ASSOCIATIONS BETWEEN VAGINAL TEMPERATURE AND INDUCED ESTRUS EXPRESSION AND FERTILITY IN LACTATING HOLSTEIN COWS

by

Liam Benjamin Polsky

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Abstract

The objective of this study was to determine the effect of vaginal temperature on levels of walking activity expressed by lactating Holstein cows following induced estrus. Lactating Holstein cows (n = 641; 41.5 ± 9.4 kg milk/d) were fitted with a leg-mounted pedometer resulting in 843 evaluated activity episodes of estrus. Vaginal temperature was monitored using thermometers, attached to an intravaginal device that recorded vaginal temperature every 10 min for 3 d. Ambient temperature and relative humidity were monitored using an external thermometer placed in the center of each pen. Milk production and body condition score (BCS) were collected at the time of thermometer insertion. All statistical analysis was performed in R. Heat stress (HS) was calculated as the percentage of time the cow had a vaginal temperature ≥39.1°C (PCT39) 9-11 d prior to timed artificial insemination (TAI), and was classified as high or low (median: 22.9%). The mean vaginal temperature was 38.9 ± 0.2°C, whereas the mean maximum and minimum vaginal temperatures were 39.7 ± 0.5°C and 38.0 ± 0.8°C, respectively, with an average amplitude 1.71 ± 0.9°C. Mean relative increase (RI) of steps/h at estrus was 237.0 ± 160 %. Animals with low BCS were associated with lower RI compared to cows with medium BCS (260.31 ± 17.45% vs. 296.42 ± 6.62%). Lower temperature and humidity (THI) values (≤ 65) were associated with greater RI compared with medium (> 65 - < 70) and high conditions (≥ 70). There was no association between PCT39 on RI. Cows who displayed greater RI at estrus had stronger associations with pregnancy per artificial insemination (P/AI) compared with low RI (27% vs. 20%) and no RI (27% vs. 12%). An interaction was observed between PCT39 and THI on P/AI, where a subpopulation of cows with high PCT39 were associated with decreased P/AI under high THI conditions, but no differences in P/AI were observed for high PCT39 cows under medium and low THI conditions

(13% vs. 24% vs. 26%). Future research should aim to refine variables related to hyperthermia as well as understanding the effects of body temperature on estrus expression and fertility.

Preface

The experiment presented in this thesis was conducted at Fazenda Colorado, in Araras, São Paulo, Brazil. The data analysis and manuscript preparation was conducted at the University of British Columbia, Vancouver. The project and associated methods within this thesis were approved by the UBC Animal Ethics Committee (Protocol A10–0290).

A version of the material in chapter 2 has been submitted for publication: Polsky, L.B., A.M.L. Madureira, E.L. Drago Filho, S. Soriano, A.F. Sica, J.L.M. Vasconcelos, and R.L.A. Cerri. 2017. Associations Between Vaginal Temperature and Induced Estrus Expression and Fertility in Lactating Holstein Cows. The paper was co-authored by my supervisor, R.L.A. Cerri and two colleagues, A.M.L. Madureira, and J.L.M. Vasconcelos. Cerri and Vasconcelos supervised, assisted in concept formation and data analysis, as well as editing and providing comments on manuscript drafts. A.M.L. Madureira, E.L. Drago Filho, S. Soriano, A.F. Sica, were involved with data collection and processing. I was the lead investigator, responsible for all major areas of concept formation, material interpretation, data collection and processing, statistical analysis, and manuscript composition.

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List of Abbreviations

AAM - Automated activity monitors

ACTH - Adrenocorticotropic hormone

AMP – Vaginal temperature amplitude

BCS – Body condition score

CIDR – Controlled internal drug release insert

CL – Corpus luteum

DIM – Days in milk

DMI – Dry mater intake

FSH – Follicle stimulating hormone

GnRH – Gonadotropin releasing hormone

HS – Heat stress

LH- Luteinizing hormone

LS –Least squares

P/AI – Pregnancy per artificial insemination

PCT39 - the percentage of time the cow spent with a vaginal temperature ≥39.1°C, 9-11 d prior to TAI

OR – Odds ratio

RI – Relative increase

RH – Relative Humidity

ROS – Reactive oxygen species

SE – Standard Error

TAI – Timed artificial insemination

T_{db} – Dry bulb temperature

THI – Temperature and Humidity Index

TMR – Total mixed ration

TRP - Transient receptor potential

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Chapter 1: Introduction

1.1 Setting the scene:

Global surface temperatures have increased 0.2°C to 0.6°C since 2000, and are projected to increase to 5.8°C by the end of the century (IPCC 2007). Global warming will influence regional precipitation patterns, marked by an increase in periodic droughts and extreme rainfall events (von Keyserlingk et al., 2013). As a result, crop yields will decline and the quality of animal feed will deteriorate. Shifting climate patterns have increased environmental temperatures experienced by dairy cows in the summer months (Honig et al., 2016). Moreover, as the dairy industry consolidates the number of farms, but continues to increase the number of high yielding production animals, there is an increased risk of suboptimal microclimate conditions within the barn (Schüller et al., 2014). Heat stress (HS) has been shown to negatively affect the profitability of the farm through reductions in the animal's reproductive capabilities and production potential (Kadzere et al., 2002), in addition to compromising the animal's welfare (Silanikove, 2000). As a result, HS has become a prevalent issue in the dairy industry, challenging producers, academics, and industry professionals.

The classic work on dairy cow HS was pioneered by Brody (1945) when agriculture temperature recording began in the United States in the early 1940's. The majority of studies examining HS today are conducted in tropical or subtropical areas (Southwest USA, Mexico, Brazil) where the effects of HS on production animals are clearly evident. However, animals housed in northern latitudes (Central Europe, Northern USA, Canada) can also experience HS, where the summer season is relatively short but warm, in addition to the persistent presence of radiant solar energy and high humidity (Schüller et al., 2014).

Yousef (1985) originally defined HS as the magnitude of forces external to the body that displace physiologic systems from the resting state. Kadzere et al. (2002) modified this definition to include all high temperature forces that induce coping mechanisms, ranging from the sub-cellular level to the whole animal, in efforts to avoid physiological dysfunction. Heat abatement strategies employed by dairy cows include increased respiration rate, drinking, sweating, and standing time (West, 2003). Cows under HS have reduced milk production, dry matter intake (DMI), and physical activity (De Rensis and Scaramuzzi, 2003; West, 2003; Schütz et al., 2008). The animal’s reproductive capabilities will decline as HS disrupts follicular development, steroidogenesis, oocyte quality, and embryo development (Honig et al., 2016). As a result, HS is responsible for annual economic losses to the US livestock production industry ranging from US$1.69 to $2.36 billion, of which $900 million are specific to the US dairy industry (St-Pierre et al., 2003). In Canada, the economic impact of a cow dying from a heat wave (3 days of environmental temperatures ≥ 32 °C) ranges from CDN$2,000 –$2,500 per animal (Bishop-Williams et al., 2015).

The objective of this literature review is to describe the effects of HS on dairy cattle production characteristics, with special attention to reproduction. This review will begin by examining how body temperature is physiologically controlled in mammals, the internal and external factors which govern the body's circadian temperature rhythm, and the current technologies used to monitor body and environmental temperatures. We will then shift into a discussion of the current state of dairy cow reproduction, and the technologies used to monitor estrus behaviour and improve fertility. Finally, how body and environmental temperatures can influence an animal's estrus behaviour and reproductive capabilities will be investigated. The majority of evidence for this review focuses on the dairy cow, but draws from other mammalian species literature when available.

1.2 Environmental and body temperature range

1.2.1 What is a cow's normal body temperature? What climate do cows prefer?

The normal body temperature of dairy cattle is reported as 38.6 ± 0.5°C (Andersson and Jónasson, 1993), with daily variation ranging 0.8°C to 1.8°C (Piccione and Refinetti, 2003). However, single temperature readings of 39.5°C and higher have been documented during the first 10 DIM (Burfeind et al., 2014). Bitman et al. (1984) first showed the diphasic circadian body temperature pattern in dairy cows. The cycle consists of; 1) lowest body temperature midnight to early morning, 2) increasing mid-morning 3) elevated body temperature in the afternoon until late evening, 4) a second nadir until the following rise the next mid-morning.

The thermoneutral zone of dairy cattle ranges from 5°C - 25°C (depending on humidity), and within these temperatures the animal generates basal levels of metabolic heat, and has minimal physiological costs to reach maximum milk production and achieve acceptable reproductive success (Kadzere et al., 2002). The animal's thermoneutral zone depends on breed, feed intake and composition, production levels, housing and management, body condition, and behaviour (Yousef, 1985).

The lower critical temperature is defined as the ambient temperature that the animal's metabolic rate must increase to in order to maintain thermal balance, and ranges from -16°C to -37°C for dairy cows (Hamada, 1971). Under cold conditions, dairy cows will increase feeding time, thyroid function, and fat metabolism, to elevate their body temperature and energy conservation (Tucker et al., 2007). Comparatively, the upper critical temperature is defined as the ambient temperature that causes the animal to exceed its thermal homeostasis, and triggers thermoregulatory mechanisms to decrease internal temperatures. The upper critical temperature for Holstein cows is reported as 25°C-26°C, which is dependent on humidity (Berman et al., 1985). When the increasing thermal load exceeds the animal's evaporative heat loss capability, the cow's body temperature will continue to rise and can lead to death if thermoregulatory mechanisms cannot displace the incremental heat (Mader et al., 2006).

1.3 Physiology of temperature detection and thermoregulation

1.3.1 How does the body detect changes in temperature?

The body detects environmental temperatures through transient receptor potential (TRP) ion channels on nerve endings of neurons located in the dermis and epidermis (Collier and Gebremedhin, 2015), which have different temperature activation thresholds (Patapoutian et al., 2003). Once the external temperature is detected, the signal is sent from the TRP neurons via the spinothalamic tract to the thalamus and the preoptic area of the hypothalamus and anterior hypothalamus (Collier and Gebremedhin, 2015). The efferent information from the hypothalamus is sent to the medulla oblongata to activate sympathetic thermoregulatory mechanisms such as cardiovascular responses, skin vasodilation, and metabolic changes (Ishiwata et al., 2001). Sensory information can travel from the thalamus to the cerebral cortex for thermal perception (Collier and Gebremedhin, 2015).

1.3.2 What factors affect body temperature?

Metabolic processes in the dairy cow used for basal maintenance, body tissue synthesis, lactogenesis, and milk secretion, generate heat and contribute to the animal's total thermal load (Kadzere et al., 2002). High (>31.6 kg/d) and low (<18.5 kg/d) producing cows generate 48.5%

and 27.3% more heat respectively than dry cows (Purwanto et al., 1990). Moreover, increasing milk yield from 35 kg/d to 45 kg/d can increase ambient temperature HS sensitivity thresholds by 5°C (Bernabucci et al., 2014). Primiparous cows have a lower sensitivity to HS compared to multiparous cows (i.e. greater environmental temperatures are need to trigger HS), likely due to the fact that primiparous animals generate less metabolic heat stemming from lower milk production. Additionally, their smaller body size promotes a great surface area to mass ratio, which improves their thermoregulatory capabilities (Bernabucci et al., 2014).

1.3.3 How does the cow thermoregulate?

The primary heat abatement strategy for most mammals is evaporative heat loss, accompanied by an increased breathing and heart rate (Kadzere et al., 2002). Evaporative heat loss from dairy cows is enhanced by increases in sweating, panting, vasodilation, radiating surface area, and drinking water. Dairy cows utilize two types of perspiration for thermoregulation. Insensible sweating causes water to leave the body at all times unless the environmental relative humidity is 100%. Secondly, thermal sweating occurs as the main evaporative cooling mechanism in response to increasing ambient temperature. The heat required to convert water into vapour (2.43 J) is the amount of heat lost when 1 mL of sweat is evaporated from the animal's skin (Kadzere et al., 2002). Panting allows heat stressed dairy cows to increase their evaporative water loss via the tongue, mouth, and lungs. Approximately 15% of endogenous heat is lost from the body core via the respiratory tract (McDowell et al., 1976). The animal's respiratory frequency will start to exceed 50-60 breaths/min when ambient temperatures exceed 25°C, and if panting persists, cows will develop respiratory alkalosis (Cook et al., 2007). The increased CO_2 output will shift the blood's bicarbonate buffer system, resulting in higher blood pH for heat stressed cows (West, 2003).

In an attempt to alleviate their internal temperature, dairy cows will decrease their feed intake which reduces the heat generated by rumen fermentation (Gauly et al., 2013), and will adjust their feeding times to periods of the day when ambient temperatures are cooler (Schneider et al., 1988). The decrease in DMI results in a decrease in milk production (Kadzere et al., 2002; De Rensis and Scaramuzzi, 2003), and is responsible for 50% of the total decrease in milk synthesis (Rhoads et al., 2009). Heat stressed cows have altered lipid and carbohydrate metabolism, hallmarked by the lack of adipose tissue mobilization, increased insulin release, and increased glucose clearance (Wheelock et al., 2010). Additionally, HS cows will increase water consumption 1.2kg/°C increase in minimum ambient temperature (West, 2003), resulting in increased water content in their rumen (Silanikove, 1989) and decreased volatile fatty acid production via altered rumen pH (Kadzere et al., 2002). Decreased DMI has detrimental effects on steroidogenesis and subsequent fertility, which will be discussed later in this review.

1.4. Monitoring body and environmental temperature

1.4.1 How can you monitor body temperature?

Several technologies can be used to continuously monitor body temperature in cattle, such as rumen boluses, thermistors implanted in the ear canal or udder, sensors on milking equipment, infrared thermography, rectal thermometers, and vaginal loggers (Vickers et al., 2010; Burfeind et al., 2014; Talukder et al., 2015).

Rumen boluses are able to continuously monitor rumen temperature via an intraruminal telemetric sensing device (AlZahal et al., 2009). By using non-invasive rumen boluses, the cow's body temperature can be continuously recorded without subjecting the animal to stressful restraint procedures that can alter temperatures (Bewley et al., 2008a). Due to the activity of rumen microorganisms, rumen-reticular temperatures are 0.5°C greater than core body temperatures (Bewley et al., 2008a). One limitation for the use of this technology is caused by the anatomical location of the bolus. When cows ingest large quantities of water, there are substantial drops in recorded temperature, ranging from 8.5 ± 0.5°C, 6.9± 0.5°C, and 2.2 ± 0.5°C for cold, warm, and hot water respectively (Bewley et al., 2008b), and the deviations from basal temperatures can last more than 3h. Moreover, the physical behaviour of the cow has been shown to influence data transmission. When cows are standing, the base stations can retrieve 50% of the bolus' transmitted data, but the transmission rate declines below 40% when the animal is lying down (Ipema et al., 2008). Future research must address the large data deviations caused by drinking and water temperature, as well as improving sensor broadcasting strength.

Tympanic membrane temperature was first measured in humans by Benzinger (1959), as primary research indicated that the rectal temperature of humans was delayed in response to immediate HS events. Scientists postulated that the anatomical location of the probe near the hypothalamus could offer more accurate core body temperature readings (Taylor et al., 2014). Despite being used in thermal biology studies (Mader et al., 2002; Mader et al., 2010), tympanic temperature sensors are heavily influenced by facial skin temperatures, practicality and invasiveness, and if the probe is in contact with the tympanic membrane (Taylor et al., 2014). Moreover, Bergen and Kennedy (2000) reported a weak correlation between vaginal and tympanic temperature (r =0.77), limiting its use as an indicator for reproductive health.

Rectal temperature is the most common sampling method for collecting body temperature due to the ease of collection, low cost of thermometers, and physiological accuracy to core body temperature. However, the accuracy of the reading has been shown to be dependent on the operator and thermometer penetration depth (Suthar et al., 2013), and the process is time consuming and labour intensive. Discontinuous sampling times may not accurately capture the animal's diurnal temperature rhythm, and restraining the animal during data collection, and the possibility of rectal injuries, may induce stress responses and influence internal body temperatures (Bewley et al., 2008a).

Vaginal temperature can be continuously monitored in dairy cows, allowing the animal's diurnal circadian rhythm to be captured. Microprocessor temperature loggers can be attached to modified vaginal controlled internal drug release inserts (CIDRs) and placed into the vaginal cavity (Vickers et al., 2010; Suthar et al., 2011; Suthar et al., 2013). Vaginal temperature loggers have been validated both in vitro and in vivo (Vickers et al., 2010), and are correlated to rectal temperatures ranging from r=0.81 (Vickers et al., 2010) to r=0.94 (Suthar et al., 2013). Moreover, there is greater blood flow to the vagina compared to the rectum, making the vagina more sensitive to changes in core body temperature (Burdick et al., 2012). Research conducted in humans has shown higher vaginal temperature sensitivity to ambient temperatures when compared to the rectum and skin (Emmanuel et al., 2000). Reproductive tract temperature has direct implications on reproductive outcomes, as Gwazdaukas et al. (1973) first reported that a 0.5°C increase in uterine temperature decreased conception rates 6.9 to 12.8%. Moreover, elevated uterine temperatures create an inhospitable environment for the oocyte, sperm, and subsequent embryo survival (De Rensis et al., 2015). Increases in uterine temperature are associated with the production of heat shock proteins by the endometrium, and reduced interferon-tau production from the conceptus (De Rensis et al., 2015), which reduces embryo attachment (Ealy et al., 1993).

Vaginal temperature has also been used to detect estrus in beef (Kyle et al., 1998) and dairy cattle (Fisher et al., 2008). Increases in vaginal temperature coinciding with the LH surge, and subsequent ovulation, range from 0.48°C (Fisher et al., 2008), to ≥1.0°C (Rajamahendran et al., 1989). Kyle et al. (1998) detected 89.4% of estrus episodes when a peak in vaginal temperature was defined as an increase of 0.4°C for 3 or more consecutive hours, using a 2 or 3 d baseline temperature. However, it is not clearly established if the rise in vaginal temperature at estrus is caused by factors regulating GnRH secretion, or by the increase in physical activity during estrus. Studies conducted on humans found that during exercise, the increase in body temperature is independent of climatic conditions, and is proportionally related to the individual's metabolic rate (Sawka et al., 2001). However, Suthar et al. (2011) documented a rise in vaginal temperatures during the preovulatory LH surge in cows housed in tie stalls, a housing system that limits activity. This restricted mobility supports the idea that activity is not the only factor governing a rise in body temperature during estrus. The authors suggest that the thermogenic effect of progesterone can increase vaginal temperature, evidenced by rises in basal temperatures coinciding with progesterone secretion during the luteal phase (Suthar et al., 2011).

1.4.2 How can we measure environmental temperature?

The temperature and humidity index (THI) is a calculated index that incorporates the effects of environmental temperature with relative humidity. The unitless index was created by Thom (1959) to describe the effect of ambient temperature on humans, but has been successful applied to describing conditions that drive HS in dairy cattle (De Rensis et al., 2015).

The THI is divided into categories that potentially indicate the level of HS, but the definitions vary between authors and conditions. Armstrong (1994) used THI <71 as a thermal comfort zone, 72-79 as mild HS, 80-90 as moderate HS, and >90 as severe HS. Comparatively, De Rensis et al. (2015) defined THI <68 outside of the thermal danger zone for cows. Mild discomfort and production characteristics begin to decline between THI 68-74, and a THI ≥75 will cause drastic decreases in reproductive performance. Bohmanova et al. (2007) demonstrated that THI equations that weigh humidity more heavily are more appropriate for humid climates, while formulas that place more emphasis on T_{db} (dry-bulb temperature) are more appropriate for dry environments. Following exposure to temperatures of 40.5°C, humans can dissipate 190% of their metabolic heat through evaporative cooling, compared to Holstein cows, which can dissipate only 105%. Most of the cooling in humans occurs from the skin, compared to cows that use both the skin and their respiratory tract (Bianca 1962). Cattle can tolerate higher temperatures at lower relative humidity since heat dissipation is depended on evaporative cooling and sweating rates (Bohmanova et al., 2007).

THI can act as a rough indicator for the effects of HS on production measures, in lieu of knowing the animal's internal body temperature. Linvill and Pardue (1992) demonstrated that milk production begins to decline when the THI continuously exceeds 74 the previous 4 d before measurement. Exposure to THI ≥73 for 1 h on the day of breeding is sufficient to decrease conception rates by 5%, and pregnancy rates of Holsteins decline from 28.5% at low THI (<70) to 14.8% at high THI (>80) (Schüller et al., 2014). Studies have shown that the conception rate will begin to decline linearly for every unit increase in mean THI on the day of breeding (Schüller et al., 2014). Moreover, Holstein cows have longer calving intervals and days open during high THI months compared to low THI months (El-Tarabany and El-Bayoumi, 2015).

1.5. Heat stress and reproduction

1.5.1 What is the relationship between heat stress and reproduction?

Heat stress has detrimental outcomes on the reproductive capabilities of dairy cows due to the direct effects on the ovaries, follicular growth, oocyte quality, steroidogenesis, estrus behaviour, uterine environment, and embryo health. A study conducted by López-Gatius et al. (2004) found that the pregnancy loss rate for cows inseminated in the warm period was 3.7 times higher compared to animals inseminated in the cool season. Ultimately, the conception rates in the summer months can decrease 20-30% when compared to winter months (which can exceed 76%) (De Rensis et al., 2002; De Rensis and Scaramuzzi, 2003).

1.5.2 How does heat stress affect the hypothalamic-ovarian axis?

The body's primary response to an external stressor is to activate the sympathoadrenal system (Tilbrook et al., 2000). Thermoreceptors in the skin detect elevated temperatures (Chowers et al., 1966), which, depending on the temperature threshold, release ACTH from the hypothalamus, elevating the glucocorticoid concentrations in the blood (Collier et al., 1982). Cortisol acts as a vasodilator to shunt blood towards the skin to facilitate heat loss. Additionally, glucocorticoids stimulate lipolysis and proteolysis in efforts to combat the chronic negative energy balance caused by decreases in DMI (Cunningham and Klien, 2007).

Cortisol secretion caused by elevated temperatures disrupts reproductive function by suppressing pulsatile GnRH secretion, the subsequent LH pulse frequency (von Borell et al., 2007), and deprives the ovaries of sufficient gonadotrophin support (Dobson and Smith, 2000). These disruptions result in reduced ovarian cyclicity, decreased estradiol production by growing follicles, which culminates in ovulation failure (Dobson and Smith, 2000; De Rensis and Scaramuzzi, 2003; López-Gatius et al., 2005a).

The effects of HS on LH plasma concentration have been inconsistent. Gauthier et al. (1986) and Ronchi et al. (2001) reported that LH concentrations were unaltered, while other authors reported increases (Roman-Ponce et al., 1981). However, the majority of the literature reports a decrease in concentrations of LH (Madan and Johnson, 1973; Wise et al., 1988a; Gilad et al., 1993). The effects of HS on the LH preovulatory surge required for ovulation also shows contrasting results. The literature claims a decrease in the LH surge for heifers (Madan and Johnson, 1973) but not for cows (Gwazdauskas et al., 1981; Rosenberg et al., 1982; Gauthier et al., 1986). The difference in findings may be attributed to the animal's preovulatory estradiol levels, as animals with low estradiol plasma concentrations (cows) have decreased tonic LH pulses, compared to animals with high plasma estradiol (heifers) who maintain basal LH pulses (Gilad et al., 1993).

Cows under HS have higher plasma FSH concentrations (Roth et al., 2000), caused by decreased plasma inhibin concentrations (Wolfenson et al., 1995). As a result, cows will have an increase in the number of medium sized follicles, earlier second wave follicular emergence, and reduced follicular dominance (Roth et al., 2000). The decreased steroidogenic capacity of heat stressed follicles is caused by lower aromatase activity in the granulosa cells, and a subsequent decrease of estradiol in the follicular fluid (Badinga et al., 1993).

Estradiol is the main hormone governing estrus behaviour and ovulation (Allrich, 1994), evidenced by the positive correlations between activity intensity, duration and plasma concentrations (Lyimo et al., 2000; Lopez et al., 2004). Plasma estradiol concentrations are negatively affected by HS (Wolfenson et al., 1995; Wolfenson et al., 1997; Roth et al., 2000) and can result in delayed ovulation and ovulation of older follicles (Wilson et al., 1998). This follicular growth profile is consistent with a follicle that has developed in a low LH environment, resulting in reduced follicular dominance, and decrease in aromatase mRNA expression (Wolfenson et al., 2000).

Corpus luteum health and the levels of circulating progesterone are essential for dairy cow fertility. Progesterone is delivered to the uterus to maintain pregnancy (Wolfenson et al., 2000), and low plasma concentrations can lead to embryo implantation failure (Lonergan, 2011). The effect of HS on progesterone secretion is inconsistent, as some authors report unchanged (Roth et al., 2000) or increased progesterone concentrations (Badinga et al., 1994; Trout et al., 1998). However, the majority of studies report that progesterone concentrations decrease in the summer months (Wise et al., 1988b; Jonsson et al., 1997; Ronchi et al., 2001; Wolfenson et al., 2002). These studies demonstrate that HS has negative chronic effects on ovarian cyclicity, as a follicle that was damaged during a HS episode will mature into an impaired corpus luteum incapable of sufficient progesterone synthesis. Moreover, progesterone concentrations are determined by the production rate by the corpus luteum and the rate of hepatic metabolism, which are both influenced by DMI (De Rensis and Scaramuzzi, 2003). High producing dairy cows have high DMI (Hommeida et al., 2004) and a faster rate of steroid metabolism compared to non-lactating cows (Sangsritavong et al., 2002). Vasconcelos et al. (2003) highlighted that feeding patterns can acutely reduce progesterone concentrations by 25%.

1.5.3 How does heat stress impair follicular development?

The physiological state of the follicle is governed by the ovarian blood supply, which through the delivery of hormones and nutrients, dictates the rate of growth, atresia, and ovulation (Honig et al., 2016). Heat stress has been shown to depress follicle dominance, causing a 2-3 day earlier emergence of the second wave of preovulatory follicles (De Rensis and Scaramuzzi, 2003).

An earlier emerging follicle will have an extended dominance period, (caused by earlier follicular emergence and delayed ovulation) resulting in the ovulation of older an older follicle, which has been associated with decreased fertility (Wolfenson et al., 2000) and reduced embryo quality (Cerri et al., 2009). Moreover, HS lowers the dominance capacity of the largest follicle, resulting in the simultaneous growth of 50% more large follicles (Wolfenson et al., 2000), and causing the increase in twinning observed during warmer months (Ryan and Boland, 1991).

1.5.4 Can heat stress impair embryo development?

Newly conceived embryos are negatively affected by HS due to the increases in reactive oxygen species (ROS) produced in the oviduct and by the embryo (Hansen, 2009), coupled by a decrease in embryonic glutathion peroxidase synthesis (Ozawa et al., 2002). However, as the embryo continues to develop, the susceptibility to the negative effects of HS declines. Heat stress exposure on day 1 following estrus will reduce the proportion of embryos that develop to blastocysts, but maternal HS on days 3, 5, and 7 had no effect on the proportion of blastocysts at day 8 (Ealy et al., 1993). There is some evidence suggesting that the embryo can develop thermotolerance to the increased environmental temperature, enabling more resistance to future thermal stress episodes (Hansen, 2009). This effect is driven by the embryo's ability to decrease the production of ROS, and increase glutathione peroxidase synthesis (Hansen, 2009). The production of heat shock proteins by the embryo can be activated at day 2 of embryonic development (Edwards and Hansen, 1997), allowing for stabilization of intracellular proteins, and inhibiting apoptosis (Brodsky and Chiosis, 2006).

The negative effects of HS on embryo development carry over to the implantation phase, as cows that become pregnant during the warm period, with singletons or twins, were 3.7 and 5.4

times more likely to miscarry, respectively (López-Gatius et al., 2004). Moreover, during day 21-30 of gestation, when blastocyst implantation occurs (Guillomot et al., 1991), exposure to THI>69 can lead to a 12% increase in pregnancy loss (García-Ispierto et al., 2006). In efforts to combat early pregnancy loss caused by HS during the follicular phase, as well as the first 7 days of embryonic development, dairy farmers have opted to perform embryo transfer over Timed AI (TAI) (Rutledge, 2001; Al-Katanani et al., 2002). Bovine embryos become more resistant to HS during their development (Ealy et al., 1993), and can be successfully transferred into recipient cows that were not detected in estrus (Al-Katanani et al., 2002). When compared to TAI, fresh *in vitro* embryos improve pregnancy rates during HS conditions (Al-Katanani et al., 2002), especially for high producing animals that are already at risk for lower conception rates (Demetrio et al., 2007). However, research has shown that rectal temperature of the recipient cow 7 d after estrus negatively influences pregnancy rate at day 25, and that recipient cows with a higher body temperature on the day of embryo transfer had a lower probability of pregnancy and higher probability of embryonic loss (Vasconcelos et al., 2006). Despite heifers being more tolerant of high ambient temperatures, they also have decreased embryo transfer pregnancy rates, and produced more poor quality embryos during exposure to HS (Chebel et al., 2008). Additional research is required to understand how the delayed effects of HS affect both the donor and recipient cow used for embryo transfer procedures.

1.6. Chronic effects of heat stress

1.6.1 Why are the negative effects of heat stress so tenacious?

While the reduction in conception rate is associated with warmer months, fertility continues to remains low in autumn months despite the fact that ambient temperatures decrease and cows are no longer exposed to immediate HS (Wolfenson et al., 2000). This phenomenon accounts for more than 1/3 of “low summer fertility syndrome” (Wolfenson et al., 2000), and is speculated to be driven by the delayed effects of HS on the ovaries and follicles (Roth et al., 1997). A 41 d period is required for small antral follicles (0.13 mm) to develop to the preovulatory size (8.56 mm) (Lussier et al., 1987), implying that antral follicles can be damaged from prior HS exposure. Estradiol concentrations collected from first wave follicular fluid during d 7 of the estrous cycle in autumn was 1/3 of the concentration found during the same stage in the winter (Wolfenson et al., 1997). The reduced steroidogenic capacity of the follicles is further evidenced by cows who were heat stressed during d 2-6 of the estrous cycle, and had medium sized follicles obtained on d 3 of the subsequent cycle. The authors found that in previously HS cows, granulosa and theca cells from medium sized follicles produced ⅓ and ¼ of the estradiol and androstendione respectively, compared with non-heat stressed cows (Roth et al., 1997). Follicles from previously heat stressed cows that were aspirated for 4 consecutive estrous cycles in the autumn showed improving quality in later cycles, suggesting that frequent follicle turnover can lead to faster emergence of healthier oocytes following HS (Roth et al., 1999). Honig et al. (2016) suggest that management practices, which shorten the estrous cycle, such as frequent barn cooling cycles, may improve the fertility of dairy cows during HS conditions by increasing blood flow to the ovaries.

1.7. Dairy cow reproduction today

1.7.1 What is estrus? How do we know that the cow is ready to be bred?

Estrus is the period of sexual receptivity in dairy cows hallmarked by ovulation. Total estrus activity, defined by the animal's cumulative activity time above a threshold, ranges 10 - 12 h (Løvendahl and Chagunda, 2010; Madureira et al., 2015a), followed by ovulation 19.4 ± 4.4 h after the end of high activity (Roelofs et al., 2005a).

The classic sign of estrus in dairy cattle is standing to be mounted by another cow (Roelofs et al., 2010), and ranges between 7 - 9 mounts for both cows and heifers (Lopez et al., 2004; Rivera et al., 2010). The duration of standing estrus ranges from 5.5 - 9.5 h (At-Taras and Spar, 2001; Lopez et al., 2004). Secondary signs include sniffing the vulva of another cow, flehmen, chin resting, mounting, and restlessness (Van Vliet and Van Eerdenburg, 1996; Roelofs et al., 2010). Historically, most farmers used visual estrus detection, but this method has become impractical due to increasing herd sizes, labour demands, and low detection efficiency (At-Taras and Sphar, 2011). As a result, TAI protocols were developed to hormonally manipulate the cow's estrus cycle (Pursley et al., 1997). These synchronization protocols negate the need for estrus detection and can improve the number of cows submitted to AI. However, pharmacological intervention in food animal production is increasingly under public scrutiny in North America (Frewer et al., 2013). In Europe, synchronization programs are not commonly used due to the cost of treatment, as well as consumer reluctance to use products from animals treated with hormones or antibiotics (Chanvallon et al., 2014).

1.7.2 How is estrus controlled?

The hypothalamus, in close conjunction with the anterior pituitary, coordinates the expression of reproductive behaviours and physiological actions based on environmental cues and hormonal input (Simerly, 2002). Estradiol and progesterone are the main hormones governing sexual behaviour in mammals (Woelders et al., 2014).

The hypothalamic nuclei governing reproductive behaviour are the Ventromedial Nucleus (VMH) and the Medial Preoptic Area (MPA), which includes the Anteroventral Periventricular Nucleus (AVPN) (Woelders et al., 2014). The VMH and MPA neurons respond to various chemosensory and environmental cues (e.g.: pheromones, mounting behaviour attempts by other cows). The AVPN plays a key role in controlling the preovulatory LH surge required for ovulation due to its connectivity to GnRH neurons (Simerly, 2002). During estrus events, estradiol is bound to the VMH and MPA through two estrogen receptors, ER_{α} and ER_{β}, which bind the hormone with equal affinity, but have different distributions in the body (Woelders et al., 2014) and varying expression during different stages of the estrus cycle (Van Eerdenburg et al., 2000). Estradiol-dependent gene expression in the hypothalamus is associated with higher intensity estrus scores (Woelders et al., 2014). van Eerdenburg et al. (2000) highlighted that control of estrus behaviour is dependent on expression of ER_{α} in the MPA and VMH, as well as estradiol concentrations high enough to elicit sexual behaviour. The efferent neurons leaving the MPA and VMH elicit motor reflexes, such as the lordosis position in female rats (Carlson, 2013) to signal receptivity for breeding.

1.7.3 Wouldn’t life be easier if the cow told us she was ready for breeding?

A major behavioural change during estrus is the increase in physical activity and restlessness exhibited by cows (Roelofs et al., 2010), as the number of steps is two to four times higher when compared to diestrus activity (Kiddy, 1997). Pedometers (automated activity monitoring systems – AAM) have emerged as an alternative estrus detection method that is based on increased activity during estrus. The AAM signal a potential breeding alert when the animal's activity levels have exceeded a predetermined threshold. AAM can be attached to the neck to capture head and neck acceleration as a physical activity index, or to the animal's legs to monitor steps, movement acceleration, and lying time/bouts.

Current research highlights the benefits of AAM which improve dairy cow conception rates. Most studies report detection rates above 80% (Roelofs et al., 2010), but can be close to 90% positive predictive value depending on which detection system is used (Madureira et al., 2015a). Based on activity increase, Aungier et al. (2012) correctly identified 72% of cows' preovulatory phases, supported by Valenza et al. (2012) who detected 71%. Most AAM are able to quantify the duration and intensity (the increase in activity relative to baseline levels) of estrus events, which have a strong positive relationship with conception risk (López-Gatius et al., 2005b; Burnett et al., 2014; Madureira et al., 2015a). AAM have more accurate estrus detection and conception rates when compared to visual detection (Peralta et al., 2005).

1.7.4 What factors influences a cow's physical activity?

A variety of management and environmental factors influence dairy cattle walking activity. Cows housed in freestall barns are 2.75 times more active during estrus than when not in estrus (Stevenson et al., 2014). Estrus behaviour is more intense when animals form sexually active groups and experience estrus at the same time (Roelofs et al., 2005b). Within a sexually active group, cows received more behavioural points, displayed more mounts and took more steps. (Roelofs et al., 2005a, 2005b). Heifers have been documented to form sexually activity groups which can facilitate more behavioural estrus (i.e.: chin resting and conspecific following) (Silper et al., 2015). Increasing parity and 1 kg increase in milk yield are associated with a 21.4% and 1.6% decrease in estrus activity, respectively (López-Gatius et al., 2005b). Lame cows will also have decreased estrus duration and intensity of walking behaviour (Walker et al., 2008; Burnett at al., 2017). Breed can significantly affect walking activity, as Jerseys have shorter duration and less intense behaviour when compared to Red Danes and Holsteins (Løvendahl and Chagunda, 2010).

Environmental factors such as rain, wind speed, temperature, and humidity significantly suppress the expression of estrus behaviour (Roelofs et al., 2010).

1.7.5 How does heat stress affect estrus activity?

Hansen and Aréchiga (1999) reported reduced estrus behaviour in heat stressed dairy cows, speculating that HS induces physical lethargy as a coping mechanism to reduce internal heat production. As a result, almost 80% of estrus episodes during elevated environmental temperatures are undetected (De Rensis et al., 2015). Walking activity during warm periods is significantly lower than during cooler temperatures (369 ± 152% vs 384 ± 156% relative increase, respectively; López-Gatius et al., 2005b), and shorter estrus episodes are documented when *B. taurus* (compared to *B. indicus*) breeds are moved to tropical areas (Orihuela, 2000). Yániz et al. (2006) found that increases in mean relative humidity higher than 95% were associated with a decrease in walking activity at estrus. Heat stress is associated with delayed ovulation of the dominant follicle, and shorter standing times (White et al., 2002), and decreased steps/h in beef cattle (Sakatani et al., 2012).

1.8 Applications of body temperature

1.8.1 Why does the farmer need to know the cow's temperature?

Body temperature is one of the most useful indicators of the relationships between the cow and environmental factors, diseases, and physiological functions such as nutrition, lactation, and reproduction. One of the major benefits of continuous vaginal temperature monitoring is the ability to capture the individual temperature variation within animals, and identify their unique temperature profile. Equipped with this information, farm managers can identify subgroups of cows that are at high risk for HS complications that may require proactive health or reproductive management. Moreover, individual temperature monitoring can be used to evaluate the cow's response to farm heat abatement strategies such as fans, misters, or sprinklers.

1.8.2 Relationship between body and environmental temperature

Some research demonstrates a weak relationship between ambient and vaginal temperature (Verwoerd et al., 2006; Suthar et al., 2012), but this finding could be attributed to the large variation in individual temperature profiles among animals, likely caused by their ability to buffer against fluctuations in ambient temperature. Moreover, the confined and cross ventilated designs of modern barns ensure stable environmental conditions, which are not always found in studies conducted in tropical climates (Kendall et al., 2006). Individual temperature variation and responses to ambient temperature could be used as criteria for genetic selection of cows more tolerant of HS.

1.9 Thesis objectives and hypotheses

Currently, activity monitors require increased scientific validation to optimize their performance and accuracy. There is a scarcity of studies that investigate physiological parameters and environmental temperature events associated with estrus activity. The objective of this research paper is to examine how vaginal and environmental temperatures, measured 9-11 d prior to estrus, can affect walking behaviour of dairy cows following induced estrus. This paper will also investigate risk factors for estrus walking behaviour as well as pregnancy per AI (P/AI). We hypothesize that as vaginal temperatures increase, estrus activity and P/AI will decrease.

Chapter 2: Associations Between Vaginal Temperature and Induced Estrus Expression and Fertility in Lactating Holstein Cows

2.1 Introduction

New precision dairy farming technologies allow for continuous monitoring of animal behaviours, which in turn can be used to improve herd health and reproduction management. Automated activity monitors (AAM) utilize behavioural increases in physical activity and restlessness displayed during estrus to correctly detect estrus (Roelofs et al., 2010) and can predict ovulation timing (Roelofs et al., 2005a; Stevenson et al., 2014). Current research highlights estrus detection rates above 87% (Aungier et al., 2012), but can be close to 90% positive predictive value depending on which detection system is used (Madureira et al., 2015a). Moreover, AAM technologies can capture the intensity and duration of estrus behaviour, as both have a strong positive relationship with conception risk (Garcia et al., 2011; Madureira et al., 2015a). AAM have been shown to improve estrus detection and conception rates when compared to visual detection (Peralta et al., 2005).

Environmental factors such as rain, wind speed, temperature, and humidity can affect the expression of estrus behaviour (Roelofs et al., 2010). Global surface temperatures have increased 0.2°C to 0.6°C since 2000, and are projected to increase to 5.8°C by the end of the century (IPCC, 2007), increasing HS concerns facing dairy producers. This is particularly worrisome given that the thermoneutral zone of dairy cattle ranges from 5°C to 25°C (dry bulb temperature). Within this temperature interval, the animal generates basal levels of metabolic heat, and has minimal physiological costs to reach maximum milk production and achieve reproductive success (Kadzere et al., 2002). Heat stress is defined as the magnitude of high temperature forces, external to the body, which cause the animal to exceed its thermoneutral zone and induce coping mechanisms to avoid physiological dysfunction (Kadzere et al., 2002).

Heat stressed cows have reduced milk production, DMI, and physical activity (De Rensis and Scaramuzzi, 2003; West, 2003; Schütz et al., 2008). Reproductive capabilities of dairy cattle are decreased as HS disrupts follicular development, steroidogenesis, oocyte quality, and embryo development (Honig et al., 2012). The major effects of HS on dairy reproduction include the lower percentage of cows detected in estrus (Thatcher and Collier, 1986), and the reduction in conception to AI (Pereira et al., 2015). Consequently, HS is responsible for annual economic losses to the US livestock production industry ranging from US$1.69 to $2.36 billion, of which $900 million are specific to the USD dairy industry (St-Pierre et al., 2003).

Vaginal temperature can be continuously monitored in dairy cows, allowing for the capture the cow's diurnal circadian rhythm without subjecting the animal to stressful restraint procedures that can alter core temperatures. Reproductive tract temperature has direct implications on reproductive outcomes, as Gwazdaukas et al. (1973) first reported that a 0.5°C increase in uterine temperature decreased conception rates 6.9% to 12.8%. However, only a dearth of studies has investigated the relationship between vaginal and environmental temperature events with estrus expression and subsequent fertility. There is large opportunity for this field to expand, particularly once more activity monitors are subjected to scientific validation to optimize their performance and accuracy.

The objective of this research was to examine how vaginal and environmental temperatures recorded 9-11 d prior to breeding can affect physical activity in lactating dairy cows following induced estrus. This paper also investigated risk factors that could affect physical activity during estrus as well as pregnancy per AI (P/AI). We hypothesized that vaginal temperature increases would coincide with environmental temperature increases and collectively cause reductions in estrus expression and P/AI.

2.2. Materials and methods

2.2.1 Location, animals and diet

This experiment was conducted from February 2014 to February 2015 on a commercial dairy farm located in Araras, São Paulo State, Brazil: 22.3605° S, 47.3798°W and 629 m elevation. At the time of the experiment, the herd size was approximately 1,700 lactating cows with a 305 d average yield of 11,438 kg per cow. Lactating Holstein cows (n = 641; 209 primiparous; 432 multiparous) that were more than 50 DIM were enrolled onto this study on a weekly basis.

Cows were housed in a cross-ventilated free stall barn with grooved concrete floors. Cows were blocked in groups of 300 animals in pens with 2 rows of deep-bedded sand stalls (1 cow/stall). Milking was performed thrice daily (at approximately 0500 h, 1300 h and 2100 h) using a 72-stall rotary parlour. Cows were fed a TMR balanced to meet or exceed the nutritional requirements of lactating dairy cows producing 45 kg/d of 3.5% fat corrected milk (NRC, 2001). Feed was delivered twice daily at approximately 0700 h and 1600 h and pushed up 3 times/d. Water and TMR were available for *ad libitum* intake.

Cows enrolled in this study were restrained in headlocks at the feed bunk after the morning milking during the performance of experimental procedures. All experimental procedures were approved by the UBC Animal Ethics Committee (Protocol A10–0290).

2.2.2 Measurement of THI and vaginal temperature.

A humidity and temperature-measuring instrument (HOBO U23 Pro v2 Relative Temperature/Humidity Data Logger, Onset® HOBO® Data Loggers, Cape Cod, Massachusetts, USA) was placed in a protected area closest to the bed or feed bunk in each pen. Temperature and humidity data was recorded every 10 min for three consecutive days. Software provided by the company (HOBOware, Onset® HOBO® Data Loggers, Cape Cod, Massachusetts, USA) was used to read the recorded temperature and humidity data. The THI was calculated using Mader et al. (2006); $\text{THI} = (0.8 \times \text{T}^{\circ}\text{C}) + [(\text{RH}/100) \times (\text{T}^{\circ}\text{C} - 14.4)] + 46.4$.

A temperature recording data logger (Thermochron iButton, Lawrenceburg, KY, USA) was coupled to an intravaginal progesterone implant (Eazi-Breed, Zoetis, São Paulo, Brazil) allowing the sensors to be in direct contact with the vaginal wall, and recorded vaginal temperature continuously every 10 minutes for 3 d, 9-11 d prior to TAI.

Body condition score (1 to 5 scale at 0.25 increments; Wildman et al., 1982), documented by trained researchers, and milk production (AfiMilk MPC milk meter, Afikim, Israel) were recorded at the time of thermometer insertion.

2.2.3 Vaginal temperature analysis

Different definitions of HS were screened to determine the most appropriate way to quantify HS: the total number of temperature bouts (number of 'episodes' when the cow's body temperature exceeded 39.1°C), peaks of highest temperature, temperature amplitude (AMP), and total heat load (area under the curve for the total time and highest temperature episode greater than 39.1°C) were calculated. There was no relationship between any of the aforementioned variables and RI, P/AI, or THI (Table 2.1) and thus will not be discussed further.

PCT39 was chosen to represent vaginal temperature due to the negative relationship between RI, P/AI, and lack of co-linearity with THI. PCT39 represents the daily time (in percentage units) an animal is under HS.

2.2.4 Reproductive program and pregnancy diagnosis

Following the expected voluntary waiting period of 55 ± 5 DIM, cows were enrolled into an ovulation synchronization protocol for first AI and following negative pregnancy diagnoses. Cows were presynchronized beginning at 32 ± 6 DIM using two injections of 25 mg (i.m.) of $PGF_{2\alpha}$ (dinoprost tromethamine; Lutalyse, Zoetis, SP, Brazil) given 14 d apart. Cows were then bred based on the protocol described by Pereira et al. (2015): Eleven d following the second $PGF_{2\alpha}$ injection, cows were bred according to the following synchronization protocol: [D-11] intravaginal progesterone implant (Eazi-Breed containing 1.9 mg of progesterone, Zoetis, SP, Brazil) + 2.0 mg (i.m.) of estradiol benzoate (Gonadiol®, Zoetis, SP, Brazil) + 100 µg (i.m.) of gonadotropin-releasing hormone (Gonadorelin Diacetate; Cystorelin®, Merial, SP, Brazil), [D-4]: 25 mg (i.m.) of dinoprost tromethamine (PGF; Lutalyse, Zoetis, SP, Brazil), [D-2]: removal of intravaginal progesterone insert + 1.0 mg (i.m.) of estradiol cypionate (ECP, E.C.P., Zoetis, SP, Brazil) + 25 mg (i.m.) of dinoprost tromethamine, [D0]: TAI performed using commercial semen.

Positive pregnancy diagnosis was determined using ultrasonography when the presence of an amniotic vesicle with viable embryo via per rectum palpation was noted (Honda HS 101V with a 5.0 MHz linear probe, Honda, Japan) 32 and 60 d after TAI.

2.2.5 Automated activity monitor data

All cows were equipped with a pedometer (Afimilk Pedometer Plus Tag, AfiMilk, Kibbutz Afikim, Israel) that was attached to one of the hind limbs within one wk of calving. Data was wirelessly transferred from tags to a database every 8 h in blocks of 2 h. Data (steps/h) was retrieved from AfiFarm Dairy Farm Management Software (AfiMilk, Kibbutz Afikim, Israel) and compiled using Excel (Microsoft Corporation, Redmond, WA).

Steps per hour were recorded on a 24 h basis and averaged into three 8 h sessions/d (0000h to 0759h, 0800 to 1559h, and 1600h to 2359h). Steps recorded during induced estrus were calculated from the 8h session with the greatest number of steps on d -1 d 0, d 1. Baseline steps/h was calculated from the average of the 8h session with the greatest number of steps on d-11, d -10, d -9 session (Figure 2.1).

2.2.6 Statistical analysis

This experiment was an observational cohort study, with a total of 843 estrus events recorded. All statistical analyses were performed using R (version .99.903; Boston, MA, USA). Descriptive statistics and normality tests were obtained using the STATS package (R Core Team, 2016). Normality was visually assessed using Q-Q plots and confirmed using the Shapiro-Wilks test. Classified variables used for analysis are described below. Milk production was classified into high or low based on the median production (41.5 kg/d). Lactation stage at thermometer insertion was classified by DIM: early (≤ 100 DIM), mid (>100 - <180 DIM), and late (≥ 180 DIM) lactation. Parity was divided into cows in first lactation, or second lactation and higher (primiparous vs. multiparous). BCS was classified as low (≤ 2.50), medium (= 2.75) and high (≥ 3.00).

The relative increase (RI) in estrus walking activity was calculated from the number of steps taken before (baseline) and after estrus induction (induced estrus steps) (steps/h) using the calculation: [(induced estrus steps/h - baseline steps/h) /baseline steps/h] * 100. RI was categorized as: no heat: (≤ 69%), low intensity (70% - 200%), and high intensity (≥ 200%) as previously described by Madureira et al., (2015b). THI was classified as low (≤ 65), medium (65-70), and high (≥ 70) by dividing THI data into 33rd percentiles. Continuous vaginal temperature data were transformed into new class variables. PCT39 is the percentage of the time during 3 d data collection when the vaginal temperature was ≥ 39.1°C, and was selected to represent the threshold indicating HS. PCT39 was classified as high or low (median: 22.9%).

Pearson's correlation coefficients, univariable relationships, and significance levels were calculated for the relationships between animal measurements (BCS, DIM, milk yield, parity and PCT39), automatic measurements (RI), and environment measurements (THI) using the HMISC package (Frank et al., 2016). Continuous data were analyzed by ANOVA using the LME4 package (Bates et al., 2015). The outcome variable was RI, and the model's fixed effects included milk production, parity, DIM, BCS, PCT39, and THI with cow as a random effect.

The P/AI was evaluated by logistic regression using the GLM package in R (R Core Team, 2016). The model built by the logistical regression included the fixed effects of RI, milk production, DIM, parity, BCS, and PCT39. Using backwards stepwise elimination variables were removed from the model using the Wald statistic criterion ($P > 0.15$). Significance was set at $P < 0.05$, and $0.05 < P < 0.10$ was considered a tendency.

2.3 Results

2.3.1 Environmental and vaginal temperature

The mean (± SD) calculated THI was 67.7 ± 3.4, with maximum and minimum values of 73.8 and 61.0 respectively. The mean (± SD) vaginal temperature during the 9-11 d recording period was 38.9 ± 0.24°C, with mean maximum and minimum temperatures of 39.7 ± 0.46°C and 38.0 ± 0.75°C, respectively. The average vaginal temperature amplitude was 1.70 ± 0.87°C. The mean PCT39 was 27.6 ± 23.1%, with a Q1 and Q3 distribution of 8.5% and 40.8%, respectively. The Pearson correlation coefficient between PCT39 and THI was r = 0.32 ($P < 0.001$). The maximum vaginal temperature and amplitude were also weakly correlated to THI (r = 0.28; ($P < 0.001$), and r = 0.17; ($P < 0.001$)), respectively.

2.3.2 Animals and description of estrus events

The AAM detected a total of 843 estrus events that were then used for the analyses of risk factors affecting RI. Estrus events were considered as 'true' events if the RI exceeded 70%. All true estrus events were followed by AI and subsequent pregnancy diagnosis performed on day 32 after AI and again on day 60 if previously diagnosed pregnant. The mean RI recorded by the monitors was 237.4 ± 160.1% and the mean baseline activity was 198.5 ± 71.5 steps/h. The average (± SD) milk production was 41.5 ± 9.4 kg/cow/d. At the time of data collection, the mean DIM was 164.3 ± 105.9 d. The percentage of animals submitted for first, second, and third AI were 26% (214/834), 19% (158/834), and 55% (462/843), respectively.

2.3.3 Factors affecting relative increase of estrus expression

The cow's BCS had a strong association with estrus behaviour, as animals with a low BCS exhibited a lower RI compared to animals with medium BCS ($P < 0.05$; Table 2.2). The DIM at the time of estrus was associated with RI as cows in early lactation showed lower RI compared to mid-lactation as well as late lactation animals ($P < 0.05$; Table 2.2). Lower THI values (≤ 65) were associated with greater RI compared with medium (> 65 - < 70) and high conditions (≥ 70) ($P < 0.01$; Table 2.2), respectively. Univariable analysis showed associations between THI on PCT39 ($P < 0.02$; Figure 2.2). Univariable analyses also showed associations between PCT39 ($P < 0.02$; Figure 2.2) and THI ($P < 0.01$; Figure 2.2) on RI, but when THI was included in the statistical model, PCT39 no longer remained statistically significant (*P* < 0.79; Table 2.2). Given that PCT39 and THI were only weakly correlated, we included both in the statistical model (r = 0.32; *P* < 0.001). There was no association noted between milk production (*P* < 0.13; Table 2.2) and a weak association between parity and RI (*P* < 0.06; Table 2.2).

2.3.4 Risk factors for pregnancy per AI

The P/AI was strongly associated with both RI expression and RI intensity. Cows that expressed high RI compared to no RI were 2.87 times more likely to conceive (27% vs. 12%; *P* < 0.01; Table 2.3). Cows that expressed more intense RI compared to low RI had a 1.54 times higher pregnancy rate (27% vs. 20%; *P* < 0.05; Table 2.3). Multiparous cows' likelihood of pregnancy was 0.66 times lower compared to primiparous cows. (20% vs. 27%; *P* < 0.03; Table 2.3). Cows in late DIM had 0.58 and 0.44 times lower pregnancy rate than cows in mid and early lactation (16% vs. 22% vs. 26%; *P* < 0.01; respectively; Table 2.3). An interaction was observed between PCT39 and THI on P/AI (*P* < 0.03; Figure 2.3). A subpopulation of cows with high PCT39 were associated with decreased P/AI under high THI conditions, but no differences in P/AI were associated with high PCT39 cows under medium and low THI conditions (13% vs. 24% vs. 26% *P* < 0.01). P/AI was not associated with BCS (*P* = 0.90; Table 2.3) or milk yield (*P* = 0.82; Table 2.3). There was no interaction between PCT39 and RI expression on P/AI (*P* = 0.76; Figure 2.4) or between THI and PCT39 on RI expression (*P* = 0.14; Figure 2.5).

2.4 Discussion

In the current study, we assessed the association between vaginal temperature and other risk factors on the RI of estrus walking behaviour, as well as P/AI, in lactating dairy cows. We assessed the animals' experience of HS by examining the percentage of time the cow's vaginal temperature ≥ 39.1°C, but found no association with estrus RI when including THI. We observed an interaction between PCT39 and THI on P/AI, by identifying a subgroup of animals that have low P/AI and high PCT39, under high THI conditions.

A primary objective of this study was to describe the association between heat load and estrus expression and fertility. Vaginal thermometers are a non-invasive method that allows body temperature to be continuously recorded without subjecting the animal to stressful restraint procedures, which can also influence body temperature. Within our data set, different metrics of temperature variation at varying thresholds were examined, including temperature bouts (number of episodes when the cow's body temperature exceeded 39.1°C), peaks of highest temperature, temperature amplitude, and total heat load (area under the curve for the total time and highest temperature episode greater than 39.1°C). Ultimately, PCT39 was chosen as the most practical measurement to represent HS due to its ability to quantify interactions between environmental stressors and activity measurements (see also Vasconcelos et al. 2011). Future research should examine new ways to explore continuous temperature measurements that quantify HS, which incorporate the animal's individual daily temperature variation and basal temperatures.

Our results found no association between BCS or milk production and P/AI and do not support the general literature that previously reported an association between increased milk production and reduced fertility (Walsh et al., 2011; Burnett et al., 2014). However, our work does identify BCS as a major risk factor for RI at estrus; animals with low BCS had a lower overall RI at estrus. Similarly, Madureira et al. (2015a) also reported that animals with low BCS had less intense estrus behaviour measured by two separate AAM, and by Løvendahl and Chagunda (2010) who noted that low postpartum BCS had a negative correlation with estrus activity. Aungier et al.

(2012) provides additional evidence that for every 0.25-point increase in BCS at estrus, the odds of a cows displaying high activity increased by 1.38.

Following parturition, nutrient requirements shift to support the increased demand for milk production, and the resulting state of negative energy balance (NEB) can last 10 to 12 wk (Butler, 2003). NEB is associated with reductions in ovarian cyclicity and reproductive functioning via suppressed pulsatile LH secretion, ovarian sensitivity to LH (Butler, 2000), and reduced plasma insulin and IGF-1 (Butler, 2003). We speculate that decreases in BCS, and the subsequent NEB, can decrease estrus activity of lactating dairy cows through an impaired endocrine-nutritional pathway. Recent studies highlight that cows with higher estrus activity had only 1ng/mL higher estradiol concentrations compared to lower intensity animals (Madureira et al., 2015a). The latter study also found no correlation between estradiol concentration and follicle diameter, suggesting that alternative mechanisms are at play during estrus behaviour. Future research should aim to elucidate the specific mechanism by which NEB decreases estrogen-dependent estrus behaviour in dairy cows.

Our results highlight an association between cows in later stages of lactation and higher RI at estrus. Although these findings are in agreement with Madureira et al. (2015a), they contradict the work of López-Gatius et al. (2005b) who found no relationship between stage of lactation and estrus activity. The differing results may be due to differences in classification of lactation stage as López-Gatius et al. (2005b) categorized animals as early (50-90), mid (91-150), and late (>150) DIM, based on nutritional status and subsequent energy balance; whereas we defined our classes based on DIM: early (≤100), mid (>100 - <180) and late (≥180) DIM. We speculate that the increased estrus intensity observed in the in cows in later stages of lactation is a consequence of lower metabolic challenge. Moreover, following the first estrus episode post-puberty, heifer estrus expression will continually increase in intensity (Silper et al., 2015). We speculate that the absence of progesterone during the first cycle postpartum (cows) and before the onset of puberty (heifers) is responsible for decreased estrus intensity.

This study found that the vaginal temperature recorded 9 to 11 d prior to induced estrus was not associated with walking RI. While the delayed effects of HS have been well documented on follicular growth (Wolfenson et al., 2000), P/AI (Pereira et al., 2013), and successful embryo transfer (Demetrio et al., 2007), it appears that there is little relation between acute internal heat stress conditions and estrus behaviour. Previous research has shown that cows that were previously heat stressed had decreased plasma estradiol and FSH, and increased inhibin concentrations compared to non-heat stressed cows during the following follicular wave (Roth et al., 2000). Further research is required to elucidate the complex interaction between heat stressed follicles, estradiol production, and walking behaviour at estrus. Sakatani et al. (2016) also found no relationship between estrus or non-estrus vaginal temperatures and walking activity in beef cows. When the vaginal temperature and THI were both included as fixed effects in our statistical model, THI was a stronger risk factor for RI expression. Our results, as well as other authors (Verwoerd et al., 2006; Suthar et al., 2012) did not demonstrate a relationship between ambient temperature or THI, and vaginal temperature. The lack of correlation between body and ambient temperature can be attributed to the large variation in individual temperature profiles among animals, likely caused by the ability to buffer against fluctuations in ambient temperature. Moreover, the confined and cross ventilated design of the barn in this study mitigated the effects of extreme environmental conditions, which is not the case in most work of this nature conducted in tropical climates (Kendall et al., 2006).

Our results show that a lower THI was associated with increased steps/h at estrus. Environmental factors such as season, temperature, day length, and photoperiod have all been shown to influence estrus behaviour in dairy cows (Orihuela, 2000). Our results are supported by Sakatani et al. (2012) who showed that average steps/h of beef cows decreased in the summer months compared to winter. Yániz et al. (2006) found that increases in mean relative humidity higher than 95% were associated with a decrease in walking activity at estrus. López-Gatius et al. (2005b) also reported that the relative increase in estrus activity was lower in the warm period when compared to the cool period (369 ± 152 vs. 384 ± 156%, respectively). Based on current findings, we speculate that the reduction in estrus behaviour is multifactorial in nature, and can be caused by increasing THI conditions, leading to reductions in DMI and altered dominant follicle profiles. Heat stress compromises the steroidogenic capacity of the dominant follicle's theca and granulosa cells, causing reduced estradiol concentrations (De Rensis et al., 2015). Plasma estradiol concentrations are negatively affected by HS (Wolfenson et al., 1995; Wolfenson et al., 1997; Roth et al., 2000) and result in delayed luteolysis and ovulation of older follicles (Wilson et al., 1998), which can compromise cattle fertility.

Current research suggests that when THI exceeds 75, production and reproduction will begin to decline (De Rensis et al., 2015). However, our results have shown that HS can decrease RI walking behaviour when THI is 68, and we speculate that the subclinical effects of HS may begin at even lower THI values. Dairy farms located in geographic areas more prone to HS would benefit from AAM, as the technology could identify behavioural changes caused by HS, while possibly improving heat detection and AI submission rates. AAM technology companies would benefit from incorporating the effects of ambient temperature into their heat detection algorithms, which would improve their accuracy by modifying alert thresholds that account for behavioural changes caused by HS.

Relative increase of walking activity was associated with improved P/AI as cows who had high RI were 2.8 times more likely to be pregnant when compared to animals who did not show a RI. Moreover, cows that exhibited high RI were 1.5 times more likely to become pregnant than low RI animals. Increases plasma E2 concentrations during the periovulatory period were expected given the timed AI protocol used, which in turn have been associated with improved P/AI in heat stressed cows (Pereira et al., 2013). The increase in estradiol for heat stressed cows may be responsible for the increased RI intensity and improved pregnancy rates. Madureira et al. (2015a) also found that cows with high estrus activity had a 35% improvement in fertility, and López-Gatius et al. (2005b) reported an improvement in fertility for every unit increase in RI walking activity. Garcia et al. (2011) reported that high estrus intensity was associated with 4.6 to 4.8 higher odds of pregnancy and calving, respectively, compared to low or medium intensity walking behaviour. Cerri et al. (2004) and Pereira et al. (2016) showed that cows that exhibit estrus at TAI and timed embryo transfer, respectively, have greater fertility and lower pregnancy loss compared with animals not in estrus. While the physiological relationship between estrus intensity and fertility remains unclear, current research suggests that animals who demonstrate more intense estrus have shorter intervals to ovulation (Van Eerdenburg et al., 2002), and low estrus expression leads to more ovulation failure and TAI asynchrony (Burnett et al., 2016).

The findings of this study showed that that decreases in P/AI were only associated with a sub-population of cows with high PCT39, under high THI conditions. The negative effect of ambient temperature on pregnancy rate has been well documented, as insemination during the warm period can led to a 0.8-fold decrease in P/AI rate (López-Gatius et al. (2005b). Furthermore, Morton et al. (2007) estimated that a daily maximum THI of 72 or more from d 35 before to d 6 after the day of breeding decreases conception rates of lactating dairy cows by 30%. Even exposure to THI ≥ 73 for 1 h on the day of breeding is sufficient to decrease the conception rate by 5%, and pregnancy rates of Holsteins significantly decline from 28.5% at low THI (< 70) to 14.8% at high THI (> 80) conditions (Schüller et al., 2014). Data from Demetrio et al. (2007) and Vasconcelos et al. (2011) also demonstrated that high body temperature measured on the day of embryo transfer had a negative effect on conception rates and embryonic retention. Despite the literature consensus, our results show that even under high THI, 9-11 d prior to breeding, there are a subgroup of cows who continue to show similar pregnancy rates as cows who are under low heat load in low THI conditions. Pereira et al. (2013) found no difference for P/AI in cows exposed to 1 bout of HS, or estrus cycle synchronization for animals exposed to 2 or more events of HS. The physiological mechanism behind maintaining high pregnancy rates under high THI conditions by sustaining low vaginal temperatures is unclear, and future research should aim to elucidate this relationship.

Table 2.1. Pearson's Correlation coefficients showing the relationship between relative increase in steps of lactating Holstein cows (*n*=641), and THI and explored metrics of vaginal temperature both recorded 9-11 d prior to breeding.

	Relative Increase (%)	THI	PCT39 (%)	Temp. Bouts	Temp. Peaks(°C)	Temp. Amp.(°C)
Relative Increase[1]						
THI[2]	-0.16***					
PCT39[3]	-0.10*	0.30***				
Temp. Bouts[4]	-0.04	0.02	0.50***			
Temp. Peaks[5]	-0.09*	0.28***	0.71***	0.30***		
Temp. Amp.[6]	-0.04	0.17***	0.22***	0.14***	0.50***	
Heat Load[7]	-0.10**	0.29***	0.87***	0.15***	0.76***	0.26***

[1] [Relative increase is calculated as: (induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100)]; [2] [THI (Temperature and Humidity Index) calculated as: (0.8 ×TºC) + [(RH/100)× (TºC − 14.4)] + 46.4(Mader et al., 2006)]; [3] [PCT39 = percentage of time (in min) 9 - 11 d prior to breeding when the cow's vaginal temperature was ≥ 39.1°C]; [4] [Temp. Bouts: number of 'episodes' when the cow's body temperature exceeded 39.1°C]; [5] [Temp. Peaks: peaks of highest temperature when the cow's body temperature exceeded 39.1°C]; [6] [Temp. Amp; temperature amplitude/phase of body temperature exceeding then returning to 39.1°C]; [7] [Heat Load; Area under the curve for the total time and highest temperature episode greater than 39.1°C].

[8] Significance levels: *P<0.5, **P< 0.01, ***P< 0.001

Table 2.2. LS Mean ± SE for relative increase (RI; %) in steps of Holstein cows (*n*=641) according to BCS, DIM, THI, PCT39, milk production, and parity.

Parameter	**Peak Relative Increase[1] (%; LS Mean ± SE)**
BCS[2]	
Low (<2.75)	260.31 ± 17.45^{a}
Medium (=2.75)	296.42 ± 6.62^{b}
High (≥3)	292.86 ± 12.32^{ab}
DIM[3]	
Early (≤100 DIM)	265.40 ± 9.90^{a}
Mid (100-180 DIM),	288.36 ± 11.58^{b}
Late (≥180 DIM)	295.75 ± 11.29^{b}
THI[4]	
Low (≤65)	313.26 ± 10.59^{a}
Medium (65-70)	276.18 ± 11.02^{b}
High (≥70)	260.06 ± 10.76^{b}
PCT39[5]	
High (≥22.90%)	284.12 ± 9.55^{a}
Low (<22.90%)	286.08 ± 18.42^{a}
Milk Production[6]	

High (≥41.50 kg/d)	282.12 ± 15.66^{a}
Low (<41.50 kg/d)	288.09 ± 13.99^{a}
Parity[7]	
Primiparous	291.41 ± 13.47^{a}
Multiparous	278.80 ± 13.47^{a}

[1][RI was calculated as [(induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100]. [2][BCS was classified based on 1 to 5 scale at 0.25 increments (Wildman et al., 1982). 3[DIM = Early: ≤100 DIM, Mid: > 100 - <180 DIM, Late ≥180]. [4][THI = low (≤ 65), medium (> 65 - < 70), and high (≥ 70). THI= $(0.8 \times T^{o}C) + [(RH/100) \times (T^{o}C - 14.4)] + 46.4$ (Mader 2006)]. [5][PCT39 = percentage of time (in min) 9-11 d prior to breeding when the cow's vaginal temperature was ≥ 39.1°C (median =22.9%)]. [6][Milk Production: Median=41.50kg/d]. [75][Parity = Primiparous: 1st lactation, Multiparous: 2nd lactation and higher]

[8a-b]Different letters indicate difference between variables within columns ($P<0.05$)

Significance was set at $P<0.05$, and $0.05 > P < 0.10$ was a tendency.

Table 2.3. Odds ratio ± 95% CI for pregnancy per AI (%) of lactating Holstein cows (*n*=641) according to milk yield, BCS, Relative Increase (RI), DIM and parity.

Parameters	OR	95% CI	P - value	Pregnancy per AI (%; n/n)
Milk yield[1] (Low vs. High)	1.03	0.70-1.54	0.82	25% (103/408); (19% 77/397)
BCS[2] (Med vs. Low)	1.1	0.54-2.25	0.33	20% (33/163); 20% (19/96)
BCS[2] (High vs. Med)	1.12	0.69-1.81	0.65	23% (128/547); 20% (33/163)
RI[3] (High vs. No heat)	2.87	1.73-4.80	<0.01	27% (124/455); 12%(21/174)
RI[3] (High vs. Low)	1.54	0.99-2.42	0.05	27% (124/455); 20%(35/177)
DIM[4] (Late vs. Early)	0.44	0.28-0.70	<0.01	16% (35/220); 26%(95/363)
DIM[4] (Late vs. Mid.)	0.58	0.35-0.96	<0.01	16% (35/220); 22% (50/223);
Parity[5] (Multi. vs. Primi)	0.66	0.45-0.95	<0.03	20% (108/538); 27% (72/267)

[1][Milk production = median: 41.50 kg/d]; [2][BCS = Low: ≤2.75, Med: =2.75, High:≥ 3]; [3][RI (relative increase) = no heat: ≤69%, low intensity: 70%-200%, high intensity: ≥200% (RI is calculated as [(induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100)]; [4][DIM = Early: ≤100 DIM, Mid: 100-180 DIM, Late ≥180 DIM]; [5][Parity = Primi: 1st lactation, Multi: 2nd lactation and higher]

[6]Significance was set at P<0.05, and 0.05 > P < 0.10 was a tendency.

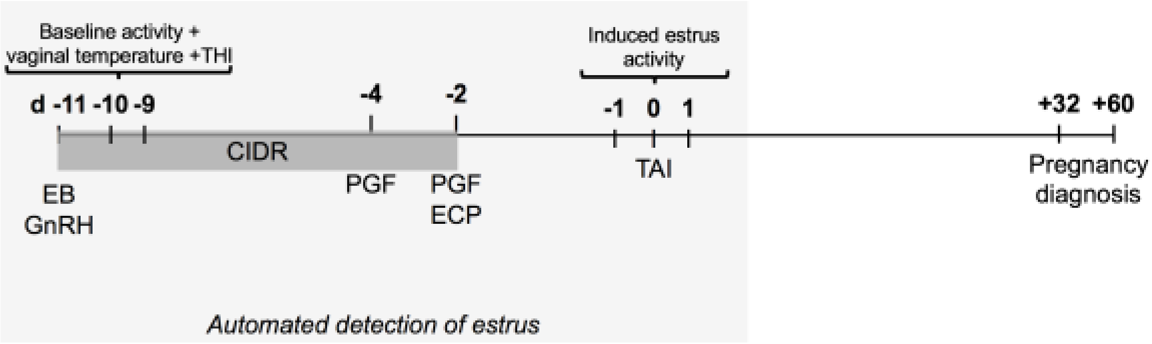
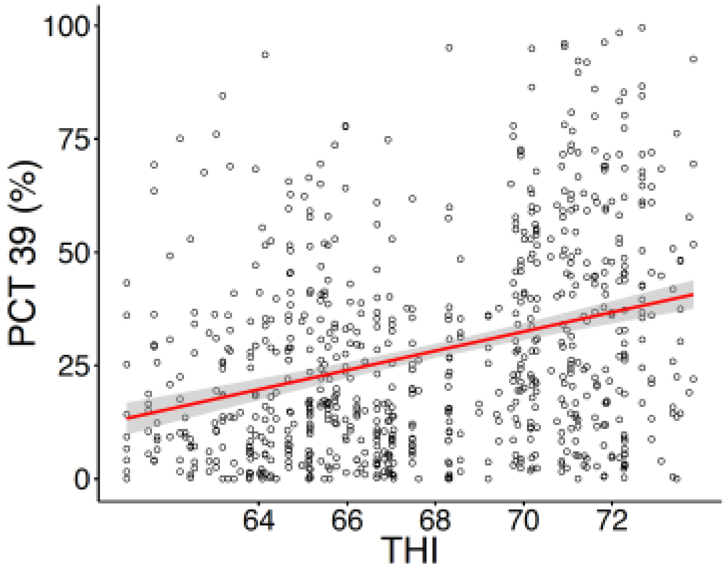


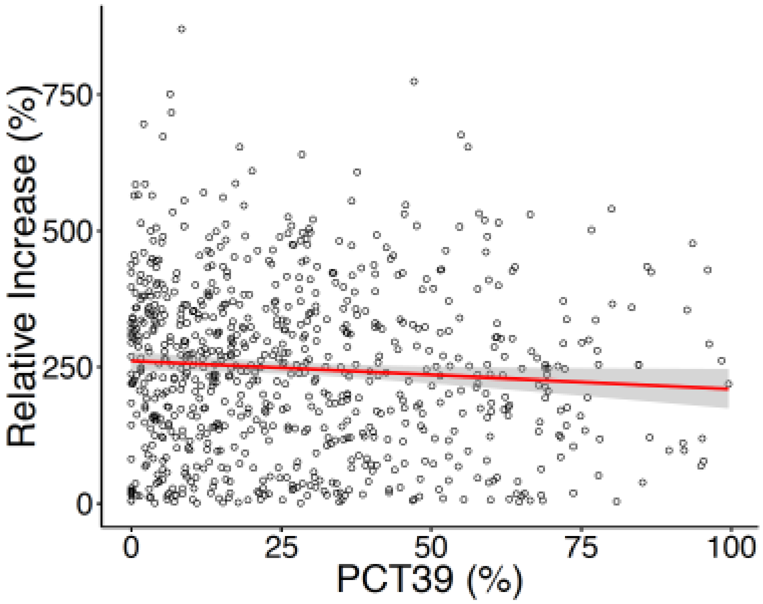
Figure 2.1. Schematic of the experimental estrus synchronization protocol redrawn from Pereira et al. (2015)**;** EB (estradiol benzoate – 2 mg, Gonadiol, Zoetis, São Paulo, Brazil), GnRH (gonadorelin diacetate - 100 µg, Cystorelin, Merial, São Paulo, Brazil), PGF (dinoprost tromethamine - 25 mg, Lutalyse, Zoetis, São Paulo, Brazil), ECP (estradiol cypionate - 1 mg, E.C.P., Zoetis, São Paulo, Brazil), CIDR (intravaginal progesterone implant - 1.9 mg progesterone; CIDR, Zoetis, São Paulo, Brazil), TAI (timed AI). Automated detection of estrus was done with Afimilk Pedometer Plus Tags and AfiFarm software (Afimilk, Kibbutz Afikim, Israel).Relative increase was calculated as [(induced estrus steps $\bar{x}$(d-1,d0,d1)/h – (baseline steps/h; $\bar{x}$(d-11,d-10,d-9)/baseline steps / h] * 100.

Figure 2.2. Pearson's correlation coefficient between A) THI and PCT39 (*P*< 0.03; r <0.01), B) PCT39 and relative increase in walking activity (*P*<0.02; r <0.01), C) THI and Relative Increase in walking activity lactating Holstein cows (*n*=641) (*P*<0.009; r <0.01).

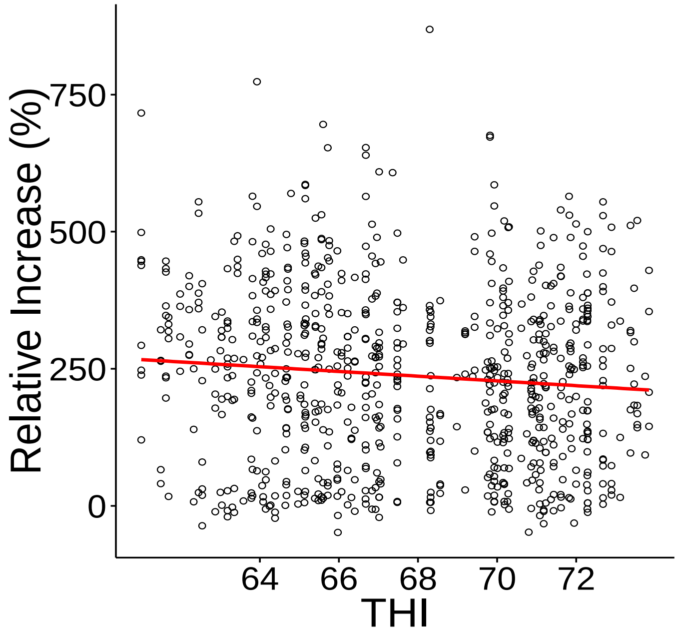
A)



B)



C)



[PCT39 = percentage of time (in min) 9 - 11 d prior to breeding when the cow's vaginal temperature was ≥ 39.1°C]; [THI (Temperature and Humidity Index) calculated as: (0.8

$\times T^{\circ}C) + [(RH/100) \times (T^{\circ}C - 14.4)] + 46.4$ (Mader et al., 2006)]; [Relative increase is calculated as: (induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100)]

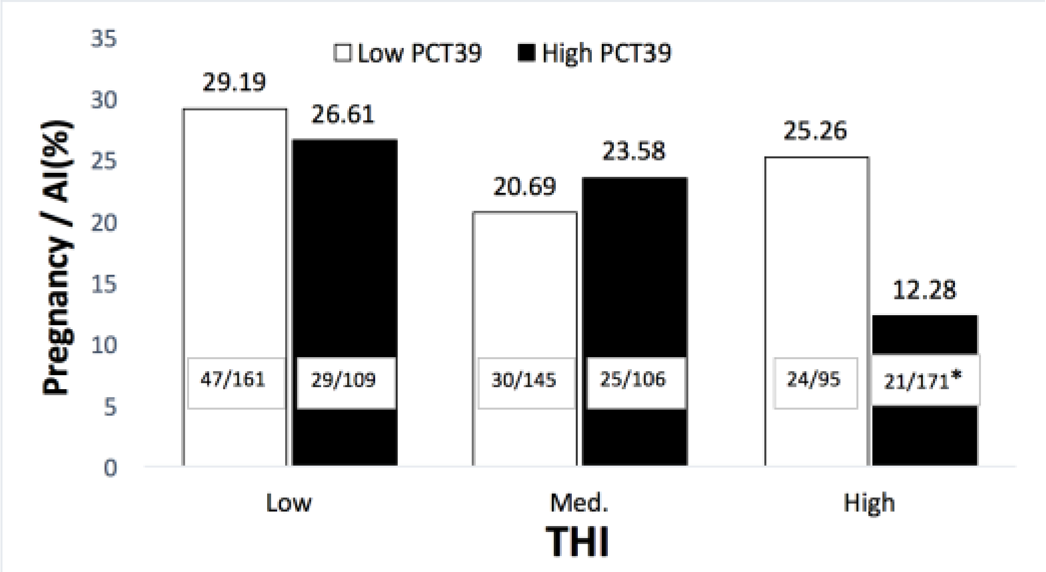


Figure 2.3. Distribution of pregnancy per AI (%) of lactating Holstein cows (*n*=641) according to the interaction between THI and PCT39. [PCT39 (percentage of time (in min) 9 - 11 d prior to breeding when the cow's vaginal temperature was ≥ 39.1°C) = median; 22.9%]; [THI = Low: ≤65, Med: 65-70 High:≥ 70]. (P < 0.03)

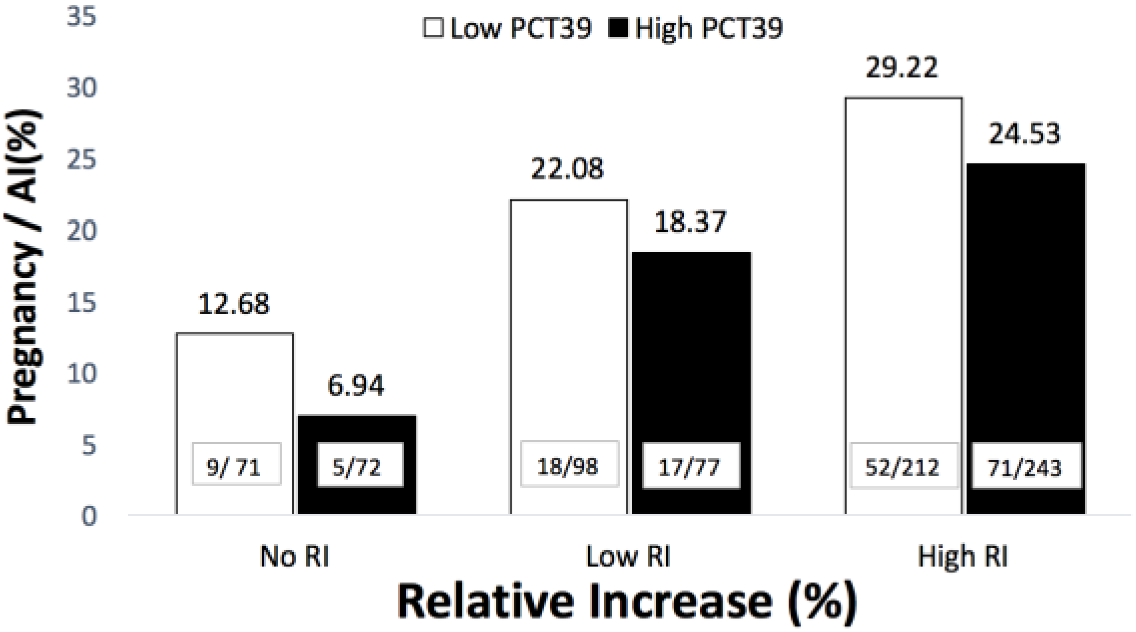


Figure 2.4. Distribution of pregnancy per AI (%) of lactating Holstein cows (*n*=641) according to the interaction between Relative Increase (RI) and PCT39**.** [PCT39 (percentage of time (in min) 9 - 11 d prior to breeding when the cow's vaginal temperature was ≥ 39.1°C) = median; 22.9%]; [Relative increase (RI) is calculated as: (induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100); RI = no heat: ≤69%, low intensity: 70%-200%, high intensity: ≥200%]. (P < 0.76)

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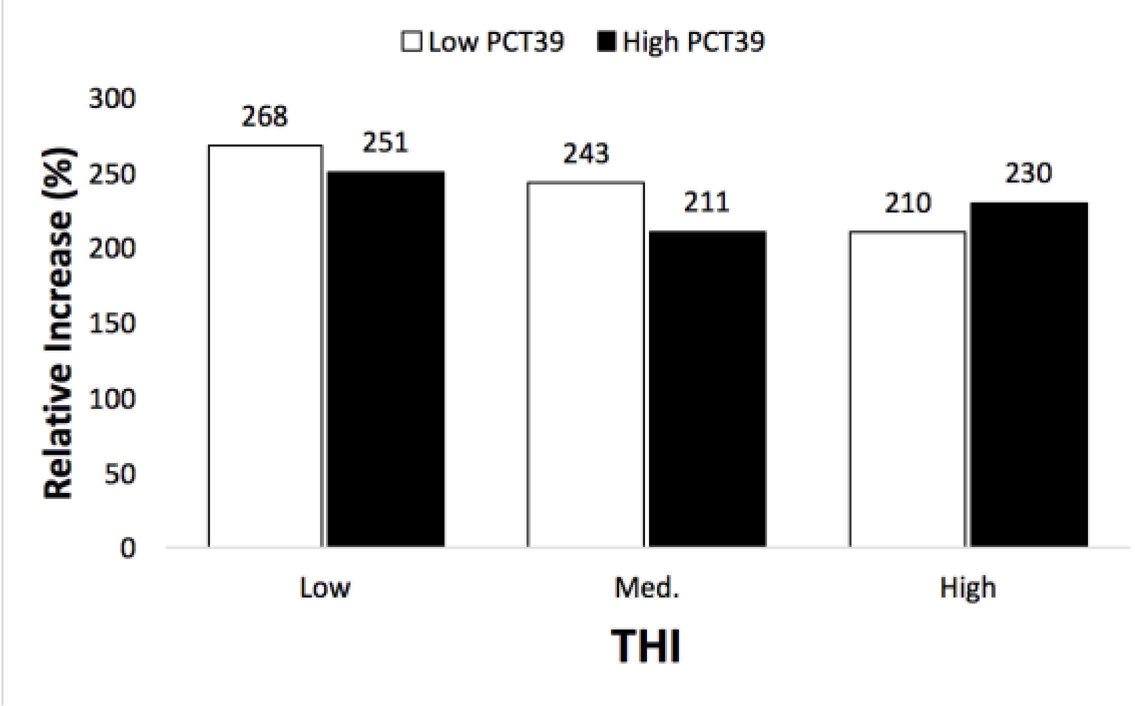

Figure 2.5. Distribution of Relative Increase (%) in walking activity of lactating Holstein cows (*n*=641) according to the interaction between THI and PCT39. [Relative increase is calculated as: (induced estrus steps/h - baseline steps/h)/baseline steps / h] * 100)]; [PCT39 = median; 22.9%]; [THI = Low: ≤65, Med: 65-70 High:≥ 70]. (P < 0.14).

Chapter 3: General Discussion and Conclusions

3.1 Summary of findings

Decreases in production yield, reproductive success, and concerns regarding animal welfare have propelled HS to the forefront of challenges facing the dairy industry. While the effects of environmental HS on reproductive parameters such as pregnancy rate, and oocyte and embryo quality are well known, there is little research documenting the effects of vaginal temperature on estrus expression and fertility. More research is required to understand risk factors which influence estrus behaviour, in efforts to validate and optimize sensor technology as a sustainable practice for reproductive management.

The aim of this thesis was to examine the relationship between vaginal and environmental temperature, and the RI in walking behaviour of Holstein cows following induced estrus. We explored different metrics of vaginal temperature in an attempt to accurately quantify the animal's experience of HS. This thesis also investigated other risk factors, such as BCS, DIM, and parity, that could affect physical activity during estrus, as well as P/AI. Vaginal temperatures were continuously monitored and documented the cow's diurnal circadian rhythm. Pedometer technology allowed for the continuous recording of cow movement and captured the intensity of estrus behaviour.

The THI, and not vaginal temperature recorded 9-11 d prior to breeding, was identified as a key risk factor for the RI in walking behaviour of dairy cows. Previous research has highlighted that cows that were previously heat stressed have decreased plasma estradiol and FSH, and increased inhibin concentrations (Roth et al., 2000), which can influence the neuro-endocrine control of estrus behaviour (Woelders et al., 2014). However, large variations in individual temperatures, complemented by the animal's thermoregulatory system, could have buffered against high ambient temperatures experienced by a portion of cows in this experiment. Different metrics of vaginal temperature at varying thresholds were examined, including temperature bouts (number of episodes when the cow's body temperature exceeded 39.1°C), peaks of highest temperature, temperature amplitude, and total heat load (area under the curve for the total time and highest temperature episode greater than 39.1°C). Ultimately, PCT39 (average percentage of time 9-11 d prior to breeding when the cow's vaginal temperature ≥ 39.1°C) was chosen as the most functional measurement to represent HS due to its ability to quantify interactions between environmental temperatures and fertility. We found an interesting interaction between PCT39 and THI on P/AI, in that decreases in P/AI were only associated with a sub-population of cows with high PCT39, under high THI conditions. Identification of groups of animals at risk for HS can lead to further genetic assessment of HS coping mechanisms, which in turn can help improve genetic selection of animals submitted to AI.

3.2 Study limitations

One of the objectives of this research was to investigate the associations between vaginal temperature and RI of estrus activity. While we have shown that vaginal temperature recorded 9-11 days prior to TAI is not associated with steps per hour at estrus, one limitation of this study is the absence of vaginal temperature data during the RI event itself. Current research demonstrates that increases in vaginal temperature coinciding with the LH surge, and subsequent ovulation, range from 0.48°C (Fisher et al., 2008) to ≥1.0°C (Rajamahendran et al., 1989), and can be used to predict estrus in beef cattle (Kyle et al., 1998). Even though univariable analysis of our data found no relationship between vaginal temperature and baseline activity measurements (measured concurrently), the hormonal control of estrus behaviour can induce physiological changes that can have thermogenic effects on the body and influence vaginal temperatures (Suthar et al., 2011). Moreover, elevated rectal temperatures (Pereira et al., 2013) and a lack of estrus behaviour (Cerri et al., 2004) at TAI have been shown to decrease pregnancy rates. Currently, there is a lack of research describing the relationship between vaginal temperature at breeding, estrus activity, and fertility in dairy cattle. Further understanding of this relationship can be used to improve automated sensor detection accuracy, in addition to utilizing vaginal temperature as a supplemental estrus detection method.

Another limitation of this study was the lack of hormone sampling during different stages of the estrus cycle, and immediately following TAI. Ideally, estradiol would have been sampled on the day of highest activity to determine any relationship between HS, follicular estradiol production, and estrus intensity. Estradiol is the trigger hormone for estrus behaviour and ovulation (Allrich, 1994), and has been shown to affect estrus activity intensity and duration (Lyimo et al., 2000; Lopez et al., 2004). In contrast, a recent study showed a poor correlation between estrus intensity and estradiol concentration in plasma, and that cows with more intense estrus activity had only 1 ng/mL higher estradiol concentrations compared to lower intensity estrus episodes (Madureira et al., 2015a). Heat stress has been shown to decrease follicle estradiol production (Wolfenson et al., 1995), but the relationship between heat stressed follicles and walking activity intensity remains unknown. Progesterone sampling during peak physical activity, as well as at TAI, would have been essential in confirming ovulation and determining the accuracy of the pedometers in measuring “true” estrus episodes.

In this study, vaginal temperature was recorded every 10 min for 3 consecutive days, 9-11 d prior to breeding, and then transformed into PCT39. Even though PCT39 can capture the individual animal’s experience of HS, it may not accurately represent the complete physiological state of the animal. There are large variations in basal body temperatures of dairy cows, in addition to daily fluctuations exceeding 1.5°C (Piccione and Refinetti, 2003), and as expected, some cows had PCT39 values of 100%. The limitations of this variable are that it does not account for the animal's peak temperature during a HS episode, or the temperature amplitude experienced during a HS cycle. While total heat load attempted to incorporate both temperature characteristics, unique temperature profiles (ie: multiple peaks for 1 HS episodes; multiple temperature slope changes per episode), and evaluating the compounding physiological effects of increasing body temperature, require complex mathematical assessment beyond our skills and prevented further investigation. Future research should take advantage of machine learning and algorithm computation to explore new temperature variables, which incorporate the animal's individual daily temperature variation, and basal temperatures, to quantify HS even more accurately.

3.3 Future directions to explore heat stress and reproduction

Although the literature examining the effects of environmental temperature on dairy cow physical activity has been clearly established, there are still many knowledge gaps that need to be addressed. An immediate follow up study would be to leave the vaginal thermometers in the animals during the entire estrus synchronization protocol, during TAI, and for 7 days following breeding. With a more comprehensive temperature profile for the animals within an experimental setting, we would be able to determine which days of follicular development are more sensitive to HS, examine the influence that synchronization protocols have on body temperature, and how the altered follicular environment affects estrus activity and fertility. Leaving the thermometers inside the vagina for an additional 7 days following breeding would provide insight into the delayed effects of HS, and how vaginal temperature may influence embryo development and implantation success.

A second future study should investigate potential genetic adaptations to HS, which allow dairy cows to maintain physiological homeostasis under elevated temperatures. The Slick Hair gene, prevalent in *B. indicus* cattle breeds, controls hair length, which is an important component for evaporative heat loss and efficient transfer of heat to the environment (Dikmen et al., 2008). Tracking potential differences in gene expression between animals that have been classified as thermo-sensitive or thermo-resistant based on their respective PCT39 value would also provide important insight. Genetic analysis may be able to uncover the coping mechanisms responsible for HS phenotypic traits, and have the potential to improve the genetic selection of cows submitted for AI. Improved understanding of subgroups of cows at risk for HS can allow for increased proactive reproductive management strategies by dairy managers. Moreover, the temporal changes in gene expression relative to the HS event could provide insight into the cow's coping mechanisms, which would aid in the application of farm heat abatement strategies (ie. fans, sprinklers, mister timing).

Future studies should also investigate the effects of HS on other physical activity measurements, such as lying behaviour, and the relationship with fertility. An increase in restlessness, such as the total number of lying bouts or total lying time (Kerbrat and Disenhaus, 2004) can be used as an estrus detection method. Analysis of standing and lying patterns of heifers in estrus revealed that the animals stand for 8 h without lying down on the day of estrus (Silper et al., 2015). However, heat stressed heifers and cows have been reported to increase their standing time and decrease lying time to expose more surface area for heat abatement, sensible water loss, and radiating surface area (Cook et al., 2007; Allen et al., 2015). Monitoring lying behaviour through AAM technology can help elucidate how HS effects estrus lying behaviour and its use as a form of estrus detection.

3.4 Conclusions

Continuous monitoring technologies can provide valuable information about the vaginal temperature and activity profiles of dairy cows, which can be used to optimize reproductive management decisions. The RI of estrus walking behaviour captured by an AAM at the time of induced estrus was associated with BCS, DIM, and THI measured 9-11 d prior to RI, but not with PCT39, milk production, or parity. Parity, DIM, and RI were identified as risk factors associated with P/AI. Cows that had more intense RI at estrus were more fertile than cows with low intensity RI or no RI. An interaction was observed between PCT39 and THI, in that decreases in P/AI were only observed in cows with high PCT39 under high THI conditions. Information obtained from automated sensor systems (AAM and thermometers) show promise as part of reproductive programs used on dairy farms as they: a) improve P/AI outcomes, b) alter the management of cows at risk for HS, and c) increase data collection of functional traits.

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