiration rate reaches approximately 60 breaths/min, because it occurs when THI reaches 68 (Zimbelman et al., 2009; Atkins et al., 2018). Several research groups demonstrated that cows exposed to heat stress conditions spend fewer hours lying down than cows under thermoneutral conditions (Cook et al., 2007; Allen et al., 2015). This effect is observed likely because heat-stressed dairy cows attempt to increase conductive heat loss, which is optimized when a greater surface area can be exposed to exchange heat (Berman, 2003). Choshniak et al. (1982) suggested that vasodilation and increased peripheral blood flow are physical responses that favor heat loss under heat stress conditions. Despite facilitating heat loss, this altered blood flow reduces blood perfusion to the liver and the mammary gland (McGuire et al., 1989; Lough et al., 1990), which ultimately impact milk production.

Measures of CBT, such as vaginal or rectal temperatures, are highly correlated with THI, and are more suitable to gauge heat stress severity than measures such as udder temperature (Kaufman et al., 2018). Core body temperature is associated with respiration rate (Atkins et al., 2018), and with reductions in DMI and milk yield in heat-stressed cows (Spiers et al., 2004). Because of these associations, CBT is commonly recorded in studies focused on heat-stressed dairy cows during the lactating and dry periods. In dry cows, in which CBT is not confounded with milk yield (Berman et al., 1985), several authors reported that CBT was approximately 0.3 ºC greater for non-cooled compared with cooled dairy cows exposed to heat stress (Karimi et al., 2015; do Amaral et al., 2009). Despite a relatively small increase in CBT, the aforementioned studies reported major reductions in subsequent lactation performance of cows that were not cooled during the dry period, indicating that minimal increases in CBT during the dry period should not be overlooked.

Dry matter intake is drastically reduced during prolonged exposure to heat stress (Spiers et al., 2004). Because nutrient intake is affected by total DMI (NRC, 2001), and its decrease precedes reduction in milk yield (West, 2003), DMI decline is usually accepted as the primary culprit of the diminished milk yield observed in heat-stressed dairy cows (Rhoads et al., 2009). Recent studies indicate, however, that reduced nutrient intake accounts for only 35 to 50% of the decrease in milk yield of dairy cows exposed to heat-stress (Rhoads et al., 2009; Wheelock et al., 2010). The precise nutrient-intake mechanisms that negatively impact milk yield under heat-stressed conditions are not well understood. It has been reported that maintenance requirements of heat-stressed dairy cows can be increased by 7 to 25% compared with cows under thermoneutral environmental conditions (NRC, 2001). Rhoads et al. (2009) suggested that a shift in post-absorptive metabolism and nutrient partitioning is likely involved in this increase of basal requirements. Others reported that increased nutrient demand to support autonomic thermoregulation mechanisms (e.g., panting, sweating), and production of heat-shock proteins also contribute to increased maintenance requirements of cows under heat stress (Tomanek, 2010; Johnson, 2017).

When DMI is insufficient to support energy requirements, dairy cows will enter into negative energy balance (**NEB**; Drackley et al., 1999). Cows in NEB present increased plasma concentrations of energy balance indicators, such as nonesterified fatty acids (**NEFA**; Baumgard et al., 2017). When exposed to chronic heat stress, however, basal insulin secretion is increased, which reduces adipose tissue mobilization as a source of energy (Baumgard and Rhoads, 2013). Therefore, heat-stressed dairy cows usually do not present increased concentration of NEB indicators compared with non-heat-stressed cows, and this effect seems to be consistent during the lactating (Rhoads et al., 2009; Wheelock et al., 2010) and non-lactating periods (Urdaz et al., 2006; Tao and Dahl, 2013). Conversely, glucose uptake by skeletal muscle is increased during prolonged exposure to heat stress, leaving less glucose available for milk synthesis (Wheelock et al., 2010). Moreover, somatotropin concentrations are usually reduced in heat-stressed cows (Mohammed and Johnson, 1985), which further prevents nutrient partitioning towards the mammary gland, resulting in reduced milk yield.

Heat Stress During the Lactating Period

Heat stress poses a major challenge for lactating dairy cows, impacting both milk production and reproductive performance (Jordan, 2003; West, 2003). Effects of heat stress on various traits of biological and economical importance are studied commonly using observational data or comparing cooled with non-cooled dairy cows exposed to an elevated THI environment.

Early work from Johnson et al. (1963) suggested that milk yield decreases 1.4 kg for each 0.55°C increase in CBT. Others later demonstrated that milk yield is reduced by 0.32 kg for each unit of increase in THI (Ingraham et al., 1979). Reduced DMI has been considered the main culprit for reduced milk yield of dairy cows exposed to heat stress (West, 2003; Spiers et al., 2004). More recent evidence, however, indicates that only 35 to 50% of the heat-stress induced decline in milk yield is explained by reduced DMI (Rhoads et al., 2009; Wheelock et al., 2010). Furthermore, because heat-stressed cows rely on energy-demanding intrinsic mechanisms to prevent hyperthermia (e.g., sweating, panting), maintenance requirements for lactating dairy cows can increase by 7 to 25% during periods of heat stress (NRC, 2001). This increased requirement occurs because altered energy partitioning to favor heat dissipation reduces energy availability to milk synthesis, ultimately reducing milk yield (West, 2003). Furthermore, it is possible that reduction in blood flow to the mammary gland observed under heat stress (Lough et al., 1990) negatively impacts nutrient uptake by the mammary gland, further decreasing milk synthesis. It has been suggested that higher milk-producing dairy cows are less resilient to heat stress because they present greater production of metabolic heat than lower milk producing cows and non-lactating cows (Purwanto et al., 1990; NRC, 2001). Indeed, findings from Berman et al. (1985) suggested that CBT increases by 0.02°C for each kg of fat-corrected milk above 24 kg.

Several experiments demonstrated that cooling systems are effective methods to reduce respiration rate and CBT (Seath and Miller, 1948; Flamenbaum et al., 1986; Honig et al., 2012) and enhance milk production (Strickland et al., 1988; Honig et al., 2012; Safa et al., 2019) of heat-stressed lactating dairy cows. Thatcher (1974) suggested that use of air conditioning increases milk yield by approximately 10% compared with non-cooled cows. This strategy, however, is not cost-effective (Hahn et al., 1969). Igono et al. (1987) later suggested an effective and profitable cooling system comprised of a combination of forced ventilation and water spray increased milk yield by approximately 2 kg/d compared with non-cooled control cows. A recent study demonstrated that lactating cows cooled during the first 7 wk of lactation resulted in increased DMI by 1.5 kg/d and milk yield by 7.4 kg/d compared with non-cooled cows exposed to heat-stressed conditions (Safa et al., 2019). In addition, Safa et al. (2019) reported that cows that were cooled during the first 7 weeks postpartum produced 1,442 kg more milk during the entire lactation compared with cows that were not cooled. Other researchers suggested that more frequent cooling sessions would further benefit lactating dairy cows already being cooled 5 times/d (Honig et al., 2012). In the previous experiment, it was suggested that cows receiving 8 cooling sessions/d produced 3.5 kg/d more milk than cows being cooled 5 times daily. Despite the proven efficacy of heat abatement systems, a cooling regimen that could provide optimal results and be implemented in large herds in various geographic regions has not yet been clearly defined.

Besides affecting DMI and milk yield, heat stress affects milk composition. Salfer et al. (2018) recently suggested that milk fat and protein contents vary across the year, with lower values during the summer than during other seasons. Corroborating with these findings, Smith et al. (2013) demonstrated that milk protein is reduced from 3.2 to 3.1% in Holstein cows exposed to heat stress. In addition to the changes in milk composition driven by reduced DMI and differences in diet composition, milk protein content may be reduced in heat-stressed dairy cows because of the increased demand of amino acids for heat-shock proteins synthesis (Collier et al., 2008; Johnson, 2017). Heat stress also has been shown to reduce milk fat content (Bernabucci et al., 2014). In contrast, Smith et al. (2013) reported increased milk fat during periods of heat stress for Holstein cows. It should be considered, however, that information regarding diet composition was not available in the study conducted by Smith et al. (2013). Therefore, conclusions from this study should be considered with caution.

Heat stress has a major negative impact on reproductive performance of dairy cows (Jordan, 2003). In an observational study, Schüller et al. (2014) reported that conception risk was reduced when mean THI was 73 for 1 h or more on the day of AI. In fact, results from this study indicate that conception was significantly decreased when average THI on the day of AI is as low as 56. Under heat-stressed conditions, oocyte quality and embryo development are impaired (Putney et al., 1988; Rivera and Hansen, 2001; Roth et al., 2001), which partially explains the reduced conception risk commonly observed during summer and early autumn (Roth et al., 2001; 2004). It has been suggested that cooling lactating cows with a combination of fans and sprinklers may improve conception risk of dairy cows exposed to heat stress (Wolfenson et al., 1988). Despite the important findings reported in this study, the sample size was small (74 cows). Nonetheless, Flamenbaum and Galon (2010) concluded that herds with intensive cooling regimens observed a smaller reduction in conception risk in lactating cows during summer compared with herds that adopted subpar cooling strategies. Altogether, these findings suggest that cooling lactating dairy cows may be beneficial to conception risk, but some reduction in fertility is expected during the warmer months of the year (Jordan, 2003).

Besides affecting conception risk, heat stress induces important alterations in the hypothalamic-pituitary-ovarian axis, which ultimately modifies ovarian follicular development and steroidal concentrations (Roth and Wolfenson, 2016). Growth of medium-sized follicles is impaired under hyperthermic conditions (Roth et al., 2000), which reduces inhibin and estradiol concentrations in plasma (Roth et al., 2000). In addition, cows exposed to heat stress have smaller preovulatory follicles (Schüller et al., 2017) and reduced LH concentration during the preovulatory surge (Wise et al., 1988). Even though it has been suggested that chronic heat stress may negatively affect luteal function (Sheldon et al., 2002), this association has not been demonstrated in other studies (Jordan, 2003). Therefore, heat-stress induced alterations in follicular dynamics result in reduced duration and intensity of estrus (Roth and Wolfenson, 2016). Indeed, estrus expression has been consistently demonstrated to be reduced under heat-stressed environment conditions (Pennington et al., 1985; Younas et al., 1993; De Rensis and Scaramuzzi, 2003; Schüller et al., 2017). Despite the vast literature demonstrating the negative association between environmental conditions and estrus expression at the cow-level, it is not clear whether herd-level traits, such as insemination risk, are affected by heat stress. A recent report suggested that insemination risk is minimally reduced during the summer compared with cooler months of the year (Scanavez et al., 2016). Further research, however, is necessary to better understand the association between thermal stress and herd-level traits of estrus detection efficiency.

Lactating dairy cows exposed to heat stress change their behavior to optimize heat loss (Polsky and Von Keyserlingk, 2017). One of the well-known behavioral changes observed in dairy cows exposed to heat stress is an increase in standing time (Cook et al., 2007; Allen et al., 2015). Increased standing time is an important risk factor for lameness (Zahner et al., 2004; Cook et al., 2007). Because lameness is a highly prevalent disorder in dairy herds (Cook and Nordlund, 2009), and has extensive deleterious effects on milk production and reproductive performance of dairy cows (Garbarino et al., 2004; Hernandez et al., 2005), it can be speculated that improved thermal balance might optimize these outcomes by reducing lameness prevalence. Very limited research exists evaluating the effects of heat stress on health of lactating dairy cows.

Heat Stress During the Dry Period

The deleterious effects of heat stress on overall performance of dairy cows is not limited to the lactating period. Several authors demonstrated that exposure to heat stress during the dry period has negative effects on milk yield in the subsequent lactation (Avendaño-Reyes et al., 2006; Adin et al., 2009; Tao et al., 2012). Early work conducted by Collier et al. (1982) demonstrated that calves born to cows that did not have access to shade during the last trimester of gestation were lighter than calves born to cows that had access to shade. In addition, this study reported a positive linear association between calves' birth weight and dams' milk yield, which provided initial indication that exposure to heat stress during the dry period can impact milk production after parturition. In a study with a larger sample size (475 cows), Urdaz et al. (2006) suggested that use of shade and fans in addition to a sprinkler system to cool dry cows resulted in 84 kg more milk in the first 60 days in milk (DIM) of the subsequent lactation than cows that only had access to sprinklers before calving. After considering the investments required for improved cooling (e.g., shades, fans, electricity), Urdaz et al. (2006) indicated that the more intensive cooling system was more profitable than use of sprinklers alone. In this study, however, dry cows were cooled only during the last 4 wk of gestation. Recent work conducted in Florida indicates that cooling dairy cows during the entire dry period is more beneficial to subsequent milk yield than cooling during part of the dry period (Fabris et al., 2019). When cooling is used during the entire dry period, milk yield is increased by 2.1 to 9.3 kg in the subsequent lactation compared with cows that were not cooled (Adin et al., 2009; do Amaral et al., 2009; Tao et al., 2011), demonstrating that heat stress during the dry period has extensive negative effects on postpartum milk yield.

The association between exposure to heat stress during the dry period and milk production after parturition was reviewed by Tao and Dahl (2013). In this review, the authors compiled data from 7 experiments, and reported a negative correlation between CBT during the dry period and milk yield during the subsequent lactation. It is not clear, however, whether such a correlation exists for individual cows managed under similar circumstances (e.g., in the same pen) and naturally have greater or lesser CBT. Nonetheless, it is well accepted that evaporative cooling systems are effective to alleviate the negative effects of heat stress on future productivity of dry cows (Tao and Dahl, 2013). In fact, economic feasibility of cooling dairy cows during the dry period has been studied, and findings from Ferreira et al. (2016) suggest that cooling dry cows is a profitable management practice for approximately 90% of cows in the U.S.

Several mechanisms have been proposed to describe how heat stress affects dry cows and postpartum milk yield. Under heat stress conditions, dry cows consistently have reduced DMI compared with non-heat-stressed counterparts (Adin et al., 2009; do Amaral et al., 2009; Tao and Dahl, 2013; Karimi et al., 2015). Reduced DMI before parturition is associated with increased risk of health disorders after calving, such as metritis (Huzzey et al., 2007), which could ultimately affect milk yield (Rajala and Gröhn, 1998). In addition to reduced DMI, heat stress during the dry period is associated with impaired placental development (Collier et al., 1982), which results in a reduced number of cells in the placenta (Early et al., 1991; Tao and Dahl, 2013). Fewer cells resulting from impaired placental development may explain the reduced concentration of placental lactogen observed in sheep exposed to chronic heat stress during late gestation (Bell et al., 1989). Because placental lactogen has an important role stimulating DMI and increasing milk yield (Byatt et al., 1992), it is possible that dairy cows with reduced exposure to placental lactogen because of heat stress may produce less milk after calving. Another hormone that has a crucial role in mammogenesis and lactogenesis is prolactin (Tucker, 2000). It has been demonstrated that prolactin concentrations are greater for heat-stressed dry cows than for their cooled counterparts (do Amaral et al., 2009). Increased circulating prolactin downregulates expression of prolactin receptors in heat-stressed dry cows (do Amaral et al., 2010; do Amaral et al., 2011; Tao and Dahl, 2013). Therefore, it is possible that reduced sensitivity of mammary tissue to prolactin because of reduced expression of prolactin receptors may have a negative impact on subsequent lactation milk yield (Dahl, 2008; Tao and Dahl, 2013). Adin et al. (2009) were the first to explore impaired mammary cell proliferation as a mechanism to explain the deleterious effects of dry-period heat-stress on postpartum milk yield. In their study, non-cooled dry cows had reduced expression of enzymes that control mammary cell proliferation compared with dry cows that had access to a heat abatement system. In an elegant experiment, Tao et al. (2011) later demonstrated that heat-stressed dry cows had a reduced rate of mammary cell proliferation approximately 20 days before parturition compared with cooled cows, despite presenting similar rates of apoptosis. In summary, the mechanisms by which heat stress during the dry period negatively affects postpartum performance are multifactorial, and include changes in behavior (e.g., DMI), placental development, hormonal profiles, and mammary development.

Very limited data exploring the association between dry-period heat stress and postpartum health and reproductive performance are available. In an observational study that included records from 2,613 multiparous cows, Thompson and Dahl (2012) suggested that incidence of mastitis and respiratory problems is greater for cows exposed to heat stress during the dry period than for cows that were dry during the coolest months of the year. It is important to consider, however, that this study compared performance of cows dried-off during the warmest months (e.g., June, July and August) with cows that were dried during the coolest months of the year (e.g., December, January, and February). It is possible that several other factors such as diet, photoperiod, and overcrowding could influence the observed results. In a controlled experiment, Urdaz et al., (2006) reported no differences in postpartum health of cows that were exposed to different cooling regimens during the last weeks of gestation. In that study, however, it is possible that important health disorders (e.g., metritis) were underdiagnosed. Nonetheless, it has been shown in controlled experiments that early-lactation immune function is reduced in cows exposed to heat stress during the dry period (do Amaral et al., 2010; 2011). Therefore, the possibility of an association between dry-period heat stress and postpartum health should not be ignored. In the study conducted by Thompson and Dahl (2012), the authors reported that cows dried off during hot months had longer days to first AI, increased number of AIs, and extended days to pregnancy compared with cows dried off during cool months of the year. Others reported similar findings comparing reproductive performance of cows that were cooled or not during the dry period (Avendaño-Reyes et al., 2006; Karimi et al., 2015). When an effective cooling method was used for dry cows, both days open and services per conception were improved compared with cows that were not cooled during the non-lactating period (Avendaño-Reyes et al., 2006). Karimi et al. (2015) reported that cooling during the dry period resulted in reduced services per conception, but no differences were detected in number of days open. This lack of difference likely happened because of insufficient experimental power to detect differences resulting from a relatively small sample size, because mean days open were 97.0 and 106.7 for cows that were cooled or not cooled during the dry period, respectively. Because reproductive performance is greatly affected by postpartum health (Santos et al., 2010; Ribeiro et al., 2013; Ribeiro et al., 2016), it is possible that the reduced fertility observed in cows that were exposed to heat stress during the dry period occurs partially because of increased risk of developing postpartum health disorders. Therefore, despite the need for larger controlled studies, it is possible that postpartum health and reproductive performance may be affected by exposure to heat stress during the non-lactating period.

In addition to the effects of dry-period heat stress on subsequent performance of the dam, several studies have focused on investigating its effects on the offspring. Heat stress during the dry period is associated with reduction in birth weight of calves (Collier et al., 1982; Adin et al., 2009; Tao et al., 2011). This effect may be partially attributed to shorter gestation length, but also to compromised fetal development (Bell et al., 1989; Tao and Dahl, 2013). Immune function also is compromised in calves born to cows exposed to heat stress during late gestation (Tao et al., 2012; Tao and Dahl, 2013). Serum IgG concentration and absorption efficiency is reduced in calves born to cows that were exposed to heat stress during the dry period (Tao et al., 2012). It has been reported that colostrum IgG concentration is reduced in cows exposed to heat stress during the dry period (Nardone et al., 1997; Karimi et al., 2015). Therefore, it is possible that the effect of maternal heat stress on calves' serum IgG concentration occurs because of subpar colostrum quality.

Recent data indicate that heat stress during the last weeks of gestation may affect culling dynamics and milk production during the first lactation of the offspring. Monteiro et al. (2016) reported that heifers born to heat-stressed dams produced on average 5.1 kg/d less milk during the first 35 wk of lactation than heifers born to cows that were cooled during the dry period. Moreover, the authors suggested that proportion of cows culled by the end of the first lactation was greater for cows exposed to heat stress in utero than for cows born to dams that were cooled during the dry period. Specific mechanisms involved in reduced productive performance and survival of cows exposed to heat stress in utero are not clearly understood. Notwithstanding, more comprehensive research is warranted to evaluate the economic losses caused by heat stress,which include the effects on lactating and dry cows, and their offspring.

Conclusions

Heat stress poses major challenges to the dairy industry, disrupting the wellbeing and productivity of cows. Under heat stress conditions, dairy cows have increased respiratory rate, sweating, and peripheral blood perfusion, in an attempt to maintain CBT within physiologic range. Dry matter intake is reduced during prolonged exposure to heat stress, which accounts for 35 to 50% of the reduction in milk yield observed in heat-stressed cows compared with cows managed in a thermoneutral environment. Major alterations in metabolism and energy partitioning increase maintenance requirements, and diminish nutrient uptake by the mammary gland of heat-stressed dairy cows, which further reduces milk production. Reproductive performance is severely affected when lactating cows are exposed to heat stress, and deleterious effects are observed when mean THI on the day of AI is as low as 56. Even though it is well accepted that estrus expression is reduced during periods of heat stress, it is not clear whether herd-level indicators of estrus-detection efficiency, such as insemination risk, are disturbed during warmer periods of the year. Exposure to heat stress during the dry period alters hormonal profiles and mammary gland development, ultimately affecting milk yield during the subsequent lactation. Despite the limited literature focused on fertility of cows exposed to heat stress during the non-lactating period, reproductive performance is likely decreased compared with cows that have access to cooling systems during the dry period.

Moreover, heat stress during the dry period affects the offspring by reducing birth weight and IgG absorption efficiency. Heifer calves born to cows exposed to heat stress during the dry period produce less milk and are more likely to be culled before the end of the first lactation than heifer calves born from cows provided heat abatement during the dry period. Limited data are available regarding the effects of heat stress on the health of cows during the lactating- and dry-periods. Evaporative cooling systems are efficient aids to ameliorate the effects of heat stress on milk yield, and to a lesser extent, reproductive efficiency of dairy cows. Because most heat abatement systems require large quantities of water to operate, their adoption may not be feasible in areas where water is scarce. In summary, heat stress is a paramount challenge to the dairy industry, and more applied research is necessary to develop strategies to offset its negative impacts on animal performance and sustainability of dairy operations.

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Chapter 2 - Association Between Four-Day Vaginal Temperature Assessment During the Dry Period and Performance in the Subsequent Lactation of Heat-Stressed Dairy Cows

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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 heat-stressed cows. A total of 105 non-lactating Holstein cows from 2 commercial 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 days in milk (DIM) of the subsequent lactation to evaluate health disorders, culling risk, 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 during 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 during 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 during 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 days of gestation.

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

INTRODUCTION

Heat stress has profound effects on 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 heat-stressed 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 during June and July 2014. Mean (± 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 during the first 4 days after enrollment. The criteria for enrollment was a forecast of maximum temperature > 32°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 forth replicates consisted of 17, 18, 24, and 30 cows, respectively. In Dairy B, a total of 15 cows were enrolled in 1 replicate. Body condition score was assessed upon 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**) insert was placed in the vagina to collect VT measurements. Vaginal temperature was assessed while cows were in the far-off pen. Loggers were calibrated for accuracy of ± 0.13°C and were fixed in the CIDR insert using a moisture-resistant flexible plastic (Parafilm®, 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 in the pens in which 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**): $THI = T - (0.55 - 0.55\ RH/100) \times (T - 58)$, where T and RH are dry bulb temperature (ºF) and relative humidity, respectively (NOAA, 1976).

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

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 veterinary protocol. Diagnosis and treatments were recorded in the on-farm management software on a daily basis by trained farm personnel. Health measures of interest in the current study were: uterine diseases (**UTD**; retained placenta and metritis) and mastitis (**MAST**). Cows that did not release fetal membranes by 24 h after calving were considered to have retained placenta. Metritis was characterized by the presence of fetid uterine discharge between 4 to 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 DIM (**MAST300**).

Reproductive Management

Estrus detection was performed by a trained technician during lockups 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 $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$ treatments were enrolled in the Cosynch-72 timed AI protocol, which consisted of a GnRH injection (Factrel, Zoetis Inc., Florham Park, NJ) followed 7 d later by $PGF_{2\alpha}$ 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. Non-pregnant cows received a $PGF_{2\alpha}$ injection to induce estrus. Cows not re-inseminated after 7 d of non-pregnancy 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 Institute 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 maximum THI during 4 d 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, 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 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 $0.05 \leq P \leq 0.10$.

RESULTS

Temperature-Humidity Index, Vaginal Temperature, and Prepartum 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 ambient maximum and minimum temperatures were 36.2 and 18.2°C for dairy A, and 33.6 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 ambient temperature, relative humidity, and THI for each replicate are described in Table 2.1.

Cows classified as HT had greater ($P < 0.01$) VT compared with LT cows (Table 2.2). In addition, primiparous cows had greater ($P < 0.01$) VT than multiparous cows (38.91 ± 0.03 vs. 38.81 ± 0.03 ºC). Vaginal temperature was not ($P = 0.43$) associated with the interaction between parity and VT category (Table 2.2). In addition, VT was not ($P = 0.46$) 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 ($P > 0.23$) differ between HT and LT cows (Table 2.2). In contrast, HT cows had decreased ($P < 0.01$) gestation length and lesser ($P < 0.01$) days spent in close-up pen than LT cows (Table 2.2). 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 2.3). No difference was detected in culling by 30 DIM. Vaginal temperature category was not (*P* ≥ 0.15) associated with incidence of MAST60 or calving in the subsequent lactation, but a tendency (*P* = 0.09) was detected for the interaction between VT category and parity to be associated with MAST300 (Table 2.3). The interaction between VT category and parity were not (*P* ≥ 0.26) associated with other health disorders, culled by 300 DIM, or calving in the subsequent lactation. Dairy was not (*P* ≥ 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 (*P* = 0.12) associated with hazard to MAST60 (Table 2.4). In contrast, hazard to UTD or MAST60 (*P* < 0.01), UTD or MAST300 (*P* < 0.01), and MAST300 (*P* < 0.04)were greater for HT compared with LT cows (Table 2.). Dairy, BCS category, and the interaction between parity and VT category did not (*P* ≥ 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 evaluating 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) 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 (±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 Figure 2.1. 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 ($P = 0.08$) to have lesser hazard of being culled than LT cows (Table 2.3). 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 ($P = 0.32$) 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 (Figure 2.2). 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) month of lactation, respectively. 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 month of lactation than primiparous cows. The interaction between VT category, parity, and month of lactation was not detected ($P = 0.15$). 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 by 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, 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 by 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 cows that were primiparous at enrollment (Figure 2.3), LT cows had greater ($P < 0.01$) and tended ($P = 0.07$) to have greater milk yield in the first and fourth month of lactation compared with HT cows, respectively. Among cows that were multiparous at enrollment (Figure 2.4), milk yield differed ($P \leq 0.05$) in the second and third month of lactation between VT category. Dairy and average maximum THI were not ($P \geq 0.34$) associated with milk yield in any of the models.

Reproductive Performance

Pregnancy per AI at first service (23.2%; $P = 0.32$) 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 did not differ ($P \geq 0.53$) between HT and LT cows (Table 2.4). 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 differ ($P \geq 0.54$) between VT category.

Mean (± SEM) and median days to first AI were 67.3 ± 1.3 and 62, respectively. Mean (± 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 (*P* = 0.24) between LT and HT cows (Table 2.3).

DISCUSSION

It is well accepted that providing shade and evaporative cooling to dry cows is an effective manner to minimize the effects of heat stress on milk production during the subsequent lactation. Economic implications of investing in these types of heat abatement strategies for dry cows was assessed recently. 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 that 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, a limited number of experiments exist that have evaluated the benefits on milk 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 to cope with heat stress after calving and it should be considered in production systems in which 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 stage of production. Promising results that used genomic selection to identify heat-tolerant and heat-susceptible dairy cows have been reported (Garner et al., 2016). Findings from the current study provide evidence that on-farm data also may be used to identify dairy cows susceptible to heat stress.

The authors hypothesized that heat-stressed 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 risk of uterine diseases or mastitis during the first 60 DIM compared with LT cows. We focused on evaluating the association between vaginal temperature and mastitis and postpartum uterine diseases because they are the most common health problems in dairy cows after calving (Santos et al., 2010). In addition, occurrence of these disorders represent significant economic losses to dairy producers because of costs associated with treatment (Heikkilä et al., 2012; Liang et al., 2016), and decreased milk production (Bell and Roberts, 2007; Schukken et al., 2009) and reproductive efficiency during lactation (Giuliodori et al., 2013; Ahmadzadeh et al., 2009). 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 risk for major postpartum disorder treatment, no differences were detected in reproductive performance between VT categories. In fact, differences in postpartum health disorders were mainly detected because of greater likelihood of HT cows to be detected with uterine disease. No differences were detected in the analysis that evaluated the occurrence of mastitis by 60 DIM.

To the authors’ knowledge, this is the first study that identified heat-stressed dry cows susceptible to be treated for postpartum disorders by only using information of prepartum VT. 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 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 collected twice daily during the prepartum period differed by only 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 another evidence that we were able to identify heat-tolerant and heat-susceptible cows 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 also may 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 before calving. 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 greater near calving, which potentially could explain temperature differences between HT and LT cows. Nevertheless, we believe that the small difference detected in gestation length at enrollment was random and did neither affect VT nor confound our research sample. 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, it is possible that HT cows had established inflammatory process while VT was assessed resulting in greater CBT. Indeed, it has been shown that locomotion disorders before calving are associated with postpartum health events during 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 be affected by postpartum disorders after calving.

An experiment conducted by Tao et al. (2011) demonstrated that compromised mammary cell proliferation is a potential cause of 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 disorders during 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 occurrence of health disorders during 60 DIM in the model, it seems that postpartum health does not confound the association between VT category and milk yield. These results corroborate conclusions from earlier findings previously discussed (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 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 pre-calving VT in cows more susceptible to be affected by heat stress. Furthermore, it is possible that the tendency for reduced milk yield of HT cows was associated with impaired mammary gland development resulting from reduced gestation length compared with LT cows. Calving earlier than expected may have impacted galactopoiesis and 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 also were 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. In order to conduct an analysis with reduced bias, the authors of the current experiment excluded cows that were removed from the herd during 300 DIM, in which no differences in milk yield in late lactation were observed. Therefore, these results indicate that the impact of heat stress in dry cows in milk yield is predominantly in early lactation. The observed tendency in hazard of culling by300 DIM of HT and LT cows is intriguing, mainly 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 4 d of the dry period when vaginal temperature was assessed. Although LT cows had lower VT than HT cows, LT cows also were 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 carry-over 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 carry-over effects on concentrations of steroid hormones in follicular fluid (Roth et al., 2001b). In the current study, first AI after parturition was conducted from late August though the first week of September. Considering that development from primordial to preovulatory follicle stage requires approximately 4 months 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 indicate that heat stress during the prepartum and periparturient period affected reproductive efficiency of cows in early lactation.

In conclusion, results from this observational study demonstrate that cows susceptible to be treated for postpartum disorders may be identified at the farm-level during the summer by evaluating body core temperature at 250 to 260 days of gestation. Differences detected in postpartum health disorders indicate that cows with greater VT before calving are more susceptible to the negative effects of heat stress than cows with lower VT. In addition, cows with greater VT before calving tended to have reduced milk yield during early lactation than 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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Table 2.1. Percentage of cows classified as having high or low temperature per replicate and ambient temperature, relative humidity, and temperature-humidity index (THI) during vaginal temperature assessment.

	Replicate number[1]				
Item	1 (Dairy A)	2 (Dairy A)	3 (Dairy A)	4 (Dairy A)	1 (Dairy B)
			%, no./no		
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

[1] Weekly cohorts of cows enrolled in the study.

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

Table 2.2. Prepartum descriptive data (mean ± SEM) of cows classified as low or high vaginal temperature before calving.

	Vaginal temperature (VT) category		*P*-value		
Item	Low temperature	High temperature	VT category[1]	Parity	Interaction
Number of cows	51	53			
Average vaginal temperature, ºC	38.71 (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

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

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

Table 2.3. 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.

	Incidence			*P*-value		
Item	Low temperature	High temperature	AOR (95% CI)	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	1.39 (0.28 – 6.96)	-	-	-
Multiparous	20.0	50.0	0.22 (0.06 – 0.88)	-	-	-
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

[1] VT 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.

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

[3] MAST60: cows diagnosed with mastitis in the first 60 days in milk.

[4] MAST300: cows diagnosed with mastitis in the first 300 days in milk.

Table 2.4. 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.

			P-value		
Item	AHR	95% CI	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			

[1] VT 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.

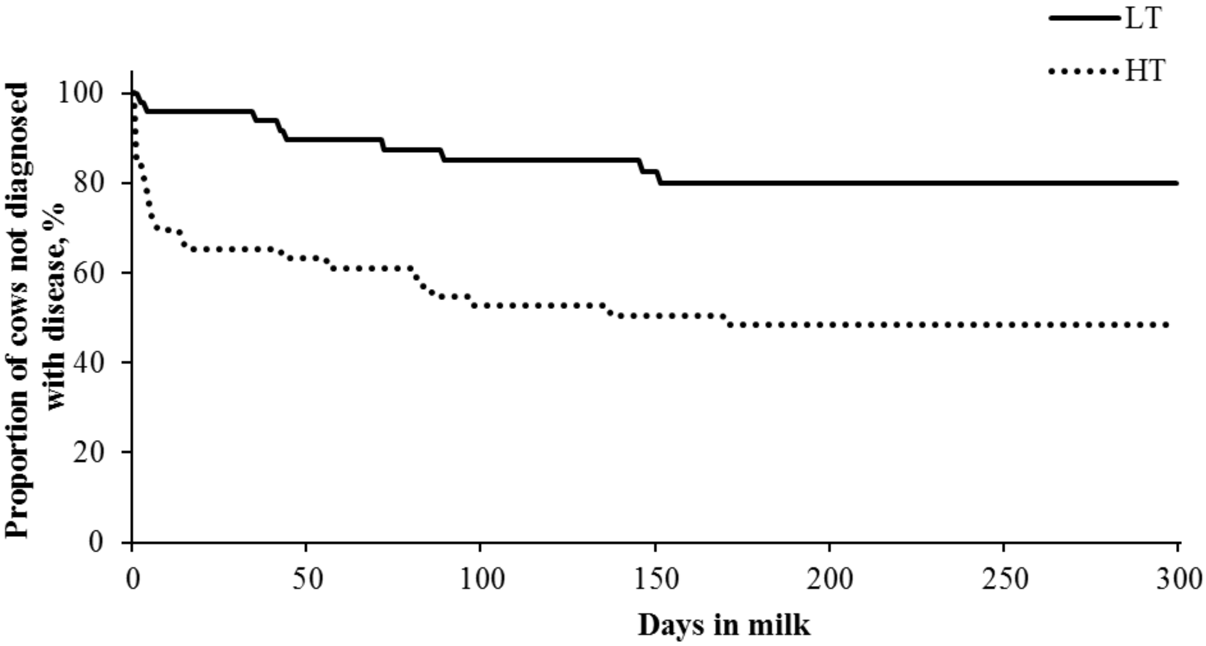

Figure 2.1. Kaplan-Meyer survival analysis for days to occurrence of uterine diseases or mastitis during the first 300 DIM for cows classified as low (LT, n = 51) or high (HT, n = 53) vaginal temperature before calving.

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 ($P < 0.01$).

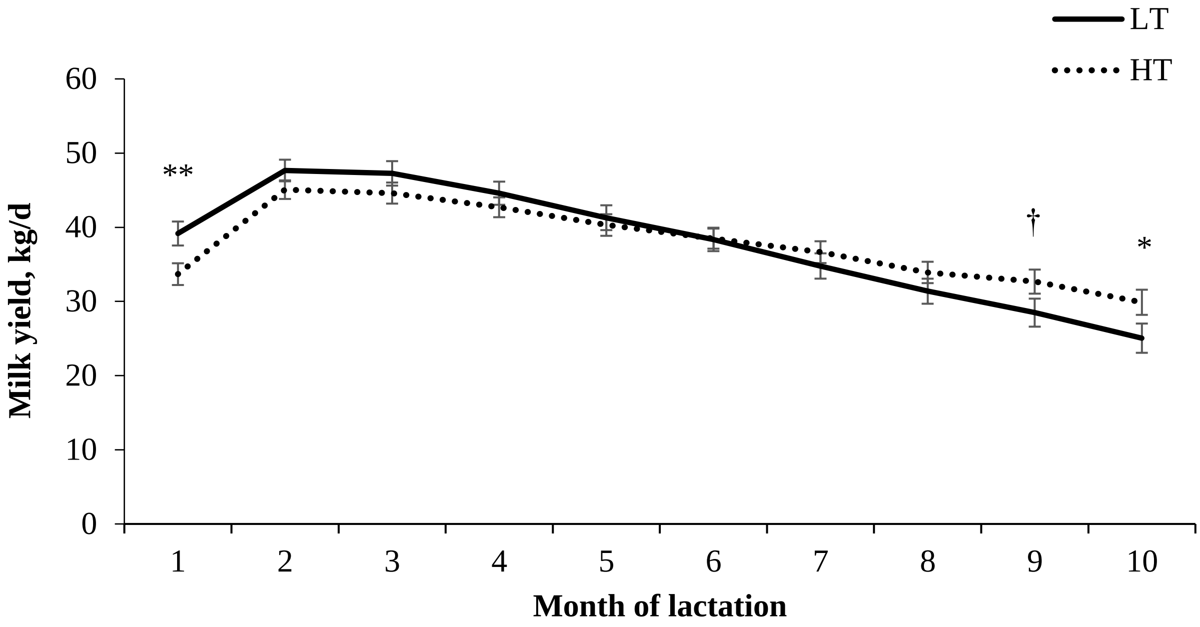

Figure 2.2. Milk yield of cows classified as low (LT, n = 51) or high (HT, n = 53) vaginal temperature before calving.

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 associated (P = 0.99) with milk yield, but the interaction between vaginal temperature and month of lactation tended (P = 0.10) to affect milk yield. Within month, pairwise differences and tendencies are represented as follows: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

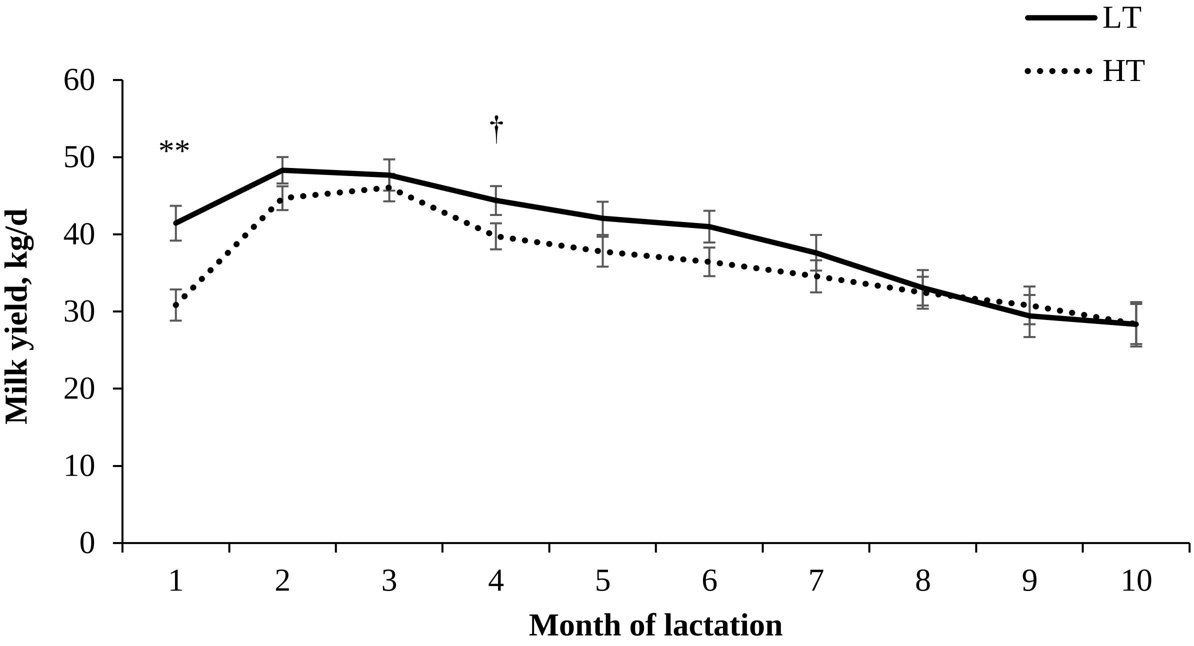

Figure 2.3. Milk yield of second-lactation cows classified as low (LT) or high (HT) vaginal temperature before calving that were not removed from the herd within 300 DIM.

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. Low temperature cows 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.

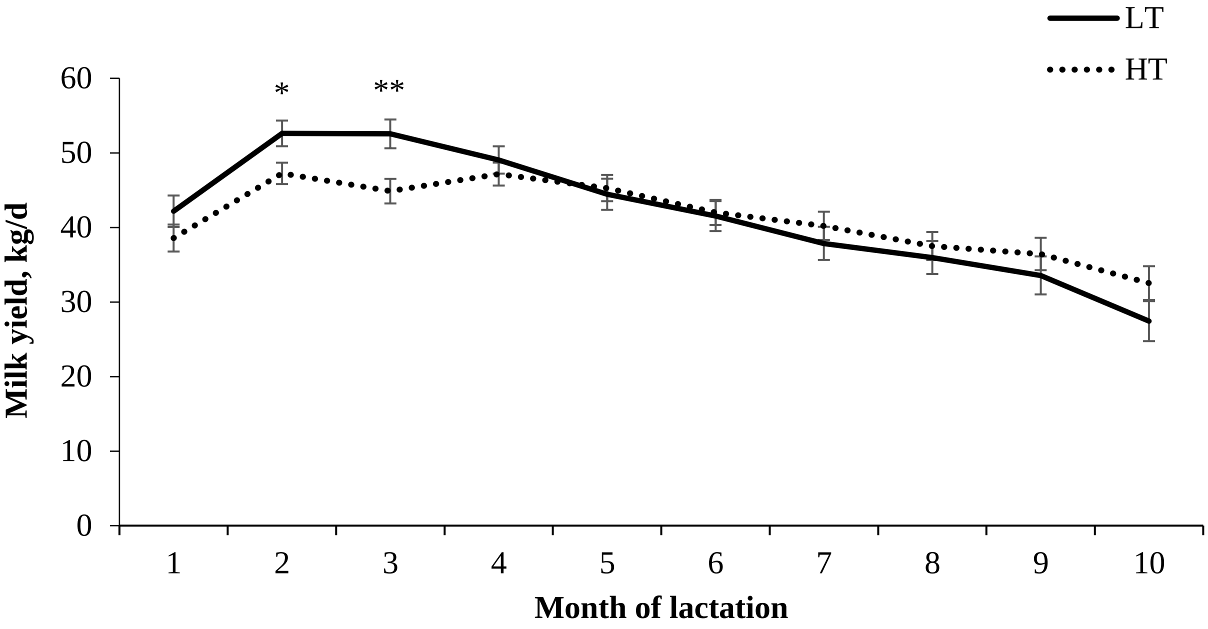

Figure 2.4. Milk yield of cows in their > third-lactation classified as low (LT) or high (HT) vaginal temperature before calving that were not removed from the herd within 300 DIM.

Low temperature cows had ($P \leq 0.01$) 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: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

Chapter 3 - Animal Factors Associated with Core Body Temperature of Non-Lactating Dairy Cows during Summer

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ABSTRACT

The primary objectives of the current study were to investigate animal factors associated with core body temperature (CBT) and to determine the time of the day in which CBT assessment best describes the magnitude of hyperthermia throughout the day of heat-stressed dry cows. The secondary objective was to develop a predictive model for CBT of dry cows. Non-lactating Holstein cows (n = 105) with 250 to 260 days of gestation from 2 commercial dairies were enrolled in the study during summer. During 4 consecutive days, CBT from all cows was recorded in 5-min intervals and average CBT was calculated for each cow. In addition, mean, maximum, minimum, and standard deviation of daily CBT were calculated and using these measures cows were categorized as having high (HT) or low temperature (LT) based on the median values. Cows carrying twins had greater ($P < 0.01$) CBT than cows bearing singletons (39.07 ± 0.07 vs. 38.84 ± 0.03 °C). Average CBT decreased ($P < 0.01$) 0.015 ± 0.004 °C for each 1-d increase in gestation length. Cows in dairy A tended ($P = 0.09$) to have lower CBT than cows in dairy B (38.91 ± 0.04 vs. 39.00 ± 0.06 °C). Season of birth, lactation number, body condition score category, previous projected 305-d mature equivalent milk yield, days in milk at dry-off, days after dry-off at enrollment, days of gestation at enrollment, and calf sex were not associated ($P > 0.12$) with CBT. Principal component analyses showed that 71% of the variance of CBT was explained by the first principal component alone, which was correlated with mean CBT (r = 0.99). Among all time points assessed, CBT recorded at 2215 h had the highest correlation with the first principal component (r = 0.93). The best agreement for classifying cows as HT or LT was between mean daily CBT and assessment at 2215 h ($k = 0.73$). The model that resulted in best predictivity (0.56) of average CBT included the following variables: dairy, gestation length, and twinning. In conclusion, findings from the present study indicate that CBT assessed between 250 and 260 days of gestation is negatively associated with gestation length and cows bearing twins have greater CBT than singletons. Our results indicate that the best time of the day to evaluate severity of heat stress in dry cows is 2215 h. Predictive models for CBT of dry cows should include dairy, twinning, and gestation length.

Key words: core body temperature, dairy cow, gestation length, heat stress, twinning

INTRODUCTION

Dairy cows exposed to heat stress during the dry period have increased core body temperature (**CBT**) and reduced milk yield in the subsequent lactation (Collier et al., 2006). Although it has been demonstrated that use of cooling systems reduces CBT of dry cows (do Amaral et al., 2009), animal factors that may be associated with thermoregulation of non-lactating cows are unknown. Body condition score (**BCS**), which is used to estimate energy requirement for dairy cattle (NRC, 2001), may affect heat dissipation. Furthermore, parity has been shown to be associated with CBT in early lactating cows (Suthar et al., 2011), and could potentially affect CBT of dry cows. Therefore, it is possible that BCS and lactation number, among other animal factors, are associated with thermoregulation capacity of dairy cows during the dry period. In most studies that evaluated the effects of heat stress during the dry period, CBT was assessed at 0730 to 0800 h or 1400 to 1500 h (Adin et al., 2009; Karimi et al., 2015; Tao et al., 2012). Nonetheless, given the circadian rhythm of CBT in dairy cows (Scanavez et al., 2016), it is not clear whether measurements obtained at those time points accurately represent severity of heat stress that dry cows undergo throughout the day.

We hypothesized that (1) BCS and lactation number, in addition to other factors, would be associated with CBT during the dry period; and (2) time of the day when CBT is assessed would interfere with heat stress severity estimation in dry cows. Our primary objectives were to investigate which factors are associated with CBT in Holstein dry cows and to determine the time of the day in which CBT assessment best describes the magnitude of hyperthermia that heat-stressed dry cows undergo throughout the day. Our secondary objective was to develop and evaluate effectiveness of a predictive model for CBT in dry cows.

MATERIALS AND METHODS

Data of animals obtained from a previous experiment (Scanavez et al., 2017) were used in the present study. All procedures reported herein were conducted under protocols 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. Dry Holstein cows with 250 to 260 d of gestation, between first and third lactation, locomotion score < 3 (Sprecher et al., 1997), and 4 functioning quarters at dry-off were eligible to be enrolled in the study. On the day of enrollment, a list of eligible cows was generated from the on-farm management software (DairyComp 305, Valley Ag Software, Tulare, CA). Cows were blocked by lactation number and randomly selected from the list. A total of 105 cows (first-lactation = 37; second-lactation = 37; and third-lactation = 31) were enrolled in the study from June 2014 through July 2014. Four replicates were performed in Dairy A (n = 90), and 1 replicate in Dairy B (n = 15). Body condition score was assessed by 1 person upon enrollment using a scale from 1 (emaciated) to 5 (obese) using 0.25-point increments (Ferguson et al., 1994). For statistical analysis, cows were classified into low BCS (**LBCS**; ≤ 3.5) or high BCS (**HBCS**; > 3.5).

At enrollment, cows were in a far-off pen and moved to a close-up pen after 260 days of gestation. In both dairies, dry cows were housed in dry-lot pens with shaded areas, and were fed once daily with a total mixed ration formulated to meet or exceed nutrient requirements (NRC, 2001). In the close-up pen, cows were fed a ration containing acidogenic salts.

Core Body Temperature and Temperature-Humidity Index Assessment

At enrollment at 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**) insert was placed in the vagina to collect CBT measurements. Loggers were calibrated for precision of ± 0.13°C (Thermodata Corporation, Milwaukee, WI) and were fixed in the CIDR inserte using a moisture-resistant flexible plastic (Parafilm®, Oshkosh, WI) and silicone aquarium sealant (Loctite®, Henkel Corporation, Rocky Hill, CT). Core body temperature was assessed every 5 min during 4 consecutive days. Time of CIDR insertion and removal were documented. After CIDR insert removal, measurements recorded by loggers were downloaded to a computer. To ensure accuracy of temperature readings, body temperature measurements collected within 1 h after CIDR insertion were excluded.

During the time CBT was being monitored, ambient temperature and humidity were recorded every 5 min in the pens in which cows were housed using loggers (HOBO U23 Pro v2; Onset Computer Corp., Pocasset, MA) fixed at 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 (ºF) and relative humidity (%), respectively (NOAA, 1976).

Statistical Analyses

Descriptive statistics were generated using PROC MEANS, PROC FREQ, and PROC HPMIXED procedures of SAS 9.4 (SAS Inst. Inc., Cary, NC). Average CBT of all readings was calculated for each cow to explore its association with various factors. Multivariable linear regression analysis was performed using the PROC GLIMIX procedure of SAS with average CBT as the dependent variable. Fixed effects tested in the model included dairy, cow's season of birth (winter, spring, summer, or fall), lactation number at enrollment (first, second, or third), BCSC (LBCS vs. HBCS), previous projected 305-d mature equivalent milk yield (**P305ME**), days in milk at dry-off (**DIMD**), days after dry-off at enrollment (**DDE**), days of gestation at enrollment (**DGE**), gestation length, twinning (yes vs. no), and calf sex (male vs. female). Interactions between dairy and lactation number, dairy and BCSC, lactation number and gestation length, and twinning and gestation length also were explored. For a second series of regression analyses, P305ME, DIMD, DDE, and DGE were transformed into categorical variables based on tertiles (**T**). These categorical variables (**P305ME T, DIMD T, DDE T, DGE T**) replaced their corresponding continuous fixed effects in the initial model. All models included all fixed effects and aforementioned interactions. Variables and interactions with P value < 0.10 were excluded from the initial model by backward elimination. Statistical significance was defined as P value < 0.05 and tendencies as $0.05 \leq P < 0.10$.

Principal component analysis was used to reduce dimensionality of CBT data. Principal components were calculated by singular value decomposition of the centered and scaled data matrix, using the prcomp function in the Stats package of R (version 3.3.0; R Core Team, 2016), and eigenvectors of the variance-covariance matrix of temperatures. Using the package stats of R, a K-means test was performed in the first 2 principal components (**PC1** and **PC2**) to verify the presence of clusters in the data. Pearson correlation coefficients (r) were calculated between single-measurements of CBT and PC1 using the Stats package of R.

Mean, maximum, minimum, and standard deviation of daily CBT were calculated for each cow. Using these measures, median values were calculated. Cows with values less than the calculated median were classified as low temperature (**LT**), and cows with values greater than or equal to the calculated median were classified as having high temperature (**HT**). Kappa Cohen reliability coefficients (κ) were calculated using the Psych package of R to evaluate agreement in classifying cows as HT or LT by using different CBT measures (e.g., single-measurement of CBT vs. mean daily CBT).

A linear model was fit using the lm function in the Stats package of R to predict CBT based on observed parameters. Fixed effects and their interactions explored in the linear regressions were tested in the prediction models. In addition to lactation number, parity (primiparous vs. multiparous) was further explored in the analyses. In order to evaluate the quality of the model, predictivity was calculated. Quality of predictions was defined by the correlation between observed and estimated average individual temperature for the testing population. Predictivity was calculated by cross validation, which was performed 500 times for each model, randomly selecting 79 animals for training the model and 25 animals for evaluating quality of predictions. Calculation of predictivity was only performed for the testing population.

RESULTS

One primiparous cow lost the temperature logger and was excluded from the study. The final study population consisted of 104 cows (n = 36, 37, 31, for first-, second-, and third-lactation cows, respectively).

Prepartum and Ambient Descriptive Data

Forty-one cows (39.4%) had singleton male calves, 54 (51.9%) had singleton female calves, and 9 cows (8.7%) bore twins. Average ± SD core body temperature was 38.83 ± 0.23 °C (Table 3.1). Previous P305ME milk yield, BCS, and gestation length were 12,976 (± 1,780) kg, 3.60 (± 0.47), and 277 (± 5.0) d, respectively. Proportion of cows born during winter, spring, summer, and fall seasons was 5.8, 15.4, 52.9, and 26.0%, respectively.

During the period of enrollment, average daily THI was 73.4 for dairy A and 74.2 for dairy B. In addition, average minimum and maximum THI were 63.9 and 81.5 for dairy A, and 65.8 and 80.6 for dairy B, respectively. Daily pattern of CBT for cows classified as having LT or HT is depicted in Figure 3.1. Further prepartum descriptive data comparing cows with LT or HT, and THI data for each replicate are reported in Scanavez et al. (2017). Average daily CBT was greater ($P < 0.01$) for HT cows than LT cows (39.01 ± 0.03 vs. 38.72 ± 0.03 °C).

Factors Associated with Core Body Temperature

In the first model, cows carrying twins had greater ($P < 0.01$) CBT compared with cows bearing singletons (39.07 ± 0.07 vs. 38.84 ± 0.03 °C). Gestation length was negatively associated ($P < 0.01$) with CBT. For each 1-d increase in gestation length, average CBT decreased 0.015 ± 0.004 °C. The interaction between twinning and gestation length was not associated ($P = 0.50$) with CBT. Cows in dairy A tended ($P = 0.09$) to have lower CBT than cows in dairy B (38.91 ± 0.04 vs. 39.00 ± 0.06 °C). Season of birth, lactation number, BCSC, P305ME, DIMD, DDE, DGE, and calf sex were not ($P > 0.12$) associated with CBT. The interactions between dairy and lactation number, dairy and BCSC, and lactation number and gestation length were not ($P > 0.12$) significant in the model.

In the second model, in which P305ME, DIMD, DDE, and DGE were transformed into categorical variables, similar findings were observed. Twinning and gestation length were associated ($P < 0.01$) and dairy tended ($P = 0.09$) to be associated with CBT. Other variables or interactions were not ($P > 0.12$) significant in the model.

Principal Component Analysis and Reliability of a Single Measurement of Core Body Temperature

The first principal component (PC1) explained 71% of the variance of CBT, and the second principal component (PC2) explained 13% (Table 3.2). Less than 17% of CBT variance was explained by the combination of all other principal components. Moreover, the K-means test showed that clustering the observations in 2 HT groups accounted for only 45.9% of the total variance in the data, denoting that no group of cows explained variance in CBT. Correlation between PC1 and mean daily CBT was 0.99. Among the 288 time-points assessed, CBT recorded at 2215 h had the highest correlation ($r = 0.93$) with PC1 (Figure 3.2).

Kappa statistics indicated substantial agreement ($k = 0.73$) between mean daily CBT and assessment at 2215 h when classifying cows into LT or HT (Table 3.3). Classification based on maximum, minimum, or standard deviation of daily CBT resulted in suboptimal agreement ($k < 0.43$) with assessment at 2215 h (Table 3.3). Agreement between mean daily CBT and single measurements across the day to classify cows into LT or HT is depicted in Figure 3.3. Among all the time-points evaluated, CBT recorded at 0715 h resulted in the poorest agreement ($k = 0.23$).

Predictive Models of Core Body Temperature

Linear models tested for predictivity of CBT are presented in Table 3.4. Variables with best predictivity of average daily CBT were gestation length and twinning (0.50 and 0.47, respectively). Body condition score category, lactation number, and parity were poor predictors of average daily CBT (predictivity < 0.21). Transforming projected milk yield and days after dry-off into categorical variables (tertiles) provided better adjustment to the models and increased predictivity by 0.07 and 0.08, respectively. Inclusion of interactions increased predictivity only marginally (data not shown). The model with best predictivity (0.56) consisted of including dairy

(P = 0.09), gestation length (P = 0.01), and twinning (P = 0.01). Estimated average daily CBT based on the model with best predictivity is illustrated in Figure 3.4.

DISCUSSION

Besides having a major economic impact to the dairy industry (St-Pierre et al., 2003), heat stress considerably affects well-being of dairy cattle (Polsky and von Keyserlingk, 2017). Hyperthermia caused by high ambient temperature induces metabolic imbalances, predisposing cows to health disorders. Considering that postpartum cows are susceptible to occurrence of diseases, research regarding the effects of heat stress on the periparturient cow is fundamental to improve animal welfare and health during summer. In a recent study conducted by our research group, we demonstrated an association between CBT during the dry period and postpartum health in the subsequent lactation (Scanavez et al., 2017). Nevertheless, it is unknown which factors are associated with CBT during the dry period. Identifying some of these factors may increase our understanding of underlying processes involved in temperature regulation of periparturient cows during periods of heat stress. In addition, determining which factors are associated with CBT may contribute to the development of strategies to manage subpopulations of cows more prone to be affected by heat stress.

Various methods have been used to evaluate magnitude of heat stress in dry cows. The most common methods consist of assessing CBT rectally (do Amaral et al., 2011; Karimi et al., 2015) or vaginally (Chen et al., 2016; Scanavez et al., 2017). Although rectal and vaginal temperatures are highly correlated (Suthar et al., 2013), vaginal temperature assessments enable monitoring of CBT in multiple time points across the day (e.g., one measurement per minute). In fact, one of the objectives of the current study was to investigate the ideal time of the day to assess CBT in dry cows. Moreover, the authors explored other potential animal factors associated with

CBT during the dry period. In order to increase its external validity, the present study was conducted at large commercial dairies, which prevented assessment of certain animal factors of interest, such as body weight and dry matter intake.

We hypothesized that CBT would be associated with lactation number because previous experiments have shown differences in CBT between lactating primiparous and multiparous cows under conditions of heat stress. In studies conducted with early-lactation Holstein cows, it was reported that primiparous cows have greater temperature than multiparous cows (Suthar et al., 2011; Wenz et al., 2011). Wenz et al. (2011), however, did not exclude cows with metabolic and infectious disorders, limiting major conclusions because health disorders may affect CBT. Nonetheless, Suthar et al. (2011) reported similar findings in an experiment that excluded cows diagnosed with clinical disorders. Even though several studies recorded CBT in mid-lactation cows, comparisons between primiparous and multiparous CBT have not been described in the literature. One could speculate that mid-lactation multiparous cows likely have greater CBT than primiparous cows because of their increased feed intake and milk yield, but studies are necessary to test this hypothesis. In a study conducted with beef cows focusing on predicting time of parturition based on CBT (Aoki et al., 2005), no differences were observed between parities. Aoki et al. (2005), however, did not include primiparous cows in their experiment. Our results indicate that CBT does not differ between primiparous and multiparous cows in a non-lactating state under conditions of heat stress. To our knowledge, no other studies have evaluated whether lactation number is associated with CBT during the dry period. Understanding whether number of lactation is associated with CBT of dry cows is critical to avoid bias when designing and interpreting research experiments and field trials.

Similar to lactation number, body condition score was not associated with CBT of dry cows. Because dairy cows with high BCS have increased caloric demands for maintenance (NRC, 2001), we hypothesized that BCS would influence thermoregulation. Indeed, in humans, increased body mass index is associated with increased body temperature (Obermeyer et al., 2017). It is possible that different DMI preceding parturition of high- and low-BCS cows influenced CBT. Cows with greater BCS have more pronounced decrease in DMI before calving (Hayirli et al., 2002; Grummer et al., 2004), which may have resulted in similar CBT between high- and low-BCS cows via reduced caloric intake.

Recent studies showed evidence that season of conception may be linked to thermal homeostasis and productive and reproductive performance during adulthood in dairy cows (Pinedo and de Vries, 2017; Ahmed et al., 2017). Calves that experienced in utero heat stress during the last 6 weeks of gestation had better capacity to thermoregulate at maturity (Ahmed et al., 2017). In addition, cows exposed to heat stress in utero presented reduced CBT in response to a heat stress challenge compared with cows that did not experience heat stress in utero. Based on these findings, we investigated the association between season of cow's birth and CBT. We classified cows based on season of birth as a proxy for heat stress exposure in utero. Our results contrast with previous findings because there was no association between season of birth and CBT. Considering that cows not exposed to heat stress in utero are more likely to complete first lactation (Monteiro et al., 2016), the authors recognize the possibility of selection bias, given the enrollment criteria used in the present study.

Herds used in this study were selected based on similar characteristics, such as breed, milk yield, housing system for dry cows, and geographic proximity. Despite a careful herd selection, dairy tended to be associated with CBT. Given that heat tolerance has a genetic component (Garner et al., 2016), differences in genetics may be the reason for cows from Dairy B to have greater CBT than cows from Dairy A. Regardless of geographic proximity of herds, differences in exposure to heat stress should not be neglected. In the previous experiment (Scanavez et al., 2017), no difference in CBT was detected between Dairy A and Dairy B. It is likely that classifying cows as having high or low CBT reduced the statistical power of the analysis. In the present study, CBT was included as a continuous variable in the analyses, which increased statistical power to detect differences.

Our results indicate that CBT is negatively associated with gestation length. Unfortunately, causality cannot be established with this dataset. It is intriguing to speculate whether CBT affects gestation length, or gestation length alters CBT. It is well-documented that CBT of beef and dairy cows is reduced in the last 24 to 48 h before parturition (Lammoglia et al., 1997; Aoki et al., 2005; Burfeind et al., 2011). Nevertheless, little is known about circadian rhythm of body temperature 2 to 3 wk before calving. Calf sex, which is associated with gestation length, was another factor explored in this study to investigate whether it influences CBT. Although gestation length of cows pregnant with female calves are approximately 1 d shorter than cows pregnant with male calves (Silva et al., 1992; Vieira-Neto et al., 2017), calf sex was not associated with CBT. In agreement with our findings, Aoki et al. (2005) did not detect differences in CBT between cows bearing male or female calves during the last 6 d of gestation. In contrast, Lammoglia et al. (1997) reported that CBT was approximately 0.3°C greater from 2 to 6 d before parturition for cows carrying male calves compared with cows carrying female calves. Nevertheless, a type II error should be considered in the latter study because only 7 cows were used in the experiment. Twinning is another risk factor for shorter gestation length. Occurrence of twinning reduces gestation length in beef and dairy cows (Echternkamp and Gregory, 1999; Vieira-Neto et al., 2017). In the current experiment, cows carrying twins had greater CBT than cows calving singletons. Based on evidence that pregnant ewes have greater CBT compared with nonpregnant ewes (Godfrey et al., 2017), we speculate that greater uterine blood flow required to support the additional calf results in increased heat production, and consequently, greater CBT. Conversely, Aoki et al. (2005) did not detect differences in CBT between cows carrying twins or singletons. In that study, however, CBT was monitored during the last week of gestation, which differs from the current study.

We investigated whether time of day at CBT assessment reflects the variation in the circadian rhythm of body temperature. Understanding the daily oscillation of CBT of dry Holstein cows is of great importance because researchers commonly use CBT assessments to determine efficiency of cooling strategies (do Amaral et al., 2009; Karimi et al., 2015). In previous experiments, CBT of dry cows was assessed in the morning, afternoon, or both. Despite the practical aspect of assessing CBT once or twice daily in commercial settings, limited assessments may lead to inaccurate representation of exposure to heat stress throughout the day because of circadian patterns of CBT (Scanavez et al., 2016). Among all CBT assessments across the day, measurements recorded at 2215 h resulted in the greatest correlation with PC1. This indicates that CBT recorded at 2215 h is a fairly precise proxy to assess variance in CBT during the entire day. In addition, our findings revealed a poor correlation between PC1 and CBT assessed early-morning or mid-afternoon. Therefore, estimating severity of heat stress to which dry cows are exposed throughout the day by recording CBT in the morning or afternoon may result in misleading conclusions. In fact, in studies that compared heat abatement systems, CBT differed between treatments in the afternoon, but not in the morning (do Amaral et al., 2009; Tao et al., 2012). This reinforces the importance of our findings, indicating that CBT recorded at these times (e.g., morning and afternoon) leads to conflicting results, and potentially, equivocal interpretation. The authors of the present study recognize the difficulty in implementing CBT monitoring programs at 2215 h in commercial dairies. For research purposes, however, recording CBT at this time of the day should be considered. These findings are of extreme importance for appropriate design and interpretation of future research projects that involve heat-stressed cows, and particularly for studies aiming to evaluate efficacy of heat abatement systems.

A secondary objective of the present study was to develop and test accuracy estimates of statistical models to predict CBT of dry Holstein cows under heat stress. These models were developed using training and validation datasets. Accuracy of the models were evaluated by comparing actual CBT values from the original dataset with values obtained from the statistical models. A similar approach has been used to evaluate models to predict carcass traits in beef cattle (Soulat et al., 2016) and methane emission levels in dairy cattle (Castro-Montoya et al., 2016). Interestingly, variables in the model with best predictivity were the same variables retained in the reduced regression analysis that evaluated factors associated with CBT. Dairy tended to influence CBT, while gestation length and twinning had a significant effect on predictivity of CBT.

In conclusion, the present study demonstrated that CBT assessed between 250 and 260 days of gestation is greater for cows carrying twins and is negatively associated with gestation length. In addition, findings from this study reveals that 2215 h is the most appropriate time of the day to evaluate severity of heat stress of dry cows during warm months. Lastly, CBT of dry cows can be estimated using statistical models that include dairy, twinning, and gestation length of dry Holstein cows. These findings are of utmost importance for the development of strategies focused on improving overall performance of subpopulations of cows affected by heat stress, which may result in reduced economic losses and improved animal well-being in dairy operations.

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Table 3.1. Descriptive data of non-lactating Holstein cows (n = 104) used in the study.

Item	Average (SD)
Core body temperature, ºC	38.83 (0.23)
Lactation number at enrollment	1.95 (0.81)
Previous projected 305-d mature equivalent milk yield, kg	12,976 (1,780)
Body condition score[1]	3.60 (0.47)
Days after calving at dry-off	325.6 (48.2)
Days after dry-off at enrollment	31.4 (11.1)
Days of gestation at enrollment	254.0 (2.8)
Gestation length, d	277.0 (5.0)

[1] Body condition score was assessed by 1 person on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) using 0.25-point increments.

Table 3.2. Principal component (PC) analysis coefficients, standard deviation of PC, and cumulative proportion of variance from PC1 to PC8 of core body temperature.

Item	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of variance	0.71	0.13	0.04	0.02	0.01	0.01	0.01	0.01
Cumulative proportion of variance	0.71	0.84	0.88	0.90	0.92	0.93	0.94	0.94
Standard deviation	14.29	6.15	3.57	2.20	2.07	1.75	1.59	1.48

Table 3.3. Cohen's Kappa reliability coefficients for classifying non-lactating Holstein cows into low- or high-core body temperature (CBT) using measurements at 2215 h and mean, maximum, minimum, or standard deviation of daily CBT.

Item	Kappa
Mean daily CBT	0.73
Maximum daily CBT	0.42
Minimum daily CBT	0.27
Standard deviation of daily CBT	0.23

Table 3.4. Predictivity mean and standard deviation of models to predict average core body temperature over 500 cross validation samples.

Model[1]	Predictivity	Standard deviation
Dairy	0.26	0.19
Lactation number	0.14	0.14
Parity	0.21	0.19
BCSC	-0.11	0.13
P305ME	0.09	0.20
P305ME T	0.16	0.17
DIMD	0.07	0.13
DIMD T	-0.17	0.14
DDE	0.15	0.15
DDE T	0.23	0.19
DGE	-0.10	0.15
DGE T	-0.05	0.16
Gestation length	0.50	0.12
Twins	0.47	0.20
Calf sex	0.18	0.18
Season	0.14	0.16
Dairy + parity	0.31	0.20
Parity + gestation length	0.48	0.13
Dairy + parity + gestation length	0.51	0.12
Dairy + parity + DIMD + gestation length	0.51	0.12
Gestation length + P305ME T + DDE T	0.52	0.12
Gestation length + DDE T	0.52	0.11
Dairy + gestation length + twins	0.56	0.14

[1] Variables tested: dairy, lactation number, parity, body condition score category[2] (BCSC), previous projected mature milk yield (P305ME), days in milk at dry-off (DIMD), days after dry-off at enrollment (DDE), days of gestation at enrollment (DGE), gestation length, twin pregnancy (twins), calf sex, and cow's season of birth (season). Continuous variables not significant in the linear regression model (P305ME, DIMD, DDE, DGE) were transformed into categorical variables based on tertiles (T).

[2] Body condition score was assessed by 1 person on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) using 0.25-point increments. Cows were classified into low BCS (LBCS; ≤ 3.5) or high BCS (HBCS; > 3.5).

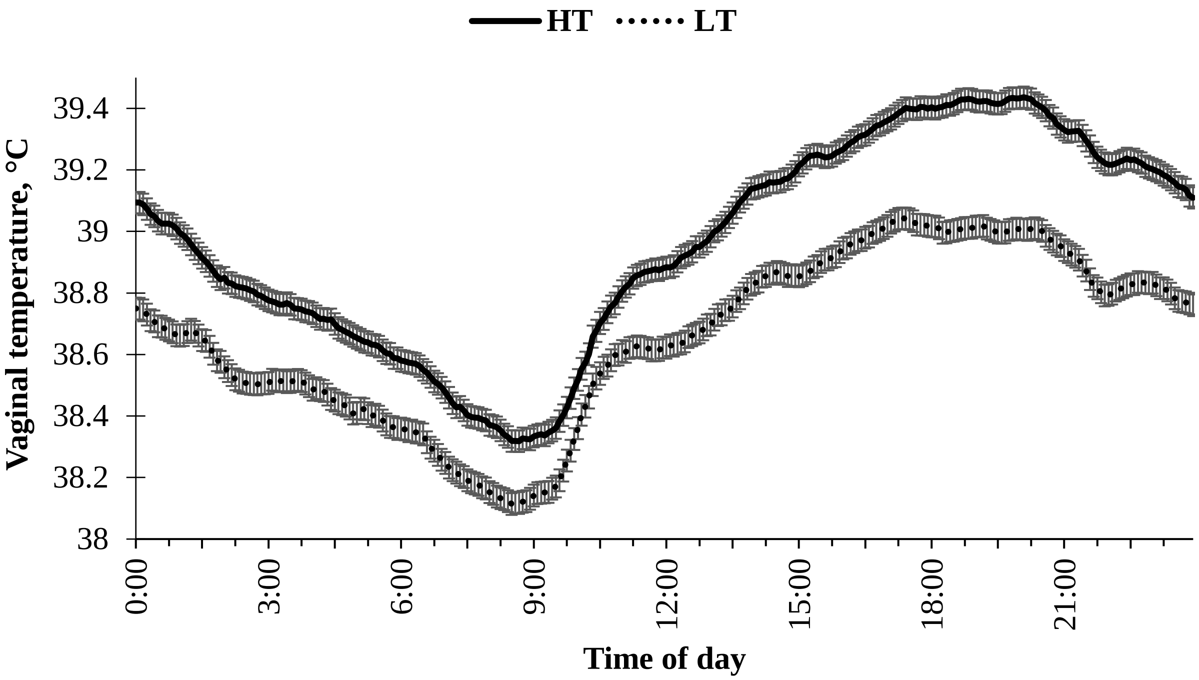

Figure 3.1. Pattern of core body temperature (CBT) measured in 5-min intervals during 4 consecutive days in 104 non-lactating dairy cows.

Error bars represent SEM. Lines represent cows with average CBT greater than (solid line – HT) or less (dotted line – LT) than the median value within parity. Average daily CBT was greater (*P* < 0.01) for HT cows than LT cows (39.01 ± 0.03 vs. 38.72 ± 0.03 °C).

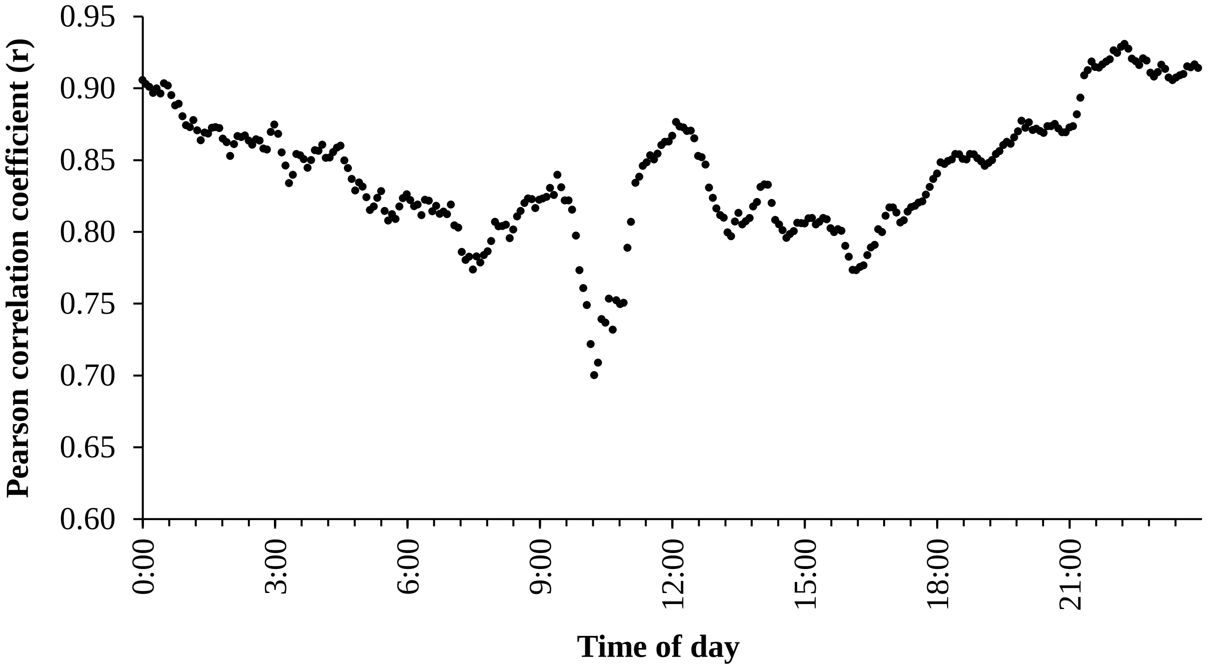

Figure 3.2. Pearson correlation coefficients (r) between single-measurements of core body temperature (CBT) and the principal component that explained the greatest proportion of the variance in CBT (PC1) of non-lactating Holstein cows.

Among the 288 single-measurements, CBT assessed at 2215 h had the highest correlation with PC1 (r = 0.93).

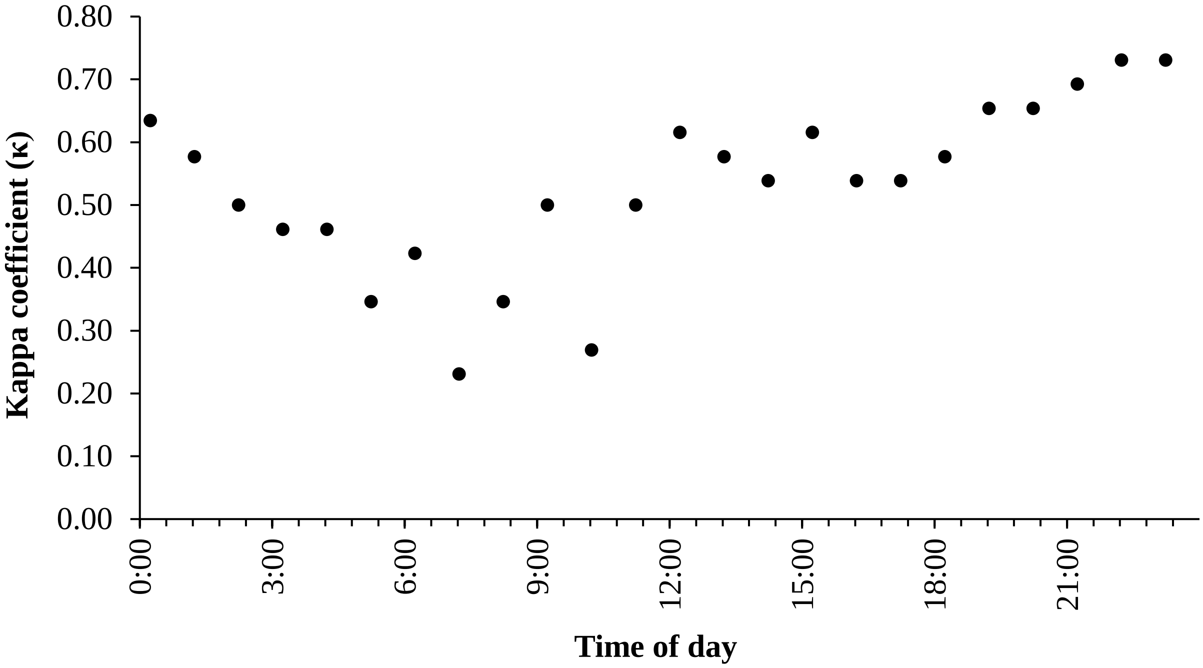

Figure 3.3. Agreement between using a single measurement of core body temperature (CBT) and mean daily CBT for classifying cows into low- or high-temperature groups.

Greatest and poorest agreement between single-measurements and mean daily CBT were at 2215 h (κ = 0.73) and 0715 h (κ = 0.23), respectively. Kappa coefficient agreement (Landis and Koch, 1977): < 0.00 = poor, 0.00 – 0.20 = slight, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial, and 0.80 – 1.00 = almost perfect agreement.

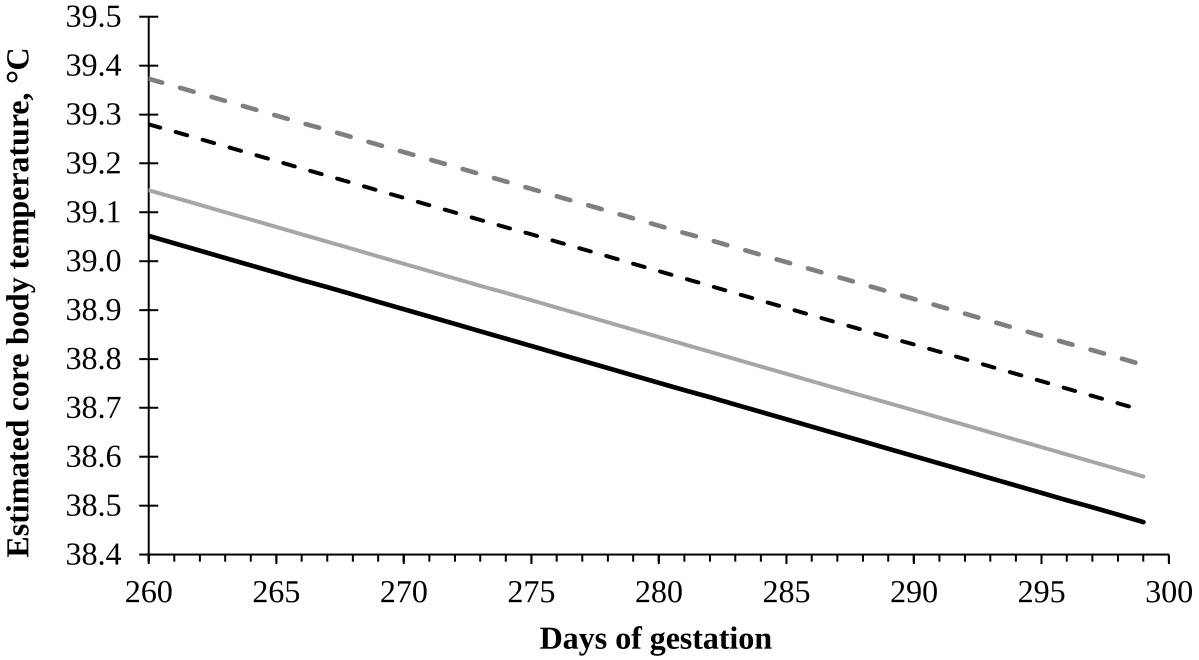

Figure 3.4. Estimated average daily core body temperature based on the final predictivity model.

Fixed effects included in the model were: dairy (*P* = 0.09), gestation length (*P* < 0.01), and pregnancy type (*P* < 0.01). Black lines represent cows from Dairy A carrying singleton (solid line) or twin (dashed line) pregnancies. Gray lines represent cows from dairy B carrying singleton (solid line) or twin (dashed line) pregnancies.

Chapter 4 - Physiological, Health, Lactation and Reproductive Traits of Cooled Dairy Cows Classified as Having High or Low Core Body Temperature during the Dry Period

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ABSTRACT

Primary objectives of this study were to compare concentrations of pregnancy-associated glycoprotein (PAG) before calving, prolactin (PRL) after calving, and energy balance indicators before and after calving in cooled cows classified as having high (HT) or low (LT) core body temperature (CBT) during the dry period. Secondary objectives were to investigate associations between dry-period CBT and likelihood of cows developing health disorders, and compare health, milk production and reproductive traits of HT and LT cows. Dry Holstein cows (n = 260) at 250 to 260 days of gestation from 3 herds were enrolled in the study during summer. Cows were provided evaporative cooling during the dry and lactating period. Vaginal temperature was recorded in 5-min intervals during 7 consecutive days and cows were classified as HT or LT. Blood samples were collected weekly from enrollment until 14 ± 3 days in milk (DIM). Additional blood samples were collected by 12 h postpartum from a subgroup of cows (n = 25) to determine PRL concentration. Cows were monitored for health disorders, milk production, and reproductive performance until 13 wk of the subsequent lactation. High temperature cows had ($P < 0.01$) shorter gestation length (273.9 ± 0.9 vs. 278.2 ± 0.9 d) and greater ($P < 0.01$) incidence of twinning (19.7 vs. 4.2%) than LT cows. Cows classified as HT had ($P = 0.02$) greater PAG concentration (134.1 ± 4.9 vs. 117.4 ± 4.9 ng/mL), but postpartum PRL concentration did not ($P = 0.55$) differ between HT and LT cows. Primiparous HT cows had ($P = 0.05$) greater prepartum nonesterified fatty acids concentration (135, 95% CI = 102 to 178 vs. 104, 95% CI = 75 to 144 mmol/dL) than primiparous LT cows, but no differences ($P = 0.72$) were observed between CBT group in multiparous cows. Concentration of β-hydroxybutyrate was ($P = 0.04$) greater for LT compared with HT cows at 7 ± 3 DIM. The quadratic effect of CBT tended ($P = 0.09$) to be associated with risk of health disorders by 60 DIM. Milk yield tended ($P = 0.10$) to be greater for LT compared with HT cows (49.3 ± 1.9

vs. 46.2 ± 1.6 kg). Pregnancy per AI at first service did not differ ($P = 0.64$) between HT and LT cows. In conclusion, HT compared with LT cows have distinctly different concentrations of PAG during late gestation and energy balance indicators during the transition period. In addition, CBT assessment during the dry period may be a useful tool to identify cows expected to have impaired health and milk yield in the subsequent lactation.

Key words: dairy cow, heat stress, late gestation, postpartum health

INTRODUCTION

Exposure to heat stress during the dry period has deleterious effects on subsequent milk yield of dairy cows (Tao and Dahl, 2013). It has been demonstrated that cooling heat-stressed dry cows reduces core body temperature (**CBT**) and improves performance during the subsequent lactation (Karimi et al., 2015). Limited data exist, however, regarding the association between CBT during the dry period and overall performance in the subsequent lactation when all cows are managed under similar conditions. Scanavez et al. (2017; 2018) reported that, when non-lactating cows are managed under similar environmental conditions, cows with high CBT have shorter gestation lengths, greater incidence of twinning, increased risk of developing health disorders, and reduced early postpartum milk yield than cows with low CBT. In the aforementioned studies, dry cows had access to shade, but no active cooling system was used. Considering the differences in performance of cows with dissimilar CBTs described by Scanavez et al. (2017; 2018), it is likely that alterations in physiological traits were also present. Physiologic traits such as blood concentrations of pregnancy-associated glycoproteins (**PAG**), prolactin (**PRL**), nonesterified fatty acids (**NEFA**) and β-hydroxybutyrate (**BHB**) have been demonstrated to differ between cows exposed or not to heat stress (do Amaral et al., 2009; Serrano et al., 2009; Tao et al., 2012). No studies, however, investigated metabolic and hormonal characteristics of periparturient cows with various CBT while managed under similar environmental conditions.

We hypothesized that cooled dairy cows with greater CBT during the dry period would have: (1) altered concentrations of PAG, PRL, NEFA, and BHB during the transition period; (2) greater incidence of health disorders; and (3) reduced milk production and reproductive performance compared with cows with lower CBT. Our primary objectives were to compare concentrations of PAG before calving, PRL concentration after calving, and concentration of energy balance indicators (NEFA and BHB) before and after calving in cooled cows classified as having high or low CBT during the dry period. Our secondary objectives were to evaluate the association between dry-period CBT and likelihood of cows developing health disorders and compare health, milk, and reproductive traits of cooled dairy cows classified as high or low CBT during the dry period.

MATERIALS AND METHODS

The procedures described herein were approved by the Kansas State University Institutional Animal Care and Use Committee.

Cows and Housing

The experiment was conducted in 3 dairy herds located in northeast Kansas from June to December 2017. Dry Holstein cows at 250 to 260 days of gestation, with 4 functional quarters, locomotion score < 3 (Sprecher et al., 1997), and without clinical disorders were included in the study. Absence of clinical signs of health disorders was determined by a trained veterinarian upon enrollment. A total of 260 cows were enrolled from June to August 2017, with 111 primiparous and 149 multiparous cows (second lactation = 106; third lactation = 43). Cows were enrolled in 5 replicates (weekly cohorts) in dairy A (n = 29), and 6 replicates in dairies B (n = 105) and C (n =

126). To ensure cows were exposed to similar environmental conditions across replicates, weather forecast of maximum temperature > 32°C during the week of enrollment was used as an enrollment criterion. Body condition score (**BCS**) was assessed at enrollment, 3 ± 3 and 14 ± 3 DIM, and recorded on a 1 (emaciated) to 5 (obese) scale with 0.25-point increments (Ferguson et al., 1994). At enrollment, cows were in the close-up pen. Cows were moved from the far-off to the close-up pen between 242 to 256 days of gestation. After enrollment, cows were followed until 13 wk of the subsequent lactation and monitored for health disorders, milk production, and reproductive performance. For cows that were sold or died before 13 wk of lactation, date of removal from the herd was recorded.

In dairy A, close-up cows were housed in a straw-based bedded pack barn with exhaust fans and evaporative cooling pads on the opposite side of the feed bunk. After calving, cows were kept in 2-row free-stalls barns equipped with fans over the stalls and sprinklers over the feed alley. In dairy B, close-up and lactating cows were housed in 2-row free-stall barns and cows were cooled with fans over the stalls and sprinklers over the feedline. Dairy C housed close-up cows in a 4-row free-stall equipped with fans and sprinklers over the feedline, whereas lactating cows were kept in a 2-row cross-ventilated barn. The 3 dairies used sand as bedding for lactating cows, cooling systems in the holding pen (dairy A = cross-ventilated system; dairies B and C = sprinklers and fans), and cows were milked thrice daily. Cows were fed total mixed rations formulated to meet or exceed nutrient requirements (NRC, 2001) for each stage of gestation (e.g., far-off and close-up) and lactation. Diets of close-up cows contained anionic salts.

Core Body Temperature and Ambient Temperature-Humidity Index

Upon enrollment, a calibrated temperature logger (iButton DS1922L, Embedded Data Systems, Lawrenceburg, KY) attached to a blank controlled internal drug release (**CIDR**) insert was placed in the vagina to record CBT. Temperature loggers were calibrated for precision of ± 0.13 °C (Thermodata Corporation, Milwaukee, WI) and were secured in the CIDR insert using silicone aquarium sealant (Loctite®, Henkel Corporation, Rocky Hill, CT). Temperature loggers were programmed to record CBT in 5-min intervals and were removed from cows after 7 d. After loggers were removed, recorded temperature data were downloaded to a computer and average CBT was calculated for each cow as described by Scanavez et al. (2017). Briefly, within dairy and parity group (primiparous and multiparous cows), median values of average CBT were calculated. Cows with CBT greater than the median value for their corresponding dairy and parity group were classified as having high temperature (**HT**), whereas cows with CBT below the median value were classified as having low temperature (**LT**).

Ambient temperature and humidity were recorded in the close-up pen using temperature loggers (HOBO U23 Pro v2, Onset Computer Corp., Pocasset, MA) located 3 m above the ground level. Measurements were recorded in 5-min intervals programmed to match the same time-points when CBT were being recorded. Data were downloaded to a computer and used to calculate temperature-humidity index (**THI**) with the equation: $THI = T - \left(0.55 - 0.55\frac{RH}{100}\right) X\ (T - 58)$, where T and RH are dry-bulb temperature (ºF) and relative humidity, respectively (NOAA, 1976).

Blood Sampling and Analyses

Blood samples were obtained by puncture of the median caudal vein or artery into 10-mL evacuated EDTA-coated tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were collected weekly from the day of enrollment until calving, and at 7 ± 3 and 14 ± 3 DIM. In dairy A, additional samples were collected by 12 h after calving for plasma and serum extraction. Samples used for serum separation were collected in 10-mL evacuated tubes with no additives (BD Vacutainer, Franklin Lakes, NJ). After collection, blood samples were placed in an insulated cooler with ice and transported to a laboratory. Samples were centrifuged at 1,200 x *g* for 15 min, plasma or serum was separated, transferred into 2 mL microcentrifuge tubes (Microstein, Midsci, St. Louis, MO) and stored in duplicates at -20°C until further analyses.

Concentrations of PAG were determined from plasma samples collected at 21 ± 3, 14 ± 3, 7 ± 3 and the last 3 days before calving by using a sandwich ELISA that was performed in a similar manner as reported elsewhere (Green et al., 2005). Briefly, the assay consisted of a combination of three anti-PAG monoclonal antibodies that served to immobilize PAGs. The monoclonal-PAG complex was detected by using an anti-PAG rabbit polyclonal antibody (ab63) and a goat anti-rabbit alkaline-phosphatase conjugate (Jackson ImmunoResearch, West Grove, PA). Product formation was measured at 405 nm on a Bio-Tek El808 plate reader. Each assay included a standard curve and a pooled sample from pregnant cows collected at d 60 of gestation. Intra- and inter-assay coefficients of variation were 6.2 and 10.5%, respectively.

Concentration of NEFA was quantified from plasma samples collected from d -21 to 14 using a colorimetric assay (Wako NEFA, Wako Diagnostics, Mountain View, CA). Intra- and inter-assay coefficient of variation were 2.82 and 6.02%, respectively. Plasma BHB concentrations were determined enzymatically (Pointe Scientific Inc., Canton, MI) from samples collected after calving. Intra- and inter-assay coefficients of variation were 2.52 and 2.98%, respectively. Additional samples collected at dairy A (by 12 h after calving; n = 25) also were analyzed for NEFA and BHB concentration.

Serum prolactin concentration was assessed in samples (n = 25) collected by 12 h after calving by RIA as previously described by Bernard et al. (1993). Intra- and inter-assay coefficients of variation were 5.03 and 4.56%, respectively. Samples were determined in duplicate in all assays.

Calving and Definition of Health Disorders

Information regarding date of calving, calf sex, twinning and stillbirth were recorded by trained farm personnel. These data were extracted from the on-farm herd management software (Dairies A and B: PCDart, DRMS, Raleigh, NC; Dairy C: DairyComp, Valley Ag Software, Tulare, CA). Singleton live calves born to cows enrolled in the study at dairies A and B were weighed immediately after birth using calibrated scales.

Health disorders of interest for this study were retained fetal membranes (**RFM**), metritis (**MTR**), mastitis (**MAST60**), and displaced abomasum (**DA**). Failure to release fetal membranes more than 24 h after calving was considered RFM. Metritis was characterized by cows presenting watery and foul-smelling vaginal discharge at per rectum palpation of the reproductive tract. A trained veterinarian from our research team examined cows for metritis at 7 ± 3 (**MTR7**) and 14 ± 3 (**MTR14**) days after calving. For further statistical analyses, cows that presented either RFM or MTR were considered to have uterine disease (**UDX**). Lactating cows presenting abnormal milk, with or without clots or swelling of the udder at any milking by 60 DIM were diagnosed as having MAST60. Lactating cows with signs of inappetence, dehydration, and reduced rumination were auscultated by a trained farm personnel using a stethoscope on the left and right flanks. During this examination, cows presenting a high-pitched metallic sound were considered a case of displaced abomasum (**DA**). Cows presenting one or more cases of the aforementioned health problems during the first 60 DIM were classified as having a health disorder (**DX60**).

Milk Yield and Culling Data

Individual milk yield was measured at each milking and recorded automatically at all 3 dairies. Average weekly milk yield was calculated for each cow by adding milk production from 7 consecutive days (from 1 to 92 DIM) and dividing by 7, totaling 13 wk.

Information of cows culled before 60 DIM (**CULL60**) were extracted from the on-farm management software. Milk yield from culled cows was included in the statistical analyses until the last week before culling.

Reproductive Management for First Insemination

Cows in dairies A and C were submitted to an ovulation synchronization protocol for first AI and were inseminated at a fixed timed at 73 ± 3 and 68 ± 3 DIM, respectively. Cows in dairy B were inseminated based on tail paint removal after 60 DIM. Cows were submitted to a presynchronization program (2 prostaglandin treatments given 14 d apart) starting at 46 ± 3 DIM. Estrus detection was performed daily during the morning based on tail paint removal. Cows not inseminated by 72 ± 3 DIM were enrolled in the Cosynch-72 timed AI protocol, and first AI occurred at 82 ± 3 DIM. Pregnancy outcome was determined by transrectal ultrasonography at 33 ± 3 (dairy A) or 35 ± 3 (dairies B and C) days after AI. Cows were considered pregnant if an embryo with a heartbeat was visualized upon evaluation of the uterine contents. Pregnancy per AI (**P/AI**) at first service was calculated by dividing the number of pregnancies confirmed after first AI by the number of inseminations performed.

Statistical Analyses

Normality of continuous data was assessed using PROC UNIVARIATE procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Core body temperature, days dry (**DDRY**), days spent in the close-up pen (**DINCU**), milk yield, days of gestation at enrollment (**DGE**), gestation length (**GL**), calf weight, and PAG concentrations were normally distributed. Days in milk at dry-off (**DIMD**) and days in milk at enrollment (**DIME**) presented a skewed distribution and were rank transformed using the PROC RANK procedure of SAS before further analyses. Likewise, distribution of NEFA and BHB residuals were non-normal, and their values were log-transformed before further analyses were conducted. Body condition score change (**BCSC**) during the dry period was calculated by subtracting BCS assessed after calving (3 ± 3 DIM) from BCS assessed at enrollment. Cows were categorized as having lost (**LBCS**), or maintained or gained BCS (**MGBCS**) during the dry period.

Dichotomous variables were analyzed using the PROC GLIMMIX procedure of SAS with binomial distribution and logit link. Continuous variables were evaluated by ANOVA using PROC GLIMMIX and PROC MEANS procedures, and repeated measures were analyzed with the MIXED procedure of SAS. The base model included parity, BCSC, pregnancy type (singleton vs. twins), CBT group, and the interaction between CBT group and pregnancy type as fixed effects. Dairy was included in the model as a random effect. In the repeated measures analyses, the following variables were also included: time (day or week), interactions between CBT group and time, CBT group and parity, time and parity, CBT group and pregnancy type, and the three-way interaction between CBT group, time, and parity. Time was nested within cow and dairy. Covariance structures (unstructured, compound symmetry, heterogeneous compound symmetry, first-order ante dependent, and first-order autoregressive) were tested and selected according to the temporal distance between data points and lowest value for Akaike Information Criteria. Nonesterified fatty acids data was analyzed separately for pre- and postpartum periods. Number of cases of health disorders during the first 60 DIM was analyzed using PROC GENMOD with Poisson distribution and log link. Variables forced to remain in models were CBT group and, when applicable, the interaction between CBT group and time.

To further investigate the associations between CBT and health disorders, and CBT and culling, additional analyses were conducted using CBT as a continuous variable. For these analyses, multivariable linear regressions were performed using the PROC GLIMIX procedure of

SAS, and the interaction between CBT and parity and the quadratic effect of CBT were added to the base model. Backward elimination was used to remove variables and interactions with *P* values > 0.10 from the initial models. Core body temperature was forced to remain in all models. Statistical significance was defined as *P* value $\leq$ 0.05 and tendencies as 0.05 < *P* $\leq$ 0.10.

RESULTS

A total of 19 cows were removed from the study (dairy A = 4; dairy B = 7; dairy C = 8). Reasons for exclusion were loss of temperature logger (n = 8) and early calving (1 wk after enrollment [n = 7]) when CBT was still being monitored. In addition, 1 cow was diagnosed not pregnant in the close-up pen 5 wk after enrollment and 3 cows were enrolled in another research study on the day of calving that could have affected milk yield. The final study population was comprised of 241 cows (dairy A = 25; dairy B = 98; dairy C = 118).

Temperature-Humidity Index and Overall Descriptive Data of Core Body Temperature Groups

During the period of enrollment, average daily THI for dairies A, B, and C were 74.2, 75.6, and 75.9, respectively. In addition, average daily minimum and maximum THI were 68.0 and 80.1 (dairy A), 68.9 and 81.3 (dairy B), and 69.6 and 81.9 (dairy C).

Core body temperature did not differ (*P* = 0.50) among herds A, B, and C, and were 38.96 ± 0.04, 39.01 ± 0.02, and 39.01 ± 0.02°C, respectively. Average CBT did not differ (*P* = 0.59) between primiparous (39.00 ± 0.02°C) and multiparous (39.01 ± 0.02°C) cows. Median values used to classify primiparous and multiparous cows into HT or LT were 39.03 and 38.90°C (dairy A), 38.99 and 38.99°C (dairy B), 39.02 and 38.99°C (dairy C), respectively.

Core body temperature was (*P* < 0.01) greater for HT than LT cows (39.15 ± 0.01 vs. 38.85 ± 0.01°C; Table 4.1). Lactation number at enrollment, previous projected 305-d mature equivalent milk yield, BCS at enrollment, and days after calving at dry-off and at enrollment did not differ between HT and LT cows ($P > 0.15$).

Proportion of singleton male (49.5%) and female (50.5%) calves born to HT and LT cows did not ($P = 0.33$) differ. Similarly, birth weight of singleton calves did not ($P = 0.52$) differ between HT and LT cows (Table 4.1), but incidence of twinning was ($P < 0.01$) greater for HT (19.7%) compared with LT cows (4.2%). Core body temperature group was not ($P > 0.24$) associated with BCSC, and BCS at 3 ± 3 and 14 ± 3 DIM.

Blood Concentration of Pregnancy-Associated Glycoproteins and Prolactin

Cows classified as having HT had ($P = 0.02$) greater plasma concentration of PAG than LT cows (Figure 4.1). In addition, PAG concentrations tended ($P = 0.07$) to be greater for primiparous than multiparous cows. Pregnancy type, BCSC, and interactions between CBT group and parity, CBT group and day, parity and day, and the three-way interaction among CBT group, day, and parity were not ($P > 0.12$) associated with PAG concentration in plasma.

Prolactin concentration by 12 h after calving did not ($P = 0.55$) differ between HT (45.1 ± 4.3 ng/mL) and LT cows (41.3 ± 4.5 ng/mL). Pregnancy type, BCSC, parity, and the interaction between parity and CBT group did not ($P > 0.30$) affect prolactin concentration.

Energy Balance Indicators

Prepartum Indicators. The three-way interaction of CBT group, parity, and day tended ($P = 0.09$) to affect prepartum NEFA concentration. In addition, a tendency ($P = 0.07$) was detected for an interaction between CBT group and parity. Such interaction occurred because primiparous HT cows had ($P = 0.05$) greater NEFA concentration than primiparous LT cows (135, 95% CI = 102 to 178 vs. 104, 95% CI = 75 to 144 mmol/dL; Figure 4.2), whereas such a difference ($P = 0.72$) was not observed in multiparous cows (Figure 4.3). The interaction between CBT group and pregnancy type affected (*P* = 0.03) NEFA concentration. For cows bearing twins, NEFA concentration tended (*P* = 0.06) to be greater for HT compared with LT cows (180, 95% CI = 133 to 242 vs. 122, 95% CI = 79 to 189 mmol/dL), whereas NEFA concentrations did not (*P* = 0.17) differ between CBT group for cows bearing singletons. In addition, concentration of NEFA was (*P* < 0.01) greater for LBCS compared with MGBCS cows.

Postpartum Indicators. Plasma concentration of NEFA after calving was not (*P* > 0.22) associated with CBT group or its interaction with day. The interaction between CBT group and parity affected (*P* < 0.01) NEFA concentrations because multiparous LT cows had (*P* = 0.01) greater concentration than multiparous HT cows (544, 95% CI = 336 to 881 vs. 409, 95% CI = 255 to 657 mmol/dL). Cows carrying twins tended (*P* = 0.06) to have greater NEFA concentration than cows carrying singletons. Body condition score change before calving, and the interactions between parity and day, and CBT group, parity, and day did not (*P* > 0.21) affect NEFA concentrations after calving.

Concentration of BHB was affected (*P* = 0.05) by the interaction between CBT group and day (Figure 4.4). In addition, the interaction between CBT group and pregnancy type tended (*P* = 0.10) to influence BHB concentration. In cows delivering singletons, BHB were 709 μmol/L (95% CI = 649 to 776) and 644 μmol/L (95% CI = 591 to 701) for HT and LT cows, respectively. Conversely, in cows delivering twins, BHB concentrations for HT and LT were 637 μmol/L (95% CI = 551 to 738) and 766 μmol/L (95% CI = 568 to 1034), respectively. Concentration of BHB was (*P* = 0.05) greater for LBCS cows than MGBCS cows.

Health Disorders

Core body temperature group was not associated (*P* > 0.10) with RFM, MTR7, MTR14, MTR, UDX, MAST60, DA, DX60, or CULL60. In addition, the interactions between CBT group and pregnancy type, and CBT group and parity were not retained in the final models that evaluated the association between CBT group and incidence of health disorders. Number of cases of health disorders per cows during the first 60 DIM, however, was greater ($P < 0.01$) for HT compared with LT cows (0.68 ± 0.07 vs. 0.18 ± 0.14). In addition, an interaction ($P < 0.01$) was detected between CBT group and pregnancy type. In cows delivering singletons, the number of health disorders during the first 60 DIM did not differ ($P = 0.36$) between CBT groups, whereas for cows carrying twins, it was ($P < 0.01$) greater for HT compared with LT cows (1.08 ± 0.13 vs. 0.01 ± 0.28). Cows bearing twins had greater ($P < 0.01$) risk of having RFM, MTR7, or MTR than cows that delivered singletons. Pregnancy type was ($P < 0.01$) associated with UDX, DA, DX60, and CULL60. Occurrence of MAST60 was not ($P > 0.11$) associated with any variables or interactions.

Associations between CBT, as a continuous measurement, and variables of interest are summarized in Table 4.2. A tendency ($P = 0.09$) for an interaction between CBT ant parity was observed (Table 4.2). Health disorders during the first 60 DIM (DX60) tended to be associated with CBT ($P = 0.09$), pregnancy type ($P = 0.09$), the interaction between CBT and pregnancy type ($P = 0.08$), and the quadratic effect of CBT ($P = 0.09$). Estimated probability of having DX60 according to CBT is depicted in Figure 4.5. Parity and the interaction between CBT and parity were not ($P > 0.42$) associated with cows having DX60.

Milk Yield and Pregnancy per AI at First Service

Milk yield tended ($P = 0.10$) to be greater for LT compared with HT cows (49.3 ± 1.9 vs. 46.2 ± 1.6 kg; Figure 4.6). Parity was not ($P = 0.51$) associated with milk yield, but its interaction with week of lactation affected ($P = 0.02$) milk production. Interactions between CBT group and parity, and CBT group and week of lactation were not ($P > 0.23$) associated with milk yield. Cows classified as MGBCS had ($P < 0.01$) greater milk yield compared with LBCS cows (49.4 ± 1.6 vs.

46.8 ± 1.6 kg). The interaction between CBT group and pregnancy type tended (*P* = 0.08) to affect milk yield. This tendency was observed because in cows carrying singletons, milk yield did not differ (*P* = 0.99) between CBT group, but in cows bearing twins, milk yield tended (*P* = 0.08) to be greater for LT than HT cows (50.5 ± 2.8 vs. 45.7 ± 1.9 kg).

Pregnancy per AI at first service did not differ (*P* = 0.72) between CBT groups (35.5 vs. 39.8% for HT and LT cows, respectively). In addition, BCSC, parity, and the interaction between parity and CBT group were not (*P* > 0.30) associated with P/AI at first service. Cows that delivered singletons tended (*P* = 0.06) to have greater P/AI than cows that delivered twins (39.8 vs. 16.7%). Pregnancy per AI was not (*P* = 0.91) affected by the interaction between CBT group and pregnancy type.

DISCUSSION

Heat stress represents a major challenge for U.S. dairy operations, with annual losses estimated at approximately $1 billion (St-Pierre et al., 2003). Indeed, this figure can be considerably greater given that recent studies demonstrated deleterious effects of heat stress on dry cows (Avendaño-Reyes et al., 2006; Tao and Dahl, 2013), representing significant economic implications (Ferreira et al., 2016). Use of evaporative cooling systems for dry cows alleviates the negative effects of heat stress on subsequent milk production. Dairy cows cooled during the entire dry period produced 2.1 to 9.3 kg/d more milk during the next lactation compared with non-cooled cows (Adin et al., 2009; do Amaral et al., 2009). In addition to optimizing profitability and performance of lactating cows, cooling during the dry period influences physiological traits, such as prolactin concentration after calving (do Amaral et al., 2009; 2011) and CBT (Avendaño-Reyes et al., 2006; Adin et al., 2009; Karimi et al., 2015). It was demonstrated recently that CBT at approximately 255 days of gestation is associated with health and productive performance during the subsequent lactation of non-cooled heat-stressed cows (Scanavez et al., 2017). Understanding other physiological characteristics of dry cows that naturally exhibit increased CBT than their counterparts housed in the same environment is key for the development of management practices targeted to improve postpartum performance of this cohort of cows. Findings from the present study indicate that several physiological traits differ between cooled cows classified as HT or LT during the dry period. Moreover, results reported herein corroborate previous evidence of an association between CBT during the dry period and health and milk production after calving.

The authors hypothesized that cooled cows with high CBT during the dry period would have different prepartum concentrations of PAG compared with cows with low CBT. Pregnancy-associated glycoproteins are produced by giant binucleated cells of the ruminant placenta and they accumulate in the bloodstream during pregnancy (Green et al., 1998). Even though the physiological functions of PAGs are not well understood (Serrano et al., 2009), concentration of PAG in maternal blood is used as an indicator of pregnancy (Green et al., 2005). In addition, it has been suggested that PAG concentrations may be an indicator of feto-placental status (Patel et al., 1997, Pohler et al., 2016) and may play a role in the release of fetal membranes after calving (Hooshmandabbasi et al., 2018). Our results indicate that concentration of PAG during the last weeks before calving is greater for HT compared with LT cows. Although it was shown in a previous report that cooling heat-stressed cows before calving increased PAG in late gestation (Thompson et al., 2013), no other studies have evaluated peripheral PAG concentration of dry cows classified as having high or low CBT. In a study conducted in Spain, it was shown that PAG concentration was greater for cows conceiving in the cooler compared with warmer periods of the year (Serrano et al., 2009). Furthermore, the authors demonstrated that lactating cows carrying twins have greater PAG concentration than cows carrying singletons (Serrano et al., 2009). Even though pregnancy type was not retained in the final model that explored the association between PAG and CBT group, twining incidence was 4.7-fold greater for HT compared with LT cows. Because heat-stressed cows with increased CBT have reduced portal blood flow (McGuire et al., 1989) and increased peripheral blood perfusion (West 2003), it is possible that PAG clearance is altered in HT cows, which may have resulted in differences in PAG concentrations between CBT groups. Nevertheless, the precise mechanisms involved in PAG metabolization and clearance remain unknown. Another speculation for the distinct profiles of PAG between HT and LT cows is a potential disruption in placental function mediated via hyperthermia.

Previous reports indicate that reducing heat load during the dry period of heat-stressed cows alters periparturient concentration of PRL (do Amaral et al., 2009; 2011). The decreased concentration of PRL of cooled compared with non-cooled dry cows may be one of the reasons why providing heat abatement during the dry period increases milk yield after calving (do Amaral et al., 2009; 2011). Contradicting our initial hypothesis, PRL concentration did not differ between HT and LT cows in the current study. This finding suggests that the physiological mechanisms involved in milk yield reduction of cows with high CBT during the dry period are independent of PRL concentration. It is important to note that a single sample after calving was collected from a subset of cows to measure PRL concentrations. It is possible that timing of blood sample collection was not ideal, given that PRL concentration is quickly reduced after calving (do Amaral et al., 2009; 2011).

Concentration of NEFA before calving have been consistently reported to be similar between cooled and non-cooled cows during the dry period (Urdaz et al., 2006; Tao and Dahl, 2013). In the current study, prepartum NEFA concentrations did not differ between multiparous HT and LT cows. Conversely, primiparous HT cows had greater concentration of NEFA than primiparous LT cows before calving, indicating more extensive mobilization of body fat of cows with high CBT. One could suggest that primiparous HT cows have a more pronounced decrease in DMI before calving, perhaps similar to cows bearing twin pregnancies, which have reduced prepartum DMI and energy balance during late gestation (Silva-del-Río et al., 2010). In fact, proportion of cows bearing twin pregnancies in the HT group was increased by 369% compared with the LT group. In lactating cows, an association between CBT and magnitude of DMI reduction has been reported (Spiers et al., 2004). Furthermore, non-cooled cows have lesser DMI than cooled cows during the dry period (do Amaral et al., 2009; Tao et al., 2011), despite no differences detected in NEFA concentrations. It is important to note that in previous studies conducted with heat-stressed dry cows with or without access to heat abatement systems (Urdaz et al., 2006; Tao and Dahl, 2013), prepartum NEFA concentrations were not reported by parity group. Differences in experimental design and lack of reports describing NEFA concentrations in heat-stressed dry cows stratified by parity limit interpretation of our results.

After calving, NEFA concentration was greater for LT than HT in multiparous cows. In addition, BHB concentration was greater for LT than HT cows at 7 DIM, regardless of parity. Concentrations of NEFA and BHB are greater during the first few weeks of lactation in cows cooled during the previous dry period compared with non-cooled cows (do Amaral et al., 2009). Such differences are attributed to the increased milk yield of cooled cows, resulting in greater mobilization of body tissues to support lactogenesis (do Amaral et al., 2009). In the current study, milk yield tended to be greater for LT compared with HT cows, which likely demanded increased energy requirements, and consequently increased concentrations of NEFA and BHB. Because nutrient demand depends on body weight and multiparous cows have greater body weight than primiparous, it is possible that metabolic challenges were more severe for multiparous LT cows, potentially explaining differences in postpartum NEFA concentrations. Collectively, we speculate that HT cows had increased concentrations of NEFA before calving as a result of decreased dry matter intake, and LT cows had greater energy balance indicators after calving because of greater metabolic challenges driven by increased milk yield.

Studies that investigated the relationship between CBT during the dry period and postpartum health are scarce. It has been reported that cows that are dried-off during summer months are more likely to present health disorders after calving than cows dried-off during winter (Thompson and Dahl, 2012). Despite the potential presence of uncontrolled confounding variables in the association reported by Thompson and Dahl (2012), it has been demonstrated that dry cows exposed to heat stress have reduced DMI (Adin et al., 2009; Tao et al., 2011). A negative relationship between DMI in late gestation and risk of occurrence of MTR after calving was reported by Huzzey et al. (2007). It is likely that reduced DMI in the periparturient period may be involved in the association between hyperthermia during the dry period and postpartum health. One of the objectives of the current study was to explore the association between CBT during the dry period and likelihood of cows developing health disorders after calving. Our results indicate that CBT group (HT vs. LT) was not associated with incidence of health disorders. These results contrast with the previous study in which cows classified as having HT during the dry period had increased risk for uterine diseases compared with LT cows (Scanavez et al., 2017). In the current study, however, cows were cooled during the dry period, whereas no heat abatement systems were used in the latter study. It is possible that use of heat abatement systems for dry cows improve periparturient immune status (do Amaral et al., 2011), thus diminishing differences in postpartum health disorders between HT and LT cows. Despite lack of differences in postpartum health disorders between CBT group, for cows bearing twins, number of cases of health disorders occurring during the first 60 DIM was greater for HT than LT cows. Thus, HT cows bearing twins likely presented more severe or longer lasting clinical cases. The quadratic effect of CBT in the analyses, which explored the relationship between CBT and occurrence of DX60, is likely the reason that individual disease differences were not detected between HT and LT cows. Nonetheless, findings from the current and the previous study (Scanavez et al., 2017) suggest there is potential merit in assessing CBT in late gestation for predicting cows at increased risk of postpartum disorders during the summer. Cows with high CBT are more likely to bear twins, which is a known risk factor for postpartum diseases and it has been shown to increase incidences of RFM and MTR by 2.8 and 6.6-fold, respectively (Hossein-Zadeh and Ardalan, 2011). In fact, Scanavez et al. (2018) reported that twinning was one of the main factors to predict CBT of dry cows. It is extremely likely that greater incidence of twinning of cows with high CBT negatively impacted postpartum health. Furthermore, it is possible that twinning and high CBT during the dry period have negative additive effects on health status of heat-stressed cows. Altogether, our results indicate that both CBT during the dry period and twinning are associated with incidence of several postpartum health disorders, despite the presence of heat abatement.

It has been consistently reported that cooling dry cows alleviates the negative effects of heat stress on milk yield during the subsequent lactation (Adin et al., 2009; do Amaral et al., 2009; Karimi et al., 2015). The current study is novel in demonstrating an association between CBT during the dry period and milk yield during the next lactation of cooled cows. Milk yield during the first 13 weeks of lactation tended to be 3.1 kg greater for LT compared with HT cows. This finding is consistent with the previous report that suggested that cows classified as LT during the dry period have greater milk yield in the first month of lactation compared with HT cows (Scanavez et al., 2017). In that study, however, no cooling methods were in place for dry cows.

We speculate that LT cows had greater mammary cell proliferation during the dry period, which resulted in greater milk yield after calving. This speculation is supported by the fact that milk yield is a function of the number of mammary cells, which is a product of cell proliferation and apoptosis (Capuco et al., 2003). Mammary cell proliferation has been demonstrated to be reduced in dry cows under heat stress (Adin et al., 2009; Tao et al., 2011), which is likely a potential mechanism involved in reduced milk yield of HT cows.

Gestation length was shorter for HT compared with LT cows. It has been demonstrated that shorter gestation length is associated with subsequent reduced milk yield (Norman et al., 2011; Vieira-Neto et al., 2017). Even though the underlying mechanisms for such association are not completely understood, Vieira-Neto et al. (2017) suggested that peak of prolactin at the onset of lactation may be altered in cows with abnormal gestation length, negatively affecting milk synthesis. In the present study, however, prolactin concentrations after calving did not differ between HT and LT cows, suggesting that a different mechanism may be the culprit for reduced milk yield of HT cows. It is possible that, because of a longer gestation length, LT cows had a more prolonged period of mammary cell proliferation before calving than HT cows, resulting in a larger number of cells at calving, favoring greater milk yield.

Cows with shorter gestation length or bearing twins have impaired reproductive performance in the subsequent lactation (Chebel et al., 2018). Although HT cows had greater incidence of twinning and shorter gestation length than LT cows, no differences were detected in P/AI for first service between CBT groups. In agreement with this finding, pregnancy outcome for first AI did not differ between non-cooled cows classified as having HT or LT during the dry period (Scanavez at al., 2017). The possibility of a type II error cannot be ignored in the current and previous study, but the objectives of the 2 experiments were not aimed to detect such differences in P/AI, which would have required a much larger sample size. Further studies with larger sample size are necessary to investigate the association between CBT during the dry period and reproductive efficiency in the subsequent lactation.

In conclusion, HT cows have distinct concentrations of PAG in late gestation and energy balance indicators during the transition period compared with LT cows managed under similar environmental conditions. In addition, cooled dairy cows classified as LT during the dry period tended to have increased milk yield in the subsequent lactation compared with their HT counterparts. Core body temperature assessment of heat-stressed cows during the dry period may be a useful tool to identify cows expected to have impaired health and reduced milk yield in the subsequent lactation. Further studies are necessary to evaluate management strategies targeted to improve health and productive performance of cows with increased CBT during the dry period.

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Table 4.1. Prepartum descriptive data (mean ± SEM), incidence of twinning, and birth weight of calves born to cows classified as having low or high core body temperature before calving.

Item	Core body temperature (CBT) group[1]		*P*-value
	Low temperature	High temperature	
Number of cows	119	122	
Average core body temperature, °C	38.85 (0.01)	39.15 (0.01)	< 0.01
Lactation number at enrollment	1.7 (0.1)	1.8 (0.1)	0.49
Previous projected 305-d mature equivalent milk yield, kg	14,123 (899)	14,108 (898)	0.95
Body condition score at enrollment [2]	3.3 (0.2)	3.2 (0.2)	0.54
Days after calving at dry-off	333.5 (5.2)	332.4 (5.1)	0.88
Days after calving at enrollment	368.5 (4.8)	367.5 (4.8)	0.89
Days of gestation at enrollment	255.2 (0.6)	255.6 (0.6)	0.16
Gestation length, d	278.2 (0.9)	273.9 (0.9)	< 0.01
Dry period length, d	59.3 (2.3)	54.7 (2.3)	< 0.01
Days spent in close-up pen	29.3 (1.3)	25.2 (1.3)	< 0.01
Calf weight, kg[3]	42.6 (1.1)	41.9 (1.1)	0.52
Twinning, %	4.2	19.7	< 0.01

[1] Core body temperature group was determined by using median values of CBT calculated separately for each parity within each dairy.

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

[3] Only singleton calves were included in the analysis.

Table 4.2. Final logistic regression models used to evaluate the association of various factors with occurrence of health disorders and culling for cows that had core body temperature (CBT) assessed between 250 and 260 days of gestation.

	Estimates of fixed effects							*P*-value			
Item[1,2]	Intercept	Parity[3]	Pregnancy type[4]	CBT[5]	Parity × CBT	Pregnancy type × CBT	CBT × CBT	Pregnancy type	CBT	Pregnancy type × CBT	CBT × CBT
RFM	13705	-	-1.60	-702.93	-	-	9.01	0.01	0.02	0.85	0.02
MTR7	9314	-	-1.06	-477.34	-	-	6.11	0.04	0.04	0.46	0.04
MTR14	10526	-0.77	-1.04	-538.77	-	-	6.89	0.04	0.02	0.22	0.02
MTR	-314	-0.59	377.52	8.01	-	-9.68	-	< 0.01	0.07	< 0.01	0.16
UDX	-205	-	248.64	5.21	-	-6.38	-	0.01	0.12	0.01	0.14
DA	-59	261.11	-2.75	1.46	-6.69	-	-	< 0.01	0.39	0.20	0.29
MAST60	-54	-	-	1.30	-	-	-	0.53	0.43	0.45	0.97
CULL60	-158	-	240.41	3.99	-	-6.20	-	0.04	0.54	0.04	0.04
DX60	8418	-	198.22	-435.85	-	-5.10	5.64	0.09	0.09	0.08	0.09

[1] Retained fetal membranes (**RFM**); metritis at 7 ± 3 (**MTR7**) or 14 ± 3 DIM (**MTR14**); metritis at 7 or 14 ± 3 DIM (**MTR**); RFM or MTR (**UDX**); displaced abomasum (**DA**); mastitis within 60 DIM (**MAST60**); culling until 60 DIM (**CULL60**); occurrence of least one health disorder within 60 DIM (**DX60**).

[2] Body condition score change was not significant in any model and was omitted from this table.

[3] Parity: primiparous and multiparous. Multiparous is the reference category (estimate = 0).

[4] Pregnancy type: singleton and twins. Cows that delivered twins are the reference category (estimate = 0).

[5] Core body temperature was assessed using an intra-vaginal temperature logger programmed to record measurements in 5-minute intervals during 7 consecutive days. Values of CBT included in the models represent average temperature calculated from all readings for each cow.

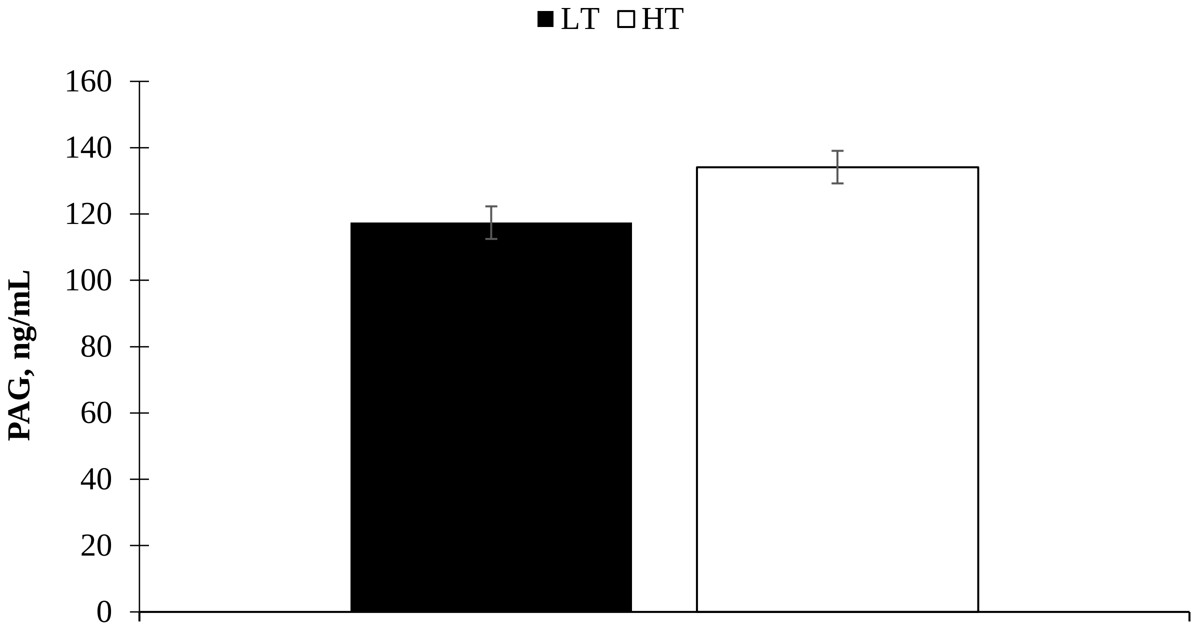

Figure 4.1. Prepartum pregnancy-associated glycoproteins (PAG) in plasma of cows classified as having low (LT; n = 119) or high (HT; n = 122) core body temperature (CBT) based on median values of average CBT within each parity and dairy.

Average CBT was 39.15 ± 0.01 and 38.85 ± 0.01°C for HT and LT cows, respectively. Error bars represent SEM. Samples were collected at 21 ± 3, 14 ± 3, 7 ± 3, and between 3 days before and the day of calving. Black bar represents LT, and the white bar represents HT cows. Concentration of PAG (*P* = 0.02) was greater for HT compared with LT cows (134.1 ± 4.9 vs. 117.4 ± 4.9 ng/mL). Day relative to calving affected (*P* < 0.01) PAG concentration but the interaction between CBT group and day was not associated (*P* = 0.74) with plasma PAG.

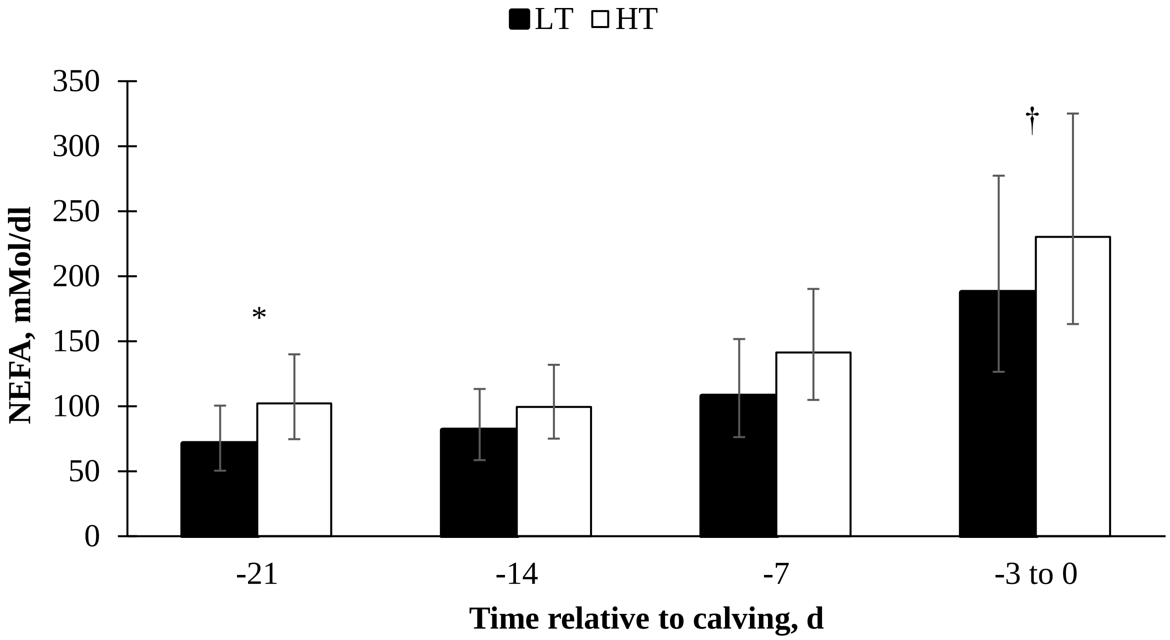

Figure 4.2. Prepartum nonesterified fatty acids (NEFA) concentration in plasma of primiparous cows classified as having low (LT, n = 119) or high (HT, n = 122) core body temperature (CBT) based on median values of average CBT within each parity and dairy.

Average CBT was 39.15 ± 0.01 and 38.85 ± 0.01°C for HT and LT cows, respectively. Error bars represent 95% confidence intervals. Samples were collected at 21 ± 3, 14 ± 3, 7 ± 3, and between 3 days before and the day of calving. Black bars represent LT, and the white bars represent HT cows. Core body temperature group was not associated ($P = 0.17$) with concentration of NEFA. Nonesterified fatty acids NEFA concentration was ($P = 0.05$) greater for HT than for LT cows (135, 95% CI = 102 to 178 vs. 104, 95% CI = 75 to 144 mmol/dL). Within a sampling day, pairwise differences and tendencies are represented as follows: † $P \leq 0.10$; * $P \leq 0.05$.

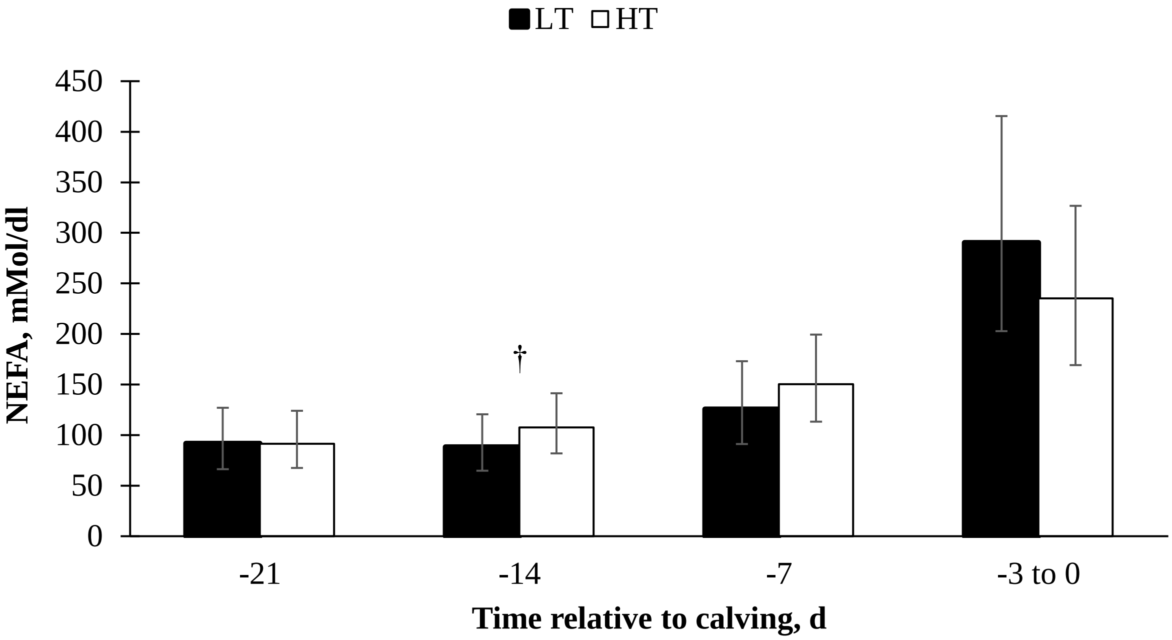

Figure 4.3. Prepartum nonesterified fatty acids (NEFA) concentration in plasma of multiparous cows classified as having low (LT, n = 119) or high (HT, n = 122) core body temperature (CBT) based on median values of average CBT within each parity and dairy.

Average CBT was 39.15 ± 0.01 and 38.85 ± 0.01°C for HT and LT cows, respectively. Error bars represent 95% confidence intervals. Samples were collected at 21 ± 3, 14 ± 3, 7 ± 3, and between 3 days before and the day of calving. Black bars represent LT, and the white bars represent HT cows. Core body temperature group was not associated ($P = 0.17$) with concentration of NEFA. Nonesterified fatty acids concentrations were ($P = 0.72$) similar between cows classified as having LT or HT. Within a sampling day, pairwise differences and tendencies are represented as follows: † $P \leq 0.10$; * $P \leq 0.05$.

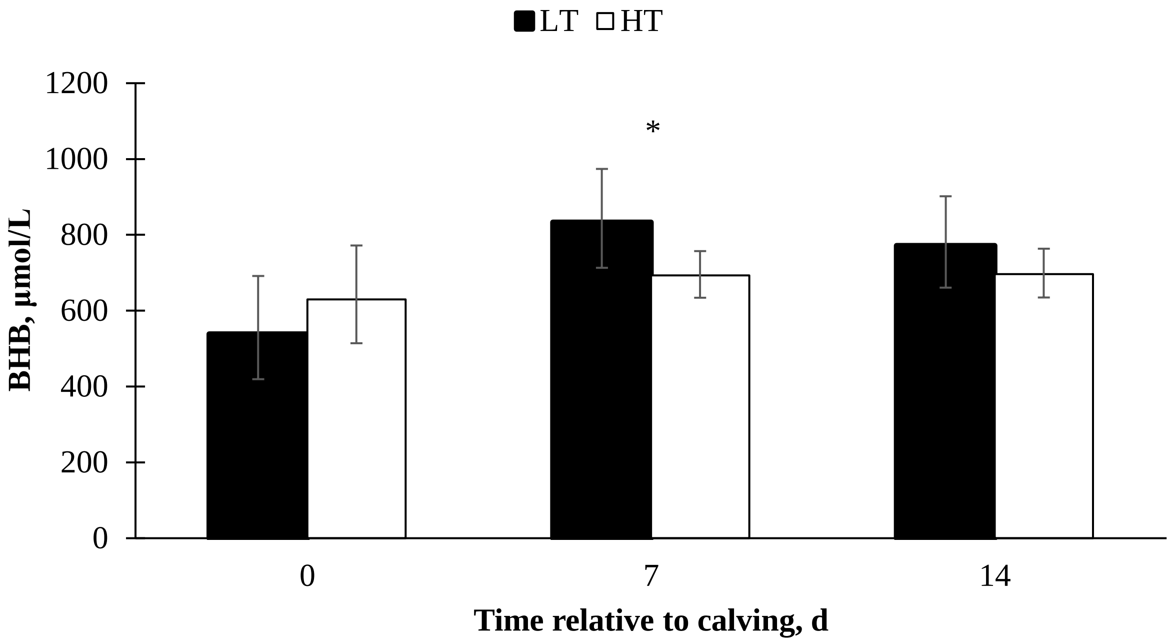

Figure 4.4. Postpartum for BHB concentration in plasma of cows classified as having low (LT; n = 119) or high (HT; n = 122) core body temperature (CBT) based on median values of average CBT within each parity and dairy.

Average CBT was 39.15 ± 0.01 and 38.85 ± 0.01°C for HT and LT cows, respectively. Error bars represent 95% confidence intervals. Samples were collected at the day of calving (d = 0), 7 ± 3, and 14 ± 3 DIM. Black bars represent LT, and the white bars represent HT cows. Core body temperature group was not (*P* = 0.64) associated with concentration of BHB. There was an interaction (*P* = 0.05) between day of sampling and CBT group. Concentration of BHB was (*P* = 0.04) greater for LT compared with HT cows in samples collected 7 ± 3 DIM (833, 95% CI = 713 to 974 vs. 693, 95% CI = 634 to 757 µmol/L). Within a sampling day, pairwise differences are represented as follows: * $P \leq 0.05$.

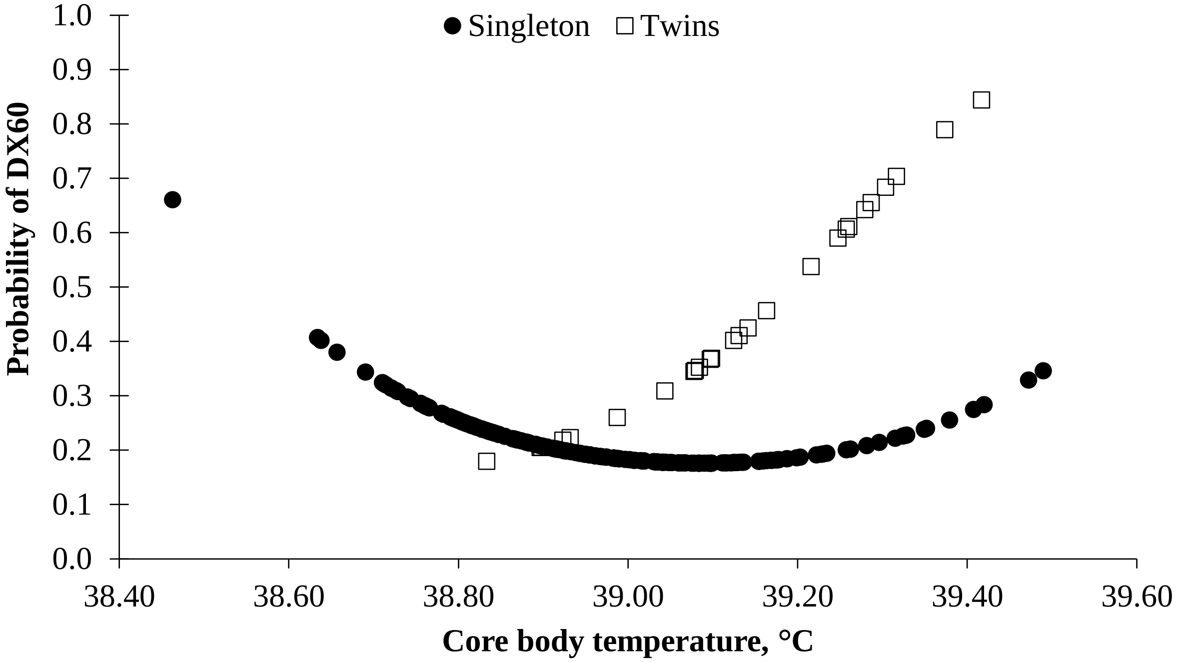

Figure 4.5. Estimated probability of cows having at least one health disorder within 60 DIM (DX60) according to the average core body temperature (CBT) and pregnancy type (singleton and twins).

Average CBT for each cow was calculated using readings obtained in 5-minute intervals for 7 consecutive days during the dry period. Health disorders of interest were retained fetal membranes, metritis at 7 or 14 ± 3 DIM, displaced abomasum, and mastitis within 60 DIM. Average CBT (*P* = 0.09) and pregnancy type (*P* = 0.09) tended to be associated with risk of DX60. The interaction between average CBT and pregnancy type tended (*P* = 0.09) to be associated with risk of DX60. A tendency (*P* = 0.09) was detected for a quadratic effect of average CBT to affect probability of DX60.

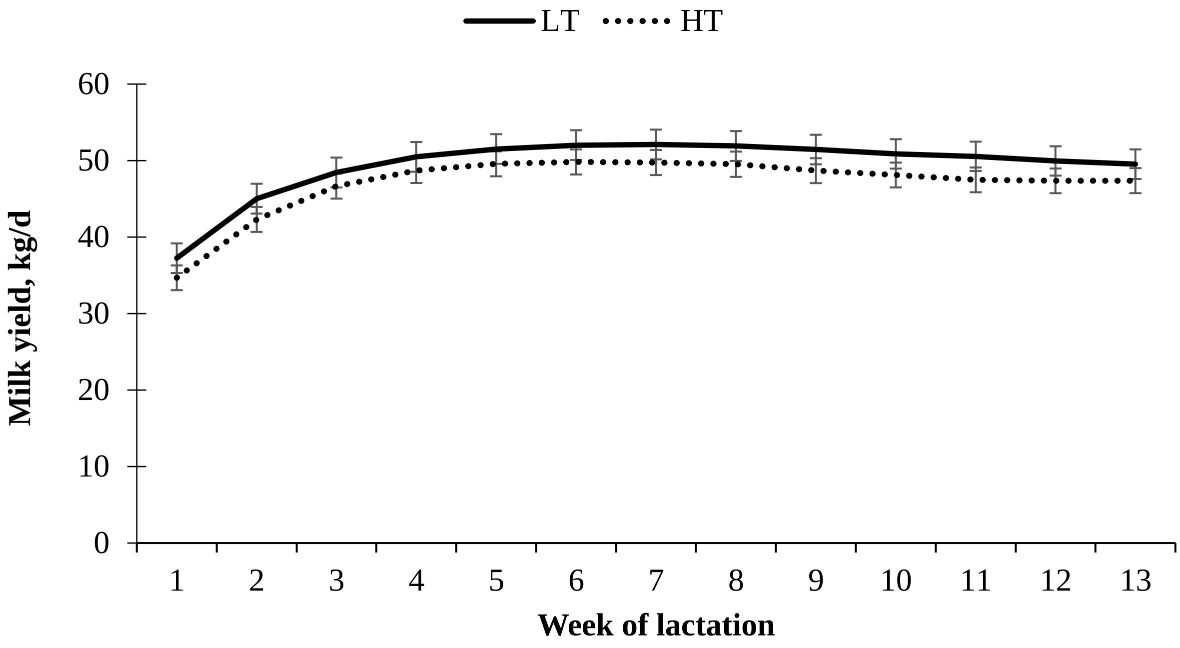

Figure 4.6. Milk yield during the first 13 weeks of lactation of cows classified as low (LT; n = 119) or high (HT; n = 122) core body temperature (CBT) based on median values of average CBT within each parity and dairy.

Average CBT was 39.15 ± 0.01 and 38.85 ± 0.01°C for HT and LT cows, respectively. Error bars represent SEM. Solid line represents LT, and the dotted line represents HT cows. Core body temperature group tended (P = 0.10) to be associated with milk yield in the first 13 weeks of lactation (LT = 49.3 ± 1.9; HT = 46.2 ± 1.6 kg). Week: $P < 0.01$; and CBT group × week: P = 0.86.

Chapter 5 - Evaluation of Seasonal Patterns and Herd-Level Traits Associated with Insemination Risk in Large Dairy Herds in Kansas

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ABSTRACT

Adequate identification of estrus is crucial to achieve satisfactory reproductive performance in dairy farms. Even though several studies evaluated expression and identification of estrus at the cow level, limited data exist regarding estrus identification parameters at the herd level. The objectives of this study were to use data from large dairy farms located in Kansas to describe temporal patterns of insemination risk (IR), and to investigate associations between IR and various herd-level factors. Nine herds that housed lactating cows in dry-lots or free-stalls were used in the study. Data from 2012 through 2017 were extracted and categorized in 21-day intervals in a total of 85 cycles, which were classified by season of the year. Mean (SD) IR was 67.6 % (4.0) and increased 0.067% (0.009) for each 21-day cycle during the period evaluated. Annual, semi-annual, and trimestral IR peaks were detected using autoregressive integrated moving average analysis. Most of these variations, however, were considered minimal and likely not of economic concern for commercial herds. Insemination risk was greatest during autumn, but did not differ among winter, spring, and summer. Insemination risk was not associated with herd milk yield per season, incidence risk of mastitis during first 21 days in milk, proportion of primiparous cows in the milking herd, or voluntary waiting period of multiparous cows. Herds that housed lactating dairy cows in dry lots had IR 2.4 percentage points greater than free-stall herds. In addition, mortality during the first 60 days in milk, and category of voluntary waiting period for primiparous cows were associated with IR. In conclusion, seasonal variability in IR was minimal, with increased values observed during the autumn. Insemination risk was greater for dry-lot than free-stall herds. In addition, reduced mortality of lactating cows by 60 days in milk and longer voluntary waiting period for primiparous cows seem to favor greater IR.

INTRODUCTION

Adequate identification of estrus is crucial to achieve satisfactory reproductive performance in dairy farms (Senger, 1994; Kinsel and Etherington, 1998). Ineffective identification of estrus has a negative impact on insemination risk (**IR**), resulting in extended intervals from parturition to first service (Stevenson and Call, 1983), increased interval between inseminations (Fricke et al., 2014), and reduced profitability in dairy operations (Cabrera, 2014). Despite the recommendation that IR should be greater than 60% for dairy herds (Mendonça, 2015), considerably poorer results have been reported (Kinsel and Etherington, 1998; Washburn et al., 2002).

Herd IR is influenced by expression and subsequent detection of estrus and can be affected by several factors. In an experiment comparing estrous behavior in two housing systems, Palmer et al. (2010) demonstrated that lactating cows housed in free-stall facilities express less evident signs of estrus than cows managed on pasture. In addition, Vailes and Britt (1990) suggested that footing surface can impact expression of estrus. Shorter duration and fewer mounting events during estrus also are associated with high milk yield (Lopez et al., 2004) and lameness (Sood and Nanda, 2006. Furthermore, expression of estrus is suppressed during periods of heat stress (Pennington et al., 1985; Younas et al., 1993; Cartmil et al., 2001; de Rensis and Scaramuzzi, 2003), which could lead to poor estrus-detection risk (Caraviello et al., 2006) and IR.

In many herds, daily observation for signs of estrus is improperly performed (Caraviello et al., 2006). In order to facilitate this task and improve accuracy of detection, various strategies have been adopted (Fricke et al., 2014), such as pressure-sensitive patches (Bading et al., 1985), pedometers (Lehrer et al., 1992), and activity monitoring systems (Jónsson et al., 2011). Although growing interest in adoption of these technologies has occurred, many large dairies in the U.S. rely on applying paint or chalk on the tail head of cows for detection of mounts received (Caraviello et al., 2006). In a recent study conducted by our research group with heat-stressed cows (Voelz et al., 2016), more than 89% of first inseminations were based on tail paint removal, which occurred between 53 and 84 days in milk. Therefore, it is plausible to infer that greater IR may be achieved in dairies with efficient reproductive management that rely largely on tail paint removal for insemination, regardless of season of the year.

Several studies evaluated expression and identification of estrus at the cow level [Palmer et al., 2010; Xu et al., 1998; Valenza et al., 2012), but limited data exist regarding estrus identification traits at the herd level (e.g., IR). It is unclear whether reduction of expressed estrus during the warm season of the year compromises IR at the herd level. A recent report (Scanavez et al., 2016) presented evidence that reduction in IR during summer is subtle in large dairies. In this study, annual average IR for 25 herds was 66% in 2015, and during the summer IR was only 2.6 percentage units worse than IR achieved during the remainder of the year. Therefore, it is possible that reduced estrual behavior of dairy cows during summer can be compensated by management practices such as applying paint daily to the tail head of cows, resulting in modest variations in IR across the year. In addition, it is not clear how housing systems for lactating dairy cows influence herd-level IR across different seasons. Because herds in the Great Plains of Kansas have a variety of housing systems, excellent reproductive performance (e.g., annual pregnancy risk greater than 22%) [Mendonça, 2015; Scanavez et al., 2016), and cows are subjected to heat stress conditions (Scanavez et al., 2016; Voelz et al., 2017), this region and cohort of herds present ideal conditions for studying herd-level aspects related to reproductive success across seasons.

The objectives of this study were to use data from large dairy farms located in Kansas to describe temporal patterns of IR, and to investigate the association between IR and several factors, including housing, milk yield, voluntary waiting period (**VWP**), proportion of primiparous cows in the milking herd, mastitis incidence, and mortality. We hypothesized that IR would be: (1) without seasonal peaks or nadirs; (2) greater in dry-lot facilities compared with free-stall herds; and (3) negatively associated with milk yield, mastitis incidence, mortality, and proportion of primiparous cows in the milking herd, and positively associated with VWP.

MATERIALS AND METHODS

Inclusion Criteria

Dairy herds located in Kansas (n = 9) with a minimum of 1,500 lactating cows, with at least 6 years of records, and were enrolled in the Kansas State University extension program to monitor herd performance were eligible to be enrolled in the study. Only herds that achieved IR ≥ 60% during 2017 were included in the study population. Among these herds, housing systems for lactating cows were either dry-lot corrals (n = 5) or free-stall facilities with access to dirt exercise lots (n = 4). All herds used artificial insemination (**AI**) as the exclusive method of breeding, and recorded information daily in on-farm management software (Dairy Comp 305, Valley Ag Software, Tulare, CA). Differences observed in reproductive management among herds enrolled included frequency of pregnancy diagnosis, VWP, DIM at which non-inseminated cows were submitted to a timed AI program, and resynch program used. Although reproductive programs were dissimilar among herds, the majority of inseminations were performed based on tail paint removal in all herds. A small proportion (3 to 32%, average = 13%) of inseminations were coded as timed AI in the management software. Minor changes occurred in reproductive management of each herd during the period analyzed in this study. These changes included alterations in VWP and resynch programs.

The researchers did not apply treatments or have contact with animals during any phase of this study. All data were extracted from a database of records from dairy herds with the permission of herd owners. Therefore, approval from an animal research ethics committee was not required for completion of this observational study.

Variables of Interest and Data Collection

Insemination risk was the outcome of interest and was calculated in Microsoft Excel (Microsoft Corp., Redmond, WA) using the following formula: IR = number of cows inseminated during a 21-day period divided by the number of cows eligible to be inseminated during the 21-day period. Explanatory variables in this formula were extracted from the on-farm management software. Cows were considered eligible to AI based on: (1) VWP of primiparous and multiparous within each herd; (2) non-pregnancy status; (3) not elected to be culled at the end of lactation; and (4) remaining in the herd for at least 11 days of the specific 21-day cycle being analyzed. All inseminations performed in a given 21-day cycle were considered for IR calculation, regardless of being performed based on detected estrus or fixed-time AI. Three herds practiced different VWP for primiparous (lactation = 1) and multiparous (lactation > 1) cows. Therefore, VWP was adjusted by parity within herd. Data from December 2012 through November 2017 were extracted and 21-day cycles were numbered from 1 to 17 for each year. Within each year, the first 21-day cycle was calculated starting on December 1st, and the 17th cycle starting on November 2nd. Cycles were categorized by season of the year based on the majority of days in a cycle occurring in a given season: winter (cycles 1 through 4); spring (cycles 5 through 9); summer (cycles 10 through 13); or autumn (cycles 14 through 17).

Explanatory variables explored were housing systems (dry-lot vs. free-stall), VWP (in days) of primiparous (**VWP-P**) and multiparous (**VWP-M**) cows, season of the year (winter, spring, summer, and autumn), proportion of primiparous cows in the milking herd (**% primiparous**), herd milk yield per season (in kg), and herd incidence risk of mastitis during the first 21 days in milk (**% mastitis**), and mortality during the first 60 days in milk (**% dead**). Proportions of primiparous cows in the milking herd and incidence risks of mastitis and mortality also were extracted in a 21-day timeframe to coincide with the 21-day cycles previously described (cycles numbered 1 to 17). For regression analyses, weighted averages of the variables were calculated for each season. Milk yield was recorded monthly at each dairy, and weighted averages were calculated for each season. Voluntary waiting periods of primiparous and multiparous cows were evaluated for each season and were categorized as: < 50, 50 to 54, 55 to 59, or ≥ 60 days in milk.

STATISTICAL ANALYSES

Descriptive Data

Continuous data were screened for normality with histograms using Stata/IC 15.1 (StataCorp LLC, College Station, TX). Mean (SD) number of cows, VWP-P, VWP-M, % primiparous, milk yield, % mastitis, and % dead during the period of the study were calculated for each herd. In addition, descriptive data stratified by production system (e.g., dry-lot or free-stall) also were explored.

Time-Series Analysis

Cycles of 21 days were selected as the measurement unit for analysis of trends and seasonal effects of IR because this interval is widely adopted in the dairy industry for monitoring IR and overall reproductive efficiency. Insemination risk in each cycle was the outcome of interest. Data from all herds were combined, and a total of 85 cycles between December 2012 and November 2017 were available for analyses. Trends and seasonal effects on IR were assessed using autoregressive integrated moving averages (**ARIMA**), as previously described (Alba et al., 2015; Arruda et al., 2018). Raw and decomposed IR by cycle data were plotted to allow for visual inspection of trends and seasonality. Autocorrelation (**ACF**) and partial autocorrelation plots were generated to assess correlations among residuals. Using ARIMA modeling with temporal trends as covariates, presence of annual, semiannual, and trimestral seasonality was tested using multivariable linear regressions models. Variables with *P*-value > 0.10 were excluded from the base model by backwards elimination. The final ARIMA model was selected based on lowest Akaike Information Criterion (**AIC**), and was assessed with the Box-Pierce test. To ensure the final model resulted in a stationary series, residuals correlations were again screened using ACF and partial ACF. Analyses were conducted using the 'forecast' package (Hyndman and Khandakar, 2008) of RStudio 1.0.44 (RStudio, Boston, MA).

Regression Model

All data used for the regression analysis were collected at the herd level. A causal diagram containing the outcome (IR), the main explanatory variable of interest (housing type), and other variables investigated was drawn to aid identification of possible confounders. Linearity between continuous predictors and IR was visually inspected using the lowess procedure of Stata, and statistically assessed using the reg procedure. Presence of multicollinearity among study variables was evaluated using the Spearman correlation coefficient. Two variables were considered correlated when the coefficient of correlation was > 0.8, which did not happen for any of the combinations of variables tested. Univariable linear regression analyses using IR as dependent variables were conducted for each predictor using dairy as random effect in Stata. Variables with *P*-value > 0.20 were deemed not significant and were not eligible to be included in the base model, unless they were identified as confounders on the causal diagram.

Remaining variables were included in the base model using dairy as a random effect, and backwards elimination was used to exclude nonsignificant variables. Statistical significance was defined as *P*-value $\leq$ 0.05 and tendencies as 0.05 < *P* $\leq$ 0.10. Distribution of best linear unbiased residuals was assessed to evaluate the final model's fit by plotting them in Stata and visually inspecting for normality.

RESULTS

Mean (SD) number of cows and IR were 4,578 (2,028) and 67.6 % (4.0), respectively. Further descriptive data stratified by housing type are presented on Table 5.1. Mean IR with a 95% confidence interval for each 21-day cycle for the herds is depicted in Figure 5.1. Insemination risk increased (*P* < 0.01) on average (SE) 0.067% (0.009) for each 21-day cycle in the time series analyzed. In addition, annual, semi-annual, and trimestral IR peaks were detected (Table 5.2). The final ARIMA model showed an overall good fit, with *P*-value = 0.83 in the Box-Pierce test. Model fit is shown in Figure 5.2.

The final multivariable mixed effect regression model showed that milk yield, % mastitis, % primiparous, and VWP-M were not associated (*P* < 0.16) with IR. The variables retained in the final multivariable regression model were housing system, season of the year, % dead, and VWP-P. Lactating dairy cows housed in dry-lots had greater (*P* > 0.01) IR than free-stall herds (Table 5.3). Insemination risk was associated with season of the year because IR was greatest during autumn. Nonetheless, IR did not differ among winter, spring, and summer (*P* > 0.16). In addition, % dead and VWP-P were associated (*P* < 0.01) with IR (Table 5.3).

DISCUSSION

Insemination risk is a key performance indicator that directly affects pregnancy risk and has a significant impact on profitability of dairy herds (Cabrera, 2014). Because previous peer-reviewed literature indicated that estrous expression is reduced during periods of heat stress (Younas et al., 1993; Cartmill et al., 2001; de Rensis and Scaramuzzi, 2003), IR is anecdotally considered to be seasonal, despite the lack of herd-level investigations to support this speculation. Recent findings (Scanavez et al., 2016) indicate that seasonal variation in IR might be minor in large dairy herds in Kansas. Nonetheless, time series analysis was not conducted in the previous report (Scanavez et al., 2016), limiting major inferences. Therefore, the present study was conducted to evaluate the existence of seasonal patterns of IR in large dairy herds by using time series analysis, and to investigate other herd-level factors associated with IR.

In the current study, overall IR for the enrolled herds showed an increasing trend during the 5-year period analyzed (2012 through 2017). Previous reports demonstrated contrasting trends, with IR reducing over time (Washburn et al., 2002). The latter study, however, was conducted using data collected between 1985 and 1999, when specific practices and technologies, such as tail painting for estrous detection or timed AI, were not yet widely employed. Substantial changes have recently occurred in the dairy industry with implementation of modern management practices focusing on improving animal health, welfare, and fertility, which likely contributed to improvements in IR observed during recent years (Scanavez et al., 2016). Contrary to our initial hypothesis, our data revealed seasonal patterns of IR, which was consistent during winter, spring, and summer, but increased during autumn. Even though the time series analysis suggested annual, semi-annual, and trimestral peaks, most of these variations seem to be minimal and likely not meaningful for commercial herds. We speculate that the increased IR during autumn might have occurred as a consequence of poor fertility commonly observed during summer (Jordan, 2003), which results in more cows eligible to be inseminated during autumn. In addition, a large proportion of calvings commonly occur during summer months in dairy herds affected by heat stress (Mendonça et al., 2017), resulting in an increased number of cows submitted to first AI during autumn. Therefore, it is possible that IR is increased after summer because of more pronounced sexual behavior resulting from a greater proportion of cows in estrus during the same period (Hurnik et al., 1975), facilitating estrous detection and increasing IR. The IR difference between summer and autumn was not nearly as large as the decrease in estrous expression previously reported in cows under heat stress (Pennington et al., 1985; Schüller et al., 2017). Therefore, we speculate that the reduced estrous expression observed at the cow level during summer months might be compensated by management practices such as daily observation of cows for signs of estrus or use of tail painting, resulting in minimal disturbance to herd-level indicators of estrous expression, such as IR.

In addition to exploring occurrence of seasonal trends, the present study was designed to investigate the relationship between herd-level characteristics and IR. As initially hypothesized, IR was greater for dry-lot compared with free-stall herds. Vailes and Britt (1990) demonstrated that mounting activity was 3 to 15 times greater when cows were in estrus on a dirt surface compared with cows housed on concrete. Vailes and Britt (1990), however, used estrogen-treated ovariectomized cows, and a small sample size, which prevents extrapolation of conclusions to commercial settings. Furthermore, Palmer et al. (2010) concluded that estrous detection using visual observation, tail painting, or radiotelemetry were less effective when cows were in free-stall facilities compared with cows in pasture paddocks. According to the latter study, such difference was observed because standing behavior was reduced in cows managed in the free-stall barns. The difference in IR between free-stall and dry-lot herds was minor in our study (–2.4 ± 0.8%). We speculate that the reduced expression of estrous behavior in free-stall herds was partially compensated by efficient estrus-detection programs, and consequently, only subtle differences were observed among cows in different housing types.

Health during the first weeks of lactation is an important determinant of reproductive success in dairy operations (Santos and Ribeiro, 2014). In the current study, however, % mastitis was not associated with herd-level IR. In research projects conducted with grazing (Ribeiro et al., 2013) and confined dairy cows (Santos et al., 2010), researchers concluded that cows having mastitis during the first postpartum weeks did not present delayed resumption of luteal activity, which supports our findings. It is important to note that others (Santos and Ribeiro, 2014; Santos et al., 2010) focused on cow-level outcomes instead of the herd-level approach used in the current study. Nevertheless, these studies clearly demonstrate the deleterious effects of other postpartum health disorders on resumption of luteal activity (Santos and Ribeiro, 2014; Santos et al., 2010), which can potentially affect IR. Another interesting finding from the current study was the negative association between % dead and IR. Considering that health status of early lactating cows likely influences % dead, it is possible that an increased percentage of cows with health disorders had negative implications on estrous expression (Rutherford et al., 2016), and ultimately, affected IR. A recent report (Mendonça and Scanavez, 2017) indicated that % dead is associated with pregnancy per AI of multiparous cows in large commercial herds. Nevertheless, to the best of our knowledge, studies focusing on the relationship between mortality during the first 60 days after calving and IR are still lacking.

Proportion of primiparous cows (% primiparous) in the lactating herd was not associated with IR of the herds used in the study. This finding is intriguing considering that primiparous cows are expected to have delayed resumption of luteal activity. Primiparous cows have greater postpartum concentrations of non-esterified fatty acids in plasma than multiparous cows (Wather et al., 2007). Increased concentration of non-esterified fatty acids is linked to negative energy balance, which is associated with delayed ovarian function (anovulation) and results in primiparous cows being more likely to be anovulatory at the end of the VWP than multiparous cows (Santos et al., 2009). Nonetheless, an increased proportion of primiparous cows did not reduce herd IR. We speculate that negative energy balance was not excessive in these herds and prevented % primiparous cows from contributing negatively to IR. Because a positive relationship exists between proportion of cows resuming luteal activity and days postpartum, VWP duration was explored as a risk factor for decreased IR. In the current study, VWP was positively associated with IR for primiparous, but not for multiparous cows. Periparturient primiparous cows are more likely to develop metritis than multiparous cows (Goshen and Shpigel, 2006), which is a risk factor for cows to not resume luteal activity (Ribeiro et al., 2013; Santos et al., 2010). Findings from the current study corroborate with results reported by Santos et al. (2009), who demonstrated that a smaller proportion of primiparous than multiparous cows resumed luteal activity by 65 days in milk. Altogether, it is likely that extending VWP for primiparous cows favors IR. In addition, use of ovulation-synchronization programs for first AI for all primiparous cows enrolled in programs with extended VWP may have caused a greater proportion of cows to be inseminated for the first time shortly after the end of the VWP, also increasing IR.

No association was detected between milk yield and IR in the study population. In a study conducted with cows with high milk yield, reduced number and shorter duration of standing events during estrus were reported (Lopez et al., 2004). Reduced intensity of sexual behavior may theoretically pose a challenge for estrous detection, given the reliance on tail paint removal in the U.S. to detect cows in estrus (Caraviello et al., 2006). The number of mounts or total duration of mounting events to trigger estrous detection aids (e.g., tail paint removal) has not been quantified and would depend on the product used. Therefore, it is possible that the negative association of increased milk yield on estrous behavior is not sufficient to decrease estrus-detection risk in herds similar to the ones enrolled in this study, resulting in satisfactory IR. Nonetheless, it is important to consider that average milk yield of the herd was used in the analyses of the current study, which differs from other reports that used a cow-level approach to study the association between productivity and estrus expression (Lopez et al., 2004; Rivera et al., 2010). The authors acknowledge the fact that milk yield was not very high in the herds studied. Therefore, the possibility that an association between IR and herd-level milk yield may exist in herds with very high milk yield cannot be ignored and should be investigated in future studies.

The authors recognize that inclusion of herds located in various geographic areas would have increased the external validity of the present study. It is important to note, however, that approximately 42% of the herds with more than 500 cows in the U.S. house lactating cows in similar facilities as the herds used in this study (NAHMS, 2016). In addition, this cohort of herds is projected to grow considerably faster than smaller herds in the future (NAHMS, 2016), which should augment the external validity of our findings. We also recognize the possibility of misclassification of mastitis cases, given that diagnoses were performed by farm personnel and clinical definition could vary slightly among herds. To the best of our knowledge, this is the first peer-reviewed report to demonstrate that various herd-level characteristics are associated with IR. In addition, findings of the current study show the inverse relationship between IR and postpartum mortality of lactating cows, which indicates the benefit of optimizing overall herd health to have a successful reproductive program.

In conclusion, seasonal variability in IR was minimal in the population studied and increased values were observed during the autumn. Insemination risk was greater for dry-lot than free-stall herds. In addition, reduced mortality of lactating cows by 60 days in milk and longer VWP for primiparous cows were positively associated with IR. Further research is warranted to evaluate the association of other factors with IR and to validate our findings in other geographic areas and production systems.

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Table 5.1. Descriptive data for the nine large Kansas dairy herds enrolled in this study. Data are presented as mean (SD, minimum, maximum).

Housing type	Number of lactating cows	Insemination risk (%)	VWP-P[1] (days)	VWP-M[2] (days)	% primiparous[3]	Milk yield (kg/cow/day)	% mastitis[4]	% dead[5]
Dry-lot	4,633 (2,637, 1,596, 9,562)	68.8 (4.2, 55.7, 74.6)	53.9 (6.5, 45.0, 65.0)	50.3 (2.5, 48.0, 58.0)	40.1 (3.0, 30.5, 46.3)	32.6 (2.1, 28.4, 36.9)	3.8 (2.5, 0.3, 14.7)	3.2 (1.7, 0.7, 8.2)
Free-stall	4,509 (773, 2,755, 5,693)	65.9 (3.3, 57.9, 75.7)	53.1 (4.0, 50.0, 65.0)	52.9 (3.7, 50.0, 65.0)	42.6 (3.5, 35.9, 49.9)	34.6 (2.2, 29.8, 39.9)	4.8 (2.6, 1.4, 15.7)	3.0 (1.2, 1.0, 7.0)
All herds	4,578 (2,028, 1,596, 9,562)	67.6 (4.0, 55.7, 75.7)	53.6 (5.5, 45.0, 65.0)	51.4 (3.3, 48.0, 65.0)	41.2 (3.4, 30.5, 49.9)	33.5 (2.3, 28.4, 39.9)	4.3 (2.6, 0.3, 15.7)	3.1 (1,5, 0.7, 8.2)

[1] Voluntary waiting period of primiparous cows.

[2] Voluntary waiting period of multiparous cows.

[3] Proportion of primiparous cows in the lactating herd.

[4] Herd incidence risk of mastitis by 21 days in milk.

[5] Mortality of lactating dairy cows during the first 60 days in milk.

Table 5.2. Final autoregressive integrated moving average (ARIMA) model used to evaluate presence of seasonal peaks in insemination risk in large dairy herds located in Kansas.

Item[1]	Fixed effect estimate	SE	*P*-value
Intercept	64.56	0.45	
Overall trend	0.07	0.01	< 0.01
Annual peaks	$1.22 \cos(2\pi t/17)$	0.21	< 0.01
	$0.58 \sin(2\pi t/17)$	0.22	< 0.01
Semi-annual peaks	$0.31 \cos(2\pi t/8.5)$	0.19	< 0.01
	$-1.27 \sin(2\pi t/8.5)$	0.19	0.11
Trimestral peaks	$0.62 \cos(2\pi t/4.25)$	0.18	0.96
	$0.01 \sin(2\pi t/4.25)$	0.18	< 0.01
Y_t	$0.84Y_{t-1}$	0.13	
	$Z_t - 0.72 Z_{t-1}$	0.15	

[1] ARIMA type = (1,0,1). AIC = 301.3.

Table 5.3. Final multivariable linear regression model used to evaluate the association of various herd-level factors and insemination risk in large dairy herds located in Kansas.

Item	Fixed effect estimate (SE)	95% CI[3] for fixed effect estimate	*P*-value
Intercept	72.4 (1.2)	70.0 to 74.8	
Housing system			
Dry-lot	Referent[a]		
Free-stall	-2.4 (0.8)[b]	-3.9 to -0.90	< 0.01
Season of the year			
Autumn	Referent[a]		
Winter	-1.79 (0.5)[b]	-2.9 to -0.7	< 0.01
Spring	-1.7 (0.5)[b]	-2.8 to -0.7	< 0.01
Summer	-2.5 (0.6)[b]	-3.6 to -1.4	< 0.01
% dead[1]	-1.1 (0.2)	-1.5 to -0.8	< 0.01
VWP- P category[2]			
45	Referent[ab]		
50	1.0 (1.2)[a]	-1.3 to 3.4	0.38
55	-0.7 (1.3)[b]	-3.2 to 1.9	0.61
60	4.0 (1.2)[c]	1.6 to 6.4	< 0.01

[1] Mortality of lactating dairy cows from parturition until 60 days in milk.

[2] Category of voluntary waiting period for primiparous cows: 45 = 45 to 49 days in milk; 50 = 50 to 54, 55 = 55 to 59; 60 = 60 to 65.

[3] Confidence interval.

[a,b,c] Values within a column-item with unlike superscript letters differ ($P < 0.05$).

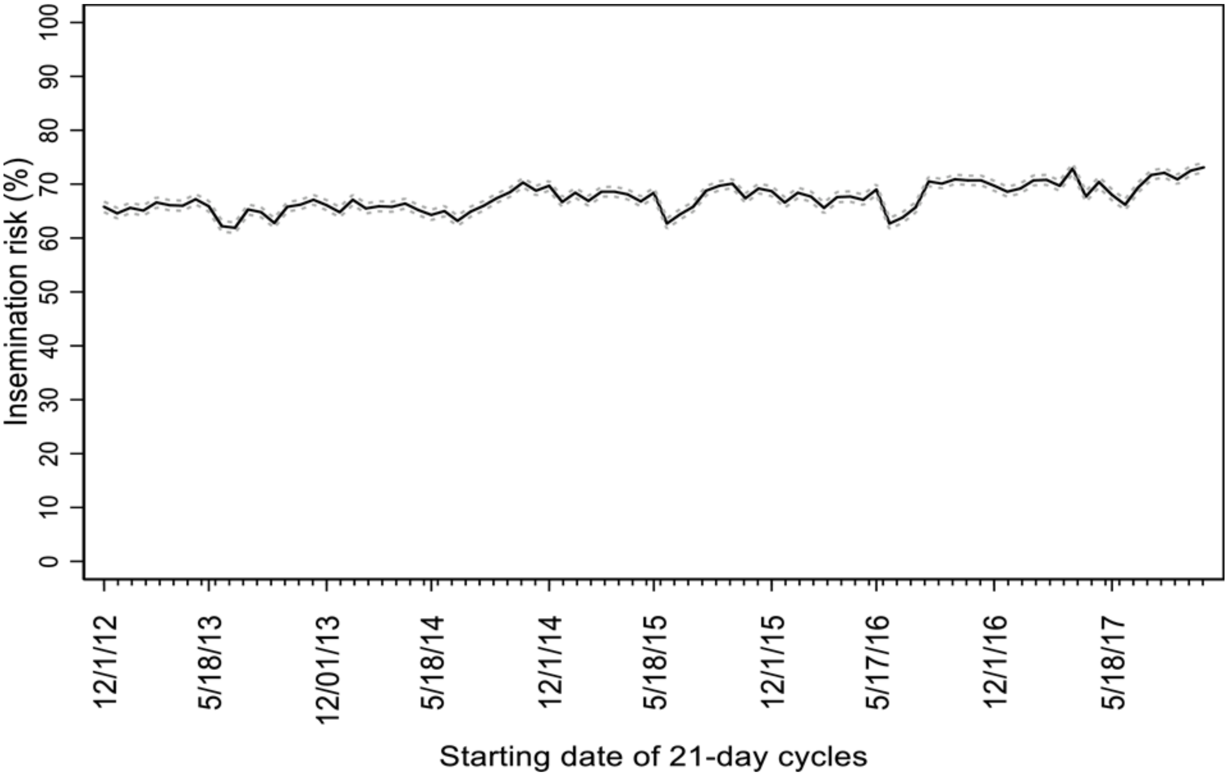

Figure 5.1. Insemination risk of 9 large dairy herds located in Kansas.

Black solid line represents mean insemination risk, and gray dotted lines represent 95% confidence interval for 21-day cycles (n = 85) starting December 1, 2012 through November 2, 2017.

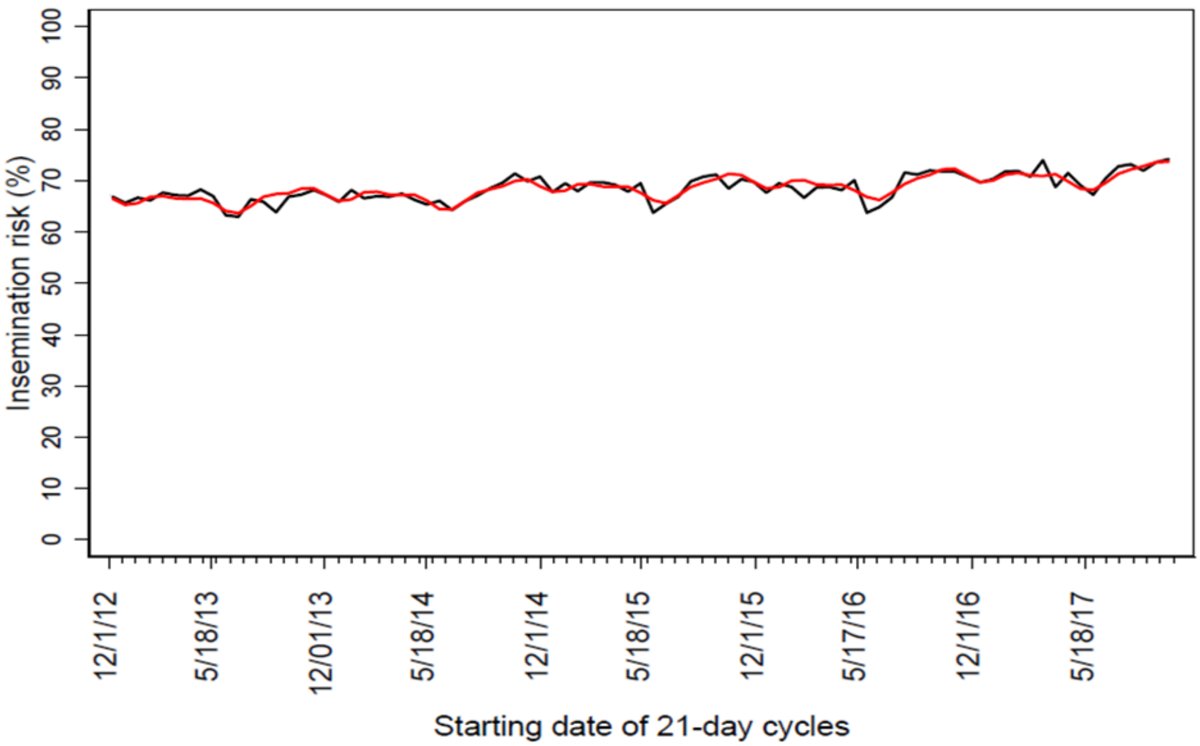

Figure 5.2. Time series model fit.

Black and red lines represent actual and estimated values for insemination risk, respectively.

Chapter 6 - Final Remarks

Findings reported in this dissertation add to the current knowledge regarding cow- and herd-level responses to heat stress in dairy herds. Studies presented herein provide compelling evidence that core body temperature of dairy cows during the dry period is a useful predictor for postpartum health and productive performance. These findings may be used to develop management strategies to offset the deleterious effects of heat stress in specific subgroups of cows more likely to be affected. In addition, this dissertation suggests that seasonal effects on herd-level insemination risk differ greatly from those traditionally reported for cow-level estrus expression. Furthermore, seasonal variation of insemination risk was studied in large commercial dairy herds to describe potential implications of heat stress and other seasonal stressors on a herd-level outcome (e.g., insemination risk).

Chapter 2 suggested that assessment of core body temperature during summer of dry cows housed in open dry-lots with 250 to 260 d of gestation may be a useful trait to identify cows more susceptible to be treated for health disorders during the subsequent lactation. In addition, cows identified as having core body temperature greater than the median value had reduced milk yield early in the next lactation compared with cows that presented temperature below this cut-off. To the best of the authors' knowledge, this was the first study to demonstrate an association between core body temperature of dry cows managed under similar environmental conditions and postpartum health and milk production. Nonetheless, core body temperature category during the dry period was not associated with reproductive performance after parturition.

Chapter 3 investigated factors associated with core body temperature in Holstein dry cows and focused on determining the ideal time of the day to assess core body temperature of heat-stressed dry cows. Furthermore, this study evaluated factors associated with core body temperature of Holstein dry cows during summer and demonstrated that core body temperature is increased in cows carrying twins and is negatively associated with gestation length. Furthermore, findings from the analyses suggest that 2215 h is the time of the day in which core body temperature assessment best describes the magnitude of hyperthermia that heat-stressed dry cows undergo throughout the day. In addition, this study indicated that core body temperature of dry Holstein cows can be estimated using statistical models that include dairy, twinning, and gestation length.

Chapter 4 aimed to compare concentration of pregnancy-associated glycoprotein, prolactin, and indicators of energy balance during the transition period in cooled cows classified as having high or low core body temperature during the dry period. In addition, this study investigated the association between core body temperature during the dry period and health, milk yield, and reproductive performance after parturition of cows cooled during the dry period. This experiment provided evidence that cows with high core body temperature during the dry period present distinct concentrations of pregnancy-associated glycoprotein and indicators of energy balance during the transition period and have impaired milk yield compared with cows that had low temperature. In addition, this experiment confirmed previous findings that indicated a negative association between core body temperature and gestation length, and greater temperature for cows carrying twins. Furthermore, core body temperature during the dry period was a useful predictor of postpartum health disorders.

Chapter 5 described temporal patterns of insemination risk in large dairy herds and investigated associations between insemination risk and herd-level traits such as housing, milk yield, and mortality. Despite the vast literature indicating that expression of estrus at the cow level is affected by heat stress, footing surface, and milk yield, associations between various factors and herd-level were explored in very few studies. Results reported in this study indicate that seasonal variation of insemination is minimal, with increased values observed during the autumn. In addition, findings from this study suggest that greater values of insemination risk are observed in dry-lot herds, in farms with low mortality of cows during the first 60 days in milk, and longer voluntary waiting period for primiparous cows. Because this study was conducted using only herds located in Kansas, extrapolation of findings to other production systems and geographic areas must be conducted with discretion.

In conclusion, findings reported herein suggest that assessment of core body temperature of Holstein cows during the dry period may be a useful tool to identify groups of cows more likely to present health disorders and impaired milk yield after parturition. This tool may be used to develop management strategies tailored to optimize postpartum health and productive performance of cows expected to present impaired performance after parturition, such as high temperature cows. In addition, insemination risk does not present important variations during summer, but it is severely affected by herd-level traits such as housing system, mortality of cows, and voluntary waiting period for primiparous cows. These findings may be useful to help dairy professionals engaged in improving reproductive performance of dairy herds to more carefully evaluate herd-level traits that prevent insemination risk to reach greater.