The Use of Infrared Thermography and Behavioural Biometrics for Estrus Detection in Dairy Cattle

By

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Abstract

Current Canadian estrus detection rates (< 40%) need to increase to reach optimal reproduction management and economic dairy production sustainability. Therefore, the general objectives of this thesis were to evaluate the use of infrared thermography (IRT) and behaviour biometrics as an estrus detection method and compare its accuracy levels with other estrus detection aids.

The initial study was designed to characterize IRT and behaviour biometrics of pregnant (Control) and cyclic synchronized (Synch) Holstein cows at 45 days in milk 5 min before, during and 5 min after milking times and to evaluate the accuracy of IRT and behaviour biometrics as estrus indicators in cyclic multiparous cows housed in a tie-stall system. Compared to baseline (i.e. luteal phase) measures, 7 different anatomical locations had increased infrared temperatures 48 and 24 h before ovulation. Additionally, behaviour biometrics change between treatments, specifically the stepping activity (treading) per day. However, Tail movement was the only behaviour that increased in frequency of movement as ovulation approached in Synch cows. Measuring thermal outputs, tail and treading behaviour can differentiate between estrus and non-estrus cows and differed between Control and Synch groups in multiparous cows.

The second study's objective was to evaluate the combined use of IRT and behaviour biometrics as an estrus alert in naturally cycling primiparous dairy cows in a tie-stall system. Radiated temperature of the vulva increased 2 days before ovulation, and changes in frequency of small hip movements 1 day before ovulation improved the accuracy of estrus detection compared to individual thermal and behavioural biometrics. Using multiple estrus detection methods reduces the error rate by increasing the specificity (Sp; true negatives) and reducing false positive alerts.

The third study aimed to characterize the biomechanics of pelvic movements, foot strikes, and tail movement using 3D-kinematics analysis in naturally cycling primiparous cows in tie-stall housed cows during the proestrus–ovulation period. In addition, changes in pelvic, foot strikes, and tail movement were observed before ovulation and during the luteal phase. Pelvic, foot and tail movements were useful indicators of ovulation 48 to 24 hours when cows are housed in confinement, such as tie-stalls, that prevent locomotory movement.

The fourth study's objectives were to develop a fully automated IRT and behaviour biometrics platform on a free-stall commercial dairy herd to demonstrate the application of estrus detection on-farm. In addition, to assess the IRT platform accuracy with other estrus detection methods already used in voluntary milking systems such as in-line milk progesterone (P_4) and accelerometer sensors tags. Skin temperature changes were associated with a decreased milk P_4 concentration at d 0 compared to d -14 and d 4. The occurrence of tail movement per IRT frame was higher at d 0 than d -14 and d 4. The sensitivity (Se) of the IRT platform was compared with the accelerometer sensors at the different time windows, Same-day, ±24 h, ±48 h, and ±72 h. Estrus alerts achieved the highest IRT accuracy in a ±48 h window relative to in-line milk progesterone estrus alerts. The accuracy of the IRT platform resulted in higher estrus detection rates compared to accelerometers but lower compared to in-line milk P_4 estrus alerts.

The fifth study was designed to identify the partial budget business analysis of IRT, Visual observation, and Ovsynch as breeding alternatives in Alberta dairy production. The secondary objective was to determine the farm profit of different estrus detection rates and evaluate IRT's prospective performance compared to Visual observation and Ovsynch as an estrus detection aid. The most cost-efficient estrus detection method was Visual observation followed by IRT, however, Ovsynch had higher economic returns in feeding cost per conception service. The return to equity

increased due to the low production cost, specifically the feeding cost of an efficient calving interval. Further, the more significant return to equity was directly associated with the highest accuracy of estrus detection using IRT.

This thesis concludes that the practical implementation of infrared thermography and behaviour biometrics to detect estrus in dairy cows is possible. The development of an infrared thermography platform provides valuable fundamental information to commercialize a novel method of estrus detection and to provide producers with an additional decision-making tool to optimize reproductive management in the context of estrus detection.

Preface

This thesis is an original work by Hector Javier Perez Marquez. The research work presented in this thesis was led by Dr Clover J. Bench from the Department of Agricultural, Food, and Nutritional Science from the University of Alberta, Edmonton, Alberta Canada. The research projects described in this thesis were approved by the University of Alberta, Animal Care and Use Committee for Livestock (ACUC-L; AUP00001652 and AUP00003266) and Lakeland College, Animal Care Committee.

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Dedications

To my hard-working wife Lucia Oliva Lopez and daughter Natalia Catalina Perez Oliva for all the understanding and support during my PhD program

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LABOR OMNIA VINCIT IMPROBUS

"Steady work overcomes all things"

Publius Vergilius Maro

Table of contents

List of tables

List of figures

List of Abbreviations

#ED	Number of Confirmed Estrus
#FN	Number of False Negatives Estrus Alerts,
#Open	Number of Cows Available to get Inseminated.
#P	Number of Confirmed Pregnancies,
#PC2nd	Number of Cows Pregnant After 2nd service
#PC3th	Number of Cows Pregnant After 3rd service
#PC4th	Number of Cows Pregnant After 4th service
%P>4	Percentage of Cows in Parity >4
%P1, 2	Percentage of cows in Parity 1-2
%P3, 4	Percentage of Cows in Parity 3-4
102$d_{VO\text{-}IRT}$	102 Days Service Interval if IRT or VO is used,
40d_{OVS}	40 Days Service Interval if OVS is used.
51$d_{VO\text{-}IRT}$	51 Days Service Interval if VO or IRT is used,
80d_{OVS}	80 Days Service Interval if OVS is used.
AED	Automated Estrus Detection Devices
AI	Artificial Insemination
AUC	Area Under the Curve
Avg	Average
CIDR	Controlled Internal Drug-Release
CL	Corpus Luteum
CR	Conception Rate
CSTags	CowManager SensOor Tags
CV	Coefficient of Variation
Davg	Daily-Radiated Temperature Average
DF	Dominant Follicle
DIM	Days in Milk
DLC	Dairy Learning Centre
DOR	Diagnostic Odds Ratio

DRTC	Dairy Research and Technology Centre
E_2	Estradiol
EBM	Estrus BenchMark
ELISA	Enzyme-Linked Immunoassay
ER	Estrus Detection Rate
Exp-ED	Expected Estrus Detection.
F	Feeding
Foot strike L	Foot Strikes Left
Foot strike R	Foot Strikes Right
FSH	Follicular Stimulating-Hormone
GnRH	Gonadotropin Releasing Hormone
HA	High Activity
HC_{day}	High Ration Cost per Day
High Ration	High Protein Content and Energy Density
HN	Herd Navigator
Hz	Herd Size,
IRT	Infrared Thermography
J Index	Youden J Index
LA	Low Activity
Lact	Lactation
LC_{day}	Low Ration Cost per Day
LH	Luteinizing Hormone
L-hip	Large Hip Movements
Low Ration	Low Protein Content and Energy Density
LR+	Positive Likelihood Ratio
LSMeans	Least Squares Means
L-tail	Large Tail Movements
LTail	Left Tail Movements
MA	Mid Activity
Max	Maximum Price
MC_{day}	Mid Ration Cost per Day

Med	Medium Price
Mid Ration	Medium Protein Content and Energy Density
Min	Minimum Price
MTail	Non-defined Direction of Tail Movement
NPV	Negative Predictive Value
NRC	National Research Council
OVS	Ovsynch
P_4	Progesterone
$PC1^{st}$	Pregnancy Cost 1^{st} Service,
$PC2^{nd}$	Pregnancy Cost After 2^{nd} service
$PC3^{th}$	Pregnancy Cost After 3^{th} service
$PC4^{th}$	Pregnancy Cost After 4^{th} service
PC_{ER}	Pregnancy Cost per Cow per Estrus Detection Rate
P_{CPDER}	Pregnancy Cost per Lactation Distribution per Estrus Detection Rate
Pelvicsl	Pelvic Shifts Left
Pelvicsr	Pelvic Shifts Right
PGF2α	Prostaglandins $F_2\alpha$
PPV	Positive Predicted Value
PR	Pregnancy Rates
Raw IR	Raw Skin Temperature
Res	Residual
Res IR	Residual Skin Temperature
RH%	Relative Air Humidity
ROC	Receiver Operating Characteristic
RTail	Right Tail Movement
Rum	Rumination
Se	Sensitivity
SEM	Standard Error Means
S-hip	Small Hip Movements
Sp	Specificity
S-tail	Small Tail Movements

SD	Standard Deviation
TAI	Fixed-Time AI
Temp	Ambient Temperature
TFC	Total Feeding Costs,
TFC_{cow1st}	Total Feeding Cost per Cow if Pregnant During 1st service
TFC_{cow2nd}	Total Feeding Cost per Cow if Pregnant During 2nd service,
TFC_{cow3th}	Total Feeding Cost per Cow if Pregnant During 3th service,
TFC_{cow4th}	Total Feeding Cost per Cow if Pregnant During 4th Service,
TFootS	Total Foot Strikes
TMR	Total Mixed Ration
$TPC4^{th}$	Total Number of Cows Pregnant After 4th services
TPelvicS	Total Pelvic Shifts
Vlips	Vulva's External Lips
VMS	Voluntary Milking System
Vnotail	Vulva Area without the Tail
VO	Visual Observation
Vtail	Vulva Area with the Tail

Chapter 1. General Introduction

Dairy production is one of the fastest-growing and essential agriculture sectors globally. The average milk consumption per capita is 100 kg of milk per year (OECD/FAO, 2019). As the world population increases in Asian and African countries, liquid milk production must increase at least 1.8% annually to supply the market's demand (OECD/FAO, 2019). In Canada, dairy production represents the second-largest Agri-Food sector with an economic contribution of 6.56 billion CAD/year and a consumption per capita of 66.68 kg of fluid milk per year (Canadian Dairy Information Centre, 2019). Canadian dairy production assures a dairy farmer's income and avoids production surpluses by implementing a supply management system to maintain a sustainable dairy production growth (Heminthavong, 2015). The Canadian dairy commission facilitates policies and framework to dairy producers and manages programs that encourage the production of quality added dairy products with the most optimum management production (Heminthavong, 2015).

The development and adoption of technologies by the dairy industry combined with improved knowledge of nutrition and genetics has resulted in yearly increases in milk yield per cow over recent decades (Lucy, 2001). Current North American dairy herds are composed of high milk yield production cows capable of supplying market demands. As a result, the population of dairy cows is consistently decreasing, and the number of dairy herds has been reduced across the world (Lucy, 2001). However, despite the advances in nutrition, genetic improvement and intensive management, the ability of dairy cows to reproduce has declined over time (<35% conception rates; Schmitt et al., 1996, longer first ovulation intervals by 47 to 67 days in milk [DIM]; Stevenson, 2001, <50% pregnancy rates; Ambrose and Colazo, 2007, and 20% of embryonic loss; Vasconcelos et al., 1999). Dairy cows must produce a calf every 12-13 months to be profitable (De Vries and Conlin, 2003); thus, cows must conceive within the first 50-60 days after parturition.

Rapid advances in genomics and reproduction management can be attributed to the implementation of artificial insemination (AI) in dairy cattle. However, an AI pitfall is the required identification of the onset of estrus; an event that is associated with changes in behaviour (Roelofs et al., 2010). These changes in behaviour before ovulation (e.g. 30.6 ± 4.4 h interval) have been well establish and defined as estrus behaviours (Roelofs et al., 2005a). The importance of detecting

estrus is that AI service achieve higher conception rates (51.1%) with an >8 to 12 h interval from onset of estrus (Dransfield et al., 1998). Dairy producers and breeding technicians using AI have been focused on observing sexual receptivity behaviours (standing to be mounted) due to their high reliability (i.e. low false positive alerts) and ease of observation (USDA, 2009). However, more recent work indicates that standing to be mounted can be as low as 50% during the peak of lactation (Peter and Bosu, 1986, Senger et al., 1994, LeBlanc, 2005). Evidence has demonstrated physiological (i.e. steroid hormone clearance) and behavioural (i.e. high eating and rumination time during the peak of lactation) challenges in dairy cows during the peak of lactation to cope with the nutritional needs to produce milk, which are indirectly associated with the display duration of estrus behaviour (< 8 h; López et al., 2004). Furthermore, intensive dairy housing such as tie-stalls has been reported to influence the expression of estrus behaviours (i.e. cows remain in-stall most of the time; Felton et al., 2012) and concrete floors (reducing mounting and standing to be mounting duration; Britt et al., 1986). Pfeiffer et al. (2020) suggested that the lack of experienced labour available and increasing herd sizes are the main reasons for failing to detect estrus by the dairy producers in intensive milk production.

Alternatives to using the observation of estrus behaviours for estrus detection are the induction of estrus and fixed time to inseminate by applying hormone-based treatments used since the 1970s (Lauderdale et al., 1974, Hafs and Manns., 1975). However, current social interest in food animal welfare has increased the preference for non-invasive practices in animal-food production (Janssen et al., 2016). Correspondingly, further advances in digital instrumentation, wider-faster internet services and interdisciplinary collaboration have produced a new generation of automated estrus detection devices that monitor dairy cow behaviour and physiological parameters (Mottram, 2000). The new era of automated estrus detection devices has led to the generation of estrus alerts that dairy producers can easily interpret and use to adjust their reproduction management (e.g. health diagnosis; Franze et al., 2012). Recently, access to technology such as infrared thermography cameras has enabled the measurement of physiological processes, expressed as changes in epidermal radiated temperature. As such, infrared thermography (IRT; Tattersall, 2016) has led to unique applications within the dairy industry. Multiple sites at a dairy barn allow images to be taken from a fixed distance, constant ambient temperature and are frequently visited by dairy cows (e.g. during milking time, water bowls, concentrate feeders, footbath, etc.), which allows consistent radiated temperature recordings.

Additionally, changes in temperature distribution in an image could be used to identify behavioural cues simultaneously (i.e. identify behaviour cues like estrus behaviour).

Hence, the objectives of this thesis work were to identify the changes in radiated temperature and behaviour biometrics in dairy cows (parous) between the proestrus and post-ovulation period. Secondly, the application of an IRT platform at a commercial dairy herd to evaluate the accuracy of radiated temperature combined with behaviour biometrics was evaluated along with other automated estrus detection technologies. Finally, to identify the economic effect of using IRT as a primary estrus detection method compared to traditional methods. Detailed objectives were:

I. To characterize behavioural and thermal biometric patterns of pregnant and non-pregnant (cyclic) cows around milking time;
II. To evaluate the accuracy of infrared thermography and behavioral biometric parameters as non-invasive estrus indicators in cyclic dairy cows in a tie-stall housing system;
III. To evaluate a combination of infrared thermography and behavioural biometrics for estrus detection in naturally cycling dairy cows in a tie-stall housing;
IV. To characterize movement biomechanics triggered by angle changes due to pelvic tilt, lateral pelvic shifts (left and right), foot strikes (left and right), and tail movements at different spatial resolution (macro, mid and micro) in naturally cycling primiparous cows in tie-stall housing as ovulation approaches;
V. To evaluate the accuracy of each behaviour biometric as an indicator of estrus in dairy cows housed in tie-stalls;
VI. Evaluate the ability of the updated automated IRT technology platform to detect estrus compared with accelerometer-based ear tags and in-line progesterone (P_4) analysis for estrus detection already used on a commercial dairy farm.
VII. To compare the partial budget analysis of IRT, Visual observation, and Ovsynch at different estrus and conception (i.e. Ovsynch) accuracy levels and pregnancy rates (PR);
VIII. To identify the financial effect (gain or loss) at different accuracy levels (Sensitivity and Specificity level) of IRT, Visual observation, and Ovsynch as

reproductive strategies using economic defaults from dairy production in Alberta Canada.

Chapter 2. Literature review

2.1 Current Dairy Industry Status

In Canada, the majority of dairy herds are located in the Eastern provinces of Quebec (5,546 herds) and Ontario (3,731 herds), followed by the Western provinces (British Columbia; 417, Alberta; 531, Saskatchewan; 159, and Manitoba; 285, Canadian Dairy Information Centre, 2017). In 2018, 73.8% of dairy operations were tie-stall systems, and 26.2% were free-stall systems in which 701 herds used automated milking systems (Canadian Dairy Information Centre, 2017). As such, milk production in Canada is at 89.8 million hectoliters (hl) with a consumption per capita of 64.99 kg of fluid milk and 38.42 kg of other dairy products (i.e. cheese, cream, yoghurt, and butter), with a limited quantity imported of 186,768 tonnes mainly of cheese, butter, and milk proteins and whey products (Canadian Dairy Information Centre, 2019). Organic milk production is 1.3 million hl a year (2018/2019) with a consistent increase in milk production and dairy producers from 2009 (869,826 hl; 206 producers) to 2019 (1,404, 837 hl; 249 producers (Canadian Dairy Information Centre, 2019).

In Canada, dairy production has been regulated under a supply management system since 1972, enforced by the Farm Products Agencies Act to adjust milk prices, production surplus, and import regulation (Heminthavong, 2015). Supply management systems operate as quota-control dairy production that allows dairy farmers to produce a permitted volume in terms of daily kilograms of butterfat (3.6 kg butterfat equal to 103.2 kg milk, Canadian Dairy Information Centre, 2019). Dairy quota value varies across provinces (e.g. 1 kg of butterfat in British Columbia costs 42,500 CAD compared to 23,000 CAD in Manitoba) and over time (14,756,549 CAD in 1998 compared to 37,531,849 CAD in 2019; Canadian Dairy Information Centre, 2019). In the quota system, milk production is controlled and the dairy industry negotiates minimum prices with dairy processors based on production costs, market prices, consumer demand and butterfat available (Heminthavong, 2015). Currently there are 10,371 quota holders in the dairy industry with the majority located in the Eastern provinces (3,446; Ontario and 4,925; Quebec, Canadian Dairy Information Centre, 2019). Supply management provides stability to the dairy industry (e.g. producers, processors, and distributors, Heminthavong, 2015).

2.2. The Challenge of the Modern Dairy Herds

Dairy producers adopted artificial insemination (AI) as a breeding strategy in most developed countries as a tool for rapid genetic selection for heritable traits of economic importance (Swan and Broster, 1976). The majority of North American dairy herds use AI (89.9%), and the remaining percentage utilize natural service (i.e. bulls) or embryo transfer (USDA, 2018). However, one of the challenges of AI is identifying the precise time for optimal AI application before ovulation occurs to achieve pregnancy.

In dairy cows, the identification of estrus like behaviours as an indication of when to inseminate is common. However, prevailing estrus detection rates (ER's) in Canadian Dairy herds are under 40% (Leblanc, 2005; Ambrose and Colazo, 2007). Surveys have shown that the majority of dairy housing systems in Canada are tie-stall (i.e. eastern regions of Ontario and Quebec) in which individual movement is restricted (Popescu et al., 2013). As a result, estrus detection is difficult since traditional behaviour cues (e.g. visual observation of standing to be mounted) are limited unless cows are moved to an open space (e.g. during exercise). Overall, poor estrus detection has led the dairy industry to adopt hormone-based estrus or ovulation synchronization protocols as a staple of breeding programs to circumvent the need for behaviour observations of estrus. However, hormone-based treatments have led to social concerns and ethical debates amongst veterinary practitioners associated with dairy production (Higgins et al., 2013) and have extra costs such as medication and specialized labour (e.g. 3.30/$PGF_2\alpha$ CAD per dose and 7.27/GnRH CAD per dose; Olynk and Wolf, 2008). Further, hormone-based synchronization protocols are invasive techniques, which cannot be certified under organic production since organic dairy herd management must utilize natural breeding methods (i.e. seasonal breeding; National Standard of Canada, 2015).

To maintain optimal milk yield profit, the dairy industry targets the first AI service approximately 60 days after calving (Groenendaal et al., 2004). However, the first 100 days in lactation coincide with peak of lactation when dry matter intake is at maximum levels. As such, milk yields have been correlated with faster estradiol (E_2) clearing due to increased metabolism, resulting in reduced expression of behaviour estrus (López et al., 2004). Additionally, negative energy balance during the pre-estrus period at peak lactation can alter ovarian dynamics (e.g. decreases in follicle growth and P_4 concentrations; < 1 ng/ml), which increases the incidence of anestrus cows in a herd (Canfield and Butler, 1990). However, no direct causation between high milk yield and low reproductive efficiency as antagonist parameters at the herd level has been

reported in the scientific literature (Bello et al., 2012). Further, research should look closely at estrus detection at the herd level since herd management can influence estrus detection greatly.

Other reasons for decreased estrus detection include environmental factors, such as housing type (i.e. tie-stalls; Kiddy, 1977; Felton et al., 2012), reduced standing to be mounted behaviour in warm weather (Hansen and Aréchiga, 1999), high stocking density and concrete flooring (Britt et al., 1986). Furthermore, multiparous cows exhibit longer durations of standing to be mounted compared to primiparous cows (At-Taras and Spahr, 2001), despite increased activity observed in primiparous cows at the beginning of estrus (Chanvallon et al., 2014). Regardless of variations in estrus behaviour, poor estrus detection can also be attributed to shortages in labour, inexperienced observers and increased dairy herd size in recent decades.

Optimum time for AI in dairy cows relative to ovulation depends on the accuracy of estrus detection strategies (Roelofs et al., 2005a). Failure to reliably or accurately detect estrus is directly associated with decreased pregnancy rates (PR) and extended calving intervals. Further, estimations demonstrate economic losses of 0.73 USD to more than 1.24 USD per extra non-pregnant day per cow after the voluntary waiting period of 50 - 60 days in milk (DIM; De Vries and Conlin, 2003). Other estimated costs per day non-pregnant were 1.80 USD (Meadows et al., 2005), 4.20 to 7.12 USD (De Vries, 2006), and 2.03 to 3.66 CAD (Liang, 2013). The daily costs increase when cows take longer to get pregnant are based on lactation number, stage of lactation, stage of gestation, prices of breeding and replacement decisions (De Vries, 2006). Additionally, non-pregnant cows/days increase feeding costs by extending the number of non-lactating days during the dry-off period (> 60 days). As a result, superior rates (>60%) of estrus detection need to be achieved in order to maintain a profitable and economically sustainable dairy industry (Galvão et al., 2013). However, the costs of different estrus detection methods and regional management costs should be considered to identify those estrus detection methods that are the most economical for dairy producers in Canada.

2.3. Estrus Detection in Dairy Cattle

In dairy cattle, estrus is often defined as the period when a cow is most receptive to copulation (onset of estrus), beginning with an increase in activity (i.e. walking, mounting, urination, and restlessness) followed by standing to be mounted (Roelofs et al., 2005a). Cows in estrus show interest in other cows (often more dominant cows; Thomas and Cert, 1989) to be

mounted where a cow in estrus stands still in a lordosis posture (i.e. urination alike posture) during mounting (Cole and Cupps, 1969). Estrus behaviours are induced by dynamic changes in circulating hormones (i.e. reproductive hormones) characteristic of the estrus period, specifically E_2.

The estrous cycle in dairy cows is characterized by cyclic changes in the morphology of reproductive organs and cow behaviour. The regression of the corpus luteum (CL) at day 18 to 19 of the estrus cycle decreases circulating concentrations of P_4 from 5 – 7 ng/mL to basal concentrations (< 2 ng/mL) during luteolysis (Sartori et al., 2004). The subtle decrease of P_4 concentrations increases preovulatory follicle diameter (~ 16.6 mm) and E_2 concentrations (> 9 pg/mL) in lactating dairy cows (Sartori et al., 2004). The presence of E_2 and absence of P_4 have been demonstrated to induce estrus for approximately 8 h (Allrich, 1994). Additionally, higher concentrations of E_2 create positive feedback on the hypothalamus that releases gonadotropin releasing hormone (GnRH). The GnRH surge creates a luteinizing hormone (LH) surge at the hypophysis to suddenly induce ovulation 28 h after the onset of estrus (Gazal et al., 1998, Burnett et al., 2018).

Improved understanding of estrus behaviour, endocrine profiles, and physiological changes at the ovary enable the development of technologies to detect estrus in dairy cows using the observation of standing heat, and secondary behaviour indicators (e.g. mounting, anogenital sniffing, chasing, chin rest; Roelofs et al., 2005a). Hormone-based protocols to allow the synchronization of estrus and fixed timed insemination without estrus detection required (Wiltbank et al., 1965). Progesterone analysis of milk samples to estimate ovulation and AI service time and automated devices to detect standing estrus, changes in activity (i.e. increased walking, decreases in eating and rumination), and rectal and vaginal body temperature (Saint-Dizier and Chastant-Maillard, 2012).

The continued estrus detection challenges have given rise to the need for rapid physiological, technological and management advances. As result, researchers and companies around the world have developed automated estrus detection systems described in further sections in an attempt to increase estrus detection rates.

2.3.1. Producer Reliance on Visual Indicators of Estrus Behaviour

Visual observation of behavioural indicators of estrus is the most common method used in Canadian dairy herds (51%) for first AI and natural service (7%; Denis-Robichaud et al., 2016) and subsequent inseminations (45%; Van Schyndel et al., 2019). Standing to be mounted (i.e. standing estrus) is the primary sign of true estrus (Hafez et al., 1969, Sprecher et al., 1995). However, secondary behavioural signs, about six hours before standing estrus, are also used for estrus detection, including anogenital sniffing, chin resting and tailgating (Roelofs et al., 2005a). A decrease in mounting behaviour (i.e. mounting by another cow) is often used to identify the end of estrus (Sveberg et al., 2011).

Estrus detection methods using visual observation of estrus behaviour can be: a) the observation of mounting events by the herdsperson, b) observation of mounting events and chin resting chalk marks by a neutralized bull (e.g. vasectomized) or the observation of rubbed tail chalk marks by mounting events (Sawyer et al., 1986). The accuracy of estrus detection using visual observation is variable and depends on the observer's methodology and training. For example, estrus detection rates using visual observation differ depending on whether cows are observed 3 times a day (~ 70%) compared to only once at a designated milking time (~ 50%; Esslemont and Bryant, 1976). A disadvantage of using standing estrus as the sole predictor for ovulation is that standing estrus may be displayed in multiple cows (sexually active groups; Price, 2008) even when only one cow is in estrus (Roelofs et al., 2005a). In addition, anogenital sniffing and chin resting also occur in other stages of the estrous cycle and, therefore, may be performed by non-estrus cows (Phillips and Schofield, 1990). Accordingly, to estrus detection rates (ER) using visual strategies can often be less than 50% (Senger, 1994) compared with other estrus detection methods (i.e. automated estrus detection ~80%; Sauls et al., 2017). Further, Felton et al. (2012) found in restricted tie-stall housing, in which cows are tethered the majority of each day, walking, mounting, or standing-to-be mounted are restricted and exacerbates estrus detection problems.

2.3.2. Estrus and Ovulation Synchronization Protocols

Estrus and ovulation synchronization protocols allow for control of the time of ovulation (i.e. ovulation synchronization) and the occurrence of estrus (i.e. estrus synchronization), thereby taking the guesswork out of the timing of AI service. Hormonal-based synchronization consists of applying prostaglandins ($PGF_{2\alpha}$) and GnRH at specified times and doses to manipulate the estrous

cycle. The GnRH regresses or ovulates the dominant follicle (promotes a LH surge) and initiates a new follicular wave (promotes follicular stimulating hormone; FSH) in the majority of cows (80-90%; Vasconcelos et al., 1999; Bello et al., 2006). Further, single or multiple injections of $PGF_{2\alpha}$ or analogues regress a responsive corpus luteum (CL; Thatcher et al., 1996, Risco and Melendez, 2011). Other products such as controlled internal drug-release inserts **(**CIDR, Eazi Breed CIDR, Zoetis Canada Inc. Kirkland, Quebec) containing progesterone that suppresses ovulation during CIDR insertion, allows for a 100% blockage of estrus and ovulation until ovulation and estrus are desired (usually 7 days) for AI without affecting fertility (Rivera et al., 2006). Cerri et al. (2011) were able to increase ovulation (i.e. oocyte quality) by giving $PGF_{2\alpha}$ combined with a CIDR, which resulted in sub-luteal concentrations of P_4. However, the accuracy average of estrus and ovulation synchronization protocols can be ~65% and can be affected by parity, milk yield and ambient temperature (Silper et al., 2017).

Ovulation synchronization (Ovsynch) allows for fixed-time AI (TAI) without the need for estrus detection widely used in North American herds (Caraviello et al., 2006). Ovsynch protocols consist of administering GnRH which causes ovulation and a new accessory CL. Subsequent $PGF_{2\alpha}$ administration regresses the corpus luteum, and a second injection of GnRH helps with the formation of a dominant follicle for later ovulation (Rivera et al., 2006). Pre-synchronization optimizes the response to GnRH in Ovsynch protocols by giving two injections of $PGF_{2\alpha}$ 14 days apart since cows may not respond to the first injection of $PGF_{2\alpha}$. The second injection is given 12 days before the first injection of GnRH for the TAI (Moreira et al., 2001). Notwithstanding the efficacy of ovulation synchronization programs, some producers dislike the need to restrain cows for injections, additional handling management, poor conception rates, and the overall absence of benefits using hormone-based protocols (Olynk and Wolf, 2008). Some researchers have also found the success of such hormonal-based strategies can be partially negated by the costs (33.1 USD; Rodgers et al., 2012) associated with the protocols (Rivera et al., 2006). In addition to the drug costs, hormone-based synchronization protocols require additional handling and specific facility requirements such as stanchions, headlocks and or squeeze chutes (Holm et al., 2008). Finally, the majority (65%) of consumers perceive negatively the use of hormone synchronizations when asked their opinion (Pieper et al., 2016). While estrus and ovulation synchronization methods aid reproduction programs, they are expensive, invasive, not well accepted by consumers, and not an option for organic producers.

2.3.2.1. The problem for organic producers

Organic dairy production is an agricultural sector that has grown steadily over the past decade. As of 2012, Canada had 218 organic farms, 10 of which are located in Alberta, Canada (Canadian Dairy Information Centre, 2012). Several criteria must be met to obtain certification as an organic dairy producer in Canada, mainly management practices (Canadian Dairy Information Centre, 2012). In particular, specific medications (e.g. antibiotics) require a milk withdrawal time documented in herd health records. However, the use of reproductive hormones (e.g. $PGF_{2\alpha}$, E_2, P_4, GnRH) to synchronize estrus and ovulation is not allowed since organic livestock management must utilize natural breeding methods (note: Organic dairy production allows the use of AI service; (National Standard of Canada, 2015).

As an alternative to organic dairy production, producers have adopted other breeds (e.g. other than Holstein cows), that can adapt to local conditions and milk production systems in Europe (e.g. organic; Lopez-Villalobos et al., 2000). Holstein-Friesian has been the breed of choice around the world for high milk production and genetic improvement. However, Holstein –Friesian cows have been selected to achieve high milk yield under intensive milking systems (i.e. higher housing, feeding, and medical requirements), which differs from organic milk production (i.e. closer to the natural environment; Ahlman, 2010). The reproductive performance of crossbred Holstein cows in organic dairy farms is higher compared to pure Holstein-Friesian cows (e.g. # AI service per conception; Holstein-Friesian 2.44, Crossbreed 1.27, conception rate using AI; Holstein-Friesian 41.3%, Cross-breed 75%, conception rate (CR) using a bull; Holstein-Friesian 32.5%, Crossbreed 75%, Rodríguez-Bermúdez., 2019). However, milk yield decreases as crossbreeds deviate from pure Holstein-Friesian (Holstein Friesian cross with Brown Swiss; 16.3 L/day, Holstein Friesian; 24.6 L/day; Rodríguez-Bermúdez et al., 2017).

Other approaches to mitigate low estrus detection suggest using natural service (e.g. dairy bull and beef bulls) to avoid the visual observation of estrus behaviours (as well as the AI services) in organic and conventional milk production. In Canada, some dairy producers combine the use of AI service with natural service (89%; Denis-Robichaud et al., 2016). However, the use of natural service in dairy production is costly (100.49 USD/cow per year) compared to TAI (67.80 USD/cow per year), primarily attributed to feeding costs (61%) to maintain a bull all year (Lima et al., 2010). Furthermore, natural service can increase the transmission of diseases, fetal dystocia in heifers,

unknown genetic merit from the bull (e.g. unknown predicted average performance of an animal's future progeny), and unsafe conditions for the farm personnel (Valergakis et al., 2007).

Organic dairies must understand the estrous cycle, optimum timing for estrus detection, and non-invasive alternative detection methods to maintain milk production and adapt to the regulations on organic farms (e.g. prohibition of hormonal-based treatments). Organic milk producers would have to use technological advances in estrus detection and or genetic selection to alleviate low reproduction performance in the absence of hormonal treatments.

2.3.3. Estrus Detection Using Milk Progesterone Concentration

A decline in the P_4 level indicates the onset of the follicular phase and subsequent ovulation (Roelofs et al., 2006). Confirmation of estrus and ovulation is achieved by observing a rise in P_4 concentration in milk or blood plasma following a period of a decreased level (King et al., 1976; Walton and King, 1986; Darwash et al., 1999). Progesterone can be found in whole milk from 0 to 50 ng/mL, and is related to the fat concentration of the milk Pope et al., 1976). As the fat concentration of milk does not change significantly from day to day, a change in P_4 concentration over time can be used to map the estrous cycle (Pope et al., 1976). The estrous cycle is characterized by a slow rise in P_4 concentration to approximately 20 ng/mL until a plateau of P_4 concentration is reached around day 8 post-ovulation. At 15-17 days post-ovulation, the P_4 concentration drops below 5 ng/mL followed by the estrus period which leads to ovulation 24- 48 h (Pope et al., 1976). Ovulation intervals using P_4 concentration in milk (<2ng/mL 30-90 h before ovulation) and plasma (<2ng/mL, 50-98 h; Roelofs et al., 2006) have been observed to be similar using an enzyme-linked immunoassay (ELISA) test which can indicate when ovulation occurred. The use of ELISA tests can be used to confirm the occurrence of estrus, however, an ELISA test kit is not an instantaneous process (i.e. ~ 5 h protocol duration; IBL America, MN, USA) and requires laboratory skills and equipment (Mottram et al., 2000). An ELISA test for on-farm use has been available since 1990 (Ridgeway Science Ltd, Alvington, Gloucs, GL15 6AH, UK). However, its application to detect estrus can be subjective (e.g. qualitative scale results provided) and can create false positives depending on the fat concertation of the milk (Mottram et al., 2000).

There are several methods to standardize the P_4 concentration in milk, such as the use of a quartz crystal microbalance to detect progesterone in solution (Koelsch et al., 1994). However, the time required (~ 4 h) to complete the process limits its use on-farm real-time situation (i.e. during

milking). An alternative approach was taken by Hart et al. (1997), who developed a screen-printed carbon electrode surface for binding antibodies allowing electrochemical readings due to the formation of an enzyme-substrate layer. However, non-progesterone components in bovine milk have also been detected with variable results (Pemberton et al., 1998). More recently in-line milk analysis systems (e.g. Herd Navigator™, DeLaval International, Tumba, Sweden & Lattec I/S, Hillerød, Denmark) have been used to monitor ovarian cyclicity, estrus detection, pregnancy diagnosis (Bruinjé and Ambrose, 2019), mastitis (lactate-dehydrogenase), urea (milk urea nitrogen) and ketosis (Beta-Hydroxybutyrate; Saint-Dizier and Chastant-Maillard, 2012). The in-line milk analysis takes at least six samples per estrous cycle starting at 21 DIM (1L each sample) and measures the P_4 concentration using a competitive immunoassay dry-stick (Yu and Maeda, 2017). The in-line milk analysis system detects the occurrence of estrus based on a drop-in P_4 (<4 ng/mL; Friggens et al., 2008). Estrus detection using in-line milk analysis has been reported to be 87.1 – 99.2% (Se) using different P_4 thresholds (i.e. <4 ng/mL instead <6 ng/mL) and prediction models (e.g. physiological state after calving and expected P_4 concertation according to days in milk; Friggens et al., 2008). However, considerable variation in the timing of P_4 decline before ovulation and abnormal estrous cycles makes monitoring P_4 concentration challenging for the prediction of AI service time. As a result, monitoring of P_4 alone is not sufficient to predict ovulation in most commercial dairy practices because of the high variation in P_4 profiles (i.e. high P_4 compared to Low P_4) between cows and it can range by 2 days between low P_4 and ovulation (Roelofs et al., 2006). Further studies are needed which combine physiological parameters such as P_4 with behaviour parameters (e.g. activity) to improve (Se–Sp) the accuracy of estrus detection and more importantly AI service timing.

2.3.4. Automated technologies to detect estrus

Automated estrus detection devices (AED) provide scheduled surveillance of dairy cows that can identify changes in behaviour frequency (i.e. number of events/per unit time) and physiological traits (e.g. temperature). Then AED run real-time analysis to detect estrus (i.e. estrus alerts) that advise farm staff when to use an AI service. As such, AED technologies can be used to supplement or replace visual observation of estrus and hormonal-based synchronization protocols.

Automated estrus detection devices are divided into three main types. First activity-monitors that measure activity intervals (i.e. walking), lying time (i.e. duration), rumination time,

feeding time (i.e. duration), neck movements, and ear movements. Activity-monitors use a 3-axis accelerometer placed either at the ear (e.g. CowManager SensOor; Agis Automatisering, Harmelen, the Netherlands), neck (e.g. HR Tag; SCR Engineers Ltd., Netanya, Israel), or at the metatarsal area of the leg (e.g. AfiTag®; S.A.E. Afikim, Kibbutz Afikim, Israël). Tri-axis acceleromenters can record rumination, feeding, resting, and activity on a given schedule (e.g. each 30 min; Dolecheck et al., 2015a) and create estrus alerts based on changes in the frequency and or duration of estrus behaviour cues. Second, mounting detectors or rump-mounted detectors (e.g. placed on rump or tail head of a cow) transmit the time and duration of each mount via radio signals to a receiver computer (e.g. Heat Watch system; DDx, Inc., Denver, CO). The system signals an estrus alert when three mounts of 2 sec or more within a 4-h period (Rorie et al., 2002). Third, core temperature loggers (e.g. DVM bolus; DVM Systems, LLC, Greeley, CO) placed in the reticulorumen using a bolus gun which record core temperature twice daily via a passive radio-frequency identification transponder. An increase in temperature due to an increase in activity creates an estrus alert. Other approaches use temperature loggers in milk to recognize higher temperatures (+ 0.3 – 0.5°C) during estrus (Maatje and Rossing, 1976, McArthur et al., 1992) compared to other stages of the estrous cycle.

Other estrus detection methods include measuring vaginal mucus impedance and electrical resistance (Kitwood et al., 1991, Zuluaga et al., 2008), volatile organic compounds using electronic nose detectors (Sanderink et al., 2017), and trained dogs capable of detecting cows in estrus using their olfactory sense (Wiegerinck et al., 2011). However, these methods described are not commercialized yet and further research regarding their feasibility on commercial dairy herds is required. The application of AED in dairy herds rely on the efficacy and practical use of technology devices in commercial farm conditions. To test the efficacy of AED, the accuracy of detecting estrus needs to be evaluated using diagnostic assessment tests (i.e. Se and Sp; Kastelic, 2006). To calculate the Se and Sp of a specific AED, a comparison between the device and a gold standard as a reference (best diagnostic test available) should be performed. In the case of estrus, gold standards often include visual observation of mounting behaviour, the disappearance of ovulatory follicles using ultrasonography, blood or milk P_4 concentration, or a combination of these methods (Dolecheck et al., 2015a). The accuracy of AED in the scientific literature is comparable to visual observations (r = 0.97; Bikker et al., 2014) with high accuracy levels reported (e.g. activity-monitors; 98.6% sensitivity, Dolecheck et al., 2015a, mounting detectors; 96% sensitivity, Rorie

et al., 2002, and temperature loggers; 50% - 84% sensitivity, Maatje and Rossing, 1976, McArthur et al., 1992). However, AED also have low Sp levels caused by false positive estrus alerts despite high Se levels (Se; ~ 80, Specificity; ~ 60%, (Dolecheck et al., 2015a). This is problematic because low Sp levels means an increased number of false positive alerts thus, Se alone is not optimal (note: an effective AED requires a high Se and high Sp).

Comparisons across AED regarding efficacy is further confounded depending on the gold standard used, data analysis (e.g. time series, univariate analysis, multivariate analysis), and the definition of estrus (e.g. confirmation by mounting events, P_4 concentration threshold, 24h prior ovulation). Other factors that affect the accuracy of AED are housing type (free-stall, tie-stalls, pasture-based), behaviour sampling and the variation of physiological parameters associated with estrus (e.g. increasing temperature, higher frequency of walking events, decreased duration of rumination etc.), and whether the algorithms used to process data are appropriate for different lactations (i.e. primiparous vs. multiparous; Dolecheck et al., 2015b). As well, some detection systems are invasive (e.g. DVM bolus) require extra capital cost, and are limited to the number of devices available in the herd (e.g. sensor tags, neck tags, pedometers). The continual improvement of AED for estrus detection is a growing field within dairy science to improve the Se-Sp level and reliability of automated estrus detection for commercial farms. The implementation of automated estrus detection technologies is part of the new trend towards “precision farming”, which aims to optimize farm management by collecting large amounts of data to improve the decision-making of dairy producers.

2.3.5. Biometric Measurements in Animal Science

Biometric systems in animal science use an individual animal’s physiological and behavioural characteristics (i.e. behaviour biometrics) to perform distinctive identification of individuals and diagnosis, recognize - classify subjects (e.g. wildlife), and detect the occurrence of a particular behaviour (Du, 2013). Additionally, biometric collection methods can measure morphological traits, inter-individual variation, and intra-individual changes over time (e.g. sensitive periods; Kuhl and Burghardt, 2013). For example, the variability and uniqueness of coat patterns, vocalizations, movement dynamics, and body measurements could provide scientists with greater understanding of physiological changes or states, which are helpful for predicting a particular process or event of importance in a livestock production setting.

Existing approaches in animal biometrics include computerized data, which can interpret information about the appearance of animals systematically (e.g. identification of leafhoppers through algorithmic formalization; Dietrich and Pooley, 1994). Algorithms describe finite, deterministic, and practical problem-solving methods, suitable for implementation as a computer program or mathematical equation (Sedgewick and Wayne, 2011). In particular, algorithms define classes of interest in a highly objective, comparable, and repeatable manner (Kuhl and Burghardt, 2013). For example, machine learning and scanning algorithms, (e.g. Naïve Bayes, Bayesian network, Decision Tree, Bootstrap aggregation, and Random forest) have been recently developed to predict conception and pregnancy in dairy cows (Shahinfar et al., 2014). Other examples use biometric parameters such as body condition score, lactation data, pregnancy status at 150 DIM, conception rate, and ambient temperature to identify the factors affecting reproductive performance in dairy cows (Caraviello et al., 2006). Similar approaches also use biometric parameters and machine learning to predict milk yield and expected phenotypic values in dairy cattle (Shahinfar et al., 2012). The use of self-learning methods, computerized algorithms and data collected daily present promising opportunities to optimize agriculture by estimating future outcomes.

Biometric measurements are part of precision dairy, aiming for economic, labour, and management efficiency by using innovative technologies as dairy herds continue to increase the number of cows (Kamphuis and Steeneveld, 2016). In addition to production and economic efficiency, automated technologies also a have positive public perception due to their non-invasive use of data modelling and self-learning algorithms that can improve animal welfare practices by avoiding additional handling. In dairy production, these types of technologies perform functions such as concentrate intake monitoring via automated feeders (Bach and Cabrera, 2017), voluntary milking systems (VMS), disease detection (e.g. somatic cell count; Hovinen and Pyörälä, 2011), controlling environmental comfort, and evaluating reproductive performance (Piwczyński et al., 2020). In particular, reproductive performance remains under the research scope worldwide due to its complexity and the variations in its management across the industry. Additionally, the greatest economic loss in dairy herds is attributed to higher culling rates (17.4%, Canadian Dairy Information Centre, 2018) due to reproductive failure. For this reason, great effort has been put into precision dairy technologies to focus on reproductive outcomes (e.g. ER, CR, PR,).

Biometrics used in dairy cattle often include chewing frequency, ruminal temperature, heart rate, animal activity, feeding duration, laying behaviour, odour, vocalizations, P_4 concentrations, milk composition, respiration rate, production management data, as discussed previously. All the parameters described above are made possible because of the development of biosensors for creating prediction models to detect estrus in dairy herds (Bewley et al., 2017). The use of biometrical sensors (e.g. AED) helps a dairy producer access more information with which to make better-informed management decisions and collect data for improved prediction models without additional labour input. However, predictive algorithms for reproductive management rely on factors and parameters recorded previously or retrospectively (i.e. data from previous years or other herds) under specific conditions that may differ by location (e.g. weather), breed, metabolic, physiological state and milk market (e.g. supply management). Herd to herd differences (e.g. management differences among herds) have also been reported to affect the Se and feasibility of AED algorithms (Caraviello et al., 2006). Data analysis at the individual level should be done using real-time monitoring and analysis to assure the accuracy of data obtained and improve outcomes. Implementation of machine learning, artificial intelligence, and data mining in combination with real-time monitoring would further make possible advanced analysis (Pietersma et al., 1998) of a dairy producer's data.

2.3.5.1 Radiated Temperature as a Biometric Measurement

Measuring body temperature is a standard method of monitoring the health status of an animal. Veterinarians and farmers measure the rectal temperature of any animal showing noticeable disease symptoms or as part of diagnostic screening protocols. However, taking core body temperature (i.e. rectal temperature) is time-consuming and disruptive for animals, especially in large herds (Hoffmann et al., 2013). Recent commercial availability of IRT provides non-invasive thermal visualization by which temperatures are monitored and recorded (Talukder et al., 2014). Radiated heat measures are displayed as a temperature distribution image (Chiang et al., 2008) that can be applied in agriculture. The non-invasiveness of IRT has led to broad research in animal science and veterinary medicine, yielding many possibilities for uses on commercial farms in the near future.

The discovery of infrared was done by Sir William Herschel (1738 – 1742) by observing sunlight going through a glass of water (Meola, 2012). Herschel measured the temperature of

visible light through a glass of water using a thermometer in each colour and discovered that the hottest temperature was above the red colour where no visible colour (infrared; previously name dark light) can be observed by the human eye (visible spectrum). The difference between the visible and IRT spectrum is the wavelength band (visible light; 0.4 – 0.75 micrometers vs infrared spectrum; 0.7-12 micrometers, Infrared Training Center, 2014). Visible light is the reflected sunlight on an object only visible in the presence of light, while infrared is the emitted heat (i.e. energy motion of atoms on the surface of an object) from an object visible through an IRT camera in the presence or absence of light (Meola, 2012). Classification of infrared is based on the wavelength (near IRT 0.7 – 2 micrometers, mid wave IRT 3 – 5 micrometers, and longwave IRT 8 – 12 micrometers). The gap between mid-infrared and long infrared (3 – 8 micrometers) is due to low atmospheric transmission (i.e. abundance of infrared absorbers such as carbon dioxide, vapour particles, methane, ozone etc.; Sheahen, 1983). Infrared thermography (IRT) cameras commonly used in animal science capture long waves (8 – 12 micrometers) and have internal camera software that can compensate for any atmospheric attenuation by a person entering information about the image, distance, ambient temperature, and relative humidity. Other factors such as the ability of each object to emit electromagnetic heat radiation (emissivity) and the absorption of radiation from the environment are also considered for any practical application of IRT.

Infrared thermography has provided real-time data for various physiological conditions in dairy cows and calves (e.g. infectious diseases, parturition, and estrus; Hoffmann et al., 2013). Talukder et al. (2014) also demonstrated that infrared thermography could predict ovulation using skin temperature changes at the vulva and muzzle. In particular, Talukder et al. (2014) found ovulation occurs 24 to 47 hours after an increase of 1° to 1.5°C from the vulva and muzzle epidermis for 8 out of 11 ovulated cows (73%) tested. Although the Se of the IRT estrus alert was higher than visual observation of estrus (67%), the Sp and positive predictive value were lower (43%) compared with visual observation of estrus (Talukder et al., 2014).

The disadvantages of IRT depend on the quality of IRT pictures recorded (e.g. focus, distance to the target, camera position), variability caused by hand-held cameras (e.g. camera vibration), whether measurements are based on single-image locations, inaccurate positions to capture data from a body region, ambient air temperature, percentage of relative humidity changes, software requirements, or the need of a person to analyze images (Hoffmann et al., 2013).

Previously, Johnson et al. (2011) found sunlight, wind, drafts as well as moisture or foreign material on the hair coat, circadian and ultradian rhythms, time of feeding, time of milking (e.g. AM – PM), laying and rumination can interfere with IRT measurements. However, work by Schaefer et al. (2012) found effective ways to measure radiated temperature attributed to the physiological process by fixing the distance, body location, and software analysis in beef cattle. Other effects such as ambient temperature, humidity and heat index can be statistically adjusted by understanding the correlation between radiated heat from a body location with ambient effects in livestock (i.e. residual radiated heat; Cook et al., 2016). The use of IRT as an estrus alert under livestock production should be combined with other parameters to increase the Sp level (e.g. reduce false positive alerts) and automated to optimize the data collection, data analysis and create estrus alerts without added labour input.

2.3.5.2. Behaviour Biometrics Measurements using 3D Kinematics

The most reliable estrus detection in dairy cattle to identify the optimum time to AI are behavioural signals associated with the onset of estrus, such as mounting behaviour and standing to be mounting. However, visual observation of estrus signals poses significant challenges for many intensive dairy farms as described in previous sections that are often due to how estrus behaviours are observed. Furthermore, most estrus detection using visual observations is subjective and varies depending on the observer's experience and time relative to the observation of estrus behaviour.

Motion capture systems (i.e. 3D kinematics) provide a complete description of body posture, extremity movements, angles of joints, and coordination (Wong and Shah, 2019). Body movements are studied using translations (i.e. geometric transformation that moves a point of a figure or space by the same distance in a given direction) and rotation (i.e. movements made about the longitudinal axis and in the transverse plane) of specific markers on the x and y axes (i.e. 2D space) and the x, y, and z axes (i.e. 3D coordinate). The placement of markers (passive markers; small reflective spheres) allow for movement to be by tracked using image analysis techniques (i.e. reflected light or infrared light; Vicon systems). In addition, markers can be identified by optical and microphone sensors (i.e. active markers) that can be identified even in the absence of light (Haslwanter, 2018). Markers are then recorded by defining their position and orientation when visible to more than one camera (i.e. a minimum of 2 cameras in the case of passive markers)

in space-fixed coordinate systems previously assigned (Haslwanter, 2018). Additionally, calculations such as acceleration, velocity, angle movements, and pendulum movements are also possible by acquiring software packages with algorithms pre-designed for specific functions (e.g. VICON system gait analysis; Python, MATLAB, ProCalc).

Current applications of kinematics use algorithms and modelling to understand human movements (i.e. gait) in biomedical science (Pfister et al., 2014). In particular, the application of 3D motion tracking has been shown to have practical applications in animal science, such as gait analysis in rodents (Wong and Shah, 2019), distal limb lameness (Weiss et al., 2017), and currently horse – rider postures (Clayton and Hobbs, 2017).

In dairy production, several milking and management systems require dairy cows to adapt to different milking, feeding, and resting routines depending on the housing type (i.e. parlours, tie-stalls, voluntary milking systems). Kinematic systems can analyze dairy cattle movement in confined spaces for its high resolution of data collection and analysis (i.e. movement within millimeters). Parameters such as the distance between markers, Euler angles (0.01 ° C), marker interactions and biomechanical modelling (Roren et al., 2013) can be easily analyzed and identify movement patterns invisible to the human eye. For example, Guesgen and Bench (2018) used kinematic analysis to characterize pelvic movement side-to-side, back–forward in tie-stalls housing and identified changes in the frequency of marker position as ovulation approached in primiparous dairy cows at a micro-movement level. Further, changes in movement frequencies can be computerized to create estrus alerts as part of an automated system even within a limited space such as that which was researched by Guesgen and Bench (2018), avoiding the housing effect (e.g. tie-stall, milking parlour stalls, and VMS robotic milking) in dairy cows estrus detection.

Research opportunities require the application of 3D kinematic analyses to commercial dairy herds without the complexity of kinematic data collection for example, adapting micro-movement analysis to an automated platform. Additionally, changes in movement frequency analyses can be adapted and combined with physiological data (e.g. IRT) to create estrus alerts in combination via an automated platform at a fixed location (e.g. milking parlour).

2.3.5.3. Infrared Thermography Combined with Behaviour Biometric Analysis

Infrared thermography has shown the ability to identify the estrus period in a variety of domesticated animals such as ewes (Barros de Freitas et al., 2018), gilts (Sykes et al., 2012), bitches (Olgaç et al., 2017), beef cows (Radigonda et al., 2017), and dairy cows (Talukder et al., 2015). However, radiated temperature readings are subject to multiple confounding factors that affect accurate readings described in previous sections. As such, the Se and Sp of estrus alerts could be influenced (e.g. increases in IRT due to environmental factors or other physiological processes) for any of the parameters described above. In Canadian dairy herds, the combination of estrus detection methods (e.g. visual observation, estrus detection aids, hormone-based synchronization) has become a common strategy to determine the timing of the first AI service (16%) and subsequent AI services (21%; Denis-Robichaud et al., 2016). Several studies achieved higher estrus detection rates by combining visual observation of estrus with the use of a neutralized bull (84.5% Se - 91% Sp) compared to visual observations alone (77% Se - 80% Sp; Sawyer et al., 1986). Similarly, Talukder et al. (2014) found estrus detection accuracy increased by combining visual observation of estrus, Estrotect patches (Genetics Australia Co-operative Ltd, Bacchus Marsh, Vic., Australia), and radiated heat (100% Se – 29% Sp) compared to radiated heat alone (73% Se – 31.7% Sp). However, the evaluation of accuracy of these combinations described above was evaluated in retrospective and under research circumstances, which can be impractical for commercial dairy herds (e.g. additional costs for having a neutralized steer, additional labour input, and impractical IRT data collection). Further diagnostic evaluation tools are required to understand the implications of lower Sp (i.e. low Sp results in high false positives) in estrus detection since it will result in missed and miss-timed AI services.

Every estrus detection method needs to be validated and its accuracy tested with a balance of Se (i.e. true positives) and Sp (i.e. true negatives; Kastelic, 2006) to demonstrate the ability of a given method to identify or diagnose estrus. The determination of the Se and Sp of a diagnostic test requires an optimum diagnostic method to use as a "gold standard" such as blood-milk P_4 level and ultrasonography to confirm ovulation retrospectively. Often in the evaluation of estrus, visual observation of standing to be mounted is used as a gold standard; however, silent-estrus and cows that fail to be mounted due to physical restrictions may not be accounted for in the evaluation of estrus. Thus, the use of an ineffective gold standard affects the results and the ultimate objective to identify which cows require AI service.

Some of the diagnostic tests include diagnostic odds ratio (DOR; ratio for a positive test result among cows in estrus relative to the odds of the test being positive if the cows are not in estrus), accuracy of test (proportion of cows who are true estrus positive and cows who are true estrus negative among the total number of cows; Shim et al., 2019), Youden J index (proportion of positive cows in estrus and cows not in estrus minus 1; Youden, 1950), and area under receiver operant characteristics (AUC – ROC; overall value of the Se in a test over all Sp values, Mandrekar, 2010).

To ensure the accuracy of estrus detection, the objective is to maximize Se since true positive alerts increase detection rates. In contrast, specificity (Sp) does not represent a clinical danger for the cow but a delay in AI service for the subsequent estrus period. Previous estrus detection studies using vulva radiated heat identified 83% (Se) of the cows in an estrus synchronization protocol, however, the Sp levels were low (~43%; Talukder et al., 2014). Another study detected 21% of cows in estrus (non-synchronized estrus), nonetheless, the combination of multiple IRT outputs from different body landmarks increased the Se to 93.7% but not the Sp (6.7%; Talukder et al., 2015). A partial budget analysis of Se and Sp levels aids decision making in a reproductive program (e.g. cost per AI service) to identify the most cost-efficient Se level (e.g. cost of missing AI service) and the economic implications of varied Sp levels (e.g. cost of miss-timed AI service).

2.4. Research Opportunities

The combination of IRT and behaviour biometrics to detect estrus in dairy cows could improve current estrus detection rates at intensive dairy systems across different housing systems (e.g. tie-stalls and free-stalls). Previous estrus detection research using IRT found significant correlations in radiated heat deviations (i.e. higher radiated heat) during estrus compared to the non-estrus period (e.g. metestrus, proestrus, diestrus; Osawa et al., 2004, Talukder et al., 2014). However, the use of IRT as an estrus alert has yielded mixed results (77% Se and 21% Sp) due to the wide variations in the application and of IRT (i.e. non-fixed location of the camera, thermal pollution in images, reduced experimental unit used, ambient temperature and relative humidity effect: Talukder 2015). As such, measuring of radiated heat could be improved by using an automated IRT platform at a fixed location (i.e. fixed distance) where dairy cows can be monitored daily.

The combination of estrus behaviour observation with bulls and hormonal-based estrus synchronization protocols had previously achieved the highest accuracy in detecting the onset of estrus (100%; Sawyer et al., 1986). However, the economic cost of combining these methods (e.g. natural service cost; 100.49 USD/cow per year and hormone-based protocol cost; 67.80 USD/cow per year, Lima et al., 2010) means it is impractical or unsustainable for reproductive programs (e.g. 56% use only visual observation at first AI service: Denis-Robichaud et al., 2016). Thus, there is an opportunity to create a highly reliable and accurate estrus detection method using combined IRT and behaviour biometrics as a low-cost alternative for the first AI service (i.e. no additional management and supplies) on commercial and organic dairy herds.

Automated infrared thermography has many advantages over other AED since IRT is more easily adapted to a wider range of applications such as stress responses (e.g. heat stress), pathological processes (e.g. lameness and respiratory infections) and metabolic disorders (e.g. ketosis; Saint-Dizier and Chastant-Maillard, 2012). Further, radiated heat and behaviour biometrics do not require additional handling or invasive techniques, thereby alleviating public concerns regarding acceptable livestock practices in Canada.

This dissertation aims to detect estrus in Canadian Holstein dairy cows using a combination of IRT outputs and measuring changes in the frequency of micro-behaviour events attributed to the estrus period within different milking systems. Additionally, to compare the accuracy and financial implications of radiated heat and behaviour biometrics with other estrus detection methods used in the Western Canadian dairy industry. As such, the general hypothesis of this dissertation is that the combination of radiated heat and behaviour biometrics serve to optimize the accuracy level of estrus detection via a non-invasive platform. It is predicted that a beta-type IRT and behaviour biometrics platform can be adapted to any milking system (e.g. pipeline milking and VMS) practically and economically. The information in this thesis contributes to the sustainability of dairy production in Western Canada and promotes the development of technology and its implementation in the dairy industry.

Chapter 3. Infrared Thermography and Behavioural Biometrics Associated with Estrus Indicators and Ovulation in Estrus Synchronized Dairy Cows Housed in Tie-stalls

3.1. Abstract

Most Canadian dairy herds operate in tie-stall housing (72 %), where estrus detection rates average 54%. The objective of this study was to evaluate infrared thermography (IRT) and behavioural biometrics as indicators of estrus in dairy cows. Eighteen cyclic multiparous cows were subjected to an estrus synchronization protocol (Synch), and eighteen pregnant cows (Control) received a sham protocol on the same schedule and frequency as the cyclic cow treatment. A decline in plasma concentrations of progesterone (P_4) and the appearance of a dominant follicle using trans-rectal ultrasonography were used as indirect indicators of estrus, and the disappearance of a dominant follicle was used to confirm ovulation. All cows were monitored via visual cameras to determine the frequency of Treading, Drinking, Neighbour interaction, Tail movement, Laying and Shifting behaviours. Infrared thermograms were recorded at the Eye, Muzzle, Cheek, Neck, Front Right Foot, Front Left Foot, Rump, Flank, Vulva Area, Tail Head, and Withers. To evaluate the accuracy of behavioural and thermal parameters, a pre-defined minimum acceptable value (i.e. threshold) for estrus alerts (> 0.30 Youden J Index and > 0.60 AUC) was used. Ovulation was confirmed in 14 (77.7%) out of 18 Synch cows. Eye, Cheek, Neck, Rump, Flank, Vulva Area and Wither thermograms exhibited higher temperatures at 48 h (Δt = + 0.30 to 1.20°C) and 24 h prior to ovulation compared with 4 days prior ovulation (Δt = 0.06 to 0.11°C) and during ovulation day (Δt = 0.03 to 0.32°C) in the Synch group. In addition, Control cows exhibited greater Treading activity per day compared with Synch cows (20.84 ± 0.39 vs 16.35 events/5min ± 0.34), and Tail movement frequency was greater in Synch cows compared with Control cows (14.84 ± 2.7 vs, 10.11 ± 4.7 events/5min). However, within Synch cows, Tail movement was the only behaviour which significantly increased in frequency 2 d before ovulation (11.81 ± 1.71 events/5 min) followed by a decrease in frequency 1 d before ovulation (4.67 ± 1.05 events/5 min) compared with ovulation day (0 d 6.10 ± 1.25 events/5 min) and during luteolysis (3 d prior to ovulation: 6.01 ± 1.25 events/5 min). Upon evaluation of all variables (thermograms and behaviour frequencies) as estrus indicators at 48 h and 24 h prior to ovulation, Treading and Tail movements before milking and nine thermal locations satisfied the pre-defined minimum

acceptable value for estrus alerts. This study demonstrates that fluctuations in radiated temperature measured at specific anatomical locations and the frequency of Tail movements and Treading behaviours can be used as non-invasive estrus alert in multiparous cows housed in a tie-stall system.

3.2. Introduction

Failure to detect behavioural estrus and false-positive estrus detection can result in missed or mistimed inseminations, leading to poor reproductive outcomes and economic losses on dairy farms (Lewis and Newman, 1984; Rae et al., 1999). Typically, ovulation occurs 24 to 30 h after the onset of standing estrus (Roelofs et al., 2005a). Specifically, the onset of standing estrus is associated with peripheral concentrations of P_4 below 0.95 nmol/L (Holman et al., 2011) and a follicular diameter greater than 15 mm (Perry et al., 2017), which is required to achieve ovulation. Visual observation of standing estrus is often cited as the industry standard for estrus detection, however, it has a 54.5% average estrus detection rate (At-Taras and Spahr, 2001). Visual methods rely on observations of cows standing to be mounted, which is easily facilitated in free-stall housing but is limited to when cows are let out for exercise in tie-stall housing systems (Michaelis et al., 2014). Alternatives to visual observation for estrus detection include the use of various electronic aids, many of which have also been found to be more accurate in free-stall housing systems (At-Taras and Spahr, 2001). The Se of estrus detection for electronic estrus detection aids has been reported within the literature for radio wave transmitters for mounting data (96%; Xu et al., 1998), activity devices attached to the leg (60%; Senger 1994), volatile organic compound electronic nose detectors (86%; Sanderink et al., 2017), and accelerometers attached to the ear or neck (83 to 87%; Al-Taras and Spahr 2001). The use of estrus detection devices combined with visual observation has been found to improve estrus detection by decreasing the difference between the retained value and the true value (error rate) when a single estrus detection method is used (34.6%) compared to when multiple estrus detection methods are used (12.5%) in free-stall systems (Firk et al., 2003), thus providing support for methods of estrus detection that measure and monitor a variety of parameters.

Overall, estrus detection methods that rely on mounting or other overt ambulatory behaviours work best in environments in which such behaviours are not restricted (e.g. free access stall systems) compared with systems in which mounting behaviour is restricted for most of the

day (e.g. tie-stall barns). For example, Felton et al. (2012) were unable to detect estrus using pedometers in a tie-stall barn despite previous studies finding them effective in free-stall systems (Roelofs et al., 2005b). An additional limitation of visual or mounting-based electronic estrus detection methods is how to detect cows ovulating in the absence of obvious signs of estrus (i.e. "silent estrus"). Because of these challenges, non-behavioural methods of detecting either estrus or ovulation, which focus on the use of elevated core body temperature during the estrus period followed by a decrease in core body temperature around the time of ovulation as indicators, have been studied (Lewis and Newman, 1984; Kyle et al., 1998; Suthar et al., 2011). For example, Redden et al. (1993) reported an increase in core body temperature of 0.6 ± 0.3 ˚C during estrus. However, the coefficient of variation for core body temperature measurements equates to 50%, making it an unreliable indicator of estrus if used in isolation of other biomarkers. Furthermore, Hoffmann et al. (2013) concluded that obtaining core body temperatures (e.g. rectal temperatures) are time-consuming and disruptive (i.e. invasive) for animals, especially within large herds.

In contrast, infrared thermography (IRT) is a non-invasive technology (Hoffmann et al., 2013) that provides real-time thermal data for the assessment of various physiological conditions in cattle (e.g. infectious diseases). Radiated heat from the body surface is measured by IRT, which is then displayed as a temperature distribution image (Chiang et al., 2008). Each pixel in a radiometric thermal image represents a temperature measurement that can be monitored, recorded and analyzed (Hurnik et al., 1985). Talukder et al. (2014) demonstrated that IRT could predict ovulation 24 to 48 h in advance based upon variations in skin temperature measured at the vulva of dairy cows housed in a free-stall system with an increase of 1.0 ˚C at a Se of 83%, Sp of 43% and a positive predictive value of 71%. In comparison, visual observation of estrus had a lower Se of 67% but a higher Sp of 86% (Talukder et al., 2014). We note that Se values, in particular, are important as an indication of how often estrus is detected when it is actually occurring (i.e. true positive detection). Several factors affect the overall accuracy of an estrus detection method, including heat stress (Yaniz et al., 2006), farm management practices (Aungier et al., 2012), milk production level (Sangsritavong et al., 2002) and type of housing (free-stalls vs tie-stalls; Palmer et al., 2010). As such, reliance on only visual methods of estrus detection, with its lower Se (Talukder et al., 2014), necessitates the search for more reliable and accurate indicators of estrus (or estrus alerts) which are not reliant on overt standing estrus cues. This is particularly important in Canada, where tie-stall housing represents 72% of dairy farms (Canadian Dairy Information

Centre, 2019), and failure to detect estrus has a direct impact on herd reproductive performance and cow longevity resulting in income losses ranging from 0.73 to > 1.24 USD per open day per cow following the voluntary waiting period of 50 days in milk (De Vries and Collin, 2003).

More recently, subtle micro–behaviour biometrics, which are thought to "prime" more overt behavioural indicators of physiological changes associated with ovulation have also been used within a tie-stall environment to assess estrus (Guesgen and Bench, 2018). The *de novo* application of combined behavioural biometrics and IRT in tie-stall housing and automated milking systems may serve to further optimize the accuracy of estrus alerts compared with visual observation methods. Therefore, the objectives of this study were to: 1) characterize behavioural and thermal biometric patterns of pregnant and cyclic cows around milking time and 2) evaluate the accuracy of infrared thermography and behavioural biometric parameters as non-invasive estrus indicators in cyclic dairy cows in a tie-stall housing system. We hypothesized that subtle changes in behaviour biometrics associated with standing estrus and fluctuations in radiated heat emitted from specific anatomical locations could differentiate the physiological status (pregnant vs cyclic and cyclic non-estrus vs cyclic estrus) in dairy cows. Our second hypothesis was that behaviour biometrics and fluctuations in radiated heat can produce estrus alerts with an estrus detection rate greater than visual estrus detection results (e.g. > 54%) reported in the scientific literature for dairy cows housed in tie-stalls.

3.3. Materials and Methods

This study was approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP 00001652) and all animals were cared for in accordance with Canadian Council of Animal Care (2009) standards and requirements.

3.3.1. Animals and Housing

Thirty-six multiparous Holstein cows (18 cyclic, 18 pregnant) were used in this study. The minimum total sample size recommended for IRT data was 6 cows and for behaviour data was 7 cows using hypothesis testing: two-sample inference - estimation of sample size and power for comparing two means (Rollin, 2016) within an α of 0.05 and a power of 0.80. Based on the availability of animals and in order to minimize disturbances during milking, cows were assigned to three replicates each of 6 cyclic and 6 pregnant cows. The study was conducted from January

to April 2016 (winter to early spring) at the Dairy Research and Technology Centre (DRTC), a tie-stall facility at the University of Alberta, Edmonton, Alberta, Canada. Cows were milked twice daily (0330-0600 and 1500-1730 h) in-stall using a pipeline milking system.

Cow parity ranged from the 2nd to 5th lactation. Cyclic cows during the study produced 44.10 ± 15.80 kg (mean ± SD) of milk per day and ranged from 45 to 55 days in milk (DIM). Pregnant cows were 185.77 ± 19.54 DIM (mean ± SD) and 90 ± 19.91 d in gestation (mean ± SD) producing 42 ± 11.30 kg (mean ± SD) of milk per day. In order to control for potential exercise-induced thermogenesis, cows were housed install for 14 d continuously with free access to water and fed a total mixed ration (TMR) once daily (0600) formulated for lactating dairy cows per NRC guidelines (National Research Council, 2001). The total mixed ration was composed of alfalfa-barley silage, rolled barley-corn, grass hay, and mineral supplements.

3.3.2. Experimental Design

Using a split-plot over time experimental design, IRT and behavioural biometric parameters between two treatments were compared: cycling (Synch) and pregnant (Control) using an equal number (n = 18) of cows per treatment (total n = 36 cows). Within the Synch treatment, behaviour and radiated temperature variables were further compared during the estrus period to non-estrus periods and ovulation day.

3.3.3. Induction of Estrus

Synch cows were subjected to a hormonal synchronization protocol to induce estrus. Synch cows were deemed healthy and at least 45 DIM at the start of the synchronization protocol. The timeline for the synchronization protocol used in the cyclic cow treatment is provided in Figure 3.1. Cows were administered gonadotropin releasing hormone (GnRH Fertiline; 100 µg, i.m.; Vetoquinol N.-A Inc., Lavaltrie QC, Canada) and intravaginal progesterone device (CIDR Zoetis Inc., Kirkland, QC, Canada Inc.) on the first day of the protocol. Prostaglandin $F_{2\alpha}$ ($PGF_2\alpha$**;** Estrumate; 500 µg, i.m; Intervet Corp. Inc., Kirkland, QC, Canada) was administrated 12 h apart during the seventh day induce luteolysis. The CIDR was removed concurrent with the first PGF2α injection (Figure 3.1). Control cows received sham injections (i.e. insertion of the needle only) to simulate GnRH (first day) and $PGF_2\alpha$ treatments (seventh day) and a CIDR device (same as Synch

cows) was inserted on the first day and removed on the seventh day to simulate the synchronization protocol applied to Synch cows (Hittinger et al., 2004; Martinez et al., 2007).

3.3.4. Blood Sampling

Blood samples were obtained from the coccygeal vein of each cow using 10 ml lithium heparin vacutainer tubes (BD Vacutainer Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for P_4 analysis. Blood samples were collected after morning and afternoon milking, starting on the sixth day of the study and continued for 5 days in Control cows and until ovulation was confirmed or for a maximum of 5 days after the second $PGF_2\alpha$ in Synch cows (Figure 3.1). Plasma was obtained by centrifuging blood samples at 3000 rpm, 4°C for 20 min and stored in 1.5 ml micro-centrifuge tubes (MCT Fisher Scientific Waltham, MA, USA) at -20°C until hormone assays were performed.

3.3.5. Progesterone Assay

Progesterone assays were performed at the endocrine laboratory of Prairie Diagnostic Services Inc. at the University of Saskatchewan (Saskatoon, Saskatchewan, Canada) using a solid-phase radioimmunoassay (ImmuChem; MP Biomedicals, LLC, Orangeburg, NY). Plasma samples were analyzed in a single assay with a duplicate analysis of every third sample. Over a concentration range of 3.20 – 33.20 nmol/L the inter-assay coefficient of variation (CV) was 10.90 - 21.70% (mean = 13.80%), and the intra-assay CV was 12.20 - 27.40% (mean = 18.10%).

3.3.6. Ultrasound Scanning

Prior to the start of the study, each cow's reproductive tract was scanned using trans-rectal ultrasonography (Ultrasound ALOKA SSD-500 3.5 MHz linear transducer ALOKA Co., LTD, Tokyo, Japan) in order to re-confirm pregnancy in Control cows that had been previously diagnosed pregnant and to confirm ovarian cyclicity in cows assigned to the Synch treatment. Cyclic cows bearing a corpus luteum at the first examination confirmed ovarian cyclicity and were selected to be placed in the Synch group. Control cows were selected between 60 and 120 d of gestation (based on breeding records) to reduce the likelihood of pregnancy loss during the study period. Ultrasound scans were conducted twice per day following morning and afternoon milking starting on the seventh day to monitor follicular growth and corpus luteum regression and to

confirm ovulation in Synch cows. Control cows were each subjected to sham rectal palpation at the same frequency and duration of ultrasound scanning as Synch cows in order to simulate the same level of invasiveness in both groups of cows. Ovulation was declared when a dominant follicle previously documented had disappeared in successive scanning sessions, followed by the appearance of a corpus luteum in the exact location as the dominant follicle. Specifically, ovulation time was declared as when the ultrasound scan found the dominant follicle had disappeared.

3.3.7. Onset of Estrus

In this study, the estrus period was defined as the period before ovulation coinciding with a decrease in peripheral concentrations of P_4 below 0.95 nmol/L (Holman et al., 2011) and a follicular diameter larger than 15 mm (Perry et al., 2017). For statistical analysis and assessment of estrus alerts, 24 h and 48 h before ovulation were evaluated since P_4 levels were expected to be below 0.95 nmol/L and follicular sizes to be greater than 15 mm 48 and 24 h before ovulation. No mounting or standing to be mounting were observed in this study since all behaviour observations occurred within each cow's tie-stall environment.

3.3.8. Infrared Thermography

Infrared radiometric thermal images were individually captured at eleven anatomical locations at 5-second intervals between each location per cow at morning and afternoon milking and at midday (a non-milking time). Infrared radiometric images of 320×240 pixels with a thermal Se of < 0.04°C at 30°C and accuracy of ± 2% of reading were recorded using a FLIR T450SC thermal camera (FLIR Systems Ltd Burlington, ON. Canada) at 11 anatomical locations; some of which have been previously investigated by other studies: Muzzle, Vulva (Talukder et al., 2014), Front Right Foot, Front Left Foot, Rump, Flank (Montanholi et al., 2008), Tail Head (InterAg, New Zealand), Eye, Cheek (Schaefer et al., 2012) Withers and Neck (Figure 3.2). Thermal images were collected for nine consecutive days, starting on the sixth day of the study until the fourteenth day, establishing a starting point for the estrus period during luteal regression and analyzing for any thermal fluctuations before and during ovulation day. To maintain a consistent camera distance, thermal images were collected at a 1 m perpendicular angle from the point of interest on each cow using a GLM15 50ft laser tool measurement (Robert Bosch Tool CO. IL, U.S.A.). Ambient temperature (°C) and percent relative air humidity (%) were recorded using a weather

meter (Kestrel 3000, Kestrel Nielsen-Kellerman Co. MN, U.S.A.) prior to each infrared recording. The ratio of energy radiated from a material (e.g. the cow's skin surface), or emissivity, was set to 0.98 to calibrate each infrared camera in accordance with manufacturer recommendations for the scanning of live tissues.

Thermal images were processed using FLIR Tools software (FLIR Systems Ltd Burlington, ON, Canada) to determine maximum, minimum and average radiated temperatures. Specifically, FLIR Tools assured the same number of pixels were recorded at each anatomical location for consistency across thermal images.

3.3.9. Behaviour Observations

To determine the frequency of behaviour events relative to ovulation, cows were monitored using digital video recordings (Swann 8-ch 960H DVR) and coded for Treading, Drinking, Tail Movement, Laying, Shifting and Neighbour Interaction as described in Table 3.1 ethogram. Surveillance cameras were mounted at a distance of 2 m behind each cow, at an angle of 45 degrees, to record each cow and her immediate surroundings. Behaviour data were recorded for 5 min continuously before, during, and after each milking and at midday for 9 days from the sixth day to the fourteenth day to coincide with IRT scans.

Each of eight observers was randomly assigned a set of videos except for three videos, which were common to all observers to evaluate inter-observer variation relative to pre-scored video standards. Inter-observer variation was evaluated using a Kappa coefficient calculated using Microsoft Excel (Microsoft Excel, 2013) and inter-observer reliability of 85% was achieved.

3.3.10. Statistical Analysis

Data were analyzed using SAS (ver 9.4, SAS, Cary, NC, USA) to identify significant behavioural and physiological parameters by day relative to ovulation. Each cow served as the experimental unit. Sample days were standardized (D -5, D -4, D -3, D -2, D -1, D 0, D 1 and D 2) using ovulation as D 0 in Synch cows. Only Synch cows in which ovulation was confirmed were included in data analysis (n = 14; 4 cycling cows did not ovulate) and compared to Control cows (n = 18). The Univariate procedure was used to evaluate data normality and to identify data outliers. Normal distribution was assessed using a Kolmogorov-Smirnov test ($P > 0.05$) in which all thermal data complied with normality assumptions, however, behavioural data did not satisfy

all normality assumptions. Models were fitted to a generalized linear mixed model using the Glimmix procedure. For all analyses, the Type 3 test was requested with the inverse (ilink) function specified. All results are presented as least squares means (LSMeans), and standard error means (SEM) calculated using a Bonferroni means separation test. Differences were considered significant if $P < 0.05$, a tendency if $0.05 \leq P < 0.10$ and not significant if $P \geq 0.10$. Peripheral concentrations of P_4 and follicular size were analyzed using Sample Day as a fixed variable.

Behaviour data were analyzed using the Glimmix procedure with a Poisson distribution and the log link function specified. Fixed variables in the model included Treatment (Control and Synch), Sample Day relative to ovulation (D -5, D -4, D -3, D -2, D -1, D 0, D 1 and D 2) and Sample Time (AM milking, Midday, PM milking), while Observer (A, AN, E, EM, H, K, and N), Replicate (1, 2 and 3), and Cow were included as random effects. Observer and Replicate were removed from the subsequent analysis as they were found not to be statistically significant. Following preliminary analysis, Treading and Tail movement behaviours were further analyzed to determine any effect of AM versus PM milking and Milking Order (Before, During and After milking) since only Treading and Tail movement were found to be statistically significant.

The maximum radiated temperature was used based on the hottest pixel in each thermogram to eliminate the potential of foreign debris to confound thermal data. Radiated heat from all anatomical locations were compared at different times of the day (i.e. AM, PM and Midday) to identify any effect if time of day relative to ovulation as well as to determine which scanning time produced the most efficient estrus alert. Additionally, pooled radiated temperature data from AM milking, PM milking and Midday thermograms (total of 3 IRT images per anatomical location) was used to run a daily-radiated temperature average (Davg) analysis for each anatomical location in order to compensate for any effect of Sample Time.

To compensate for the impact of ambient temperature on radiated temperature, the residual radiated temperature was calculated by comparing the observed radiated heat from each anatomical location compared to a predicted radiated temperature (Cook et al., 2016). To predict radiated temperature from each anatomical location, a simple linear regression ($Y = a + bX$) was run per cow. To do this, ambient temperature was assigned as the independent variable (X axis) and the observed radiated temperature was assigned as the dependent variable (Y axis). Potential confounding environmental effects were thereby eliminated in order to obtain radiated temperature changes relative to the estrus period according to the following equation:

Radiated heat res = *Radiated heat obs* - *Radiated heat pred* (Cook et al., 2016)

All thermogram data for the Vulva Area, Tail head, Rump, Flank, Front Right Foot, Front Left Foot, Muzzle, Eye, Cheek, Neck, and Withers were analyzed using the Glimmix procedure with a normal distribution specified. Experimental unit was identified as a random effect. Fixed effects in the model included Treatment, Replicate, Relative Humidity and Ambient Temperature, Sample Day, and Sample Time**.**

Receiver operating characteristic (ROC) curves were constructed using MedCalc Statistical Software (ver 16.4.3 MedCalc software Ltd. Ostend, Belgium) to evaluate the proportion of true positive estrus (sensitivity) and the incidence of true negative estrus (specificity) as a measure of estrus detection accuracy. As previously indicated, the estrus period was determined based on the concentration of P_4 (< 0.95 nmol/L; Holman et al., 2011) and follicular size (> 15 mm) as reported by Perry et al. (2017). For ROC curve analysis, data from Synch cows were binomially categorized based on estrus period (1) *vs.* non-estrus (0) for each Sample Day (D-4 to D 0). Estrus indicators were evaluated using the optimum reference value as an estrus alert for each variable, first at 48 h prior to ovulation and again at 24 h prior to ovulation for all Sample Days and to compare the optimum estrus alerts by estrus days (48 h vs 24h). Maximum and residual radiated temperature data were evaluated for all anatomical locations at AM, PM, and Midday and using Davg for both 24 h and 48 h estrus periods. Similar to radiated temperature, all behaviours were tested Before, During and After milking for both estrus alert periods evaluated. Referent test values were determined when Se and Sp were at similar values since Se and Sp are inversely related, which means at a higher sensitivity, Sp would decrease (Obuchowski, 2005). This provided a standardized way of choosing a referent among different tests. We calculated measures of test performance at the level of the referent with the highest Youden J Index (J Index) serving as the optimum referent. The Youden Index gives equal weight to false positive and false negative estrus values with a range of -1 to 1 for each variable (behavioural and radiated temperature). The area under the curve (AUC) was calculated as an accessory classification analysis as an indicator of the most accurate estrus alert variables. The minimum acceptable value (i.e. threshold) for estrus alerts were pre-determined as 0.30 J Index and 0.60 AUC for all IRT and behavioural variables based on the percentage estrus detection rate for 0.30 J Index (a Sp of estrus detection rate 55%)

which is comparable to visual observation estrus detection rates previously cited in the scientific literature. Follicular size and P_4 concentrations were evaluated using ROC curves and used as the basis of comparison (i.e. as the most efficient methods to detect estrus) for the evaluation of behavioural biometrics and radiated temperature variables.

3.4. Results

Control cows had higher concentrations of P_4 compared with Synch cows ($P \leq 0.01$). However, P_4 concentrations within Control cows did not differ significantly by Sample Day (first day; 25.84 nmol/L ± 2.20, second day; 27.42 nmol/L ± 1.55, third day; 23.22 nmol/L ± 1.58, fourth day; 25.28 nmol/L ± 1.61, fifth day: 22.05 nmol/L ± 1.61) during the study ($P > 0.15$). Progesterone concentration decreased (- 8.83 pmol/L ± 2.64) on D -3 before ovulation, and regression of the corpus luteum was confirmed in all 18 Synch cows. The highest P_4 concentration was observed at 120 h prior to ovulation (D -5; 11.20 nmol/L ± 2.70) and then decreased at 48 h (D -2; 0.77 nmol/L ± 0.44) and 24 h (D -1; 0.09 nmol/L ± 0.15) with the lowest concentration (0.02 nmol/L ± 0.12) on day of ovulation (D 0). Non-ovulating Synch cows during the study period had higher P_4 concentrations (1.25 nmol/L ± 1.07) during the expected time of estrus (48 and 24 h).

3.4.1. Ovarian Ultrasonography

Ovarian ultrasonography confirmed ovulation in 14 (77.7%) out of the 18 cows synchronized during the study period. One cow (7.1%) ovulated within 1 d, 3 (21.4%) within 2 d, 3 (21.4%) within 3 d, 4 (28.5%) within 4 d, and 3 cows (21.4%) ovulated within 5 d of CIDR removal. Follicular size was largest at 2 d (17.84 mm ± 1.39) and 1 d (19.16 mm ± 1.38) prior to ovulation compared with days preceding CIDR removal (D -5: 15 ± 2.65, D -4: 16.91 ± 1.53 and D -3 17 ± 1.55). Synch cows that did not ovulate had a larger follicular size (25.50 mm ± 2.62) than the rest of the Synch group (19.16 mm ± 1.38) at 48 and 24 h prior to ovulation.

3.4.2. Behaviour Frequencies

The frequency of Treading ($P = 0.01$) and Tail movement ($P = 0.01$) differed between Synch and Control cows. Control cows exhibited greater Treading activity per day ($P = 0.01$) compared with Synch cows (20.84 ± 0.39 vs. 16.35 ± 0.34 events/5min), respectively. However,

Treading activity did not change significantly in Synch cows in the days leading up to ovulation ($P = 0.59$).

Tail movement frequency was greater in Synch cows ($P < 0.01$) compared with Control cows during the 2 days preceding ovulation (14.84 ± 2.7 vs, 10.11 ± 4.7 events/5min), respectively. Furthermore, Synch cows exhibited consistent tail movement frequencies during the pre-milking time on D -5 and D -3 (7.50 ± 4.99 and 7 ± 1.97 events/5min). However, an increase in Tail movement frequency was observed on D -4 (13.42 ± 3.57 events/5min), and again 48 h before ovulation (D -2: 11.81 ± 1.71 events/5min), followed by a decrease in tail movements approximately 24 h prior to ovulation (D -1: 4.67 ± 1.05 events/5min) and during ovulation (D 0: 6.18 ± 1.20 events/5min). Control and Synch cows exhibited no significant differences for days relative to ovulation for Drinking ($P = 0.45$), Neighbour Interaction ($P = 0.47$), Laying ($P = 0.96$) or Shifting behaviours ($P = 0.40$).

3.4.3. Radiated Temperature

Raw radiated temperature differed between Control and Synch cows ($P < 0.05$) for 6 IRT locations (Table 3.2). Other variables found to influence radiated temperature in both groups included ambient temperature and Sample Time per day ($P < 0.05$). However, no interactions between fixed variables were found. Synch cows exhibited an increase in radiated temperature during the PM milking period in the days approaching ovulation (D -2 and D -1; $P < 0.05$) while Control cows did not ($P > 0.10$). Specifically, nine anatomical locations (Vulva Area, Tail Head, Muzzle, Front Left Foot, Front Right Foot, Rump, Cheek, Neck and Withers) exhibited an increase in radiated temperature during the last 48 h (+0.30 to 1.20°C; $P < 0.05$) prior to ovulation (D -2 and D -1; $P < 0.05$) while the remaining locations (i.e. Eye and Flank) did not show any changes in radiated temperature (+0.10 to 0.20°C; $P > 0.10$) within Synch cows. No statistical differences in thermal data were obtained during the AM milking, Midday or Davg within the Synch treatment group for the days approaching ovulation.

3.4.4. Receiver Operating Characteristics Curve Analysis

Referent values for each estrus alert (24 h and 48 h) as well as the corresponding J Index, sensitivity, Sp and AUC for P_4, Follicular size, IRT and behavioural parameters are presented in Table 3.3. The highest J Index and AUC values were reached when follicular sizes were greater

than 18.25 mm (0.55 J Index; 0.79 AUC) 48 h prior to confirmed ovulation. Drinking, Shifting, Neighbour Interaction and Lying Down behaviours events did not meet the pre-determined performance threshold (< 0.30 J Index and < 0.60 AUC) and were dropped from further analysis. Tests for the performance of raw radiated temperatures were found to be diagnostically relevant for estrus detection, although the test performances varied among anatomical locations. Thermal data obtained from the Tail Head, Front Left Foot, Front Right Foot, Eye and Muzzle did not satisfy pre-set minimum performance values (< 0.30 J index; < 0.60 AUC), nor did any IRT parameters obtained during the AM milking period. Overall, Residual IRT data yielded higher performance results for most IRT locations at all Sample Times and for both estrus alert periods evaluated. Residual IRT data obtained at Midday and during the PM milking period were characterized by an increase in radiated temperature compared with Residual IRT during the AM milking, which exhibited a decrease relative to the referent test value, which satisfied the performance test to create an estrus alert for both periods (24 h and 48 h). Neither Muzzle nor Tail Head residual radiated temperature satisfied the minimum performance standards for either estrus period at any sample time (Table 3.3).

3.5. Discussion

The first objective of this research was to characterize behavioural and thermal biometric patterns of pregnant and cyclic cows around milking time since the application of IRT and behaviour biometrics may help identify non-estrus cows in addition to cows in estrus for use as an estrus alert. Our results found that infrared thermography and behaviour biometrics do indeed differ between pregnant-cyclic and estrus-non estrus dairy cows.

The difference in radiated temperature between Control and Synch groups is attributed to the increase in radiated temperature in cyclic cows during the estrus period. Synch cows exhibited higher mean IRT temperatures for the Vulva Area, Muzzle, Eye and Withers compared with Control cows during the study period. Thermal biometrics revealed an increase in radiated temperature of +0.50 and 1.20°C 48 h prior to ovulation at several body landmarks, opposite to the significant decrease in P_4 during the same time frame prior to ovulation (i.e. during the estrus period), which agrees with the findings of Talukder et al. (2014). One possible explanation may be the increase in radiated temperature could be associated with increased activity prior to ovulation (Walton and King et al., 1986). However, during this study, Control and Synch cows

remained in a tie-stall environment in which overt movements were limited. Additionally, Frascarolo et al. (1990) demonstrated that the presence of P_4 inhibits warm-sensitive neurons and activates cold-sensitive neurons in the anterior hypothalamic area in rats. This same progesterone mechanism could explain why pregnant cows did not show higher radiated temperatures compared with Synch cows during estrus in the current study. It has also been previously postulated that estradiol, the LH surge and the GnRH hypothalamic response in cyclic cows may increase radiated temperature (Talukder et al., 2014), however these parameters were not measured in the current study.

Some of the challenges with infrared thermography measurements are rises in temperature due to of physical activity, feed intake and variations in the ambient environment. Variations in focus, distance, and camera angle (Talukder et al., 2014), cleanliness of the skin surface and subjectivity in the manual processing of thermal images can also affect radiated temperature data. However, every attempt was made in the present study to minimize these factors. To identify radiated temperature changes throughout the estrous cycle, Sample Time and ambient temperature effects were accounted for by calculating residual radiated temperature for each IRT measurement (Cook et al., 2016). Overall, the residual temperature was found to be of greater diagnostic relevance compared with raw temperature data. Specifically, changes in radiated temperature 48 h and 24 h prior to ovulation were compared to a luteal regression time frame (D -5 to D -3) and ovulation period (D 0) by setting a specific referent test value dependent upon the variable.

All behavioural parameters were compared by treatment wherein Treading frequency was the only behaviour variable to be found greater in pregnant cows. One possible explanation for increased Treading of the back legs in Control cows could be the result of either increased fluid retention or discomfort in the legs as gestation progressed. It is also impossible to rule out that Treading behaviour increases in anticipation of milking in both Control and Synch cows, since a decrease in Treading is observed post-milking. However, the drop in Treading frequency observed during estrus within Synch cows could be due to an overall decrease in activity observed during the onset of estrus (e.g. standing to be mounted, Sveberg et al., 2011) Control cows did not experience. As such, Treading behaviour may have multiple causal factors, including discomfort, anticipation of milking, or the onset of estrus. In contrast, Tail movement frequency did not vary relative to milking time and exhibited a distinct frequency as ovulation approached in Synch cows. In particular, Tail movement frequency exhibited a characteristic pattern prior to ovulation in

cyclic cows. Specifically, 48 h prior to ovulation, Synch cows exhibited increased tail activity followed by a decrease in tail activity 24 h prior to ovulation. One possible explanation for this is that estrus behaviour is characterized by increased activity followed by the onset of standing estrus (i.e. the receptive period). Estrus has been described in two stages: an increase in activity (e.g. walking, mounting, and restlessness) and the appearance of secondary signs (e.g. vaginal mucus discharge) during the first 6 h of the estrus period and, secondly, the onset of standing to be mounted which is characterized by a decrease in activity in the last 3 hours of estrus (Sveberg et al., 2011). In this regard, our findings appear to be similar to those described by Sveberg (2011), and the increase in tail movement on D -2 could be explained as part of restlessness associated with the onset of estrus. Another possible explanation for the increase in tail movements on D -2 is that vulva swelling 48 hours prior to ovulation may have led to temporary discomfort, thereby resulting in increased tail movements, which then subsided. Sumiyoshi et al. (2014) reported swelling of the vulva, contraction of the uterus and uterine horns during the peak of estradiol 24 to 30 hours prior to ovulation followed by intravaginal relaxation 6 to 18 h prior to ovulation, which fits with the timing of the peak in tail movements observed in the current study. Additionally, Fricke (2014) reported an increase in activity using an activity monitoring system (Heatime®) after a second PGF2α injection and a follicle >10 mm in diameter in a Presynch-Ovsynch protocol, which also coincides with our results. However, we cannot rule out the possibility that the increase in Tail movement frequency 48 h prior to ovulation results from a Type 1 statistical error because Tail movement frequency at D -2 is higher than most other days, not just around the day of ovulation. In contrast, P_4 concentrations in the days leading up to ovulation are highly organized. Thus, we cannot state that the peak in tail movement frequency 48 h prior to ovulation is itself, predictive of ovulation. However, the decrease in Tail movement frequency on D -1 may be the better estrus indicator, as tail movements become more still as estrus approaches. Similar to the findings of Guesgen and Bench (2018), it may not be an increase in behaviour frequency, but the subsequent decrease in frequency that is the better estrus alert. Anecdotally, members of the research team noted that when tail movement frequency dipped the day prior to confirmed ovulation, Synch cows tended to be still overall and to move their tail to one side, and hold it there, to expose the vulva.

It should be noted that, the present study did not differentiate between various types of tail events (e.g. left-right movements) or identify the magnitude of movements (e.g. distance and

velocity) during the estrus period. Additional factors which can affect tail movements include staff presence, the proximity of the milking machine, or inter-cow variation attributed to age, previous experiences, seasonal effects (e.g. presence of flies), or physical attributes (e.g. tail docking or broken tail) that can make it difficult to detect more subtle changes in tail movements if used as the sole indicator of estrus. Further, the inter-observer reliability of 85% (Kappa-coefficient) was just below of the acceptable value for behavior observations (90%). The current study found Tail movement frequency very challenging to score due to rapid movements and a large number of events. For that reason, the statistic analysis may underestimate the influence of Tail movement frequency in the experiment. While we are not aware of any of the above influencing our findings, it is possible that the observed increase in Tail movement frequency on D -4 could have been as a result of the injection of $PGF_2\alpha$. Burne et al. (2002) found that $PGF_2\alpha$ increases libido and behaviours following treatment, thus it is not unexpected to see an increase in movement within the cyclic cows in the current study on D -5 and D -4 when the prostaglandin was injected 12 hours apart.

The second objective of this research was to evaluate the accuracy of infrared thermography and behavioural biometric parameters as non-invasive estrus indicators in cyclic dairy cows in a tie-stall housing system. Our results found changes in both thermal and behavioural parameters 48 h and 24 h prior to confirmed ovulation, during the same period when P_4 concentrations are decreasing, and the appearance of the dominant follicle occurs. However, radiated heat and behaviour frequencies were found to increase and decrease in the days leading up to ovulation, suggesting a characteristic sequence of thermal and behaviour events, not merely a single event or value to serve as an indicator of estrus.

To evaluate all thermal and behavioural biometric variables for estrus detection purposes, J Index was used to represent the relative contribution of true estrus and non-estrus test results by expressing proportions within their respective groups (estrus and non-estrus). Decisions regarding behavioural and radiated temperature variable referent values can be adjusted depending on the specific breeding management strategy and factors being monitored. Given that, the number of non-estrus days is limited to a few days within the current study, this could have been reflected in lower Sp results. However, because the current study's focus is on 'test performance' and the ability to find true positives, an emphasis has been placed on Se results. As such, the inclusion of additional non-estrus days would be expected to have had little effect on positive predictive values.

In the case of estrus diagnosis, higher true positives are weighted heavier compared with true negatives since artificial insemination is not as expensive as missing when to inseminate, and the integrity of the cows is not in jeopardy.

The optimal referent test values for this study were identified according to J Index (> 0.30) and AUC values (> 0.60) for both behaviour and IRT data, in order to ensure an estrus detection rate of 55% (similar to visual observation) for comparison purposes. For radiated temperature, the test of performance using ROC curves varied by anatomical location, Sample Day, and the estrus detection period (48 h vs 24 h). Five anatomical locations were identified as diagnostically significant using raw thermal data as a 24 h estrus alert, and four anatomical locations as a 48 h estrus alert. Adjustments to eliminate ambient environmental effects on radiated temperature were accomplished via the calculation of residual temperatures. In effect, the residual temperature can be regarded as that fraction of radiated temperature that is not accounted for by thermoregulation to the environment (Cook et al., 2016). In the present study, the residual temperature is the fraction of radiated temperature most likely associated with hormone-induced temperature changes during estrus. The residual temperature variable increased the number of estrus alerts per anatomical locations with J Index > 0.30 and AUC > 0.60 (Figure 3.4).

Shifting behaviour, Neighbour Interaction, Laying and Drinking events did not present J Index values above 0.30, which suggests they are not practical for estrus detection application. Explanations of poor tests of performance for Shifting, Neighbour Interactions, Laying and Drinking events may be attributed to the frequency of data collection and proximity to milking events. Around milking (5 min before, 5 min during and 5 min after), most cows tended to increase stepping (Treading) and Tail movements while Shifting, Neighbour Interaction, Drinking and Laying behaviours may be more accurately described as sporadic events. In contrast, based on ROC analysis, 3 of 12 Tail movement frequency and 1of 12 Treading movement frequency Sample Times met the J Index > 0.30 and AUC > 0.60 criteria. As such, Tail and Treading movements comply with the requirements of biometrical parameters that can detect estrus within a window of 24 h. In particular, Tail movement frequency was found to be more effective more often, however capturing tail movements in a herd of cows is time-consuming and impractical in-barn unless these parameters can be captured and analyzed using an automated system. As such, our findings suggest it is worth further research into the combined use of infrared thermal data and subtle tail

movements in naturally cycling cows and over the complete estrous cycle as an indicator of estrus as part of an automated infrared technology platform.

3.6. Conclusions

The first objective of this research was to characterize behavioural and thermal biometric patterns of pregnant and cyclic cows around milking time in a tie-stall housing system. We hypothesized that subtle changes in behaviour biometrics associated with lordosis standing estrus and fluctuations in radiated heat emitted from specific anatomical locations can differentiate the physiological status (pregnant vs cyclic and cyclic non-estrus vs cyclic estrus) in dairy cows. The results of the current study found both thermal and behavioural differences between pregnant and cyclic cows. Radiated temperatures from the Vulva Area, Muzzle, Cheek, Eye, Neck and Withers and Treading frequency during milking increased in cyclic cows in the days approaching ovulation compared with pregnant cows. The second objective of this research was to evaluate the accuracy of infrared thermography and behavioural biometric parameters as non-invasive estrus indicators in cyclic dairy cows in a tie-stall housing system. Our second hypothesis was that behaviour biometrics and fluctuations in radiated heat are able to produce estrus alerts with an estrus detection rate greater than visual estrus detection rates (e.g. > 55%) using a J Index > 0.30 and AUC > 0.60. Multiple behaviour (e.g. Tail and Treading movements) and thermal (e.g. Residual) biometrics were found to meet the pre-determined criteria as estrus alerts. Specifically, fluctuations in radiated temperature from the vulva, muzzle, eye, neck, cheek, withers, in addition to tail movements prior to ovulation, were associated with estrus indicators in tie-stalled cows.

3.7. Acknowledgments

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University of Alberta Dairy Research and Technology Centre staff who provided assistance with day-to-day animal care and handling.

Table 3.1. Ethogram of cow behaviours observed during AM and PM milking and at midday. Continuous behaviour sampling was conducted 5 minutes before, during, and after milking.

Behaviour	Description
Treading	Cow lifts either left or right back foot and returns foot to the floor. Can be a full step or semi-step (only one foot moves but the other remains in its position).
Drinking	Cow approaches the water bowl and proceeds to place snout in water bowl.
Neighbour Interactions	Any kind (e.g. pushing, licking, scratching etc.) of contact between one cow with either the cow on its left or right side.
Tail movement	Each tail movement from side to side.
Shifting	Cow changes position without any foot movement (step).
Laying	Cow is sternally or laterally recumbent.

Table 3.2. Treatment comparison of the average radiated temperature from all anatomical locations.

	Control		Synch		
IRT location	LSMeans	SEM	LSMeans	SEM	P-Value
Vulva area	34.86	0.05	35.32	0.05	0.01
Tailhead	32.19	0.07	31.99	0.07	0.06
Rump	32.87	0.06	33.01	0.06	0.13
Flank	33.21	0.07	33.04	0.07	0.07
Left foot	30.73	0.11	30.88	0.11	0.35
Righ foot	30.85	0.12	31.00	0.11	0.36
Muzzle	32.51	0.09	33.46	0.09	0.01
Cheek	32.31	0.08	31.22	0.08	0.01
Eye	35.69	0.04	35.99	0.04	0.01
Neck	33.61	0.07	33.32	0.07	0.02
Withers	31.84	0.09	32.34	0.09	0.01

Overall least square means (LSMeans) ± standard error mean (SEM) and P-Values of the radiated temperature between the two treatments (Control and Synch) per anatomical location.

Table 3.3. Test of performance results for progesterone (P_4), Follicular size, behavioural and IRT parameters from Synch cows using ROC curve analysis. Significant results are presented (>0.3 J Index and 0.6 AUC) for all parameters (P_4, Follicular size, IRT and Behavioral biometrics) at different sample times and estrus periods (24 & 48 h prior ovulation). The J index represents the relative contribution of true estrus and non-estrus test results by expressing proportions within their respective groups (estrus and non-estrus). The optimum balance reference point was identified as the highest J index value and AUC and reflects the efficiency of each variable by expressing the true estrus (sensitivity) and true negatives (specificity).

Estrus Alert at 24 h						
Parameter	S.time[1]	J index[2]	R. Value[3]	Sensitivity	Specificity	AUC[4]
Progesterone (P_4)	AM&PM	0.35	≤0.15	90.91	44.34	0.66
Follicular size	AM&PM	0.39	≥16.50	63.64	54.74	0.65
Treading	A[5]	0.38	≤16.00	76.92	60.98	0.65
Tail movement	B[6]	0.34	≤4.00	69.23	64.44	0.68
Tail movement	A[5]	0.38	≤6.00	76.92	60.98	0.67
Vulva area raw IRT	Midday	0.33	>35.90	53.85	79.17	0.65
Rump raw IRT	Midday	0.31	>32.20	100.00	31.25	0.63
Cheek raw IRT	PM	0.34	>32.80	53.85	80.00	0.65
Neck raw IRT	PM	0.47	>33.60	84.62	62.75	0.76
Neck raw IRT	Davg[7]	0.38	>33.50	69.23	68.63	0.64
Flank res IRT	PM	0.40	≤0.19	100.00	40.43	0.60
Cheek res IRT	PM	0.45	>0.01	84.62	60.00	0.75
Neck res IRT	PM	0.43	>-0.03	92.31	50.98	0.70
Withers res IRT	AM	0.30	≤-0.53	46.15	84.31	0.64
Withers res IRT	Midday	0.30	>0.30	53.85	76.60	0.62
Estrus Alert at 48 h						
Progesterone (P_4)	AM&PM	0.38	≤0.06	84.62	53.85	0.66
Follicular size	AM&PM	0.55	>18.25	76.92	78.49	0.79
Vulva area raw IRT	PM	0.36	>36.40	46.15	90.20	0.70
Rump raw IRT	PM	0.42	>33.60	76.92	64.71	0.62
Flank raw IRT	Midday	0.35	>32.90	84.62	50.00	0.69
Withers raw IRT	Midday	0.40	>33.40	61.54	78.72	0.64
Vulva area res IRT	PM	0.35	>0.49	38.46	96.08	0.66
Rump res IRT	Davg[7]	0.42	≤-0.33	53.85	88.24	0.63
Flank res IRT	Davg[7]	0.46	≤-0.59	53.85	92.16	0.74
Front right foot res	Davg[7]	0.39	≤0.08	92.31	47.06	0.67
Front left foot res	Midday	0.33	>0.25	76.92	56.25	0.63
Front left foot res	Davg[7]	0.34	≤-0.22	61.54	72.55	0.62
Eye res IRT	AM	0.32	>-0.04	84.62	47.06	0.68
Eye res IRT	Davg[7]	0.30	≤-0.13	53.85	76.47	0.65

Abbreviations: [1]S. time = Sample time; [2]J Index = Youden index output from ROC curve analysis; [3]R. value = Optimum reference value identified at highest J index; [4]AUC: Area under the curve; [5]A = Behaviour data collected 5 min Before AM milking; [6]B = Behaviour data collected 5 min Before PM milking; [7]Davg = Radiated temperature average per day.

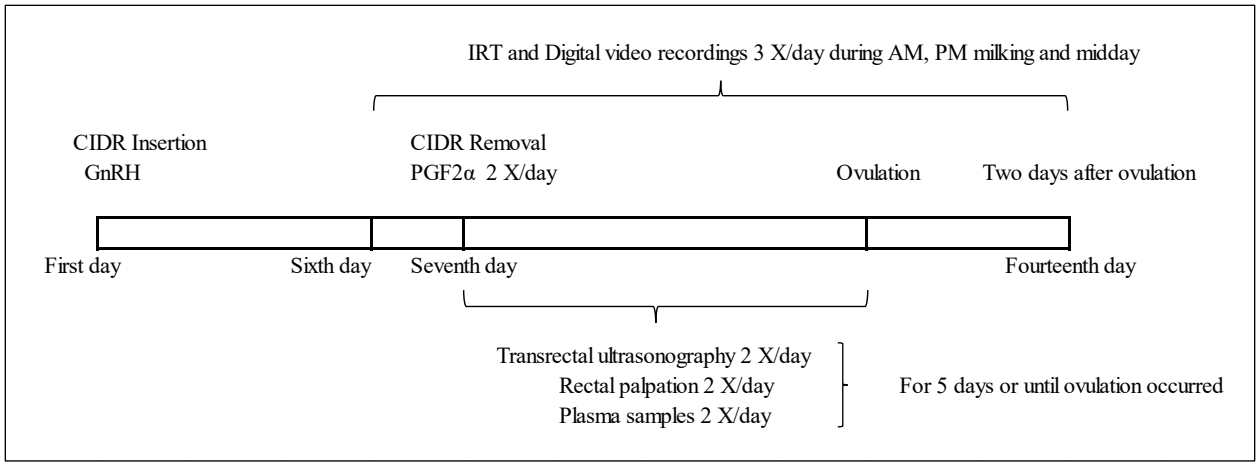

Figure 3.1. Timeline of experimental events. Gonadotropin releasing hormone (GnRH) was given (first day of protocol) to synchronize follicular development in Synch group and a sham injection (insertion of a needle only) to the Control group and an intravaginal progesterone device (CIDR) was inserted concurrently in both groups. Two injections of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) were given 12 h apart to induce luteolysis on the seventh day in Synch cows and sham injections on the same frequency as Synch group were given to the Control group. Trans-rectal ovarian ultrasonography was used to measure dominant follicles and confirm ovulation in Synch cows, whereas Control cows were subjected to palpation per rectum. Plasma samples 12 h apart (0600 and 1800) were obtained to determine P_4 concentrations from Synch and Control cows. Infrared Thermography (IRT) and digital video recordings were performed to measure changes in behaviour frequencies and skin radiated temperatures on days approaching ovulation from the sixth day until two days after ovulation.

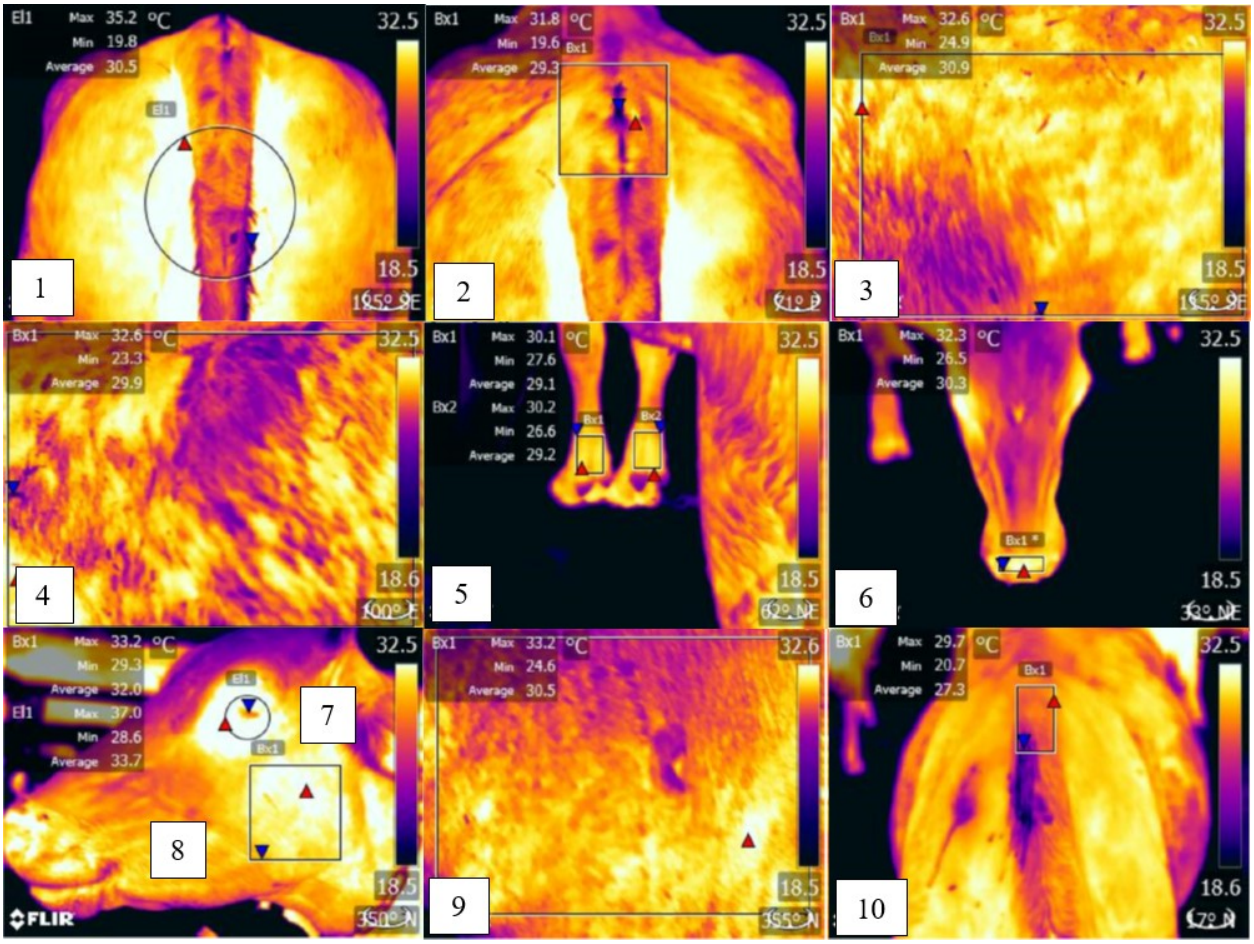

Figure 3.2. Sample thermal images from ten anatomical locations including: the Vulva area (1), Tail head (2), Rump (3), Flank (4), Front right foot and Front left foot (5), Muzzle (6), Eye (7), Cheek (8), Neck (9) and Withers (10). The squares and circles in the thermal pictures represent the area used to identify the maximum radiated temperature for each anatomical location.

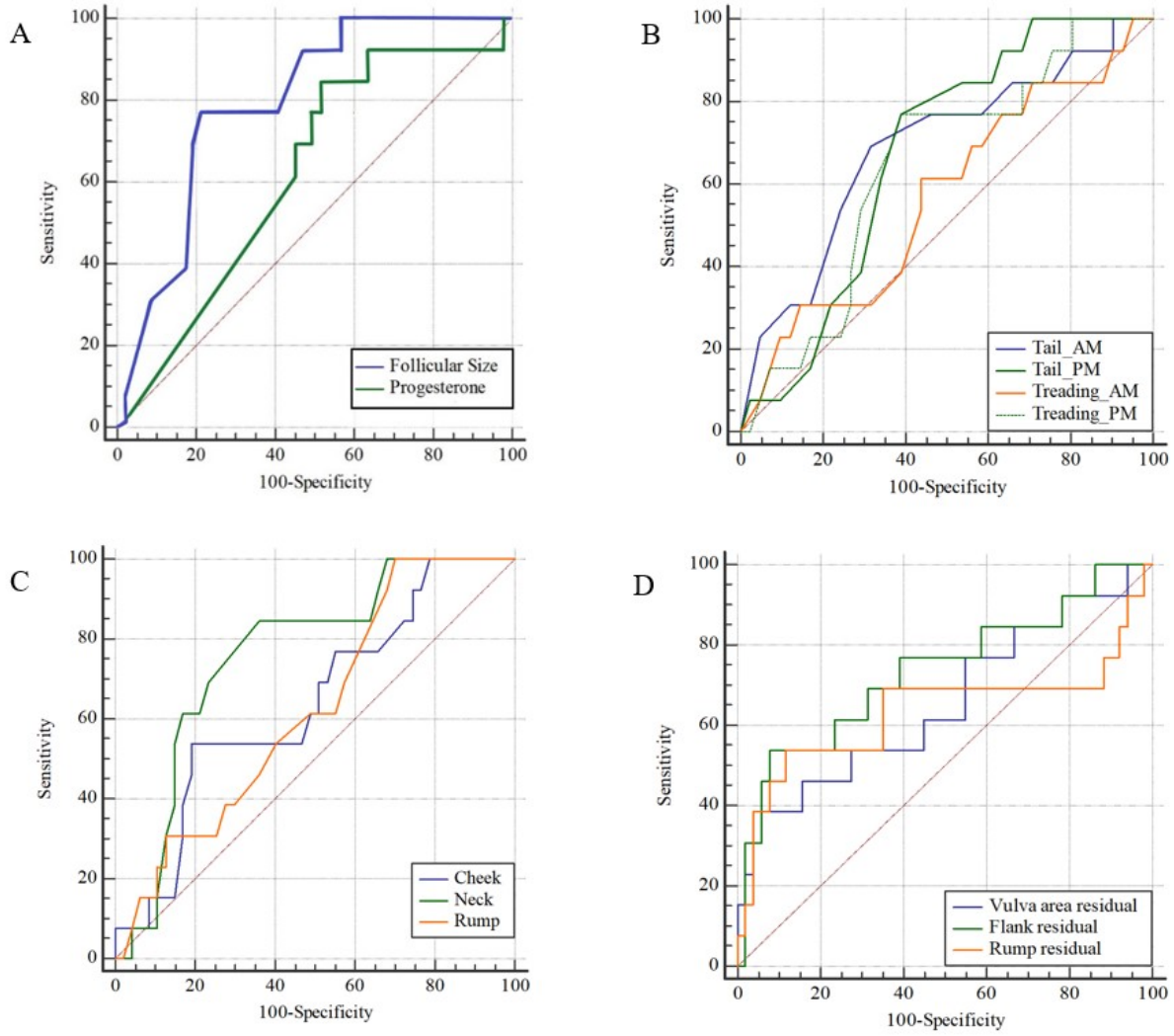

Figure 3.3. Receiver Operating Characteristics (ROC) curves comparison in Synch cows of Progesterone (P_4; P = 0.01) concentrations and Follicular size D -2 (P = 0.01) during estrus period preceding ovulation (A); Tail movements (P = 0.04) and Treading frequencies before milking (P = 0.08) comparison during estrus period D -1 (B); Raw thermal measurements from Cheek during D -1 (P = 0.07), Neck during D -2 (P = 0.01) and Rump during D -2 (P = 0.19) (C); and Residual thermal measurements from Vulva area (P = 0.1), Flank (P = 0.01), and Rump at D -2 (P = 0.24; Figure D).

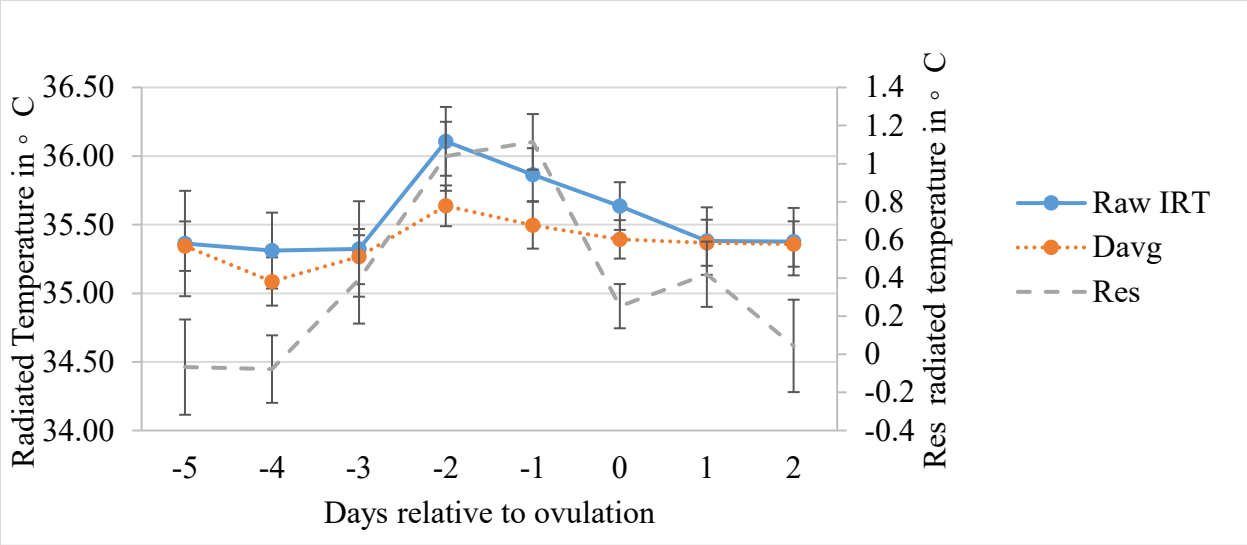

Figure 3.4. Radiated temperature increase of different thermal data measurements on days approaching to ovulation (D 0) in Synch cows for the Vulva area during the afternoon milking. Day -2 and D -1 coincided with estrus (following decreases on P_4 concentrations and follicular diameter). Raw IRT (P = 0.01) and standard error mean (SEM) temperature is compared with the daily average (P = 0.18) of radiated temperature (Davg) and SEM as well as the residual temperature (P = 0.01) Res; Residual IRT temperature is calculated by subtracting the estimated IRT temperature based on ambient temperature and observed IRT temperature from the actual radiated temperature of each anatomical location).

Chapter 4. Evaluation of Infrared Thermography Combined with Behaviour Biometrics for Estrus Detection in Naturally Cycling Dairy Cows

4.1. Abstract

Low estrus detection rates (< 50%) are associated with extended calving intervals, low economic profit and reduced longevity in Holstein dairy cows. The objective of this study was to evaluate the accuracy of infrared thermography and behaviour biometrics combined as potential estrus alerts in naturally (not induced) cycling dairy cows housed in a tie-stall barn. Eighteen first lactation cows were subjected to transrectal ultrasonography to determine spontaneous ovulation. The dominant follicle (DF) disappearance was used retrospectively as an indirect indicator of ovulation and to establish the estrus period (48-24 h prior the DF disappearance). Raw skin temperature (Raw IR) and residual skin temperature (Res IR) were recorded using an infrared camera at the Vulva area with the tail (Vtail), Vulva area without the tail (Vnotail), and Vulva's outer lips (Vlips) at AM and PM milking from Day 14 until two days after ovulation was confirmed. Behaviour biometrics were recorded on the same schedule as the infrared scan. Behaviour biometrics included large hip movements (L-hip), small hip movements (S-hip), large tail movements (L-tail) and small tail movements (S-tail) to compare behavioural changes between estrus and non-estrus periods. Significant increases in Raw IR skin temperature were observed two days prior to ovulation (Vtail; 35.93 ± 0.27˚C, Vnotail; 35.59 ± 0.27˚C, and Vlips; 35.35 ± 0.27˚C) compared to d -5 (Proestrus; Vtail; 35.29 ± 0.27˚C, Vnotail; 34.93 ± 0.31˚C, and Vlips; 34.68 ± 0.27˚C). No significant changes were found for behavioural parameters except S-hip movements, which increased at two days before ovulation (d -2; 11.13 ± 1.44 Events/5min) compared to d -5 (7.30 ± 1.02 Events/5min). To evaluate the accuracy of thermal and behaviour biometrics, receiver operating characteristics (ROC) curve analysis were performed using Youden index (YJ), diagnostic odds ratio (DOR), positive likelihood ratio (LR+), Se, Sp, and Positive predicted value (PPV) to score the estrus alerts. The greatest accuracy achieved using thermal parameters were for Res IR Vtail PM (YJ = 0.34) and L-hip PM (YJ = 0.27) for behaviour biometrics. Combining thermal and behaviour parameters did not improve the YJ index score but reduced the false-positive occurrence observed by increasing the DOR (26.62), LR+ (12.47), Sp (0.97) and PPV (0.90) in a Res IR Vtail PM, S-hip AM, S-hip PM combination. The combination of thermal and

Chapter 4 has been accepted for publication and formatted following ANIMAL guidelines.

behavioural parameters increased the accuracy of estrus detection compared to either thermal or behavioural biometrics, independently in naturally cycling cows during milking.

4.2. Introduction

The incidence of false-negatives and false-positives in many estrus detection methods contributes to extended artificial insemination (AI) intervals, calving delays, poor economic outcomes and decreased longevity in dairy cows (Mayo, 2015; Giordano et al., 2015). The visual observation of cows standing-to-be-mounted is the most reliable estrus detection method and one of the most commonly used for the first AI service (51%; Denis-Robichaud et al., 2016) due to its low incidence of false-positive estrus detection (Glencross, et al., 1981; Sprecher et al., 1995) in North American herds (USDA, 2009). However, estrus detection rates based on visual observation have a low incidence of true positive estrus alerts per cows in estrus (37 – 54%; Van Eerdenburg et al., 1996; Sakaguchi, 2010).

Physiological reasons for reduced estrus detection rates include the reduction of estrus period duration in Holstein cows (from 18 to less than 8 h) over the last 50 year (Reames et al., 2011), negative correlations with higher milk yield (López et al., 2005), reduced estrus behaviour (e.g. restlessness and mounting behaviour) in extreme ambient temperatures (e.g. hot and cold temperatures; Collier et al., 2006) negative energy balance (Grummer and Rastani, 2003) and the differences in estrus behaviour (e.g. frequency and duration) between multiparous and primiparous cows (López-Gatitus et al., 2005; Chanvallon et al., 2014). The ability to detect estrus using visual observation of cows standing to be mounted is also significantly limited or non-existent in tie-stall housing environments (Felton et al., 2012) compared with free-stalls or pasture-based herds result of cows being tethered while in their stall. Further, 72% of dairy herds in Canada are housed in tie-stall barns (Canadian Dairy Information Centre, 2019).

Good reproductive management relies on accurate monitoring and detection of estrus cues, which are used as indicators of when to inseminate a dairy cow. Research shows that the most cost-efficient time to AI is from 60 to 70 DIM for multiparous and approximately 105 days in milk for primiparous cows (De Vries, 2006) to maintain an optimal calving interval (12 to 13 months; Stevenson et al., 2014). Extended calving interval leads to an increase of 1.00 USD (primiparous) and 1.80 USD (multiparous) in costs for every extra day a cow remains non-pregnant. Further,

these costs increase to 6.00 USD if a cow is open during late lactation (≥ 160 days in milk; Meadows et al., 2005).

Advances in estrus detection rates (> 50%) have been achieved through the use of automated estrus detection devices which continuously monitor physiological and behavioural parameters to detect estrus without additional labour input (Rutten et al., 2014). Automated estrus detection consists of sensors and algorithms that create estrus alerts for proper AI service. Automated estrus detection devices can be divided into activity monitors (e.g. rumination time, laying bouts, walking, ear movements; Løvendahl and Chagunda, 2010; Aungier et al., 2012), mounting detectors (e.g. mounting counts and mounting duration; Xu et al., 1998; Sauls et al., 2017), body core temperature loggers (e.g. reticulo-rumen, vagina, ear and milk temperature; Fordham et al., 1988; Fisher et al., 2008), and analysis of P_4 concentrations in milk (Delwiche et al., 2001; Adriaens et al., 2017). However, most automated estrus detection devices are designed for application in free-stall situations and often fail to detect estrus or require additional handling such as moving cows to an outside pen if reared in tie-stall housing. Most dairy producers have adopted the combined use of various estrus detection methods, usually estrus detection devices with visual observations. However, no detailed analyses have been performed to describe the effect on accuracy by combining different estrus detection methods (Firk et al., 2002).

Live organisms emit electromagnetic radiation (thermal radiation; Boyd, 1983), some of which can be measured using infrared thermography cameras. This energy can be emitted, reflected or transmitted. In particular, animals and humans are susceptible to heat loss (e.g. conduction, convection, radiation, evaporation and respiration) in the environment (Berz, 2007). Changes (e.g. increases or decreases) in the amount of heat loss can indicate different physiological processes. For example, infrared has been used in dairy cattle to measure skin temperature changes to monitor udder health status (Sathiyabarathi et al., 2016), heat stress (Daltro et al., 2017), qualitative differences in cattle lameness (Novotna et al., 2019), and early lactation diseases (e.g. ketosis, metritis, and milk fever; Macmillan et al., 2019). Several studies report increased skin temperature at the vulva associated with the estrus period, serving as an estrus alert (Osawa et al., 2004, Talukder et al., 2014, Perez Marquez et al., 2019). Further, estrus detection using infrared cameras have been used to detect estrus and ovulation regardless of housing type in multiparous cows (71% in free-stalls; Talukder et al., 2014, ≥ 50% in tie-stalls; Perez Marquez et al., 2019). Nevertheless, debris present on the animal can potentially influence thermal radiation by masking

actual thermal readings (mixed results; Sykes et al., 2012). In addition, it is difficult to standardize the conditions for the use of handheld infrared cameras due to variations in the angle and distance between the camera and target, which can also affect thermal readings (Talukder et al., 2014). Thus, whenever infrared is used, careful consideration must be given to ensure all debris are cleared away, and conditions are kept standardized.

Biomechanical movements have also been reported as useful biometric parameters to identify different physiological processes in humans (Jain et al., 2004). Similarly, in tie-stall housed dairy cows, changes in restless behaviour as measured using < 20 mm hip movements (e.g. back-forward and left-right) prior to ovulation have also been demonstrated using 3D-kinematics (Guesgen and Bench, 2018). Infrared technology in beef cattle has also been able to measure an individual animal's behavioural frequencies using an automated RFID-IR platform (Cook et al., 2016). The above research demonstrated that behaviour frequencies could be measured by analyzing changes in thermal distribution within a thermal image by comparing the thermal radiation from a target with a colder background. Based on the above findings, the objective of the present study was to evaluate a combination of thermal and behavioural biometrics as estrus alerts at the estimated estrus period (48–24h prior ovulation) in naturally cycling dairy cows in a tie-stall housing. We hypothesized that behaviour biometrics using the hip and tail regions combined with infrared metrics from the vulva area would increase the accuracy compared to these same parameters utilized in isolation as indicators of the estimated estrus period.

4.3. Materials and Methods

The current study was conducted from June to October 2016 (summer-fall) at the University of Alberta's, Dairy Research and Technology Centre (DRTC), a 146-cow tie-stall facility located in Edmonton, Alberta, Canada. The study evaluated 18 naturally cycling (not induced by hormone interventions) primiparous Holstein cows following a hypothesis testing: two-sample inference estimation of sample size and power using two means (Rollin, 2016) with an $\alpha = 0.50$ and a power = 0.90. The minimum required number of cows was seven. However, in anticipation of excluding some cows due to abnormal estrous cycles and postpartum disease, 18 cows were assigned to the study. Cows were averaging 43 ± 2 days in milk (± SD) and producing 27.3 ± 5.63 kg (Mean ± SD) of milk per day at the beginning of the study. During the study period, cows were housed indoors for 31 ± 6 d continuously with no access to an outside pen to avoid

variations in infrared measurements associated with exposure to the outside environment. Cows were milked twice daily (0330-0600 and 1500-1730) in-stall using a pipeline milking system. Free access was given to water and a total mixed ration (TMR) based on NRC guidelines (National Research Council, 2001) for lactating dairy cows. The main ingredients of the TMR were alfalfa-barley silage, rolled barley-corn, grass hay, and mineral supplements.

4.3.1 Experimental Design

The current study followed a split-plot over time experimental design that compared thermal and behaviour biometrics during the proestrus stage (baseline), the expected estrus period, the day of ovulation and two days post-ovulation for all eighteen cows (n = 18). Each cow served as the experimental unit. Cows were assigned to the study if the presence of a corpus luteum (CL) was confirmed by transrectal ultrasonography (ALOKA SSD-500 scanner fitted with a 7.5 MHz linear array transducer, ALOKA Co., LTD, Tokyo, Japan) by the same technician throughout the study. Ovarian mapping was conducted every other day until CL regression was evident, followed by the disappearance of a dominant follicle (DF) which indicated the occurrence of ovulation. Once each cow ovulated (Day 0) and the presence of a new CL was confirmed subsequently, ultrasound scanning was resumed every other day (1700) from d 7 to d 13 and daily scans from d 14 until confirmation of subsequent ovulation (d 0) and the appearance of new CL (Figure 4.1). Dominant follicles and CL diameters were measured in mm using built-in callipers and recorded for left, and right ovaries to determine follicular growth, monitor the presence of DF and CL regression.

4.3.2 Milk Sampling and Estradiol Assay

Milk samples were obtained directly by teat stripping, discarding the first two strips during both milking times (AM and PM) following the same schedule of data collection from thermal and behaviour biometrics (Figure 4.1). Milk samples (10 mL) were collected from cows at each milking into 35 mL snap-seal containers (Fisher Scientific Company, Ottawa, ON, Canada). Samples then were transferred to 10 mL plain Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), centrifuged at 1940 x g, at 4°C for 20 min to remove milk fat, and skim milk samples were stored in two 5 ml plastic tubes (MCT Fisher Scientific, Waltham, MA, USA) at -20°C until estradiol (E_2) assays were performed.

Skim milk samples (100-µL) were analyzed using an estradiol ELISA kit (IBL America, MN, USA) in a single assay with duplicate analysis. Grgurevič et al. (2016) and Snoj et al. (2017) previously validated a direct bovine milk sample E_2 analysis in a plasma serum ELISA kit. The estradiol assay kit had a standard range of 3 – 200 pg/mL with a Se of <1.399 pg/mL and cross-reactivity with the structurally related compounds of estrone (0.2%), estriol (0.05%) and fulvestrant (0.3%). The range in E_2 concentration was 6.85 to 47.82 pg/mL with an inter-assay coefficient of variation of 9.33% at 65.64 pg/mL and an intra-assay coefficient of variation of 5.57% at 16.15 pg/mL. Estradiol daily means were calculated (AM + PM samples/2) to match ovarian structure data (e.g. CL and DF). However, E_2 concentration peaks were found individually (e.g. each cow) if the E_2 concentration per Sample day were greater than two SD plus the mean.

4.3.3. Infrared Thermography

Thermal images were captured at 4 frames/sec for a total of 5 min from a distance of 1 m perpendicular to the caudal-dorsal side of the cows during the morning (AM) and afternoon milking (PM). To compare the expected estrus period (48-24 h before ovulation) with the proestrus stage (baseline), thermal images were recorded starting on d 14 of the estrous cycle until two days after ovulation. An A310 thermal camera (FLIR Vision Systems Ltd. Burlington, ON, Canada) was used to record infrared images of 320 x 240 pixels. The thermal Se of the camera was 0.05 °C at 30°C, with a measurement range of −20°C to 120°C, and accuracy of ±2°C or 2% of the measured temperature. Thermal images were collected using Vacca2 software (Animal InfraMetrics Inc. Lacombe, AB, Canada). A laptop computer (ThinkPad, Lenovo Group Limited, Haidian District, Beijing, China), with Vacca2 software and an infrared camera powered by a 12-volt battery with a 1000-Watt inverter (MotoMaster, Canadian Tire Co. Toronto, ON, Canada) were placed on a wheeled cart that could easily be moved from stall to stall during data collection (Figure 3.2). A GLM15 50ft laser measurement tool (Robert Bosch Tool Co. IL, USA.) was used to ensure a consistent distance between the camera and cows. Ambient temperature (°C) and percent relative air humidity (Rh%) were recorded using a hygrometer (Kestrel Nielsen-Kellerman Co. MN, USA.). Emissivity was set to 0.98 following manufacturers recommendations for live tissues (e.g. cow's skin surface).

Thermal images were processed using FLIR ResearchIR software (FLIR Systems Ltd Burlington, ON. Canada) to determine the maximum, minimum, and average (SD) skin

temperature output of each area of interest from a selected frame per cow sample collection. Areas in the thermal images that defined the Vulva area with the tail (Vtail), Vulva area without the tail (Vnotail), and Vulva's external lips (Vlips) were predetermined using a standardized ellipse (Vtail and Vnotail) and a free-drawing tool (Vlips) in the FLIR ResearchIR software and was standardized for all images (Figure 4.3).

4.3.4. Behaviour Observations

To determine the frequency of behaviour events as ovulation approached, behaviour biometrics were scored using thermal frames at each milking number of Events/5min at 4 frames/sec. The same person performed behaviour observations to eliminate inter-observer variation. Behaviour frequencies were determined during the same period as infrared scanning (d 14 until two days after ovulation). Behaviour biometric data were categorized into large and small movements from hip and tail. Hip small-movements (S-hip) were defined as any movements side to side (e.g. left – right) within 10 cm from the rest position (standing still), and hip large-movements (L-hip) were defined as any movement beyond 10 cm from the rest position. Tail events were categorized as a small-tail movement (S-tail) when the tail movement was within the rear thermal area of the cow, and large-tail movement (L-tail) as any tail movement outside the cow's thermal area of each cow (Figure 4.4).

4.4. Statistical Analysis

Behavioural and thermal biometrics were analyzed using SAS software (SAS ver 9.4, Cary, NC, USA). Sample days were standardized (d -5, d -4, d -3, d -2, d -1, d 0, d 1 and d 2) using ovulation as d 0 to compare baseline with pre (d -1 to d -5) and post-ovulation (d 1 to d 2) days. Proc Univariate was used to test normality assumptions using a Kolmogorov-Smirnov test ($P > 0.05$). All thermal data complied with normality assumptions however, behaviour data did not satisfy normality assumptions and were found to have a Poisson distribution. Models were fitted using a Generalized Linear Mixed Model approach (Proc Glimmix). A Bonferroni separation test was used to present results in Least-Square means (LSMeans), SEM and the statement of ar(1) to account for the lack of independence and homogeneity in the data. Results were considered significant if $P < 0.05$, tendency if $P \geq 0.05$ and < 0.10 and P values ≥ 0.10 were considered not significant. Non-significant fixed variables were eliminated from subsequent statistical models.

Pearson correlation coefficients analysis (Proc Corr) were performed to identify possible associations between E_2, thermal, and behaviour biometrics on days approaching ovulation.

4.4.1. Infrared Thermography Analysis

Maximum skin temperature was used in all data analyses to eliminate sources of variation on the surface of the vulva (e.g. min and average temperature). To compensate for the effect of environmental factors on skin temperature, residual skin temperature (Res IR) was calculated following Cook et al. (2016) methodology by subtracting the predicted skin temperature from the observed skin temperature (Raw IR). The Raw IR for each cow from Vlips, Vtail, and Vnotail as well Res IR dependent variables (Vlips, Vtail and Vnotail) were examined using Kolmogorov-Smirnov test ($P > 0.05$). Thermal data were found to follow normality assumptions, and no outliers were identified. Fixed variable was Sample day relative to ovulation (d -5, d -4, d -3, d -2, d -1, d 0, d 1, and d 2,) and the model was tested using a Type 3 test with the inverse (ilink) function specified and the statement of ar(1) to account for the lack of independent and homogeneous data.

4.4.2. Behavioural Data Analysis

To analyze the frequency of behaviours, the fixed variables were Sample day relative to ovulation (d -5, d -4, d -3, d -2, d -1, d 0, d 1, and d 2,) and Sample time (AM milking, and PM milking) while Cow was identified as a random statement with a Poisson distribution specified.

4.4.3. Accuracy Evaluation

To evaluate the performance of thermal and behavioural biometrics as a potential estrus alert, receiver operating characteristics (ROC) curve analyses were performed to identify the most optimum reference value (threshold value) for each thermal and behaviour variable. To evaluate each variable, the period between the presence of a DF (> 15 mm diameter) and that of a regressing CL (< 20 mm diameter; Perry et al., 2017, Burnett et al., 2018), 48-24 h before the disappearance of the DF were used as indirect indicators of the estrus period retrospectively. A balanced proportion of Se (probability of testing positive when estrus occurred) and Sp (probability of testing negative in the absence of estrus) were used to identify the optimum reference value. To summarize the level of accuracy for each biometrical parameter, a Youden J index (YJ = (True positives / (True positives + False negatives) + True negatives/ (True negatives – False negatives))

- 1) was used to give equal weight to false positive and false negative values ranging from 0 (e.g. worthless test) to 1 (e.g. perfect test). Additional evaluation tools were added to the evaluation of performance such as the positive predictive value (PPV = True positives / (True positives + False positives)) or percentage of cows with a positive test that were in estrus, the negative predictive value (NPV = True negatives / (True negatives + False negatives)) or percentage of cows with a negative test that were non-estrus, and the effectiveness or proportion of all test results that were positive results.

Parallel to ROC curve analyses, estrus alerts were also evaluated by calculating the diagnostic odds ratio (DOR) to identify the odds of a positive test if the cows were in estrus relative to the odds of a test being positive if the cow was not in estrus (DOR = (True positives / False positives) / (False negatives/True negatives)). The DOR ranges from 0 to infinity, thus a higher DOR is indicative of a higher estrus alert test performance. The DOR analyses were also measured the likelihood ratio of the test, the probability of the test to be correct (LR+ = sensitivity/1-specificity) vs the probability of the test to be negative result (LR- = 1- sensitivity/ specificity) to identify the occurrence of a true positive compared to the true negative test. Efficiency was calculated as the probability that all tests are correct (Efficiency = (True positives + True negatives) / (True positives + True negatives + False Positives + False negatives)).

Raw IR and Res IR from Vtail, Vnotail and Vlips were evaluated for AM and PM milking separately due to the significant differences between skin temperature during AM and PM results found in a previous experiment (Perez Marquez et al., 2019). Similarly, all behaviour biometrics were analyzed for both Sample times (AM and PM). The test of accuracy was performed for all variables individually, and further evaluations were performed with multiple thermal parameters, multiple behavioural biometrics and combined thermal and behavioural parameters. To combine multiple infrared parameters and behaviour biometrics, the parameters were evaluated retrospectively. The “True Estrus Positive alert” was determined when all variables were positive (infrared, behaviour, and physiological) 48 - 24 h before ovulation (d -2 and d -1). If the infrared and behaviour parameters flagged an estrus alert outside the 48 - 24 h (d -2 and d -1) before the ovulation window was defined as a “False positive estrus alert.” A True negative estrus alert was when all variables were negative at a non-estrus period (baseline, ovulation and post-ovulation). The False Negative alert was declared if only one or multiple parameters did not flag an estrus

alert at the expected estrus period (d -2 and d -1). The same rule was applied for all infrared and behaviour biometrics combinations.

4.5. Results

4.5.1. Physiological Parameters

The average length of estrous cycles was 21.66 ± 3.09 (mean ± SD) days ranging from 17 to 31 days. Changes in the size of the DF and CL and the concentration of E_2 over the pre-estrus, estrus and post-ovulation periods are shown in Figure 4.5. Regression of the CL was confirmed after reduction of CL diameter started at d -4 (d -5: 22.9 ± 1.03 mm to d -4: 21 ± 1.03 mm), and the smallest CL diameter was reported at d -1 (12.13 ± 1.00 mm). Dominant follicle diameter was at its largest measurement during d -1 (17.41 ± 0.64 mm) compared with d -5 (12.38 ± 0.66 mm), which disappeared on the day of ovulation (d 0). Higher E_2 concentrations were found in 12 cows out of 18 used, however, only eight cows had E_2 concentration peaks during the study; three cows at d -2, two cows at d -1, three cows at d 0. Mean concentrations of E_2 in skimmed milk peaked during the AM Sample time d 0 (17.43 ± 1.76 pg/mL) compared with the PM d 0 (15.98 ± 1.68 pg/mL) and proestrus (d -4 15.70 ± 1.67 pg/mL). However, E_2 concentrations started to increase at d -2 (16.38 ± 1.71 pg/mL) compared with d -4 (15.70 ± 1.67 pg/mL), and d -3 (15.8 ± 1.68 pg/mL see Figure 4.5). No correlations (positive or negative) were found between E_2 concentrations and DF diameter ($P = 0.51$, $r = -0.06$). However, a negative correlation was found between CL diameter ($P = 0.05$, $r = -0.22$) and E_2 concentrations.

4.5.2. Changes in Skin Temperature

The University of Alberta DRTC facility experienced minimal daily variation in ambient temperature and relative air humidity during the study period (temperature; 14.05 ± 3.06°C, relative air humidity; 68.86% ± 6.94 (mean ± SD)). The relationship between ambient and animal skin temperature was an average $r = 0.62$ ($P = 0.32$).

Changes in Raw IR at the vulva were significant ($P < 0.05$) at PM milking compared to AM milking on days leading to ovulation (see Figure 4.6A and B). Significant differences were also observed by Sample day for Res IR results (Figure 4.6C and D). Specifically, an increase in skin temperature was observed during d -2 PM milking compared to baseline and ovulation day, however, no interactions between Sample day and Sample time were found ($P > 0.10$). An increase

in skin temperature was also observed in Res IR, with an increase at d -2 of 0.51 ± 0.23˚C compared to d -5 and d 0 PM scan days (Figure 4.6D). However, changes in Res IR had less variation between vulva measurements compared with the Raw IR (Figure 4.6D). No correlations (positive or negative) were found between the peak of skimmed milk E_2 concentration and Raw/Res IR increases ($r > 0.10$).

4.5.3. Behaviour Frequencies

The frequency of hip and tail movements did not differ between AM and PM milking times. Further, there were no changes in tail movements over the sampling period of d -5 to d 2 (Figure 7A). However, S-hip and L-hip movements tended to increase over sampling periods but only for the AM milking ($P = 0.07$ and 0.06, respectively). S-hip movements increased ($P < 0.01$) over the sample period during the PM milking (Figure 7B). No interactions were found ($P > 0.10$) between Sample day and Sample time in behaviour parameters.

4.5.4. Accuracy Evaluation Results

Optimum reference values (i.e. threshold value) with Se and Sp level (i.e. highest value of True estrus positives and True estrus negatives) and corresponding YJ index for all thermal and behavioural parameters are presented in Table 4.1. Residual IR Vtail during PM milking (YJ = 0.34) yielded the highest scores, which coincided with the highest DOR score (DOR = 4.58), LR+ (1.85), NPV (0.82), Efficiency (0.66), and was the second highest scoring test for PPV (0.50) and Sp (0.50). Thermal and behaviour biometrics at AM milking did not result in the same diagnostic performance compared with thermal and behaviour biometrics at PM milking (Table 4.1), except for S-hip during the AM milking. Overall, S-hip and L-hip movement frequency changes were the most relevant behaviour biometric for use as part of an estrus diagnostic test.

One hundred and twenty possible combinations between behaviour and thermal parameters were evaluated at different Sample times (AM–PM) using the optimum reference value for each parameter. However, only the 20 combinations with the highest scores are presented in Table 4.2. The highest YJ score was found for Raw IR Vlips PM-Res IR Vnotail PM (YJ = 0.35) with a balanced Se (0.65) and Sp (0.70) but a lower DOR (4.83). The highest DOR (42.05) was observed for Res IR Vlips, PM S-hip, AM S-hip PM and provided the highest PPV (0.94), Sp (0.99), LR+ (15.35) but a low Se (0.22) and YJ (0.20). Greater Efficiency (0.77) was found in Res IR Vtail PM

+ S-hip AM + S-hip PM with an YJ (0.32), DOR (26.62), LR+ (12.47), Sp (0.97) and PPV (0.90) compared to other combinations (see Table 4.2). Additional, the number of cows flagged in estrus with higher DOR (Res IR Vlips, PM S-hip, AM S-hip PM) were only four cows (True estrus positive), but 0 False Positives alerts compared to Res IR Vtail PM + S-hip AM + S-hip PM (DOR = 26.62; 7 cows in estrus with 1 False Positive alerts). In contrast, the highest YJ (Raw IR Vlips PM + Res IR Vnotail PM = 0.35) found 12 cows in flagged estrus with 20 False Positive alerts.

4.6. Discussion

4.6.1. Physiological Associations with Thermal Radiation Fluctuations

Ambient temperature and percentage of relative air humidity were maintained consistently through the summer–spring season inside the DRTC barn that may explain the lack of any effect of ambient temperature on our infrared readings. However, this relationship was not observed in all animals, with some animals exhibiting significant relationship whilst other animals did not. This was most likely due to the environmental monitor being placed in a fixed position in the barn, and thus, the data recorded was unrepresentative of some of the cow stalls. Furthermore, some animals were in closer proximity to air circulation fans compared to others, which likely would have affected by ambient air temperatures. When ambient temperature and animal skin temperature were pooled, there was no ambient temperature and relative air humidity on animal skin temperature ($P > 0.10$). Other studies using infrared technology found air circulation, solar loading, camera distance, emissivity, and percentage of relative air humidity influencing infrared readings (Cook et al., 2016, Perez Marquez et al., 2019).

The differences in thermal data found between AM and PM measurements could be attributed to the thermogenic effect of the heat increment of feeding. Feeding took place at 0600 h, after AM milking was finished and infrared images had been recorded, which may explain the lower infrared readings found at the AM Sample time because animals had not yet been offered fresh fed in the morning. Similar temperature changes after feeding intake were found by Montanholi et al. (2009), and Freetly et al. (2006). Another factor potentially affecting infrared readings was the lower air temperature found in the barn during the AM milking compared to PM milking. Despite the lack of correlation between ambient temperature and animal skin temperature, animals thermoregulate to their environment, and thus, ambient temperatures in the PM might have affected thermoregulation resulting in higher thermal radiation. Another possibility is that

circadian rhythms in body core temperature have been widely reported in humans (Costa et al., 2018), sheep (D'Alterio et al., 2011), and dairy cattle (Berry et al., 2003), and this may be the case in dairy cows such that the overall greater activity during the day results in higher skin temperature in PM images. Other studies confirmed that the increase of activity during the day could increase the volume of blood circulating specifically at the skin level (Cramer et al., 2019, Rahim et al., 2018), and higher infrared 48 h prior ovulation were found during PM milking compared to AM milking in a tie-stalls (Perez Marquez et al., 2019).

The fluctuations between d -5 to d 2 of the estrous cycle (proestrus - estrus – ovulation – post-ovulation) in Raw and Res IR coincided with greater DF diameter (> 15 mm), regression of the CL (< 20 mm) and E_2 concentrations in skim milk (17.43 pg/mL) at d -1. However, the highest increases in skin temperature observed on d -2 did not match with the larger DF diameter and the peak of E_2 concentrations at d -1. The peak in skin temperature coincided with the interaction CL (regression) - DF (development) at d -2 (see Figure 4.4). No significant correlations existed between E_2 and skin temperature increases. Potential explanations with the changes in infrared during the presence of larger DF and increases of E_2 have been related to the increase of physical activity at the onset of estrus (Oshi et al., 2006) reported in other estrus detection studies on tie-stall herds (Kennedy and Ingalls, 1995, Guesgen and Bench, 2018). Thermal fluctuations may be related to the changes in endocrine profile during the follicular phase (e.g. gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, estradiol–progesterone interaction, cortisol levels etc.) effect in skin physiology (Frascarolo et al., 1990). Other studies suggested that thermogenesis is associated with estradiol release during estrus in visceral fat and skeletal muscle through adaptive thermogenesis (Brown et al., 2010, Clarke et al., 2013). The increased activity that precedes the standing to be mounted and the changes in the blood perfusion at the vaginal and vulva area had been reported as major factors that increase the temperature in the vulva (Oshi et al., 2006). Similar results to this study have found increases in vagina temperature 24 hours prior to ovulation, followed by a decrease in blood flow during ovulation (Hassan et al., 2017).

Thermal areas (Vtail, Vnotail, and Vlips) did not differ significantly when measuring Raw and Res IR. However, even when all the thermal areas followed similar thermal patterns (e.g. increases and decreases), Vlips temperatures were slightly lower skin temperature compared to Vtail and Vnotail. The low Vlips skin temperatures can be attributed to the presence of feces, and urine in the outer lips of the vulva, which creates a moisture environment as the tail does not allow

the lips to dry-off. Note: The vulva's outer lips were not clean or dried-off in the current study, as it would not be feasible under barn conditions. Additionally, the maximum skin temperatures were found in pixels around the vulva area, which explains why Vtail and Vnotail were able to identify the changes in skin temperature similarly as Vlips.

4.6.2. Behavioural Changes during the Expected Estrus Period

Behavioural data were continuously recorded using an "all-occurrence sampling" at AM and PM milking times to identify temporal changes in events (Lehner, 1996) in the days leading to ovulation. In the current study, we did not find differences between milking times in the frequency of any behaviour events. In a previous, study we found increases in restless behaviours before milking compared to during and after milking (Perez Marquez et al., 2019), which can be an indication of discomfort, or in anticipation of milking (Metz-Stefanowska et al., 1992).

S-hip movements were higher at the d -2 compared to the proestrus period and ovulation day at the PM milking, and there was a tendency for S-hip during AM milking to be higher ($P = 0.07$). Changes in S-hip movements at the PM milking may be related to increased activity during the estrus period in dairy cows. Restless behaviour (such as the number of steps and shifting of weight shifting between legs) has been reported with the potential use to detect differences in standing comfort and as a response to lameness (Chapinal et al., 2009). Similar to the present study, Guesgen and Bench (2018) identified micro-movements in the pelvis 24 h before ovulation in naturally cycling dairy cows in tie-stall housing using 3D kinematic analysis. However, in other studies (Valenza et al., 2012; Burnett et al., 2018), the interval from increased activity to ovulation was approximately 24 h. The different intervals in the increase of activity to ovulation may be due to the differences in data collection periods such as the 24 h window between ultrasonography in the current study vs 12 h window between ultrasonography in Burnett et al. (2018), different behaviours, and housing type (e.g. tie-stalls vs free-stalls). Additionally, behaviour data collection in the present study only occurred during milking compared with other studies in which activity bouts, for example, were assessed using activity monitors on cows housed in free-stalls (during non-milking time). Other behaviours such as S-tail and L-tail did not differ as ovulation approached. Some of the factors that may have affected our results were the potential for miscalculation of tail movements due to the velocity of tail movements being faster than could be captured by the frame rate of the camera (4 frames/sec) and the inter and intra-cow variation. Other

factors that may affect the overall tail movements can be related to the presence of flies during the months of summer and early fall at the study location. Frantz et al. (2019) reported changes in tail movement caused by fly population and this was associated with footstep movements, which indicates that the tail frequency of tail movements may not be entirely attributed to the restless behaviour at the estrus period. However, the presence of flies was mitigated in the present study by placing flytraps inside the barn.

4.6.3. Individual Estrus Alerts vs Combined Estrus Alerts

Estrus alerts were constructed using the changes in of Raw IR, the Res IR, and changes in the frequencies of hip and tail movements during milking. The evaluation of accuracy for thermal data identified differences between AM and PM Sample times, with a higher score obtained during PM scanning. The changes in skin temperature of the vulva as ovulation approach is consistent with a previous study reporting the same response in synchronized multiparous cows (Perez Marquez et al., 2019). The YJ index resulted in a positive test to identify estrus for thermal data. However, the positive test balances the proportion of false positives and false negatives (e.g. high true-positive estrus alerts have high false-positive proportions). This means that optimum reference values can be adjusted depending on the objective of the diagnostic test (e.g. cost per AI relative to pregnancy cost).

Furthermore, by comparing Raw IR and Res IR we observed that Res IR had higher scores (Table 4.1). Residual IR accounted for thermal regulation to ambient temperature, which may have resulted in a closer approximation to thermal radiated attributed to physiological process such as the estrus period. Further, the combination of infrared parameters increased the YJ index (≤ 0.30) during PM scanning. One reason for this is that the more parameters used in an estrus alert reduced the error rate attributed to the false positives and false negatives estrus occurrence. Mainly, Res IR Vtail, Res IR Vlips, Raw IR Vlips PM and Raw IR Vtail PM Res Vlips were found with the highest YJ index for all combinations, which means that infrared parameters are particularly better to identify estrus with a balance Se and Sp. However, the balanced proportion of Se and Sp often results in adding a proportion of false negative and false positive that should have to be tested in practical circumstances (e.g. cost per arterial insemination relative to the cost of pregnancy). Additionally, the accuracy of the infrared camera ($\pm$ 0.45°C) could influence the occurrence in false-positive alerts and explain the unknown increase in raw and residual skin temperature during

the project; nevertheless, the use of a black body during infrared recording can help to estimate the error rate in a particular scanning session. Regardless of a higher YJ index in infrared combinations, the DOR analyses did not show higher results (3.84 - 4.97) probably for the balanced Se and Sp, which in estrus detection, the number of true negative estrus are higher than true estrus positives since estrus occurred once in the 21-day estrous cycle.

None of the behaviour biometrics showed considerable differences between AM and PM evaluation of accuracy results, which may be, explained by the lack of significant differences in behaviour frequencies between milking schedules. Higher accuracy scores were achieved for hip movements during AM and PM milking. The lack of differences in tail movements can be related to the lack of tail movement during milking time while Perez Marquez et al. (2019) reported an increase in tail movement as milking time approached, tail movements tended to decrease or be absent during milking. The combination of behaviour biometrics did not improve the YJ score or DOR except for S-hip, L-hip, L-tail with a higher DOR and PPV. The combination of these three behaviours correctly identified the total of true negatives occurrence and false positive, however, the Se was low.

The combinations between thermal and behavioural biometrics resulted in higher Efficiency, PPV, DOR and in some cases YJ index. The explanations to these results may be the complementary information as parameters are added (true positives can be confirmed if more than one parameter coincided), for example; the highest combinations consisted of higher scores from individual evaluations (behavioural and thermal) which decrease the error rate. Similar results were found by Hoffmann et al. (2013), by looking at activity monitor estrus detection methods combined with visual observations. The additive effects of using multiple methods in a diagnosis reduce the error rate by eliminating the number of false positives and increasing the identification of true negatives occurrence.

The current study objective was to compare and contrast the combined thermal and behaviour biometrics as estrus alerts at an estimated estrus period. Our null hypothesis states that no changes in the accuracy were expected between estrus alerts using thermal and behaviour biometrics individually, compared to combined thermal-behaviour estrus alerts. We rejected the null hypotheses since adding behaviour parameters to thermal estrus alerts reduced the number of false positive and both false negative tests. Additionally, residual thermal measurements were found to be more accurate compared to raw thermal measurements for estrus detection from the

vulva area. Infrared thermography from Vtail, Vnotail and Vlips followed the same patterns of fluctuation on the days leading up to ovulation. The resolution in small hip movements was essential to distinguish the estrus period from the proestrus stage and ovulation day compared to large hip movements in a tie-stall. The data suggest that the use of multiple parameters has utility as an estrus detection method by combining infrared data from the vulva and small-hip movements during milking in primiparous cows housed in a tie-stall barn.

4.7. Ethics Approval

This study was approved by the Animal Care and Use Committee: Livestock (University of Alberta, Edmonton Alberta, Canada, approval number: AUP00001652) under the Canadian Council of Animal Care Standards and requirements (2009).

4.8. Author Contributions

H. J. Perez Marquez contributed with the data collection, statistical analysis and manuscript drafts. D. J. Ambrose was involved in experimental design, provided advice regarding the reproductive physiology aspects, and assisted with ultrasonography data collection and manuscript reviews and edits. A. L. Schaefer contributed with expertise in the IR thermography outputs and interpretation. N. J. Cook assisted with data analysis, estradiol analysis and manuscript edits. C. J. Bench was involved in experimental design, objectives and hypothesis formulation, as well as manuscript revisions and edits.

4.9. Acknowledgements

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Table 4.1. Accuracy evaluation of thermal and behaviour biometrics as individual parameters for all primiparous cows using a receiver operating characteristics (ROC) and a diagnostic of odds ratio (DOR).

Parameter	Stime[1]	Threshold	Se[2]	Sp[3]	Efficiency	PPV[4]	NPV[5]	YJ[6]	DOR[7]	LR+[8]	LR-[9]
CL[10]	--	<12.06	0.37	0.97	0.84	0.78	0.85	0.34	19.54	12.71	0.65
DF[11]	--	>15.90	0.89	0.61	0.67	0.39	0.95	0.50	13.22	2.29	0.17
E_2[12]	--	>17.05	0.37	0.70	0.63	0.25	0.80	0.06	1.33	1.21	0.91
Vtail[13]	AM	>34.16	0.76	0.27	0.44	0.36	0.7	0.02	1.27	1.03	0.91
Res Vtail	AM	>0.18	0.51	0.59	0.57	0.41	0.71	0.11	1.66	1.26	0.82
Vnotail[14]	AM	>34.80	0.54	0.58	0.57	0.41	0.72	0.12	1.75	1.28	0.8
Res Vnotail	AM	>0.73	0.78	0.28	0.46	0.37	0.73	0.07	1.6	1.09	0.77
Vlips[15]	AM	>34.71	0.24	0.82	0.63	0.43	0.68	0.06	1.62	1.33	0.93
Res Vlips	AM	>-1.67	0.81	0.29	0.46	0.37	0.74	0.09	1.66	1.13	0.68
Vtail	PM	>35.28	0.68	0.52	0.58	0.43	0.77	0.2	2.48	1.41	0.62
Res Vtail	PM	>0.14	0.73	0.61	0.66	0.5	0.82	0.34	4.58	1.85	0.45
Vnotail	PM	>35.00	0.62	0.56	0.59	0.44	0.75	0.19	2.31	1.42	0.67
Res Vnotail	PM	>-0.14	0.76	0.51	0.6	0.45	0.81	0.26	3.55	1.54	0.48
Vlips	PM	>34.10	0.78	0.44	0.56	0.43	0.81	0.22	3.14	1.39	0.5
Res Vlips	PM	>0.23	0.57	0.7	0.67	0.51	0.77	0.27	3.42	1.92	0.61
S-tail[16]	AM	>33	0	0.94	0.62	0	0.65	-0.06	0	0	1.06
L-tail[17]	AM	>49	0	0.94	0.62	0	0.65	-0.06	0	0	1.06
S-tail	PM	>13	0.22	0.83	0.62	0.40	0.67	0.05	1.38	1.3	0.94
L-tail	PM	>44	0.03	0.96	0.64	0.25	0.66	-0.02	0.64	0.65	1.02
S-hip[18]	PM	>6	0.86	0.37	0.54	0.41	0.84	0.23	3.66	1.37	0.37
L-hip[19]	PM	>38	0.44	0.83	0.7	0.57	0.74	0.27	3.87	2.59	0.67
S-hip	AM	>13	0.47	0.79	0.68	0.53	0.74	0.26	3.28	2.2	0.67
L-hip	AM	>29	0.53	0.54	0.54	0.37	0.69	0.07	1.33	1.15	0.87

Abbreviations: [1]Stime = Sample time (AM = 3 AM and PM = 3 PM); [2]Se = Sensitivity; [3]Sp = Specificity; [4]PPV = Positive predicted value; [5]NPV = Negative predicted value; [6]YJ = Youden J index; [7]DOR = Diagnostic odds ratio; [8]LR+ = Positive likelihood ratio; [9]LR- = Negative likelihood ratio; [10]CL = Corpus luteum; [11]DF = Dominant follicle; [12]E_2 = Estradiol; [13]Vtail = Skin temperature from the vulva with tail; [14]Vlips = Skin temperature from the vulva's external lips; [15]Vnotail = Skin temperature from the vulva without tail; [16]S-tail = Small tail movements within the rear thermal area of the cow; [17]L-tail = Large tail movements outside the cow's thermal area of each cow; [18]S-hip = Hip small movements side to side (e.g. left – right) within 10 cm from the rest position (e.g. standing still); [19]L-hip = Hip large movements beyond 10 cm from the rest position.

Table 4.2. Accuracy evaluation of combined thermal and behaviour biometrics for all primiparous cows using a receiver operating characteristics (ROC) and a diagnostic of odds ratio (DOR). Only significant results (> 0.30 YJ or > 1.00 DOR) from combinations within and between thermal and behavioural biometrics.

Combined Parameters	Stime[1]	Se[2]	Sp[3]	Efficiency	PPV[4]	NPV[5]	YJ[6]	DOR[7]	LR+[8]	LR-[9]
Res IR Vtail + Res IR Vlips	PM	0.73	0.56	0.63	0.47	0.81	0.29	3.84	1.67	0.48
Vlips – Res IR Vnotail – Res IR Vlips	PM	0.57	0.75	0.69	0.55	0.78	0.31	4.24	2.24	0.58
Vlips – Res IR Vtail – Res IR Vlips	PM	0.57	0.77	0.71	0.58	0.78	0.34	4.97	2.52	0.56
Vtail – Vlips – Res IR Vtail – Res IR Vlips	PM	0.51	0.77	0.69	0.56	0.76	0.29	3.99	2.28	0.63
Vlips – Res IR Vlips	PM	0.57	0.73	0.69	0.54	0.77	0.30	3.94	2.12	0.59
Vlips – Res IR Vtail	PM	0.65	0.70	0.69	0.54	0.80	0.35	4.83	2.19	0.50
Vlips – Res IR Vlips	PM	0.57	0.73	0.69	0.54	0.77	0.30	3.94	2.12	0.59
S-hip - L-hip	AM	0.97	0.06	0.38	0.35	0.90	0.03	4.94	1.03	0.48
S-hip - S-tail	AM	0.97	0.06	0.38	0.35	0.90	0.03	4.94	1.03	0.48
S-hip - L-tail	AM	0.97	0.07	0.39	0.36	0.92	0.04	6.13	1.05	0.38
L-hip - L-tail	AM	0.97	0.06	0.38	0.35	0.90	0.03	4.94	1.03	0.48
L-hip - S-tail	AM	0.97	0.06	0.38	0.35	0.90	0.03	4.94	1.03	0.48
S-hip - L-hip – L-tail	AM	0.16	0.99	0.71	0.93	0.70	0.15	30.05	11.51	0.85
Raw IR[10]	PM	0.54	0.62	0.60	0.44	0.73	0.16	2.09	1.42	0.74
Res IR[11]	PM	0.57	0.76	0.70	0.57	0.78	0.33	4.58	2.37	0.57
Res IR VtailPM - S-hipAM - S-hipPM	--	0.35	0.97	0.77	0.90	0.75	0.32	26.62	12.47	0.67
Res IR VnotailPM - S-hipAM - S-hipPM	--	0.35	0.96	0.76	0.84	0.74	0.31	15.74	8.32	0.68
Res IR VlipsPM - S-hipAM - S-hipPM	--	0.22	0.99	0.73	0.94	0.71	0.20	42.05	15.35	0.79
VtailPM - S-hipAM-S-hipPM	--	0.32	0.96	0.75	0.83	0.74	0.28	13.98	7.68	0.71
VnotailPM - S-hipAM - S-hipPM	--	0.27	0.97	0.74	0.88	0.72	0.24	18.36	9.59	0.75
Vlips - S-hipAM - S-hipPM	--	0.32	0.93	0.73	0.74	0.73	0.25	7.54	4.61	0.73

Abbreviations: [1]Stime = Sample time (AM = 3 AM and PM = 3 PM); [2]Se = Sensitivity; [3]Sp = Specificity; [4]PPV = Positive predicted value; [5]NPV = Negative predicted value; [6]YJ = Youden J index; [7]DOR = Diagnostic odds ratio; [8]LR+ = Positive likelihood ratio; [9]LR- = Negative likelihood ratio; [10]Raw IR = Combination between all the Raw IR parameters in a given Sample time (AM – PM); [11]Res IR = Combination between all the Res IR parameters in a given Sample time (AM – PM).

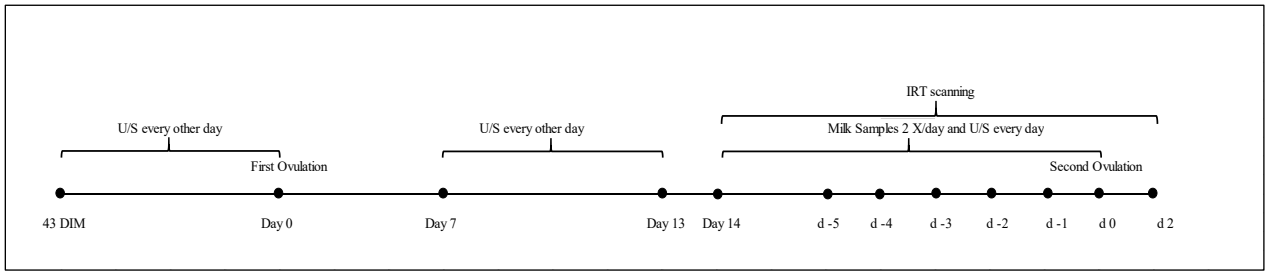

Figure 4.1. Experimental timeline. Transrectal ultrasonography (U/S) was performed every other day from 43 ± 2 days in milk (DIM) until the first ovulation (Day 0), which resumed every other day from Day 7 to Day 13 to monitor ovarian dynamics. From Day 14 until two days after the second ovulation (d 0), U/S was performed once daily. Simultaneously, infrared thermography (IRT) was performed and thermal frames were recorded during morning and afternoon (AM-PM) milking to record the maximum skin temperature and the frequency of event for hip and tail behaviours (Events/5min). Additionally, milk samples were collected from Day 14 until d 0 to determine peripheral estradiol concentrations.

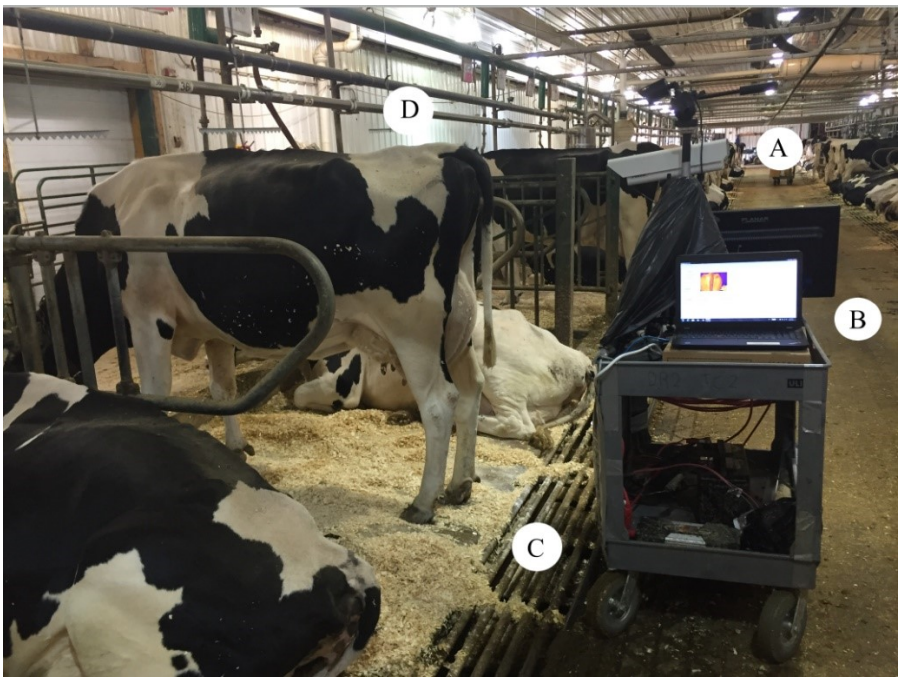

Figure 4.2. Infrared thermography cart. A) A310 thermal camera protected in a camera case with a perpendicular angle facing the vulva area with 1 m. B) Laptop with Vacca2 frame puller software (Animal InfraMetrics) connected to the thermal camera via Ethernet cable. C) Power source (12 volts battery with a 1000-watt power inverter). D) Primiparous dairy cow at her stall.

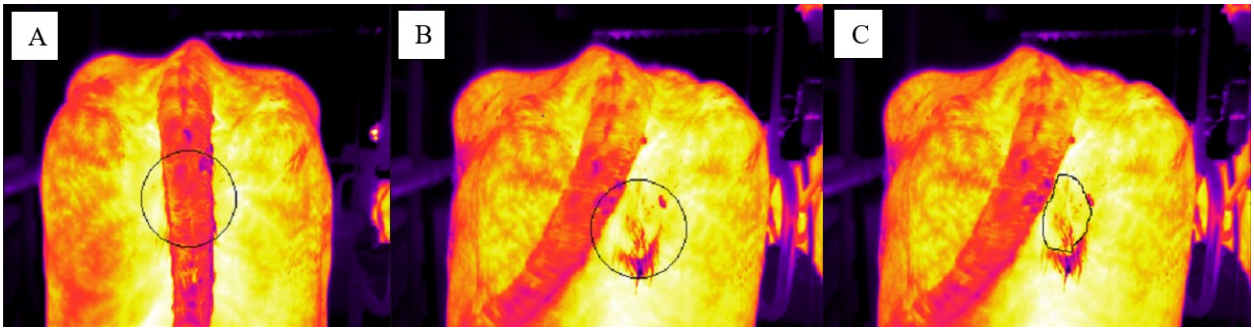

Figure 4.3. Vulva area with the tail (Vtail; A), Vulva area with vulva exposed (Vnotail; B) and Vulva's external lips (Vlips; C). The ellipses (A and B), and hand draw area (C) were consistently used to record the same number of pixels through all the thermal images to identify the maximum skin temperature for each image.

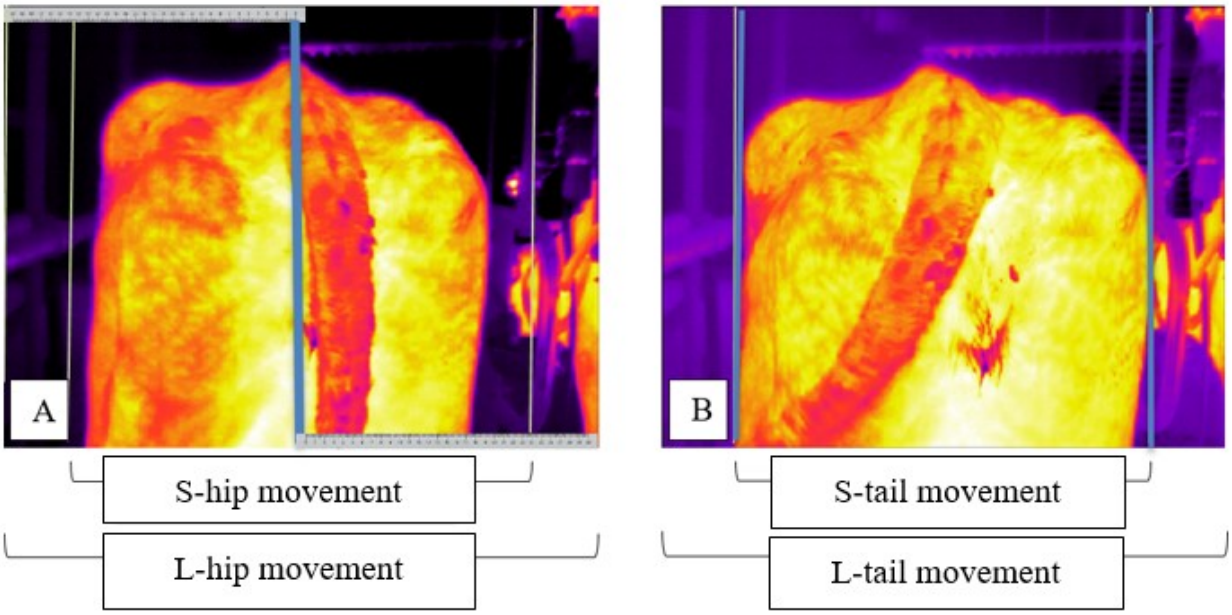

Figure 4.4. Hip movement frequency (A), were divided into S-hip (any event within 10 cm side to side using tail head as a middle reference point), and L-hip (all events beyond 10 cm side to side using tail head as a middle reference point). Tail movements (B) were similarly divided into S-tail (tail events inside the thermal shape of the cow within the frame), and L-tail (tail events outside the thermal shape of the cow).

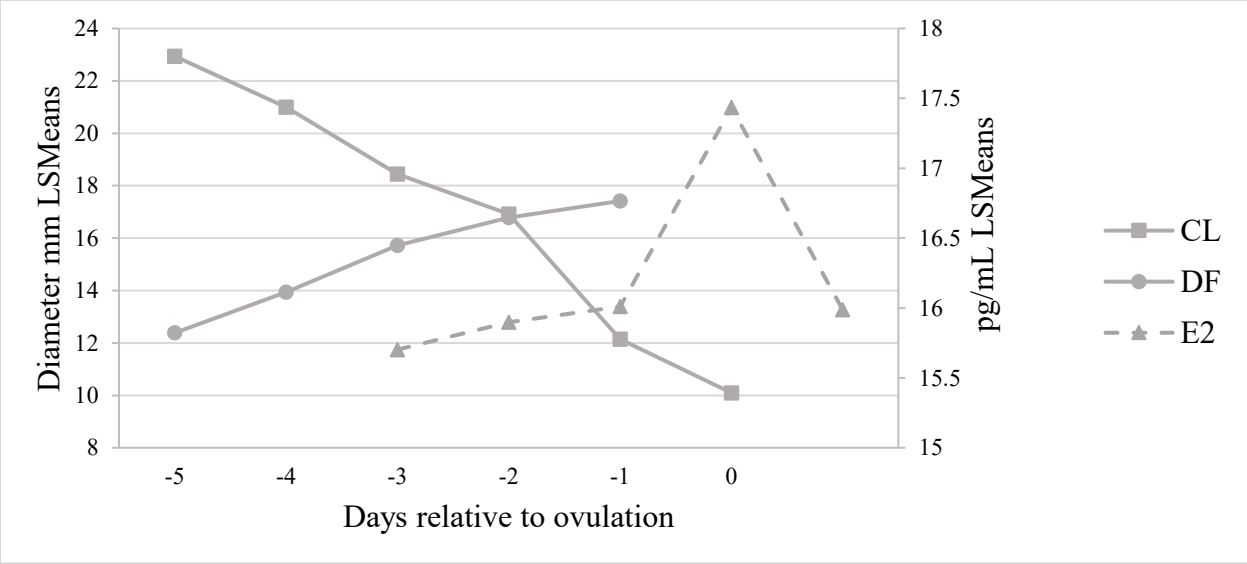

Figure 4.5. Diameter in mm (Least-square Means; LSMeans) of ovarian structures and estradiol (E_2) concentrations in skimmed milk as ovulation approaches. Corpus luteum (CL) started to regress at d -4 until the lowest diameter during ovulation. Dominant follicle (DF) diameter was at its largest on d -1, however, the peak of E_2 was found until d 0. A weak negative correlation was found between the CL diameter and E_2 concentrations ($P = 0.05$, $r = -0.22$); however, no significant correlations were found between the DF diameter and E_2 concentrations ($P = 0.51$, $r = -0.06$).

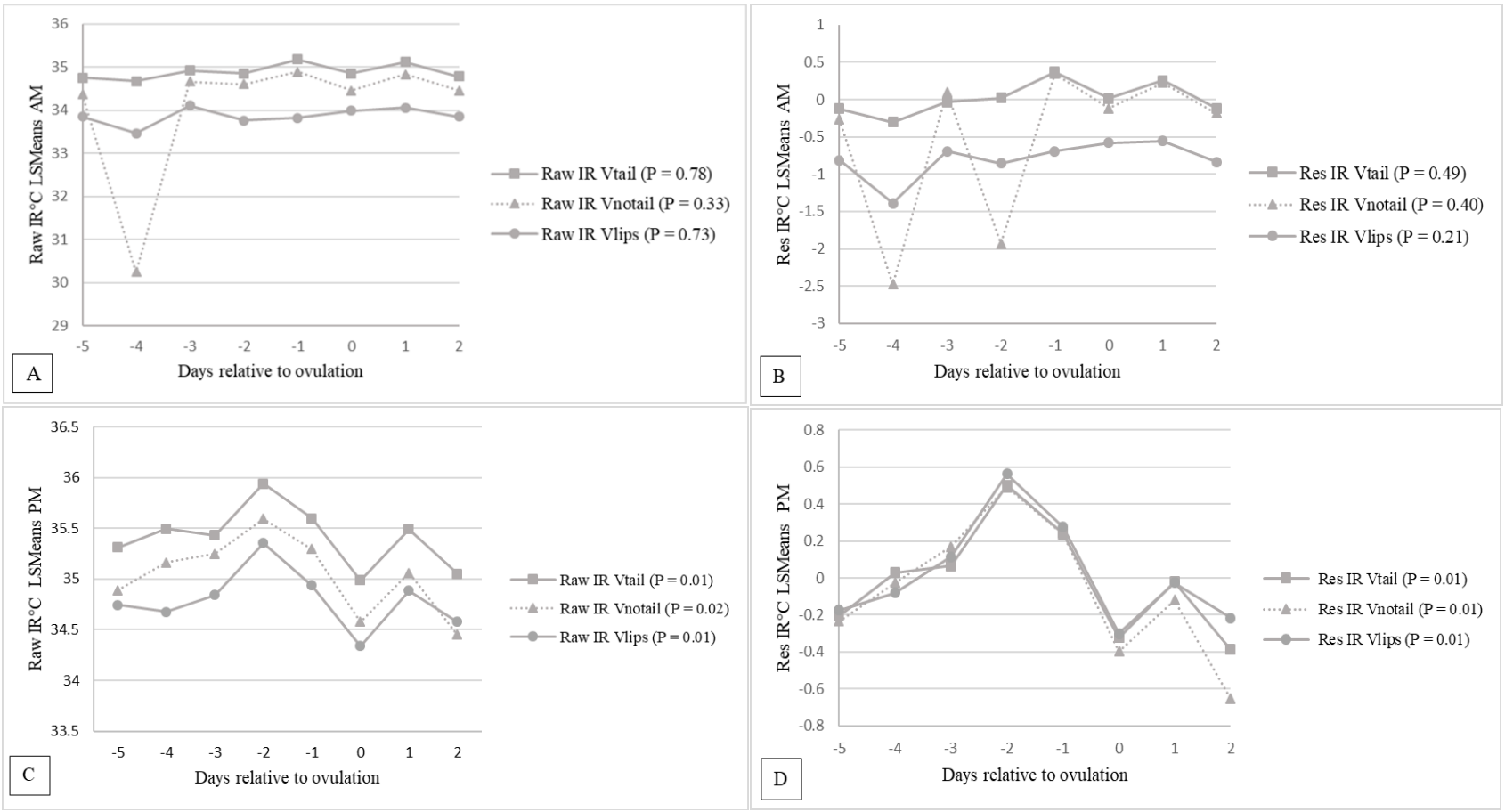

Figure 4.6. Raw IR (AM; A, PM; C) and Res IR (AM; B, PM; D) from Vtail, Vnotail, and Vlips (see Table 4.1 for abbreviations) during milking as ovulation approached. Thermal increases were observed in both infrared thermography (IRT) measurements specifically during d -2 and significant decreases during d 0 which coincided with E_2 concentrations and DF diameter. However, by accounting for ambient temperature (subtracting the predicted skin temperature based on ambient temperature and the observed skin temperature) Res IR data were more consistent compared to Raw IR.

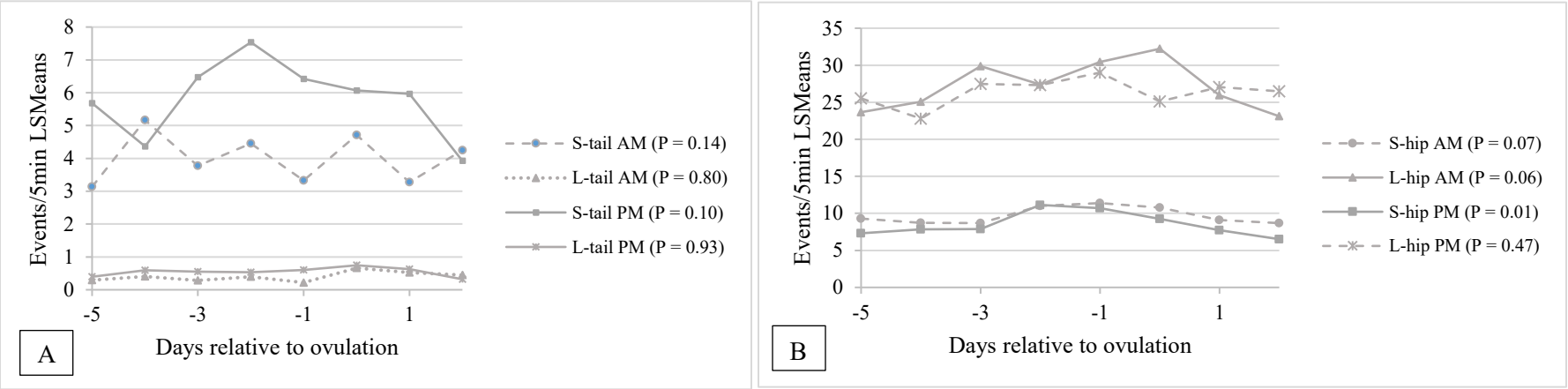

Figure 4.7. Behavioural measurements followed a change in frequency of events (Events/5min, Least-square Means; LSMeans). A) Increases in d -2 in S-hip during PM milking time (P = 0.01) and AM milking (P = 0.07) were observed followed by a decrease after ovulation day. Changes in L-hip AM during days relative to ovulation resulted in a tendency (see Table 4.1 for abbreviations). However, PM milking did not follow a pattern relative to ovulation. B) Changes in tail movement were observed in S-tail AM-PM compared to L-tail, however, none of the tail frequencies of event were statistically significant (P ≥ 0.10).

Chapter 5. Characterization of Pelvic, Foot and Tail Biometrics Using 3D-Kinematic Analysis during The Proestrus-Ovulation Period in Naturally Cycling Primiparous Dairy Cows Housed in a Tie-stall System

5.1. Abstract

The objective of this study was to investigate 3D-kinematics as a method for determining if primiparous dairy cows display differences in behaviour biometrics during the estrus period as ovulation approaches in a tie-stall system. The second objective was to evaluate the accuracy of behaviour biometrics as estrus alerts. Fourteen primiparous dairy cows (n = 14) were studied as part of a split-plot over time design. 3D-kinematic assessment took place during proestrus-estrus-ovulation period, Follicular diameter, corpus luteum (CL) diameter, and estradiol (E_2) concentrations served as physiological parameters to indirectly estimate the estrus period (d -1). The frequency of Pelvic tilts, Pelvic shifts left (Pelvicsl), Pelvic shifts right (Pelvicsr), Total pelvic shifts; TPelvicS), Foot strikes left (Foot strike L), Foot strikes right (Foot strike R), and Total feet strikes (TFootS) were recorded. Additionally, the frequency of Micro tail left (TailLMicro), and right (TailRMicro) movements, Middle tail left (TailLMid), and right (TailRMid) movements, Macro tail left (TailLMacro), right (TailRMacro) movements were also recorded. This study's overall length of estrous cycle in primiparous dairy cows was 21.66 ± 3.09 (LSMeans ± SEM days). The largest Follicular diameter (LSMeans ± SEM; 17.04 ± 0.59 mm) and E_2 (17.43 ± 1.76 pg/mL) occurred 24 h before ovulation. The frequency of some behaviour biometrics increased (LSMeans ± SEM Events/5min) at d -2 including Pelvic tilts (19.75 ± 8.67 Events/5min), Pelvicsl (20.26 ± 13.64), and TPelvicS (20.82 ± 8.79) compared to baseline (d -4, Pelvic tilt; 13.10 ± 8.32, Pelvicsl; 3.71 ± 2.52, and TPelvicS; 6.34 ± 2.72). Other significant patterns observed include a decrease at d -1 in the frequency of TFootS (9.86 ± 1.98), TTailMicro (7.30 ± 3.62), TTailMid (1. 82 ± 1.01), and TTailMacro (1.66 ± 1.01) movements followed by an increase in frequency at d -4 (TFootS; 14.44 ± 2.78, TTailMicro; 14.57 ± 7.23, TTailMid; 6.07 ± 3.27, and TTailMacro; 1.84 ± 1.12). The accuracy of each behaviour biometric as a potential estrus alert was analyzed using J index values with balance Se – Sp (J index, Se–Sp) levels (ROC curves analysis). Feet strikes had the greatest accuracy (0.50; 0.90-0.6) followed by Pelvic tilts (0.37; 0.78-0.59), Foot strikes L (0.33; 0.44-0.89), TailLMid (0.30; 1.00–0.30), TailLMacro (0.41; 1.0–0.41) and TFootS (0.34;

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0.67-0.68). Our results indicate that naturally cycling, primiparous dairy cows housed in tie-stalls exhibit behavioural fluctuations as the estrus period approaches, which can be used as estrus alerts.

5.2. Introduction

Economic success in dairy production is attributed to accurate estrus detection and reproductive management (Gröhn and Rajala-Schultz 2000) between 70 and 100 days after parturition (Stangaferro et al., 2018). The importance of detecting the onset of estrus is directly associated with pregnancy achieved per artificial insemination (AI) service (Burnett et al., 2018). Pregnancy per AI service is highest at 8 h after the onset of estrus in primiparous dairy cows (LeRoy et al., 2017). However, inaccurate estrus detection results in missed or mistimed insemination that often requires a second (> 40%) or even third (7%) AI service (Denis-Robichaud et al., 2016).

In Canada, the overall estrus detection rate is below 40% (LeBlanc, 2005), nevertheless, in tie-stalls barns can be lower as 19% via visual observation (Kennedy and Ingals, 1995). Some of the causes of not detecting estrus include low peripheral concentrations of estradiol (E_2) in lactating dairy cows (López et al., 2004), which have been correlated with reduced mounting behaviour durations (from 16 to 8 h). Subsequently, reduced estrus duration decreases the likelihood of estrus detection via visual observation (~56%; Fricke et al., 2014) in Holstein cattle. Other factors that affect the expression of estrus include confined-space, concrete flooring, slippery conditions and cows not having access to outdoors dirt pens (Stevenson, 2001) which, requires extra cow handling (e.g. access to outdoor pens) and labour input to observe mounting behaviour daily. In addition, cows of different parities (primiparous vs multiparous) differ in estrus expression (e.g. higher intensity and duration in primiparous cows) and pregnancy rate per AI service (higher in multiparous cows; At-Taras and Sphar, 2001), which can also lead to inaccurate estrus detection.

Activity-monitors have partially accomplished attempts to optimize estrus detection (70 – 80%, using pedometers; Kiddy, 1977), rectal temperature using mercury thermometers, (59%, Walton and King, 1986), and intra-vaginal temperature loggers (69%, Redden et al., 1993) in tie-stalls. However, more recent studies found contradictory results using pedometers (AfiMilk Pedometers Plus Tag, Felton et al., 2012) and the wide coefficient of variation in core body temperature, (50%, Redden et al., 1993), making automated indicators of estrus unreliable in tie-

stalls. Additionally, reproductive technologies have partially addressed estrus detection challenges by using hormone-based treatments to induce estrus and or induce ovulation (e.g. fixed time ovulation). Nevertheless, the use of hormone-based injections is invasive, increasingly lacks social acceptance and is less cost-effective (~6 per cow USD) compared with estrus detection methods (0.15/cow USD; Galvão et al., 2013). In contrast, estrus detection based on radiated temperature from the vulva achieved 71% positive predictive value (as a measure of accuracy) with 83% Se and 43% Sp within pasture-based dairies (Talukder et al., 2014), 53% in tie-stall housing (Perez Marquez et al., 2019), and 24% estrus detection when using motion and image analysis (Du-Ming and Ching-Ying, 2014). However, none of the above activity devices has been successful in detecting estrus using behaviour parameters within a confined space (e.g. tie-stall), addresses the difference in parity (primiparous – multiparous), and mitigates the low Se of radiated temperature.

New biometric-based methodologies include the analysis of biomechanical features by using quantified algorithms and image analysis to track reflective markers using an optical motion system (Siebert et al., 2018). Biometric techniques that include three-dimensional fields (i.e. X, Y, and Z) can analyze movements precisely to identify biomechanical abnormalities (Winter, 1990) and gait in animal science (Biewener and Patek, 2003). Guesgen and Bench (2018) demonstrated 3D-kinematics can also be used to identify "micro-lordosis" movements (e.g. small pelvic side to side and front and back movements) in tie-stall dairy heifers during a 24 h window prior to ovulation day. Additional information, such as posture angles, complement these types of biomechanical data by analyzing the articulation of movements via spatial reference axes for proximal and distal body segments (Keefe et al., 2008) to measure behaviour biometrics that can be used for diagnostic purposes (e.g. estrus detection) in livestock production. Estrus detection requires a flagging system in order to identify cows in estrus, which in most cases uses a reference value (e.g. threshold or cutoff value) based in a biometrical parameter(s). Further, the ideal estrus detection test discriminates unerringly between estrus (true positives) and non-estrus (true negatives) and is capable of flagging cows daily. In addition, estrus detection methods require some sort of scoring index to evaluate the accuracy of an estrus alert. The most common diagnostic analysis for evaluating accuracy is a receiver operating characteristics (ROC) curve, which uses a balanced proportion of Se and Sp and an accuracy index (J = sensitivity + specificity – 1; Youden, 1950), the higher in the index, the higher the accuracy achieved. Therefore, the primary objective of this study was to characterize movement biomechanics triggered by angle changes due to pelvic

tilt, lateral pelvic shifts (left and right), foot strikes (left and right), and tail movements at different sizes (macro, mid and micro) in naturally cycling primiparous cows in tie-stall housing as ovulation approaches. The second objective was to evaluate the accuracy of each behaviour biometric as an indicator of estrus in dairy cows housed in tie-stalls. We hypothesized that behaviour biometric changes will occur as ovulation approaches (during the estimated estrus period) compared to the proestrus stage (baseline) in naturally cycling primiparous cows. More specifically, we predict that 3D kinematics can be used to detect subtle changes in postural angles associated with pelvic tilting, tail movements, and foot strikes. Further, estrus alerts can be created by identifying optimum reference values based on the frequency of these changes in postural angles during estrus that can be used to differentiate between the estrus period, proestrus, and ovulation days in naturally cycling primiparous dairy cows housed in a tie-stall.

5.3. Materials and Methods

5.3.1. Animals and Housing

The University of Alberta Animal Care and Use Committee for Livestock reviewed and approved this research (AUP 1652) and ensured all animals were cared for in accordance with the Canadian Council of Animal Care (2009) standards and requirements for farm animals in research. The experiment was conducted at the Dairy Research and Technology Centre of the University of Alberta, Edmonton, Alberta, Canada, from June to October 2016.

Fourteen primiparous Holstein cows were housed in individual stalls for 31 ± 6 d (mean ± SD) continuously with access to a total mixed ration based on barley and silage, barley-maize grain and alfalfa or grass hay following the National Research Council guidelines (National Research Council, 2001) and freshwater *ad libitum*. Cows were milked twice daily (0330-0600 and 1500-1730 h) in-stall using a pipeline milking system. Primiparous cows were in their first lactation and producing 27.3 ± 5.63 kg (mean ± SD) of milk per day.

5.3.2. Experimental Design

The current study followed a split-plot over time experimental design using each primiparous cow as an experimental unit. Cow behaviour biometrics were compared during the proestrus stage (baseline), 72 – 24 h before ovulation and on ovulation day.

5.3.2.1. Spontaneous Estrus and Ovarian Mapping

All first lactating cows were scanned using trans-rectal ultrasonography (Ultrasound ALOKA SSD-500 3.5 MHz linear transducer ALOKA Co., LTD, Tokyo, Japan) at 43 days in milk (DIM). The presence of a corpus luteum (CL) during ovarian mapping determined the cyclicity of primiparous cows, and only cyclic cows were included in the study. Ovarian mapping was used to measure CL regression (CL diameter), and follicular development (Follicular diameter). Further disappearance of the dominant follicle and the appearance of a subsequent CL confirmed the ovulation day. Cows were scanned every other day starting at 43 DIM until ovulation (d 0). Once the first ovulation was confirmed, trans-rectal ultrasonography continued from d 7 to d 13 every other day and daily scans from d 14 until the confirmation of subsequent ovulation (Figure 5.1).

5.3.2.2. Milk Sampling and Estradiol Assay

Milk samples were obtained at each milking time (AM-PM) using a 30 ml milk collection container for E_2 analysis. Milk samples were collected by striping the left front teat and discarding the first two milk strips starting at d 14 until two days after subsequent ovulation. Ten out of the 30 ml of milk were placed in 10 ml plain vacutainer tubes (BD Vacutainer Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and centrifuged at 1940.2 x g, at 4°C for 20 min to remove the milk fat (Skim milk) and stored in 5 ml plastic tubes (MCT Fisher Scientific Waltham, MA, USA) at -20°C until E_2 assays were performed.

Estradiol analysis was performed at the endocrine laboratory of the Lacombe Research and Development Centre for Agriculture and Agri-Food Canada (Lacombe, Alberta, Canada) using an enzyme-linked immunosorbent assay based on the principle of competitive binding for plasma and serum (sensitive ELISA kit; IB78239, IBL America, Minneapolis, MN, USA). The estradiol sensitive assay kit had a standard range of 0/3.0 – 200.0 pg/mL with a Se of <1.399 pg/mL. Skim milk samples (100 µL sample volume) were analyzed using a plasma and serum estradiol sensitive ELISA kit following the user's manual and previous validations using milk, in plasma assays (Grgurevič et al., 2016 and Snoj et al., 2017) in a single assay with duplicate (parallel) analysis. To assure the reliability of the estimates for antibody concentrations in skim milk the covariance of variation (CV) range was set as 0 to 15% following Plikaytis et al. (1994) recommendations. The detection range was 6.85 to 47.82 pg/mL) with an inter-assay CV of 9.75% (average of CV from the standard curve) and an intra-assay CV of 5.57%.

5.3.2.3. 3D-kinematic Setup and Recording

The current study followed the same platform specifications (hardware and software) previously described in Guesgen and Bench (2018). Precisely for this study, a skeleton template was constructed using 20 reflective 14 mm markers (Life Science Basic Kit, Vicon Motion Systems Ltd., Denver, Colorado; Figure 5.2A) set using a primiparous cow not participating in the study. Markers were adhered to pre-determined body landmarks, including at the third coccygeal vertebrae, sixth coccygeal vertebrae, right coxal tuberosity, left coxal tuberosity (ilium of the pelvis), right ischial tuberosity, left ischial tuberosity (ischium of the pelvis), femoral greater trochanter, femoral body, femoral lateral epicondyle, tibia lateral condyle, tibia's body, tarsal calcaneal tuber and metatarsal body in left and right sides. Using Nexus software (Bonita, Vicon Motion Systems Ltd., Denver Colorado), each marker was labelled and connected following the cow's morphology to build segments (e.g. Hip, Spine, Legs and Tail; Figure 5.2B and 2C) and recorded for 1 min in a standing position. The skeleton template was then further applied to all experimental units.

Prior to beginning the 3D-kinematic assessment, each cow was habituated to the kinematic recording area and marker placement to minimize potential confounding effects during the experiment (full details in Guesgen and Bench, 2018). For 5 days, each cow was walked from its home stall to the kinematic testing area by one of two handlers. The cow would stand in the testing area for 10 min each day. To avoid neighbour interactions and possible removal of markers, all neighbouring cows in the kinematic area were removed. The same two people handled the cows throughout the experiment. Habituation and placement of the markers took approximately 1 min. For consistency, kinematic assessment took place during the same period as during habituation.

Kinematic recordings took place at d 14 after the first ovulation until 2 days after confirming a subsequent ovulation between 0900 and 1430 to avoid milking times and ovarian mapping (1700). Before placing kinematic markers, all cows were brushed to remove dust and debris, the location of each kinematic marker was marked using tail paint then the 20 markers were set using 2"x2" pieces of adhesive polypropylene red tape (Acklands Grainger, Thornhill, ON, Canada). Kinematic recordings started with a 20s calibration clip to calibrate the skeleton template with the cow in the kinematic recording area, then a 15 min recording was captured. The middle 5 min were used for data processing to avoid effects from the environment (during the first 5min) and restless behaviours due to boredom (last 5 min). All markers were removed and cleaned using

cotton with isopropyl alcohol 90% and marker locations were tail painted to ensure consistent marker placement in subsequent trials at the completion of kinematic recording.

5.3.2.4. Kinematic Data Processing

After kinematic data collection, each clip was reconstructed and labelled using Nexus software "Reconstruct" pipeline and a skeleton template (Figure 5.2C). The middle 5 min of each clip was selected to be reconstructed (30,000 – 60.000 frames), the skeleton was calibrated using "Skeleton ROM" pipeline. Segments were created using the Butterworth filter pipeline (Figure 5.2D) and exported in ASCII and C3D format. Using ProCalc 1.2.1 (Vicon Motion Systems Ltd., Denver, Colorado), 16 behaviour variables were created loading the C3D files from Nexus software, as shown in Table 5.1. Each behaviour contained a simple angle constructed using two vectors (A & B), each vector was made using two labelled markers from the skeleton (e.g. vector A; tibia's body – tarsal calcaneal tuberosity and vector B; metatarsal body – tarsal calcaneal tuberosity). The vertex of each angle was located in the articulation joint between vector A and vector B. To calculate the number of events per angle changes variable, a 3D workspace was created and the parameters for each variable (e.g. movement biomechanics) were specified (Table 5.1) during a resting position at baseline (e.g. proestrus) for each cow (Figure 5.3). All postural event changes were triggered by a change in angle position from the resting average position (average of non-movement postural position angle during 5 minutes) acquired at baseline (e.g. proestrus stage) for each primiparous cow (Table 5.1). The postural angle changes in the tail movement were further divided depending on angle change size in, Macro (> 100°), Mid (between 95 – 100°) and Micro (between 85 – 94°. The number of events for each behaviour variable were then exported to an Excel (Microsoft Office, 2016) spreadsheet.

5.4. Data Analysis

The frequency of postural angle changes for all behaviours were analyzed using a generalized linear mixed model (Proc Glimmix) in SAS (v 9.4, SAS Institute Inc., North Carolina, USA). Normality assumptions were assessed using a Kolmogorov-Smirnov test ($P < 0.05$) for all behaviour data, however, behaviour data did not satisfy normality assumptions ($P > 0.05$) and a Poisson distribution was identified. For data analysis, Sample days were standardized (d -4, d -3, d -2, d -1 and d 0) using the disappearance of the dominant follicle and subtle appearance of a CL

as d 0 and proestrus stage as d -4. The generalized linear mixed model included "Sample Day" as a fixed variable, Cow as a random statement, and a Poisson distribution with a log link function specified. For all behaviour variables, a Type 3 test was requested with the inverse (ilink) function specified and the statement of ar(1) to account for the lack of independent and homogeneous data. All results are presented as least squares means (LSMeans) and standard error means (SEM) calculated using a Bonferroni means separation test. Differences were considered significant if $P < 0.05$, a tendency if $0.05 \leq P < 0.10$ and not significant if $P \geq 0.10$. Additionally, peripheral concentrations of E_2, Follicular and CL diameter were analyzed using Sample Day as a fixed variable. Pearson, correlation coefficient analysis was used to identify possible associations between E_2 and behaviour biometrics as well as associations between the different behaviour biometrics on days approaching ovulation. For this study, a strong positive correlation was considered as > 0.80, moderate > 0.50 but < 0.80 and weak < 0.50, a strong negative correlation was considered as < -0.80, moderate < -0.50 but > -0.80 and weak < -0.50.

In addition to Sample Day relative to ovulation, all behavioural parameters were evaluated using a Receiver Operating Characteristic (ROC) curve using (Proc Logistic) SAS software (ver 9.4, SAS, Cary, NC, USA). Estrus alerts were evaluated by using the most optimum reference value (i.e. threshold value), which resulted from a balanced proportion of true positive (sensitivity) and true negative (specificity) occurrence. Behavioural biometrics were evaluated using d -1 (-24 h) as the estimated estrus period following the highest Follicular diameter (> 15 mm), CL regression (< 20 mm), and peripheral E_2 concentration (> 16 pg/mL; Perry et al., 2017). Additionally, all estrus alerts using behavioural biometrics were evaluated at d -2 (- 48 h) and the peak of E_2 concentration in skim milk of each primiparous cows. The minimum acceptable value for estrus alerts was pre-determined using a Youden J Index (J Index) of 0.30 to ensure an estrus detection rate greater than 50%. Follicular and CL diameter and E_2 concentration were evaluated using ROC curves and used as the physiological basis of comparison (e.g. as the most accurate methods to detect ovulation) to evaluate all behavioural biometrics.

5.5. Results and Discussion

5.5.1. Ovulation Occurrence, Ovarian Structures and Estradiol Concentrations

Estrous cycle length was 21.66 ± 3.09 (LSMeans ± SEM) days with a range of 18 to 27 days. The largest Follicular diameters were found at d -1 (17.04 ± 0.59 mm) compared to d -4

(13.87 ± 0.53 mm) and CL diameter gradually decreased from d -4 (22.45 ± 0.95 mm) to d -1 (15.70 ± 0.87 mm). Estradiol skim milk concentrations peaked at d -1 (17.43 ± 1.76 pg/mL) compared to d -4 (15.70 ± 1.67 pg/mL) and d 0 (15.98 ± 1.68 pg/mL). However, E_2 skim milk concentrations did not change (P = 0.98) on the days leading to ovulation compared to Follicular (P = 0.01) or CL (P = 0.01) diameters. Similarly, Perry et al. (2014) found no relationship between Follicular diameter and peak of E_2 concentrations in plasma. As such, Follicular diameter found during d -1 was used as an indirect physiological indication of estrus (Ireland et al., 1982, Kruip et al., 1985).

5.5.2. Behaviour Biometric Analysis for Days Preceding Ovulation

The first objective of this study was to characterize the frequency of angle changes due to pelvic tilting, pelvic shifts (left and right), foot strikes (left and right), and tail movements (macro-mid-micro) as behaviour biometrics for the detection of estrus during the estimated estrus period. We hypothesized that there would be changes in the frequency of postural angle changes from baseline as ovulation approaches in primiparous dairy cows in a tie-stall housing.

The frequency of angle changes associated with Pelvic tilting (P = 0.01), Pelvicsl (P = 0.01), Pelvicsr (P = 0.01), and TPelvicS (P = 0.01, Figure 5.4A and Table 5.2) differed significantly on days leading to ovulation. In particular, the occurrence of Pelvic tilt, Pelvicsl, Pelvicsr and TPelvicS biometric changes increased on d -2 compared to d -4 and ovulation day (Table 5.2). The increase in pelvic movements at d -2 could represent the increase in activity at the beginning of the estrus period and the decrease of frequency of changes in postural angles event during d -1, similarly to the low activity during standing to be mounted behaviour hours before ovulation. Moderate positive correlations (P = 0.01, r = 0.63) were found between E_2 skim milk concentrations and the frequency of Pelvic tilt changes, which may indicate the possible effect of E_2 on restless behaviour. The increase in Pelvic tilt frequency as ovulation approached could also be explained by the onset of lordosis postures, which further coincides with an increase in pelvic shifting (back – forward and left to right) previously reported by Guesgen and Bench (2018). Similar sexual posture associations were found by Pfaff (1999) who was able to trigger lordosis behaviour with a simple stimulus (e.g. hand touch) by administrating estrogen to female rats. However, none of the cows in the current study had physical contact with other cows or staff members during the 3D-kinematic assessment. As such, Pelvic tilt changes may indicate a form of

sexual fidgeting without stimuli as a means of expressing sexual receptivity postures (i.e. subtle visual cue similar to lordosis or standing to be mounted).

Total foot strikes tended (P = 0.07) to decrease around d -1 compared to baseline (see Table 5.2) and was found to be significant when left, and right foot strikes were analyzed separately (Foot strike L; P = 0.03 and Foot strike R; P = 0.01). Foot strikes decreased from proestrus (d -4) with the lowest frequency of changes in postural angles events at d -2 and d -1 (Figure 5.4B). No significant correlations were found between physiological parameters (Follicular and CL diameter), except for a weak negative correlation between E_2 concentrations and Foot strike L (P = 0.04; r = -0.43, Table 5.3). Dairy cows housed in tie-stall systems often move the back legs in a particular way (i.e. treading behaviour up and down), which may be related to pre-milking-milking anticipation (Kézér et al., 2015). However, the current study did not assess movement biomechanics during milking time. The decrease in Foot strikes during d -1 compared to baseline is contradictory to an expected increase in activity (i.e. walking activity) in dairy cows before ovulation (i.e. mounting events; Van Eerdenburg et al., 1996 and mounting events of 2 h blocks twice per day; Aungier et al., 2015) and tie-stalls during milking time (Perez Marquez et al., 2019). Some explanations for these contradictory results may be the inability of angle changes to differentiate between Foot strikes (up – down), the stepping behaviour (pendulum forward – backward) and balancing movement while scratching. Other tie-stall studies have also reported non-significant changes in leg movements before ovulation using pedometers (Felton et al., 2012), which suggests that cows do not express significant leg movements in a confined space during estrus. Another potential confounding effect may have been the tubing fencing on the right side of the kinematic stall while the stall was empty on the left side, which may explain why leg movements that require larger space occurred more often on the left side. Further studies should focus more on differentiating between stepping and striking behaviours and record at milking time when foot strikes are more often observed as this would likely provide more clarity regarding the frequency of postural angles changes during the estrus period.

The frequency of tail movements increased at d -2 followed by a decrease at d -1 compared to baseline (Table 5.2), which was similarly observed in Pelvic tilt and Pelvic shifts (left and right). In particular, TailLMacro movements increased (P = 0.01) on the day of ovulation. TailRMacro also tended to increase in movement at d -2 (P = 0.06), however, pooling of Tail macro movements left, and right (TailTMacro) did not change as ovulation approached (P = 0.40). Changes in tail

movements differed as the movement size decreased (Figure 5.4C). TailTMid movements and TailTMicro movements were greater (P = 0.01) than TailTMacro movements (P = 0.40). Specifically, TailLMid and TailRMid frequencies decreased from baseline to d -1. TailRMid movements increased at ovulation day compared to the reduction observed in TailLMid during ovulation day (Table 5.2). Tail Micro movements (left, right and total) decreased on d -3, increased at d -2, and decreased at d -1 and d 0 compared to baseline (Figure 5.4C). Micro tail biometrics resulted in higher frequencies of angle changes (TTailMicro, 18.60 ± 3.31 events/5min) compared to macro tails biometrics (TTailMacro, 4.64 ± 1.00 events/5min). As a result, changes in postural angles can be easily measured in micro-behaviours using Kinematics analysis.

Possible explanations for the changes in tail movement patterns could be related to the increased movement associated with mounting and general activity, which have been widely reported during the estrus period when E_2 peripheral concentrations peak and a dominant follicle is present ($R^2 = 0.96$; Aungier et al., 2015). Similar results were also found by Guesgen and Bench (2018) in which increases in micro, restless behaviours associated with the pelvis (e.g. forward-backward and side to side) preceded ovulation. The possible causation of the increase of tail movements during the estrus period could be attributed to changes in the physiology of the reproductive tract, such as swelling of the vulva and the presence of fluid in the uterus and vagina. Cows may rub (macro tail movements) and subtly expose the vulva during the estrus period (micro tail movements). Similar restless tail behaviour has been observed during physiological changes in the reproductive tract, such as that seen pre-parturition (Miedema et al., 2011, Sumiyoshi et al., 2014).

The significant correlations between Foot strikes and Tail movements (Table 5.4) may be due to the changes in standing postures and the reflex driven by the sacral-coccygeal part of the sympathetic trunk of the bovine. However, seasonality (e.g. summer), the presence of flies, working staff, tail injuries (i.e. broken or frostbite tails), and 3D-kinematic markers may also influence Tail movements. However, these were unlikely in this study because barn ventilation, a two-week acclimation to the kinematic markers and the absence of tail injuries minimized these potential confounding effects.

5.3.3. The Accuracy of Behaviour Biometrics Compared with Physiological Estrus Indicators

The second objective of this study was to evaluate the accuracy of specific behaviour biometrics (pelvic, foot and tail movements) as estrus alerts in dairy cows housed in tie-stalls. We hypothesized that estrus alerts can be created by identifying threshold values based on changes in postural angles during estrus that can differentiate between proestrus, estrus and ovulation days in naturally cycling primiparous dairy cows housed in a tie-stalls.

The accuracy (as measured using balanced Se – Sp and J index) of all behavioural biometrics are expressed in Table 5.3. The J index of 0.30 score achieved at least 50% of true positive estrus, any parameter with a J index greater than 0.30 was reported to have statistical significance for this study. Notably, behavioural biometrics with a J index over 0.30 included Pelvic tilt, Foot strike L, TailLMacro (-24 h before ovulation), Foot strike L, TPelvicS, (-48 h before ovulation), Foot strike R, and TFootS (during the peak of E_2 concentrations, see Table 5.3). Regardless of the precision of 3D-Kinematics to identify behavioural biometric changes in postural angles, not all parameters complied with the minimum J index value for the accuracy evaluation, which indicates that not all behaviour biometrics measured are useful to detect estrus.

Follicle and CL diameters gave a close estimation of when estrus and ovulation may occur. In fact, the Follicular diameter was the most accurate parameter to estimated estrus time (1.00 Se, J Index 0.61) followed by E_2 (0.89 Se, J index 0.44). However, the use of ultrasonography to detect estrus is not practical since it requires professional training, is time-consuming, and disruptive to dairy cows to use daily. Further, the peak of E_2 can useful as an indicator of estrus and ovulation. Nevertheless, ELISA assays require laboratory conditions to obtain consistent and reliable results. As such, physiological parameters should be used as an indication of the estrus period and ovulation confirmation but not as estrus detection methods.

Behaviour biometrics with the highest J index were TFootS and Foot strike R using to the peak of E_2. The highest scores found by TFootS and Foot strike R can be explained as the expected decrease in activity during the standing to be mounted in the late stage of the estrus period when the E_2 peak in concentrations occur. Other significant accuracy scores were Pelvic tilt, Foot strikes L and TailLMacro, TailLMid, and TFootS at -24 h before ovulation. An explanation for these results may be the increase in frequencies of changes in postural angles size in micro-movements used to detect Pelvic tilt movement. Further Pelvic tilt movements may only be present during sexual receptivity compared to tail and foot movements that may be present at other times as well. Additionally, Foot strikes L and TailLMacro, TailLMid, and TFootS achieved higher accuracy

scores, and were characterized by decreases in the frequency of postural angle changes during the estrus period. One possible explanation is that it may be easier to create an estrus alert when the frequency of changes in postural angles decrease from baseline rather than increase, which may explain why activity monitors fail to detect estrus in a tie-stall since activity monitors flag estrus occurrence based on increases in activity. The evaluation of accuracy did not find higher J index values at 48 h prior to ovulation except for Foot strike L in which the frequency of events at d -2 was lowest for Foot strike L and no other behaviour biometrics were observed to have the lowest activity during d -2.

During this study, behaviour biometrics were characterized for use as estrus alerts during the four days before ovulation, which is a critical time when estrus should occur. This study intended to use 3D-kinematisc as an estrus detection method and to provide information for use in current estrus detection aids. Using kinematics can also develop newer methods to better understand of behavioural changes on days leading to ovulation in a confined space (e.g. stall).

5.4. Conclusions

We accepted our hypothesis that 3D-kinematics is an objective and precise method to measure changes (increases and decreases) in the frequency in postural angles due to pelvic tilting, pelvic shifts (left, right, and total pelvic shifts), foot strikes (left, right, and total foot strikes), and tail movements (macro-mid-micro) during the four days before ovulation. Behaviour events triggered by the position of anatomical angles can be measured in different sizes depending on the environment or housing type; such as tie-stalls. Additionally, the highest accuracy evaluations using 3D-Kinematics were found at the peak of E_2 (TFootS; and Foot strike R) followed by 24 h (Pelvic tilt, Foot strikes L, TailLMacro, TailLMid, TFootS) and 48 h (Foot strike L) before ovulation. As such, the use of pelvic, foot and tail biometrics may be useful as indicators of ovulation during the peak of estradiol, in addition to 48 to 24 hours in advance.

5.5. Acknowledgements

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Table 5.1. Behaviour biometric description based on segment angle movements from specific body landmarks in a 5 min 3D-kinematic recording at 100 frames/sec (30,000 frames total).

Behaviour biometrics	Description
Pelvic tilt	Number of events triggered by 2˚ increase of an angle change from the average angle position (175.05˚ ± 0.81) at baseline (D -4) between the lumbar spine marker (1st arm) and the model markers between ishium right side and ishium left side (2nd arm) and the model point between tubar coxa right side and tubar coxa left side as a vertex
Pelvic shift left (Pelvicsl)	Number of events triggered by 2˚ decrease of an angle change from the average angle position (150.83˚ ± 2.83) at baseline (D -4) between the lumbar spine marker (1st arm) and straight direction from tubar coxa right and ishium left side (2nd arm) and ishium left side as a vertex
Pelvic shift right (Pelvicsr)	Number of events triggered by 2˚ decrease of an angle change from the average angle position (172.24˚ ± 0.49) at baseline (D -4) between the lumbar spine marker (1st arm) and straight direction from tubar coxa left and ishium right side (2nd arm) and ishium right side as a vertex
Total pelvic shift (TPelvicS)	Total of events summed between Pelvic shift left and Pelvic shift right events
Foot strike left (Foot strike L)	Number of events triggered by 10 ˚ decrease of an angle change from the average angle position at baseline (D -4) between the tibia's body marker form the left leg (1st arm) and metatarsal body left (2nd arm) using the tarsal calcaneal tuber left side as a vertex
Foot strike right (Foot strike R)	Number of events triggered by 10 ˚ decrease of an angle change from the average angle position at baseline (D -4) between the tibia's body marker form the right leg (1st arm) and metatarsal body right (2nd arm) using the tarsal calcaneal tuber right side as a vertex
Total feet strikes (TFootS)	Total of events summed between Foot strike left and Foot strike right events
Tail macro left (TailLMacro)	Number of events beyond >100˚ angle change from the rest angle position (67.30 ± 9.52) between the model point (Caudal spine – tail head) and ishium right (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Tail macro right (TailRMacro)	Number of events beyond >100˚ angle change from the rest angle position (67.57 ± 7.55) between the model point (Caudal spine – tail head) and ishium left (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Total tail macro (TailTMacro)	Total of events summed between Tail macro left and Tail macro right events
Tail mid left (TailLMid)	Number of events between 95˚- 100˚ angle change from the rest angle position (67.30 ± 9.52) between the model point (Caudal spine – tail head) and ishium right (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Tail mid right (TailRMid)	Number of events between 95˚- 100˚ angle change from the rest angle position (67.57 ± 7.55) between the model point (Caudal spine – tail head) and ishium left (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Total tail mid (TailTMid)	Total of events summed between Tail mid left and Tail mid right events
Tail micro left (TailLMicro)	Number of events between 85˚- 94˚ angle change from the rest angle position (67.30 ± 9.52) between the model point (Caudal spine – tail head) and ishium right (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Tail micro right (TailRMicro)	Number of events between 85˚- 94˚ angle change from the rest angle position (67.57 ± 7.55) between the model point (Caudal spine – tail head) and ishium left (1st arm) and the distal tail marker (2nd arm) using model point (Caudal spine – tail head) as a vertex
Total tail micro (TailTMicro)	Total of events summed between Tail micro left and Tail micro right events

Table 5.2. Least Square Means and standard error means (LSMeans ± SEM) at baseline, d -2, d -1, d 0 with the corresponding significance level (P – value) for all behaviour biometrics.

Behaviour biometrics	Baseline[1]	d -2[2]	d -1[3]	d 0[4]	P - value
Pelvic tilt	13.10 ± 8.32	19.75 ± 8.67	17.43 ± 11.06	11.75 ± 7.47	0.01
Pelvicsl	3.71 ± 2.52	20.26 ± 13.64	10.64 ± 7.17	3.48 ± 2.36	0.01
Pelvicsr	3.50 ±1.82	0.38 ± 0.13	0.04 ± 0.05	0.81 ± 0.45	0.01
Foot strike L	6.30 ± 1.24	4.13 ± 0.90	4.75 ± 1.00	7.43 ± 1.42	0.03
Foot strike R	8.05 ± 1.88	6.67 ± 1.65	5.08 ± 1.26	4.98 ± 1.24	0.01
TailLMacro	0.93 ± 0.54	1.14 ± 0.63	0.11 ± 0.10	3.21 ± 1.69	0.01
TailRMacro	1.91 ± 1.04	0.87 ± 0.52	1.85 ± 1.01	0.97 ± 0.55	0.06
TailTMacro	1.84 ± 1.12	2.47 ± 1.48	1.66 ± 1.01	1.87 ± 1.13	0.40
TailLMid	2.01 ± 1.32	1.20 ± 0.79	0.25 ± 0.19	1.42 ± 0.94	0.01
TailRMid	3.22 ± 1.42	2.74 ± 1.22	2.06 ± 0.93	4.46 ± 1.94	0.01
TailTMid	6.07 ± 3.2	3.44 ± 1.87	1.82 ± 1.01	3.84 ± 2.09	0.01
TailLMicro	7.21 ± 4.20	4.75 ± 2.76	2.64 ± 1.55	5.81 ± 3.37	0.01
TailRMicro	10.33 ± 4.76	3.83 ± 1.80	3.96 ± 1.86	7.92 ± 3.65	0.01
TailTMicro	14.57 ± 7.23	10.06 ± 4.97	7.30 ± 3.62	15.33 ± 7.54	0.01
TpelvicS	6.34 ± 2.72	20.82 ± 8.79	8.08 ± 3.45	6.58 ± 2.83	0.01
TFootS	14.44 ± 2.78	11.61 ± 2.36	9.86 ± 1.98	12.35 ± 2.42	0.07

Abbreviations: [1]Baseline: luteal phase, d -4; [2]d -2: 48 h prior ovulation; [3]d -1: 24 h prior ovulation, estimated estrus period; [4]d 0: Ovulation day.

Table 5.3. Test of performance results for estrus alerts at d -1, d -2, and the peak of E_2 for the estrus indicators and the behavioural biometrics with a balance Se – Sp.

		d -1[1]				d -2[2]				Peak E_2[3]		
Behaviour biometrics	OTV[4]	Se[5]	Sp[6]	J index[7]	OTV	Se	Sp	J index	OTV	Se	Sp	J index
Follicular diameter	16.00	1.00	0.61	0.61	14.50	0.89	0.50	0.39	0.10	0.33	0.83	0.17
CL	12.06	1.00	0.28	0.28	14.01	1.00	0.35	0.35	14.00	0.63	0.74	0.37
E2	17.06	0.11	1.00	0.11	11.69	0.89	0.28	0.17	13.67	0.89	0.56	0.44
Pelvic tilt	33.14	0.78	0.59	0.37	14.00	0.13	1.00	0.13	46.73	0.86	0.36	0.22
Pelvic shif left	22.84	0.63	0.48	0.11	139.10	0.29	1.00	0.29	42.95	0.77	0.52	0.30
Pelvic shif right	34.78	1.00	0.09	0.09	6.21	1.00	0.21	0.21	82.61	0.43	0.75	0.18
Foot strike left	51.08	0.44	0.89	0.33	29.00	1.00	0.31	0.31	49.12	0.88	0.39	0.26
Foot strike right	12.52	0.22	0.97	0.19	9.18	0.88	0.29	0.16	4.99	0.88	0.66	0.53
TailLMacro	2.92	1.00	0.41	0.41	3.16	1.00	0.09	0.09	5.20	0.44	0.73	0.17
TailRMacro	14.25	0.63	0.59	0.21	10.73	0.78	0.36	0.14	9.19	0.33	0.82	0.15
TailTMacro	16.73	1.00	0.23	0.23	12.57	0.88	0.35	0.23	23.23	1.00	0.26	0.26
TailLMid	7.89	1.00	0.30	0.30	13.51	0.22	0.91	0.13	4.71	0.78	0.4	0.18
TailRMid	21.14	0.44	0.74	0.19	37.29	0.78	0.44	0.22	65.22	1.00	0.21	0.21
TailTMid	21.74	1.00	0.21	0.21	24.61	1.00	0.25	0.25	38.26	1.00	0.26	0.26
TailLMicro	119.29	0.56	0.73	0.28	222.06	0.33	0.85	0.18	250.36	1.00	0.21	0.21
TailRMicro	77.66	0.56	0.67	0.22	56.05	0.89	0.34	0.23	78.92	0.22	0.94	0.16
TailTMicro	158.00	0.88	0.41	0.29	172.39	1.00	0.24	0.24	221.44	0.88	0.39	0.27
T pelvic Shift	21.72	0.78	0.29	0.06	39.82	0.22	1.00	0.22	38.42	0.77	0.55	0.33
T foot strike	49.21	0.67	0.68	0.34	85.22	1.00	0.29	0.29	16.63	0.88	0.63	0.50

Abbreviations: [1]d -1: Estrus alert at d -1, 24 h before ovulation; [2]d -2: Estrus alert at d -2, 48 h before ovulation; [3]Peak E_2: Estrus alert during the peak of E_2 (d -1 – d 0); [4]OTV: Optimum threshold value (Events/5min); [5]Se: Sensitivity (True positives); [6]Sp: Specificity (True Negatives); [7]J index: Youden J index (Performance of the diagnostic test).

Table 5.4. Significant Pearson correlation coefficients between E_2 measurements and behaviour biometrics with a P-value and direction association.

	Outputs	E_2	Pelvic tilt	Foot strike L	FootstrikeR	TFootS	TailRMacro	TailRMid	TailLMicro	TailTMicro	TPelvicS
	R[1]	1	0.64	0.52	0.6	-0.11	0.52	0.63	0.59	-0.55	0.52
E_2	P-value		0.01	0.02	0.01	0.63	0.02	0.01	0.01	0.01	0.02
	N[2]	22	20	21	21	22.00	21	21	21	22	22
Pelvic tilt	R	0.64	1	-0.21	-0.2	-0.14	0.05	-0.08	-0.2	0.23	-0.12
	P-value	0.01		0.38	0.4	0.56	0.85	0.74	0.4	0.31	0.6
	N	20	22	21	21	20.00	21	21	21	22	22
Foot strike L	R	0.52	-0.21	1	0.95	0.05	0.4	0.65	-0.01	-0.52	0.64
	P-value	0.02	0.38		0.01	0.83	0.09	0.01	0.97	0.02	0.01
	N	21	21	21	21	21.00	20	20	20	21	21
Foot strike R	R	0.6	-0.2	0.95	1	0.02	0.33	0.62	0.11	-0.51	0.86
	P-value	0.01	0.4	0.01		0.93	0.16	0.01	0.66	0.02	0.01
	N	21	21	21	21	21.00	20	20	20	21	21
TailRMacro	R	0.52	0.05	0.4	0.33	0.80	1	0.91	0.54	-0.65	-0.02
	P-value	0.02	0.85	0.09	0.16	0.01		0.01	0.02	0.01	0.97
	N	21	21	20	20	21.00	21	21	20	21	21
TailRMid	R	0.63	-0.08	0.65	0.62	0.93	0.91	1	0.57	-0.74	0.23
	P-value	0.01	0.74	0.01	0.01	0.01	0.01		0.01	0.01	0.34
	N	21	21	20	20	21.00	21	21	20	21	21
TailLMicro	R	0.59	-0.2	-0.01	0.11	0.53	0.54	0.57	1	-0.31	0.15
	P-value	0.01	0.4	0.97	0.66	0.01	0.02	0.01		0.19	0.54
	N	21	21	20	20	21.00	20	20	21	21	21
TailTMicro	R	-0.55	0.23	-0.52	-0.51	-0.17	-0.65	-0.74	-0.31	1	-0.26
	P-value	0.01	0.31	0.02	0.02	0.44	0.01	0.01	0.19		0.25
	N	22	22	21	21	22.00	21	21	21	22	22
T pelvic S	R	0.52	-0.12	0.64	0.86	-0.06	-0.02	0.23	0.15	-0.26	1
	P-value	0.02	0.6	0.01	0.01	0.79	0.97	0.34	0.54	0.25	
	N	22	22	21	21	22.00	21	21	21	22	22

Abbreviations: [1]R: R value; [2]N: Number of observations.

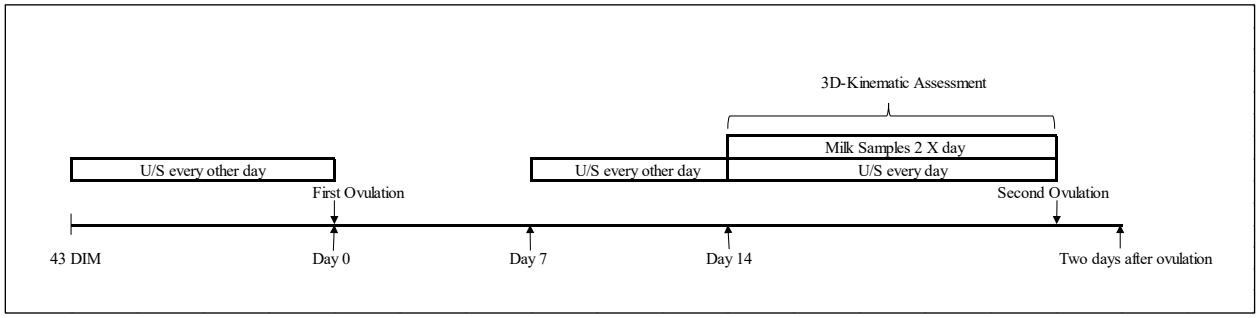

Figure 5.1. Spontaneous ovulation protocol and study timeline.

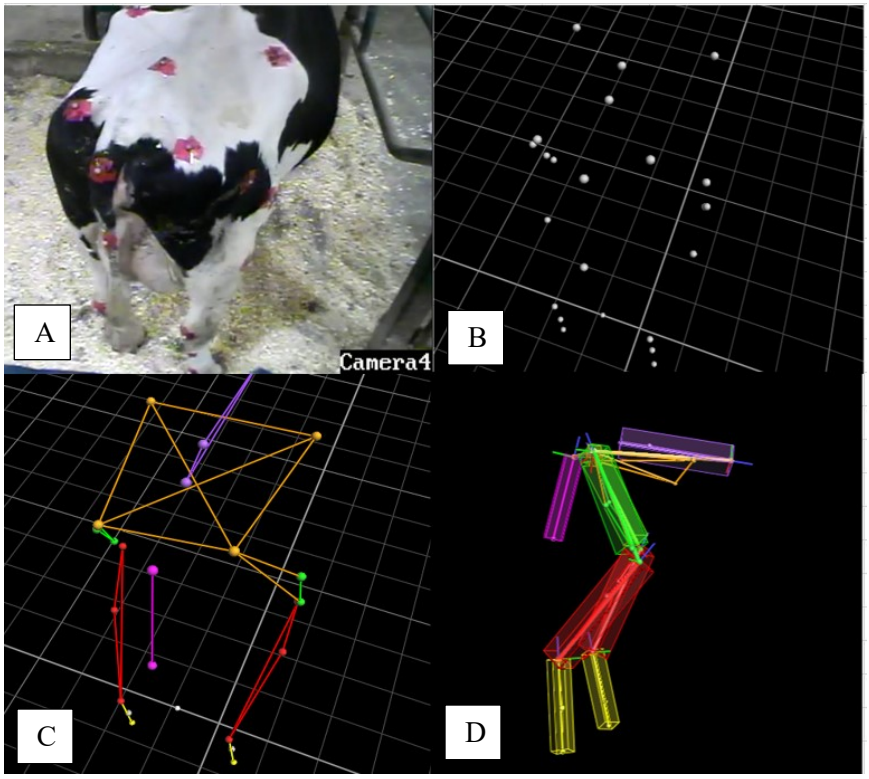

Figure 5.2. 3D-Kinematic assessment. A) Reflective marker locations in the posterior area of a lactating dairy cow. B) Reflective markers in a 3-dimentional field after been recorded using Nexus 2.7. C) Labelling (TCR = tubar coxa right side, SacSpine = sacral-spine, CaudSpine = caudal-spine, IshL = ishium left side, ProxTail = Proximal-tail, DisTail = distal-tail, TarsalR = tarsal calcaneal tuber right, and MetaTR = metatarsal body right side) and segment reconstruction (Reconstruct pipeline). D) Filtering process, linearizing the predictions and measurements of reflective markers about their mean.

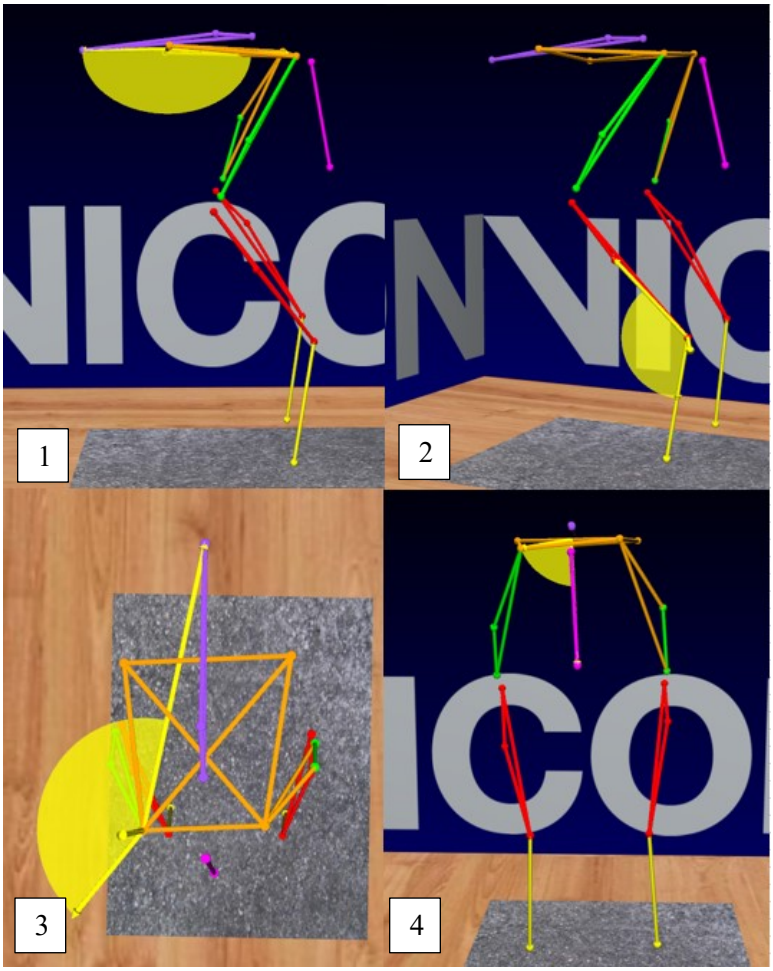

Figure 5.3. Angle locations (arms and vertex) for each behaviour biometric (left side) during baseline (d -4) in a rest position. 1) Pelvic Tilt angle, 2) Feet strike left (Foot strike L) angle, 3) Pelvic shift left (Pelvicsl), and 4) Tail angle left side (Macro, Mid, and Micro).

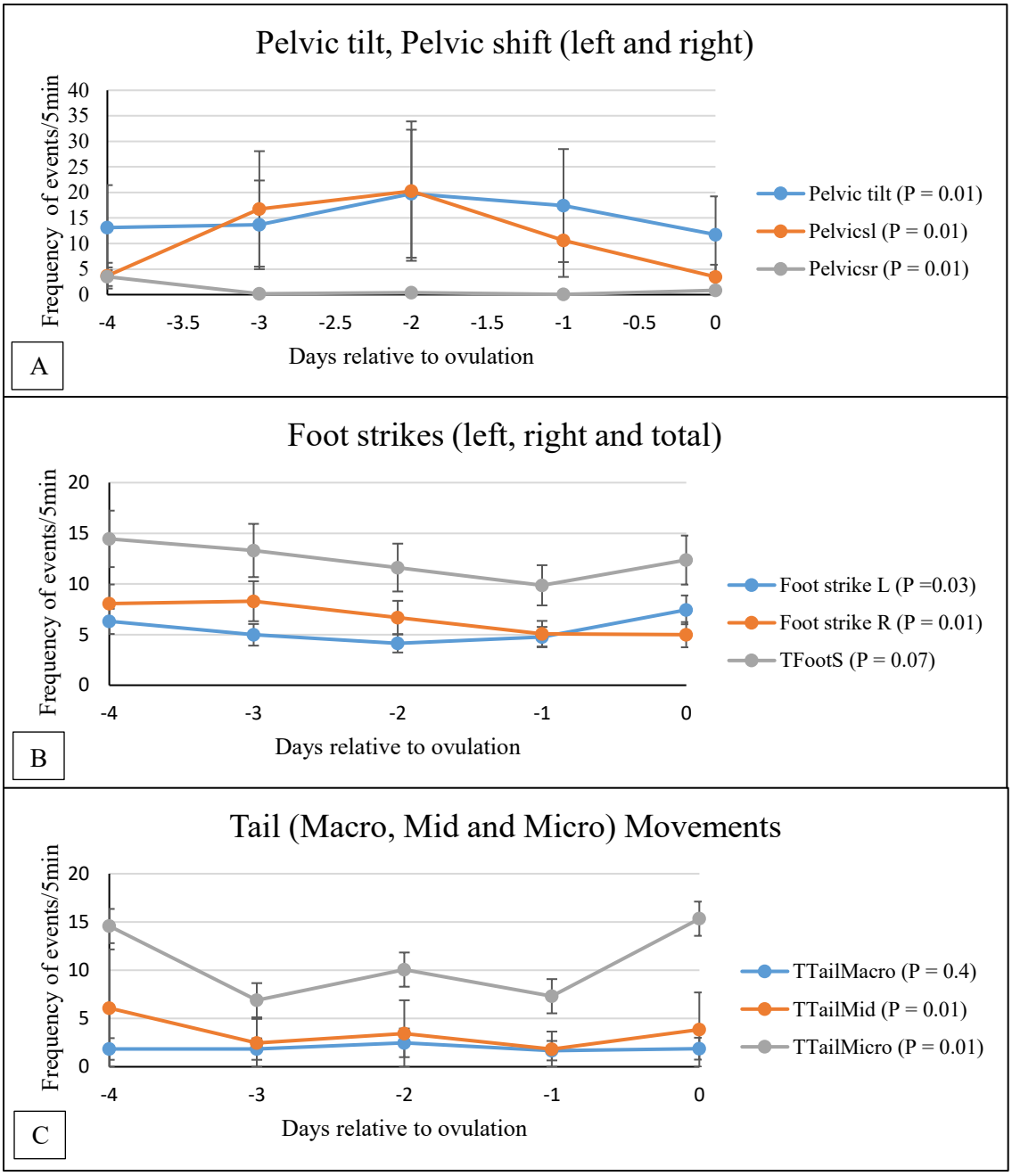

Figure 5.4. Behaviour biometrics Least square means ± standard error mean (LSMeans ± SEM) of frequency of movements in a 5 min of 3D-Kinematic recording period. A) Pelvic tilt with a significant level (Pt; P = 0.01), Pelvic shift left (Psl; P = 0.01), Pelvic shift right (Psr; P = 0.01). B) Foot strike left (Fsl; P = 0.03), Foot strike right (Fsr; P = 0.01), Total foot strikes (TFS; P = 0.07). C) Total tail movements at different resolution, Total tail macro (TTMacro; P = 0.4), Total tail mid (TTMid; P = 0.01) and Total tail micro (TTMicro; P = 0.01).

Chapter 6. Automated Infrared and Tail Movement Tracking as an Estrus Detection Method in a Commercial Voluntary Milking System

6.1. Abstract

The implementation of automated technologies aims to optimize reproductive performance by accurately detecting estrus. The primary objective of this study was to develop an automated infrared thermography platform (Estrus BenchMark™; EBM) capable of measuring skin temperature and tail movements as a means of flagging cows in estrus. The second objective was to compare the accuracy of the IRT platform to detect estrus with in-line milk progesterone (P_4) analysis (Herd Navigator™; HN) and a 3-axis accelerometer system (CowManager SensOor™ tags; CSTags) used in a commercial dairy herd. Data were collected on forty-six cows starting at 45 days in milk (DIM) until 120 DIM. In-line milk P_4 was determined automatically (at set intervals) throughout the study period and cows were flagged in estrus when P_4 fell below 5 ng/mL. The CSTags and EBM true positive estrus alerts (Se %) were compared to HN estrus alerts at different time windows (Same-day, ±24 h, ±48 h, and ±72 h). The EBM was able to collect skin temperature and tail movement data (left tail movements; LTail, right tail movement; RTail, and pooled tail movement; MTail) in real time. Skin temperature changes were associated with a decrease in milk P_4 concentration (Least-Squares Means; LSMeans) at d 0 (P_4; 3.51 ± 0.05 pg/mL, Skin temperature; 33.31 ± 2.38°C) compared to d -14 (P_4; 20.22 ± 0.73 pg/mL, Skin temperature; 32.05 ± 3.77°C) and d 4 (P_4; 10.98 ± 0.66 pg/mL, Skin temperature; 31.67 ± 3.48°C). The prevalence of tail movements per IRT scanning (8 frames/milking) was greater ($P = 0.01$) at d 0 (LTail; 62.50%, MTail; 68.75%, and RTail; 56.25%) compared to d -14 (LTail;15.62, MTail; 6.25%, and RTail;15.62%), and d 4 (LTail; 9.37%, MTail; 9.37%, and RTail; 12.5%). The Se of EBM was compared with CSTags at different time-windows relative to estrus (d 0), Same-day (CSTags; 6%, EBM; 42%), ±24 h (CSTags; 23%, EBM; 50%), ±48 h (CSTags; 43%, EBM; 58%), and ±72 h (CSTags; 44%, EBM; 56%). The highest EBM Youden index (0.45), diagnostic odds ratio (9.04), and efficiency (0.77) were achieved by estrus alerts within ±48 h window relative to HN estrus alerts. The accuracy of EBM proved to be able to measure skin temperature and tail movements at each milking occurrence and to identify fluctuations in skin temperature and tail movement as the estrus period approached. The accuracy of EBM resulted in higher estrus detection rates compared to accelerometers but lower compared to HN estrus alerts.

Chapter 7 has been formatted following the Animal Journal.

6.2. Introduction

The adaptation of precision technologies in dairy farming has increased in the last three decades. The use of automated sensors that can track physiological and secondary estrus behaviour parameters have achieved superior estrus detection rates (~80%; Roelofs et al., 2017, Schweinzer et al., 2019) compared to the visual observation of standing to be mounted (~50%; Gaude et al., 2017, Mayo et al., 2019). Automated estrus detection sensors can be divided into in-line milk progesterone (P_4) analysis, 3-axis accelerometers, mounting detectors, digital-video monitoring, temperature loggers, vaginal-fluid electric impedance or conductivity, and vaginal olfactory-essence detectors. Automated estrus detection sensors aim to optimize the estrus detection interval to artificial insemination (AI) service at the most economically efficient period (from 60 to 70 days in milk; De Vries, 2006). Furthermore, the use of automated estrus detection sensors has been able to shorten the time to pregnancy (85 DIM; Neves et al., 2012) and increase overall herd milk production (9,000 kg/year) compared to visual observation methods (7,000 kg/year; Pfeiffer et al., 2020). However, the evaluation of most automated sensors (Se: 95% Sp; 96.38%; accuracy: 95.20%; Dolecheck, 2015b) has been tested under research conditions (e.g. controlled environment, eliminating abnormal ovarian dynamics, unhealthy cows, and/or hormone-based synchronized cows) that may not be representative of commercial dairy production. Additionally, the absence of economic analysis to demonstrate any profit advantages of automated estrus detection systems has created uncertainties, which has affected whether dairy producers are comfortable implementing new technologies (Ayinde et al., 2014). As such, the implementation of automated devices in commercial dairy herds is lower (10% in North American herds; Denis-Robichaud et al., 2016, and 20% in European herds; Steeneveld and Hogeveen, 2014) compared to the percentage of dairy producers using visual observation of estrus and hormone-based synchronization protocols.

Some of the reasons for lower estrus detection within intensive dairy systems can be divided into various environmental (e.g. flooring, warm ambient temperatures, and housing type) categories. Felton et al. (2012) found pedometers are not able to adequately detect estrus in cows housed in tie-stalls continuously. Differences in flooring (e.g. concrete and dirt) has also been reported to influence estrus duration (dirt; 13.8 h, concrete; 9.8 h), total mounts (dirt; 7, concrete; 3.2 events/30min) and average number of events of standing to be mounted (dirt; 3.8, concrete;

2.7 events/30min; Britt et al., 1985). Additionally, warm temperatures (> 30°C) can affect follicular dynamics and steroid hormone concentrations and extend carry over effects (e.g. abnormal effects) up to 21 days after exposure to temperatures above 30°C (Roth et al., 2001). Other factors affecting estrus expression are ovarian cysts (2.7 to 30%; Borsberry and Dobson, 1989, Kesler and Garverick, 1982) after parturition when dairy cows experience metabolic challenges. Additionally, estrus detection rates can be influenced by herd management (e.g. a non-experienced herds person, increased labour expenses, etc.) and large herd size (Saint-Dizier and Chastant-Maillard, 2012). Lastly, ovulation intervals under healthy and normal length cycles can vary among individuals (e.g. multiparous 22.1 h, primiparous; 18.7 h; Stevenson et al., 2014). As such, different algorithmic predictions must account for intra- and inter-individual variation.

The use of estrus technologies in animal production has led to a variety of newer and more accessible tools such as infrared thermography (IRT) cameras. Thermal cameras sense the amount of thermal radiation emitted by any object above 0°K and convert it to a measurable temperature (°C; Meola, 2012). Skin temperature measured with IRT cameras has been useful for detecting physiological process-related amounts of vascular circulation and heat exchange from live organisms to the environment (Harper, 2000). For example, in animal science, IRT cameras have been helpful in diagnosing laminitis in Zebu cattle (dos Santos Sousa et al., 2020), febrile responses in piglets (Cook et al., 2015), and stress responses in poultry (Weimer et al., 2020). In dairy cows, skin temperature fluctuations predicted ovulation 2 d prior in cows housed in tie-stalls (Perez Marquez et al., 2019) and free-stall barns (Talukder et al., 2014). However, IRT's use as an estrus detection method has so far not been perceived as feasible under commercial barn circumstances. Some of the challenges associated with in-barn IRT are the need for a fixed location where all cyclic cows can be scanned consistently without labour input (Cook et al., 2016). Additionally, ambient temperature effects need to be adjusted in IRT skin temperatures (Loughmiller et al., 2001) and false-positive estrus alerts need to be screened by combining multiple parameters that can be adopted in the same data analysis such as behavioural parameters (Perez Marquez et al., 2021).

The primary objective of the present research was to develop an automated platform capable of capturing IRT frames (Estrus BenchMark™; EBM) as cows exit a voluntary milking robot. Specifically, to measure skin temperature at the vulva and tail movements following every milking event. The second objective was to compare estrus alert accuracy using EBM compared

to in-line milk P_4 and the increase in activity using accelerometer ear tag sensors. It is hypothesized that the EBM system will measure skin temperature and tail movements consistently in order to identify fluctuations associated with the estrus period in an automated manner. It was predicted that the accuracy of EBM would be equal to other automated estrus detection methods already commercialized and used by dairy producers.

6.3. Materials and Methods

The current study was conducted from February to October 2020 (Winter-Fall) at the Lakeland College Dairy Learning Centre (DLC), a 121-cow free-stall facility located in Vermilion, Alberta, Canada. The DLC barn is divided into a parallel parlour (70 cows) and voluntary milking system (VMS; 51 cows). In total, the current study attempted to gather thermal and behavioural biometrics in 72 Holstein lactating cows (21 primiparous and 51 multiparous) located in the DLC barn's VMS side. However, 26 cows were eventually moved to the parlour side due to apathetic behaviour (i.e. refusal to enter the milking robot) towards the VMS milking robot during the study period and were subsequently removed from the project as a result (Note: 46 cows were included in the final data analysis; 10 primiparous, 36 multiparous [2-6 lactations]). Cows averaged 29.34 ± 13.21 (mean ± SD) days in milk (DIM) and were producing 43.92 ± 9.85 kg per day with an average of 2.79 milking robot visits a day. Free access to water was provided and a total mixed ration based on NRC guidelines (National Research Council, 2001) for lactating dairy cows. The main ingredients of the TMR were corn silage, rolled barley-corn, grass hay, and mineral supplements.

6.3.1. Experimental Design

A split plot over time experimental design was used to compare changes in skin temperature and the occurrence of tail movements with in-line milk progesterone (P_4) and eating, ruminating, high, mid, and low activity durations using ear accelerometers for each experimental unit (n = 46) between 45 to 120 DIM (Figure 6.1).

6.3.2. In-line Milk Progesterone Analysis

Milk P_4 was analyzed using the Herd Navigator System (HN; Herd Navigator System™ DeLaval International, Tumba, Sweden & Lattec I/S, Hillerød, Denmark) to monitor ovarian

activity and estrus occurrences. Herd Navigator samples (~1L each) were commenced at day 21 after calving with repeated sampling determined automatically by the HN system (at set intervals; every 2 d) determined by fluctuations (increases or decreases) on P_4 concentration curve and the estrous cycle phase. For P_4 analysis, the HN system uses an immunoassay biosensor dry-stick described by Pemberton et al. (1998). Raw P_4 values are adjusted based on algorithms to compensate for ambient temperature and percentage of air humidity between milk samples (e.g. changes in ambient temperature and relative air humidity can affect dry-sticks and misrepresent P_4 values; Jørgensen et al., 2016). The results were sent to management software (DeLaval DelproTM, International, Tumba, Sweden) where adjusted P_4 concentrations and estrus alerts were displayed. In-line milk P_4 triggered an estrus alert if milk P_4 concentrations were below 5ng/mL with an accuracy of 99% of estrus confirmations with a Se of 93.3% and a Sp of 93.7% previously reported (Friggens et al., 2008).

6.3.3. Behaviour Accelerometer

Behaviour parameter durations were measured using a 3-axis accelerometer ear tag sensor (CowManager SensOor™; CSTags, Agis Automatisering, Harmelen, Netherlands) for the following behaviours: rumination (Rum), feeding (F), resting (Res), low activity (LA), mid activity (MA), and high activity (HA). CowManager SensOor tags were placed on each cow's left ear 25 to 120 DIM to measure changes in the duration of behaviours as estrus approached. Behaviours were measured as total duration (hours) of each behaviour duration over 24 h (TD/24 h) periods. Estrus alerts were triggered when increases in HA duration and decreases in Rum duration were observed on an individual basis, as previously validated by Bikker et al. (2014) and reviewed by Dolechek et al. (2015). CowManager sensor tags also measured the temperature of skin surface at the left ear of each cow (°C).

6.3.4. Estrus Detection Using Infrared Thermography and Behaviour Biometrics

Skin temperature and behaviour biometrics from the vulva area were measured using an infrared thermography (IRT) platform (Estrus BenchMark™, Animal InfraMetrics, Lacombe, AB, Canada). The Estrus BenchMark (EBM) camera was placed on top of a VMS robotic milker facing the robot's exit alleyway at a 45° angle 2 m from the expected target (i.e. the vulva area of an exiting cow). The EBM platform camera was a FLIR A35 thermal model (FLIR Vision Systems

Ltd. Burlington, ON, Canada) with a resolution of 320 × 256 pixels, -25°C to 135°C temperature range and accuracy of ± 5°C or 5% of the measured temperature. The A35 thermal camera had a Se of 0.05°C at 30°C and was connected to a Lenovo laptop (ThinkPad, Lenovo Group Limited, Haidian District, Beijing, China) via Cat 5 cable. Ambient temperature (°C) and percent relative air humidity (Rh %) were recorded every 30 min (average) for the duration of the study period using a temperature logger (iButtonLink, LLC, Whitewater, WI, United States) located inside and outside the VMS robot. Twenty frames (i.e. IRT pictures) per cow were captured as each cow exited the VMS robot in order to determine the categorical occurrence (e.g. nominal observations; Yes/No) of tail movement (Tail movement to the left; LTail, Tail movement to the right; RTail, and pooled tail movement; MTail Figure 6.2). The EBM platform signaled an estrus alert when skin temperature increased (+1°C) more than the average expected during a non-estrus stage and the vulva was exposed based on the assessment of tail movements from IRT images (note: every cow was followed from 45 DIM to calculate a non-estrus skin temperature). Raw thermal and behavioural data were adjusted to mitigate ambient temperature effects using the Cook et al. (2016) methodology and the elimination of thermal outliers due to other physiological processes (e.g. post-vaccination fever and mastitis fever).

6.3.5. Statistical Analysis

6.3.5.1. Skin Temperature and Behaviour Biometrics

Thermal data were analyzed using SAS software (SAS ver 9.4, Cary, NC, USA). Sample days were standardized (d -21, to d -1, and d 0) using the HN estrus alert as d 0 to compare the non-estrus period (baseline; d -21 to d -1) with the estrus period (d 0) identified via thermal and behavioural parameters. Proc Univariate was used to test normality assumptions using a Kolmogorov-Smirnov test ($P > 0.05$). The thermal data complied with normality assumptions and models were fitted using a Generalized Linear Mixed Model approach (Proc Glimmix). Fixed variables were estrous sample day (Sample day) relative to the estrus period noted as d 0 (d -21 to d 0), Lactation (Lact; Primiparous – Multiparous), Health status (Health: mastitis and vaccination events), Ambient temperature (Temp), and Relative air humidity (RH%) to identify non-estrus and estrus related fluctuations in skin temperature.

Tail movement data (LTail, RTail, and MTail) were analyzed to identify the occurrence (Yes/No) of vulva exposure (vulva exposure required tail movement to be exhibited) during the

baseline period (d -7, d -14, d 1, and d 4) compared with the estrus period (d 0). Proc logistic was used to identify the likelihood of vulva exposure at d 0 using different tail movements (LTail, RTail, and MTail) in the model. Additionally, other fixed variables (Health, Temp, RH% and Lact) were analyzed to identify additional factors affecting vulva exposure.

6.3.5.2. Accelerometer Analysis

Non-activity, Rum, EAT, MA, and HA total duration were analyzed to identify significant changes between non-estrus and estrus periods (defined as d 0) using HN as standard. Behaviour durations were pooled into h in a 24 h period (e.g. 5/24 h) results to match the Milk P_4 and EBM results. The model included estrous Sample day and Lact with a Poisson distribution specified. Note: IRT and behaviour duration data were tested using a Type 3 test with the inverse (ilink) function specified and the statement of ar(1) to account for a lack of independent and homogeneous data. Differences were considered significant if $P < 0.05$, a tendency if $0.05 \leq P < 0.10$ and not significant if $P \geq 0.10$. Milk P_4 concentrations were analyzed using Sample Day as a fixed variable.

6.3.5.3. Evaluation of Estrus BenchMark Estrus Alerts

Each system generated an independent estrus alert based on its own proprietary criteria (to define d 0 for each system), then the agreement between estrus alert (i.e. d 0) across methods was compared. The HN's estrus alert was defined as when the milk P_4 was lower than the average threshold (<5 ng/mL) confirmed by the DeLaval Delpro system. The drop in milk P_4 (indirect physiological association of estrus) was associated with the occurrence of estrus (Bikker et al., 2014, Dolechek et al., 2015). The estrus alerts created by HN were considered as the only confirmed estrus occurrence and further used as the confirmation of estrus day. To evaluate accuracy of EBM, the number of cows detected in estrus was divided by the number of cows confirmed in estrus (Sensitivity) by HN and CStags, HN (separately), and CStags (separately). The Sp (i.e. true negatives) was calculated as the number of cows detected to not be in estrus divided by the number of non-estrus cows confirmed by HN–CStags, HN (separately), and CStags (separately). Additional diagnostic tools such as positive predicted value (PPV), negative predicted value (NPV), Youden J index (YJ), diagnostic odds ratio (DOR), positive likelihood ratio (+LR), negative likelihood ratio (-LR) and efficiency (Efficiency) were calculated to corroborate the diagnostic tests.

Diagnostic evaluation formulas:

PPV = (True positives / (True positives + False positives))

NPV = (True negatives / (True negatives + False negatives))

YJ = (True positives / (True positives + False negatives) + True negatives/ (True negatives – False negatives)) - 1)

DOR = ((True positives / False positives) / (False negatives/True negatives))

+LR = (Sensitivity/1- Specificity)

-LR = (1- Sensitivity/ Specificity)

Efficiency = ((True positives + True negatives) / (True positives + True negatives + False Positives + False negatives))

6.4. Results

6.4.1. Estrus Occurrence and Milk Progesterone Concentrations

Ninety-eight estrus alerts were created by the HN system, and 97 estrus alerts by the CStags system for an average of 2.13 per cow. Six estrus alerts coincided the same day (6.10%), 24 within a 24 h window (23.40%), 48 within a 48 h window (43.20%), and 49 within a 76 h window (44.10%). The overall milk P_4 concentration on HN estrus alert day (d 0) was 3.51 ± 0.05 pg/mL (mean ± SE). Milk P_4 concentrations started to increase at d -7 (11.94 ± 0.79 pg/mL) to peaking during d -14 (20.22 ± 0.73 pg/mL), remained low at d 1 (3.18 ± 0.16 pg/mL) and d 4 (10.98 ± 0.66 pg/mL). Additionally, the HN system provided a prevalence of abnormal ovarian dynamics based on milk P_4 concentrations, which noted 34.78% luteal cysts, 36.95% prolonged anestrus, and 28.26% follicular cysts. Other diseases that could affect estrus were mastitis (8.69%), metritis (8.69%), lameness (4.34%), ketosis (2.17%), and leucosis (2.17%) which were recorded by farm staff and within the veterinary records.

6.4.2. Skin and Ear Temperature

The overall ambient temperature at Lakeland DLC from February to September was 18.18 ± 0.05°C and 69.89 ± 12.15% relative air temperature (mean ± SE). Inside the VMS robotic milking room, the ambient temperature was 19.96 ± 0.04°C, and relative air humidity was 70.07 ± 0.14%. The coldest ambient temperature (9.43°C) and lowest relative air humidity (26.96%) were recorded in February (winter season), and the warmest ambient temperature (31.18°C) and

highest relative air humidity (100%) in August (summer season). The fluctuation in ambient temperature at different seasons affected (P = 0.01) cow skin temperature inside the DLC barn (R^2 = 0.96; see Figure 6.4). However, other parameters such as Lact (P = 0.36) and Health (P = 0.73) did not have a significant effect. On the other hand, skin temperature relative to estrus day tended (P = 0.09) to increase at d 0 (33.31 ± 2.38°C) compared to d -14 (32.05 ± 3.77°C), d -7 (31.95 ± 3.37°C), d 1 (31.20 ± 3.49°C) and d 4 (31.67 3.48°C, see Figure 6.5).

In addition to skin temperature via IRT recording, ear temperature was measured by the CStags system, with no significant (P = 0.99) results as the estrus day approach. However, the ambient temperature had a significant effect (R^2 = 0.95) on ear temperature readings. The highest ear temperature was identified at d -14 (30.16 ± 0.34°C) compared to d 0 (29.81 ± 0.34°C), d -7 (29.99 ± 0.34°C), d 1 (29.68 ± 0.44°C) and d 4 (29.74 ± 0.37°C).

6.4.3. Behaviour Biometric Results

Total duration of behaviours obtained from the CSTags did not change significantly when pooled daily (P > 0.05) during the d 0 compared to non-estrus days. Regardless of the non-statistical significance, behavioural differences were observed as cows approached d 0, as Figure 6.3 shows. In addition, all behaviours were significantly affected by Lact group (P = 0.01). Behaviours that exhibited a decrease at d 0 were NA (5.84 TD/24 h) and Rum (9.45 TD/24 h) compared to d -14 (NA; 6.58 TD/24 h, and Rum; 9.67 TD/24 h). Behaviours that resulted in an increase in duration at d 0 were MA (1.73 TD/24 h) and HA (2.37 TD/24 h) compared to d -14 (MA; 1.91 TD/24 h, and HA; 2.86 TD/24 h). Contrary to that which was expected, Eat behaviour duration did not decrease during d 0 (4.11 TD/24 h) compared to d -14 (4.30 TD/24 h). However, when comparing Eat total duration between Lact groups, a non-significant decrease was observed at d 0 in Multiparous cows (d 0; 3.60, d -14; 3.68 TD/24 h) compared to Primiparous cows (d 0; 4.30, d -14; 4.20 TD/24 h).

The occurrence of vulva exposure (EBM) per Sample day resulted in significant changes (P = 0.01) between d 0 and base line days (see Figure 6.6). Vulva exposure (LTail, RTail, and MTail) was observed in 34 cows out of 42 (88.18; Chi-Sq) and the most accurate (proportion of correct predictions) tail movement was MT (0.94; Accuracy) compared to LT (0.80; Accuracy), and RT (0.90; Accuracy). The greatest prevalence percentage was found at d 0 for LTail (62%), MTail (68%), and RTail (56%) compared to d -7 (LTail; 18%, MTail; 9%, RTail; 9%) and d 4

(LTail; 9%, MTail; 9%, RTail; 12%). The effect of Temp, RH%, Lact, and Health did not affect vulva exposure ($P > 0.05$).

6.4.4. Estrus Detection Accuracy

Percentage of agreement between estrus alerts was 6.10% between HN and CStags on the same day (d 0), 23.40% with a ± 24 h window, 43.20% with a ± 48 h window, and 44.10% with a ± 72 h window. When comparing the EBM system to a combined estrus alert HN – CStags (same day) resulted in 33.33% estrus detection rate (Se; 33.33% and Sp of (85.46%). The Se of EBM increased with an HN – CStags estrus alert window of ± 48 h (34.37%) and ± 72 h (35.38%) with the exception of ± 24 h (33.33%). When the EBM estrus alerts were compared to HN alone, the Se increased to 41.82% (same day), 50.22% (± 24 h), 57.90% (± 48 h), 56.49% (± 72 h). Similar results were observed comparing EBM and CStags 38.20% (same day), 43.51% (± 24 h), 50.00% (± 48 h), 57.95% (± 72 h). Estrus BenchMark achieved the highest accuracy when comparing to HN – CStags at ± 48 h (Efficiency; 0.74, YJ; 0.19, and DOI; 3.09), HN ± 48 h (Efficiency; 0.77, YJ; 0.44, and DOI; 9.03), and CStags at ± 72 h (Efficiency; 0.76, YJ; 0.44, and DOI; 8.93. see Table 6.1)

6.5. Discussion

6.5.1. Milk Progesterone Profile and CowManager Sensor Tags Estrus Alerts

In-line milk P_4 estrus alerts did not coincide with CSTags for most estrus alert timing (Same day), nevertheless, within a 48 – 72 h window, estrus alerts coincided approximately 40% of the time. One explanation for these results may be attributed to the variation in ovulation interval observed between the increase in activity at the onset of estrus and the expected ovulation observed in other studies (24 h; Van Eerdenburg et al., 2002, Burnett et al., 2020). Additionally, the interval between the drop in plasma P_4 (< 5ng/mL) and ovulation occurrence can be as long as 48 h (Perez Marquez et al., 2019), which may explain some of the non-agreement between estrus alerts based on P_4 and increases in activity on the same day (HN and CSTags). Furthermore, the AI interval varies by estrus detection method used. For example, for HN AI usually occurs within 4 d after the milk P_4 decreases (Bruinjé et al., 2017) compared to accelerometers, in which AI usually occurs 28.7 h after activity increased (Valenza et al., 2012). Another potential explanation is the incidence of silent estrus (i.e. regression of the CL and ovulation without exhibiting sexual receptivity

behaviours). Ranasinghe et al. (2010) found the incidence of silent estrus to be 55.2% of cows tested using accelerometers after the voluntary waiting period (~50 days after calving). Given this, the poor estrus alert agreement between HN and CSTags systems could be attributed to physiological (e.g. silent estrus) and health factors mentioned above that are not representative of the accuracy of CSTags to detect estrus.

6.5.2. Temperature Changes at the Estrus Period

The ambient temperature and relative air humidity fluctuated between the winter, summer and fall seasons. The differences in ambient temperature affected IRT measures (EBM) and ear temperature (CSTags) by masking temperature changes due to physiological changes during the estrus period, specifically in hot temperatures. The effect of ambient temperatures was observed especially with regard to 'raw' IRT measures where the SEM was as high as ± 3.77°C. The explanation for this variation could be the absence of heat elimination related to metabolic processes in dairy cows (e.g. digestion, protein synthesis, blood circulation, and muscle movement; Bertocchi et al., 2014) due to summer ambient conditions (> 31°C and relative air humidity of 100%). However, skin temperature was still able to distinguish the estrus period from pre-estrus and post-estrus stages using ambient temperature adjustments as described by Cook et al. (2016) and Loughmiller et al. (2001) despite fluctuations in ambient temperature and relative humidity. Changes in skin temperature during the estrus period have been described previously in the literature (Talukder et al., 2014; Perez Marquez et al., 2019).

6.5.3. Behaviour Total Duration in the Estrus Period

Behavioural durations obtained from the CSTags were observed to fluctuate between non-estrus stages and the estrus period, particularly high activity and rumination 2 days prior to estrus, during estrus day and 2 days post-estrus. However, these results were not significant ($P > 0.05$) and the observed between pre-estrus, estrus, and post-estrus period could be attributed to the differences between lactation groups (i.e. primiparous – multiparous). Differences in rumination and high activity patterns between lactation groups can be attributed to differences in morphological composition (e.g. smaller and lighter body shape in primiparous compared to multiparous) and their respective rumen capacities (e.g. higher in multiparous compared to primiparous cows). Several studies report that differences in total rumination duration by lactation

parity are due to differences in dry matter intake (e.g. higher in multiparous than primiparous cows; Maekawa et al., 2002, Kowsar et al., 2008). The expected inter-individual differences within parity, the reduced behaviour expression in hot temperatures (Collier et al., 2006), and physiological-health conditions could explain the absence of any differences between non-estrus and the estrus period using 3-axis accelerometers (e.g. heat production in a larger cow with increased milk yield compared to a small cow with decreased milk yield; larger cows move less in hot temperatures to avoid heat production, which 3-axis accelerometers cannot differentiate).

6.5.4. Behaviour Occurrence during the Estrus Period Compared to Non-estrus Periods

Vulva exposure (i.e. tail movement) data were measured in a categorical (as discrete behaviour signals; yes/no) manner using thermal frames captured as each cow left the robotic milker. The automated identification of vulva exposure using thermal distribution changes across frames complemented this project's first objective. The prevalence of vulva exposure increased as tail movement increased during the estrus day compared to other estrous cycle stages. These findings are similar to previous study findings by the same co-authors that measured tail movements (Perez Marquez et al., 2019, Perez Marquez et al., 2020). One explanation for greater vulva exposure at the estrus period (d 0) may be related to other well-documented accessory sexual receptivity behaviours (vulva sniffing, urine sniffing, vaginal mucus discharge, and increased tail wagging; Price, 2008) found in cattle (*Bos Taurus and Bos Indicus*) that have a similar function. Specifically, moving the tail to the side allows vulvar olfactory compounds (e.g. alcohols, amines and aromatic alkanes) produced in the cervicovaginal mucus during estrus to be transmitted airborne to stimulate the bull's flehmen response (Klemm et al., 1987).

In the current study, vulva exposure was measured by evaluating tail movements to the left and right to identify side preference movements during the estrus period. Tail movements were observed to be higher when the tail moved to the right compared to the left, as Figure 6.6 shows. Nevertheless, the difference between tail movement direction was not statistically significant, and vulva exposure increased when the results were pooled (MT). Furthermore, vulva exposure was not affected by lactation group or ambient temperature. One possible explanation may be that primiparous and multiparous cows have similar tail morphologies and movement expressions (e.g. similar size between cows and non-required mobilization of energy).

6.5.5. Accuracy of the Infrared Thermography Platform

The study's second objective was to compare the accuracy of estrus detection alerts made by the EBM with estrus alerts from other automated estrus detection systems (e.g. CSTags accelerometers and HN in-line milk P_4 analysis). The EBM coincided with the CSTags ~50% but showed less agreement with HN (~30%) using a HN-CSTags combination. The use of infrared thermography to detect estrus and ovulation occurrence has been previously demonstrated in different dairy milking systems (e.g. tie-stalls; Perez Marquez et al., 2020, pasture-based; Talukder et al., 2014) and beef cattle systems (Vicentini et al., 2020). However, previous research predicted estrus in an experimental setting would fail to translate to commercial farm settings due to the extensive use of labour input to collect IRT pictures and conduct data analysis. However, the current study was able to provide proof of concept of the ability of EBM to continuously collect data for 8 months (February to September) and to detect estrus without labour input. Furthermore, all data processing, analysis, and storage were able to be conducted remotely (e.g. the Lakeland DLC was located in Vermilion, Alberta and all data processing was done remotely in Edmonton, Alberta via an ethernet connection). The current study also demonstrated that automated technologies are able to monitor an entire herd daily by strategically locating IRT cameras and using software that can simultaneously measure skin temperature and behaviour biometrics in a non-invasive manner. Furthermore, an increased demand for dairy products, labour shortage in developed countries, larger herds, access to water sources (Britt et al., 2017) and consumer demand for humane animal care practices require the dairy industry to optimize herd management (Boogaard et al., 2011). One potential solution is to promote sustainable dairy production by implementing non-invasive automated technologies such as the EBM.

The estrus alert agreement between EBM and HN's in-line milk progesterone analysis was around 50% (Se). A possible explanation may be attributed to the incidence of silent estrus (50%; Isobe et al., 2004), reduced estrous cycles (27.3%; Royal et al., 2000) and/or anovular cows after parturition (~25% at 60 to 70 d postpartum; Sartori et al., 2017) often experienced in dairy herds. In all abnormal ovarian cases, P_4 concentrations may decrease (< 5ng/mL) and flag as an estrus alert in HN systems. The presence of abnormal cycles and anestrus were confirmed by the HN system, however, in the present study, ovarian dynamics (corpus luteum and follicular diameters) were not measured via ultrasonography to confirm the oestrous cycle's ovulation or viability (e.g. quality of estrous cycle based on ovarian dynamics) of ovulation.

The EBM coincided with CSTags system estrus alerts at a higher Se (58%) when the estrus alert window was extended to ±72 h. Some explanations for this result include intra-individual behavioural variation (e.g. parity group; At-Taras and Spahr, 2001), potential environmental effects on estrus alerts (e.g. ambient temperature, relative air humidity, and seasonality: Hansen and Fuquay, 2016) or physiological abnormalities. Additionally, the expression of sexual receptivity behaviours (e.g. increase in activity, female-female mounting) is often observed at the onset of estrus (e.g. proestrus stage; Dolecheck et al., 2015b) compared to standing to be mounted (Roelofs et al., 2005a). In terms of estrus detection, the estrus alerts could be further adapted following the events leading to ovulation to identify the ovulation interval along with the onset of estrus. However, further research should account for the interval between an EBM estrus alert and AI service and AI service to ovulation (Note: the current study did not consider AI service parameters).

The accuracy levels of EBM were greater than the accelerometer system used in this study (~50%) and the overall estrus detection rates in Canada (< 40%; Leblanc, 2005) as evaluated by commercial dairy herds. However, the EBM's accuracy in detecting estrus also differed in the diagnostic parameters analyzed. The variation between Se (58%) and Sp (87%) can be attributed to the larger number of negative tests (i.e. non-estrus days) compared with the number of positive tests in the estrous cycle (e.g. larger number of non-estrus days compared to one estrus day). The greatest accuracy of EBM was observed during the ±48 h window compared to HN and ±72 h compared to CSTags. In both diagnostic tests, the total number of negative estrus tests were 29,033 (HN) and 29,059 (CSTags) compared to the total number of frames of positive estrus tests, which were 465 (HN) and 439 (CSTags). These results gave us an indication that the discrimination of negative tests is as important as detecting positive tests. False-positive alerts can result in mistimed AI service, which is more costly (i.e. cost of failing to inseminate plus the cost of AI service; $12.53 US net loss per cow) compared to failure to identify estrus (i.e. missed AI service; 4.44 USD net loss per cow, De Vries and Collin, 2003). However, it is essential to identify the most cost-efficient balance between Se (70%; Inchaisri et al., 2010), Sp (75%; Perez Marquez et al., 2021), and how much the Se can be decreased without losing profit. To increase the Se of the EBM, the software should analyze different machine learning algorithms (e.g. Decision Tree, Random Forest, Naive Bayes, etc.), other ambient temperature adjustments, and compare-contrast estrus alerts with pregnancy-calving outputs.

6.6. Conclusions

In conclusion, the current study developed an automated platform capable of capturing IRT frames and measuring skin temperature at the vulva as well as tail movements after every milking event without additional labour input. Therefore, we accept the null hypothesis that the IRT platform was able to measure skin temperature and tail movements consistently and to identify fluctuations associated with the estrus period in an automated manner. Furthermore, the IRT platform was able to distinguish between left and right tail movements and measure skin temperature for each cow exiting the robotic milking system. Additionally, the EBM platform's accuracy in detecting estrus was competitive compared to the accelerometer system used simultaneously during the study period. Our results suggest that the EBM platform can be an alternative to detect estrus for dairy producers and aide dairy reproductive management.

6.7. Acknowledgments

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Table 6.1. Evaluation of estrus alerts flagged by Estrus BenchMark (EBM) and confirmed by Herd Navigator (HN), CowManager SensOor Tags (CSTags) and a combination of HN-CSTags at different estrus confirmation time-windows (Same day, ±24 h, ±48 h and ±72 h).

	Same day			±24 h[1]			±48 h[2]			±72 h[3]		
	HN[4]	CSTags[5]	HN-CStag[6]	HN	CSTags	HN-CStags	HN	CSTags	HN-CStags	HN	CSTags	HN-CStags
No. frames	29836	29836	3276	29836	29836	3276	29836	29836	3276	29836	29836	3276
EBM+[7]	243	183	21	341	228	21	465	286	22	439	408	23
No. Estrus	581	479	63	679	524	63	803	572	64	777	704	65
No. Non-estrus	29255	29357	3213	29157	29312	3213	29033	29264	3212	29059	29132	3211
Sensitivity	0.42	0.38	0.33	0.50	0.44	0.33	0.58	0.50	0.34	0.56	0.58	0.35
Specificity	0.86	0.86	0.87	0.86	0.86	0.85	0.87	0.86	0.85	0.87	0.87	0.86
Efficiency	0.76	0.75	0.76	0.76	0.76	0.74	0.77	0.76	0.74	0.77	0.77	0.74
PPV[8]	0.06	0.04	0.05	0.08	0.05	0.04	0.11	0.07	0.05	0.10	0.09	0.05
NPV[9]	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.98	0.99	0.99	0.98
YJ[10]	0.28	0.24	0.20	0.37	0.30	0.19	0.45	0.36	0.20	0.43	0.45	0.21
DOR[11]	4.46	3.79	3.26	6.42	4.77	2.94	9.04	6.27	3.09	8.47	8.94	3.23
LR+[12]	3.02	2.72	2.51	3.70	3.13	2.29	4.38	3.64	2.37	4.25	4.34	2.44
LR-[13]	0.68	0.72	0.77	0.58	0.66	0.78	0.49	0.58	0.77	0.50	0.49	0.76

Abbreviations: [1]±24 h = Estrus detection alerts within a 24 h window from the day confirmed; [2]±48 h = Estrus detection alerts within a 48 h window from the day confirmed; [3]±72 h = Estrus detection alerts within a 72 h window from the day confirmed; HN[4] = Herd Navigator; CSTags[5] = CowManager sensor tags; HN-CSTags[6] = Herd Navigator and CowManager sensor tags estrus alerts; [7]EBM+ = Estrus positive alerts detected by Estrus Bench Mark system confirmed by HN, CSTags, and HN-CSTags PPV[8]; Positive predicted value; NPV[9] = Negative predicted value; YJ[10] = Youden J index; DOR[11] = Diagnostic odds ratio; LR+[12] = Positive likelihood ratio; LR-[13] = Negative likelihood ratio.

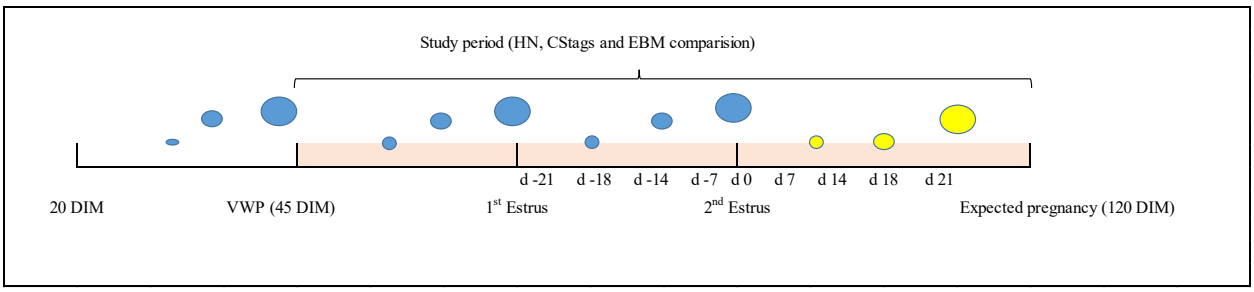

Figure 6.1. Study timeline, estrous cycles expected (blue circles), and expected increase in progesterone (P_4) after estrus alerts (yellow circles). Cows were monitored by Herd Navigator (HN; in-line milk progesterone analysis), CowManager sensor tags (CStags; accelerometer) and Estrus BenchMark (EBM; skin temperature combined with behaviour biometrics). All the cows were included in the study after the voluntary waiting period (VWP) at 45 days in milk (DIM) until 120 DIM to ensure at least 2 estrous cycles. Milk P_4, estrus duration, skin temperature and vulva exposure were compared as the estrus period-approached (d -14, d -7, d 0, d 1, d 4).

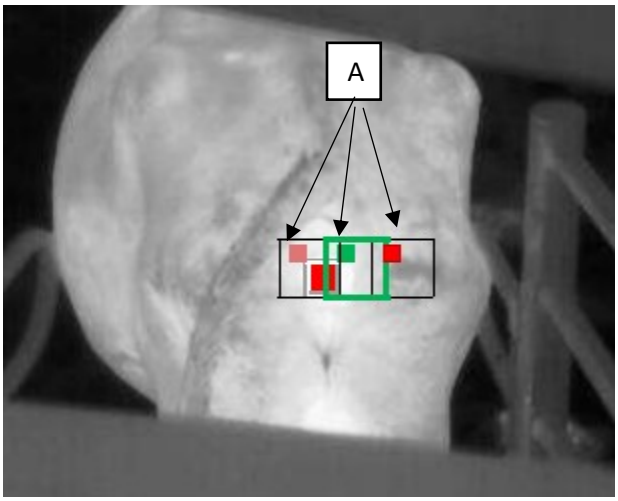

Figure 6.2. Sample thermal frame capture as a cow exited the VMS robot. Maximum skin temperature (A) was measured (red squares) and the tail movement occurrence (tail movements to the left, right and non-directional movement) was capture using the changes in the skin temperature distribution in left, right and mid squares.

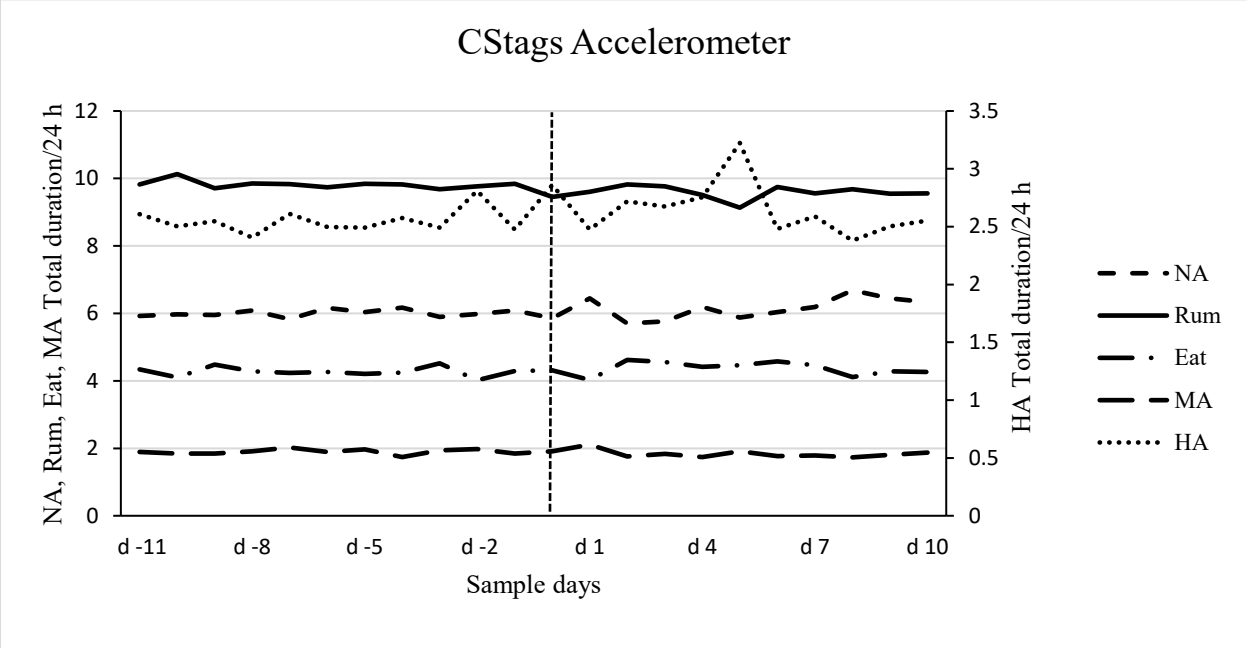

Figure 6.3. The overall total duration (hours; TD/24 h) of non-active (NA), rumination (Rum), eating (Eat), mid active (MA), and high active (HA) as the estrus period (d 0; vertical dotted line) approaches. Fluctuations in HA and Rum can be observed at d -2 until d 2 with an increase of HA and a decreased Rum at d 0 and d 5. However, no significant changes in Sample day ($P > 0.05$) were observed when behaviours are pooled in least-squared means for all the cows in the study period (n = 46).

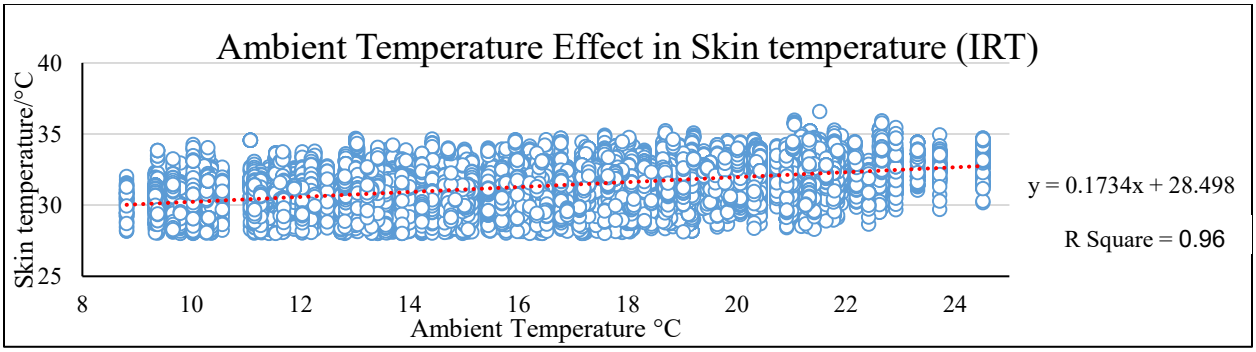

Figure 6.4. The effect of ambient temperature (Temp) in skin temperature recorded (vulva maximum temperature; blue dots) with infrared cameras (IRT) resulted significant ($P = 0.01$) and highly predictable using Temp ($r = 0.96$; $Y = 0.1734x + 28.49$). The linear regression analysis was performed to account the effect of Temp in skin temperatures following Cook et al. (2016) methodology.

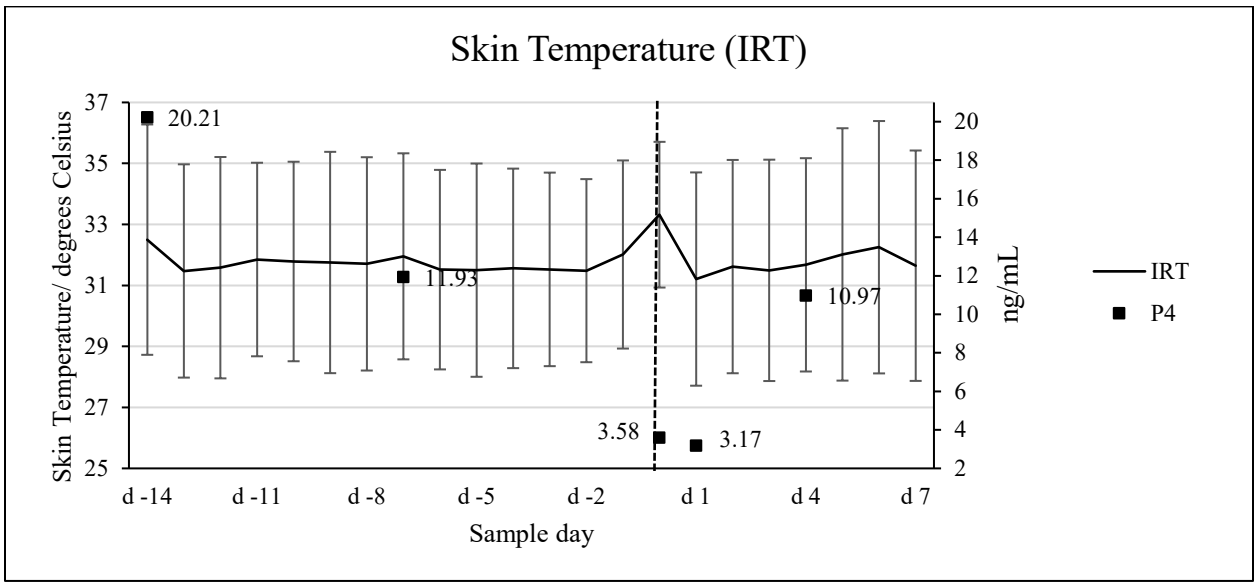

Figure 6.5. Increases (P = 0.07) in skin temperature (Leas-Square Means; LSMeans) were associated with the lowest concentrations of milk progesterone (P_4) concentrations (LSMeans) at d 0 (vertical dotted line). No significant fluctuations were found in other Sample days (e.g. d -14, d -7, d 1, and d 4).

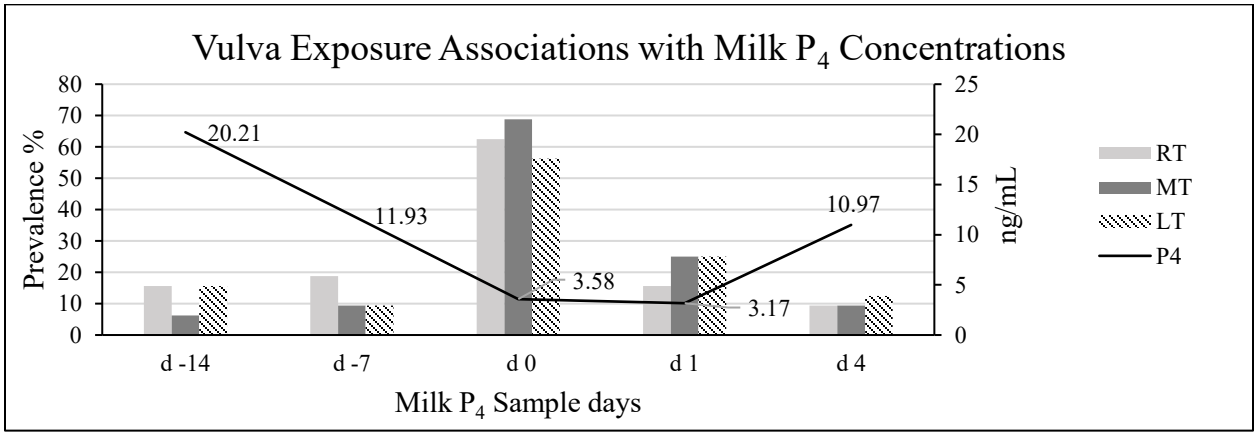

Figure 6.6. The prevalence (percentage of cow exposing vulva) of tail movements, tail movement to the left (LTail), tail movement to the right (RTail), and non-directional tail movement (MTail) were compared with the milk progesterone (P_4) Sample days. The greatest prevalence of LTail, RTail, and MTail coincided with low milk P_4 during the estrus period (d 0). No significant differences were found (P< 0.05) between tail movements parameters, however, the differences between Sample days were significant (LTail; P = 0.01, RTail; P = 0.01, MTail; P = 0.01).

Chapter 7. Business Analysis of Infrared Thermography, Visual Observation, and Ovsynch as Breeding Strategies in Alberta Dairies

7.1 Abstract

The dairy industry is searching for new technologies to address low (<50%) estrus detection. However, the lack of information on economic benefits regarding new technology implementation has led some dairy producers to continue using conventional estrus detection methods (e.g. visual observation of standing to be mounted). The objective of this study was to compare the costs of infrared thermography (IRT), visual observation (VO) and ovulation synchronization (Ovsynch: OVS) as breeding strategies at different accuracy levels (Sensitivity-Specificity) and pregnancy rates (PR). The costs associated with Breeding, Feeding, Operation Costs, Return to Equity and Culling Risk per estrus detection rate (ER; 30-100%, conception rate for OVS; 30-100%), PR (PR per Parity group; 1-2 (50%), 3-4 (43%), and >4 (41%)), and ER accuracy were used to determine the financial benefit of each breeding method. Breeding Cost results (CAD/cow) showed a greater cost associated with using OVS (138.99) compared to VO (115.78), and IRT (127.69). Pregnancy Costs were affected by Breeding Cost; however, ER had a significant effect on PR expense for each method: IRT (ER 30%; 210.38-100%; 132.19), VO (ER 30%; 205.93-100%; 129.39), and OVS (ER 30%; 247.21-100%; 155.33). The minimum Se level with a positive Financial Effect for IRT and VO was 60% with Sp of 100% and for OVS was Se 65% and Sp 100%. However, if the Se was 100%, a positive Financial Effect was observed even with a Sp of 85% for IRT and 75% for VO. Culling Risk was reduced if ER increased differently depending on parity group. The implementation of IRT as an estrus detection method yields a competitive breeding cost compared to VO and OVS. Further, breeding methods must accomplish at least ~60% accuracy to have a positive net return.

7.2. Introduction

Although reproduction is critical to dairy production, there is little analysis regarding the relative costs and benefits of different management practices. One of the goals of a dairy reproductive protocol is to maintain a calving interval between 12 to 13 months in order to maintain sustainable milk production (Call et al., 1978, Evans et al., 2006). However, a 12 to 13 months calving interval requires reproductive programs to breed cows 60 days after calving (USDA, 2009). The extension of the optimum breeding period results in economic losses per extra open

day (non-pregnant days) of ~1.00 USD in early parities and greater than 1.00 USD for older cows (De Vries and Conlin, 2003). Nevertheless, economic losses associated with failing to inseminate artificially (AI) at 60 days after calving are often unknown to the dairy producer because such losses do not represent instant cash gains or losses (e.g. gains or losses not visible until the year later; Call et al., 1978).

The majority of dairy herds in North America utilize AI (89.3%) or AI in combination with natural service (51.5%) as standard breeding methods (USDA, 2018). However, in Canada, 97% of dairy producers use AI service, which requires the detection of estrus or the application of hormone-based protocols (Denis-Robichaud et al., 2016). The most commonly (51%; Denis-Robichaud et al., 2016) used estrus detection method for the first AI service in dairy cows is the visual observation (VO) of mounting and standing to be mounted events for at least 30 min by an experienced herdsperson (Britt et al., 1986, Roelofs et al., 2004). However, the rate of estrus detection via visual observation is below 50% (Senger et al., 1994,), leading to unclear economic benefits using VO. As such, the dairy industry has utilized hormone-based synchronization protocols, such as Ovsynch (OVS), that can induce ovulation and set a time for AI without the need for estrus detection. Ovsynch protocols have shorter AI service intervals if cows do not get pregnant (VO: 42 d; OVS: < 32 d) and involve reduced labour input (e.g. no visual observation time required; Pursley et al., 1997). However, OVS protocols require multiple injections (i.e. GnRH and $PGF_2\alpha$; Pursley et al., 1995), professional training, extra supplies (i.e. drugs and syringes) and expertise from veterinarians. Furthermore, similar pregnancy rates using OVS protocols (37%) are observed compared to VO methods (39%; Pursley et al., 1997), as such, no clear increases in fertility rates are observed.

More recently, automated technologies have grown in popularity for estrus diagnosis. For example, commercially available automated technologies can measure changes in activity (i.e. walking, laying down, rumination, eating, mounting) and cow temperature relative to the estrus period (Løvendahl and Changuda, 2010). Some advantages to the use of automated estrus detection technologies are they do not require extensive labour input, they provide real-time analysis, and can measure a wide variety of parameters (e.g. behavioural and physiological), thereby improving the accuracy of estrus detection (e.g. accelerometers; > 80%) as compared to visual observation (At-Taras and Spahr, 2001). Although, the accuracy of automated estrus detection varies in the literature from < 50% to > 90% depending on the algorithms, housing type, ambient temperature, parity group, and methodology used (as reviewed by Bruyère et al., 2012). Wide variation in

accuracy across automated estrus detection, in addition to a lack of information regarding financial benefits has kept the overall adoption of automated estrus detection technologies low within the dairy industry (below 25% on dairy farms) compared to traditional (51% visual observation) methods in North America (Denis-Robichaud et al., 2016).

Access to technology such as infrared thermography (IRT) allows the agriculture industry to measure thermal radiation from animals and to better understand biological processes. Thermal radiation is the exchange of heat between two bodies (i.e. live organisms and inanimate objects) or the environment without physical contact (Edwards et al., 1979, Modest, 1993). Thermal emissions change if the skin and epidermis undergo dilatation or vasoconstriction of blood vessels, if there is skin damage, or if there are changes in thermal neural sensory (e.g. skin response to cold or hot temperatures; Davy, 1977). In cattle, IRT has been used to diagnose inflammation due to bacterial infections (Spire et al., 1999), bovine respiratory disease in calves (Schaefer et al., 2007), defective spermatozoa in bulls (Kastelic et al., 1996), estrus detection (Hurnik et al., 1985), and the periods before and after ovulation (Perez Marquez et al., 2019). However, IRT must be feasible under commercial dairy systems for adoption as an estrus detection method. Therefore, the overall intent of this paper is to evaluate the economic feasibility of IRT as an automated estrus detection method in a commercial dairy herds.

The economic effects of estrus detection are not easy to identify because calving intervals are extended from days to months until the dairy producer perceives the economic outcomes (positive or negative; Britt, 1985). Previous studies found increases in yearly net return to equity by increasing estrus detection accuracy from 35% (-95.09 USD per cow/year) to 65% (6.95 USD per cow/year; De Vries and Conlin, 2003). The primary variables influencing estrus detection economic effects are milk production, calf losses, replacement costs, and pregnancy value (Britt, 1985). In particular, estrus detection accuracy has a direct effect on pregnancy cost because of the breeding cost per eligible cow, breeding cost of mistimed insemination and the probability of pregnancy per AI service (De Vries, 2006). Additionally, low estrus detection accuracy indirectly affects culling decisions at the farm level and longer calving intervals, high AI service costs, and reduced yearly net returns by increasing replacement costs (Evans et al., 2006). Despite the economic evaluations regarding estrus detection effects, regional variables such as herd size, labour costs, industry targets (e.g. organic production, supply management, milk yield etc.), and simulated economic data from other regions (e.g. East-West coast Canada, U.S. data, etc.) can result in broad variation between studies (Pfeiffer et al., 2020). The present study focuses on the

economics of dairy production in the province of Alberta, Canada, to eliminate the regional variations associated with dairy production management. The first objective of this study was to compare the costs of breeding via IRT (estrus detection) with the costs of traditional breeding (detection of estrus via visual observation; VO and hormone intervention via Ovsynch protocol: OVS) at different estrus detection rates (ER for IRT and VO) and conception rates (only for OVS) and pregnancy rates (PR). The second objective was to identify the financial effects (gain or loss) of the same techniques at different accuracy levels (Se and Sp level) for IRT, VO, and OVS as breeding strategies using economic defaults from dairy production in Alberta, Canada.

7.2. Material and Methods

7.2.1. Data

The current study used the annual average costs of dairy production activities, inventories, capital purchases, milk sales and feed purchases from the *Economics of Milk Production in Alberta* Edition 2009 - 2017 years from Economics and Competitiveness Branch, Economics Section, Alberta Agriculture and Forestry. Data were generated from surveys of ninety-seven dairy producers across Alberta for 2009 – 2017 using a systematic random sampling for every month for the years 2009 to 2017.

In this study, a dairy enterprise included just the activities related to dairy production (i.e. lactating cows, dry cows and heifers replacements). The dairy enterprise was divided into Gross Income (Milk sales, Pool Adjustments, Miscellaneous Receipts, Net Cattle Sales, Net Inventory Changes, and Gross Income), Total Other Costs (Bedding and supplies, Veterinary service and medicines, Milk hauling, Producer's Fees, Utilities, Fuels, Machinery repairs, and Miscellaneous), Labour (Hired labour, Family labour, and Total labour costs), Reproductive Management Costs (breeding costs, and pregnancy cost), and Feeding Costs. Data were left as fixed in the annual figures for the following variables:

A) Gross Income (Milk sales, Pool Adjustments, Miscellaneous receipts, Net cattle sales, Net inventory changes),
B) Total Other Costs (Bedding and supplies, Veterinary service and medicines, Milk hauling, Producer's Fees, Utilities, Fuels, Machinery repairs, and Miscellaneous),
C) Labour Costs (Hired labour and Family labour; see Table 7.1). The herd size used was the average herd size found in Alberta dairies in 2017 (166 head) ranging from

59 to 737 dairy cows in the herds surveyed. Note: grain and hay production (exogenous production) section of the farms were not included in the dairy enterprise.

For this study, data from 2009 to 2017 were obtained in order to track costs and revenues over time to model a representative dairy farm enterprise in Alberta, Canada, using Alberta Agriculture and Forestry data (The Economics of Milk Production in Alberta). Dependent variables from 2009 to 2017, such as Breeding Costs (Figure 7.1A), Feed Costs, Total Other Costs, Labour costs, Total Capital Costs (Figure 7.1B), and Gross Income, Total Operating Costs, Total Production Costs, and Return to Equity (Figure 7.1C), are presented in Figure 7.1(A-C) to demonstrate the average changes over time. To identify the economic differences between estrus detection methods and OVS, a detailed analysis was made for Breeding Cost, Pregnancy Cost, Feed Cost, Return to Equity, Culling Risk, and Financial Effect of Estrus Detection at different Accuracy Levels.

7.3.2. Breeding Costs

The default costs associated with breeding services were calculated by identifying the cost of AI supplies (e.g. AI sleeves, AI labour, and semen), veterinarian costs associated with breeding (fresh check and pregnancy diagnosis), miscellaneous costs (propylene gloves, paper towels, lubricant), and costs associated with the identification of estrus and hormone-based synchronization methods. The historical Breeding Costs through the years 2009-2017 varied primarily due to the cost of semen (i.e. per AI straw) with a yearly increase of 2.61%. Semen prices were obtained from local semen suppliers (personal communication; SEMEX Canada), and the median price was used for this study (30 CAD/AI straw; see Table 7.2).

The Breeding Cost calculated for the use of infrared thermography (IRT; identification of estrus using radiated temperature and behaviour biometrics; Perez Marquez et al., 2020). The IRT costs were calculated by summing the platform's components (IRT camera A300; 17,600 CAD, RFID reader; 1,750 CAD, and Hardware/Software; 3,000 CAD) with an amortization period of 120 months and interest payments of 5%. The capital cost's annual fee was then divided by the herd size (166 head) to identify IRT use per cow/breeding-service. Visual observation (**VO**; observation of mounting behaviour and standing to be mounted; Sawyer et al., 1986) was calculated as the total time observing cows (30 min daily) by a herdsperson multiplied by the average hourly salary in Alberta dairies (21.58 CAD/hour). The ovulation synchronization method used for this study was the Ovsynch method (OVS; two sets of Gonadotropin Release Hormone

(GnRH) and one Prostaglandin ($PGF_2\alpha$) injections 2mL each; Lucy et al., 1986). Ovsynch costs were calculated by summing the cost of each injection (medicines (each GnRH; 6.50 CAD and $PGF_2\alpha$; 5.50 CAD), syringes, and needles; 5.00 CAD, Felton C. personal communication) plus herdsperson labour input (5 min/cow). Default Breeding Costs (Semen Costs, AI supplies, AI labour, Vet fresh check and Vet Pregnancy check) were added to the Breeding Costs per breeding strategy in order to obtain the per cow costs (CAD/Cow) and per herd cost (CAD/herd) in a yearly cycle (Table 7.3).

7.3.3. Pregnancy Costs

The costs associated with pregnancy (Pregnancy Cost) were calculated using ERs at 30% to 100% per Breeding Costs per predicted number of cows getting pregnant (PR; pregnancy rate) in a specific Parity (number of calving events) group (Parity 1-2, Parity 3-4, and Parity >4). Note: In the case of OVS, no estrus detection is required; the Conception Rate (the number of pregnant cows divided by the total AI service) was used. Parity groups and PR averages (lactation 1-2; 50%, lactation 3-4; 43% and lactation >4; 31%) in Alberta herds were taken from Ambrose and Colazo (2007). In addition, the distribution of Parity groups in a herd was estimated using the average of the distribution of Parity groups (mean ± SEM) from two Alberta herds (lactation 1-2; 57.37% ± 6, lactation 3-4; 38.52% ± 3 and lactation >4; 4.09% ± 1; University of Alberta-Dairy Research and Technology Centre and Lakeland College-Dairy Learning Centre). To calculate the Pregnancy Costs, the following formulas were used:

ER = *(#ED/ Exp-ED) 100,*

where ER = Estrus Detection Rate, *#ED* = number of confirmed estrus, *Exp-ED* = expected estrus detection.

PR = *(#P/#Open) 100*

where PR = Pregnancy Rate, *#P* = number of confirmed pregnancies, *#Open* = number of cows available to get inseminated.

$PC1^{st}$ = *(Hz * ER/100) Breeding Cost,*

where $PC1^{st}$ = Pregnancy Cost 1^{st} service, *Hz* = herd size, *ER* = Estrus Detection Rate

#$PC1^{st}$ = *(Hz * ER/ 100) * PR/ 100,*

where #PC1st = number of cows pregnant after 1st service, *Hz* = Herd size, *ER* = Estrus Detection Rate , *PR* = Pregnancy Rate

PC2nd = *(Hz* - #PC1st*) * ER/ 100) * Breeding Cost,*
where PC2nd = Pregnancy Cost after 2nd service, *Hz* = Herd size, #PC1st = number of cows pregnant after 1st service, *ER* = Estrus Detection Rate

#PC2nd = *(([Hz* - #PC1st*] * ER)/100) * PR/100,*
where #PC2nd = Number of cows pregnant after 2nd service, *ER* = Estrus Detection Rate, *PR* = Pregnancy Rate

PC3th = *((Hz* – [#PC1st + #PC2nd]*) ER/100) * Breeding Cost,*
where PC3th = Pregnancy Cost after 3th service, #PC1st = Number of cows pregnant after the 1st service, #PC2nd = Number of cows pregnant after the 2nd service, *ER* = Estrus Detection Rate, *Breeding Cost* = Breeding Cost per estrus detection method

#PC3th = *(((Hz* –[#PC1st + #PC2nd]*) * ER)/100) * PR/ 100,*
where #PC3th = Number of cows pregnant after 3rd service, Hz = Herd size, #PC1st = number of cows pregnant after the 1st service, #PC2nd = Number of cows pregnant after the 2nd service, *ER* = Estrus Detection Rate, *PR* = Pregnancy Rate

PC4th = *((Hz* – [#PC1st + #PC2nd + #PC3th] * *ER/100) * Breeding Cost,*
where PC4th = Pregnancy Cost after 4th service, *Hz* = Herd size, #PC1st = Number of cows pregnant after the 1st service, #PC2nd = Number of cows pregnant after the 2nd service, #PC3th = Number of cows pregnant after the 3th service, *ER* = Estrus Detection Rate, *Breeding Cost* = Breeding Cost per estrus detection method

#PC4th = *((((Hz* – *([*#PC1st - #PC2nd - #PC3th*]) * ER)/100 * PR/100,*
where #PC4th = Number of cows pregnant after 4th service, *Hz* = Herd size, #PC1st = number of cows pregnant after the 1st service, #PC2nd = Number of cows pregnant after the 2nd service, #PC3th = Number of cows pregnant after the 3th service, *ER* = Estrus Detection Rate, *PR* = Pregnancy Rate

PC_{ER} = *Sum* PC1st *to* PC4th

where PC_{ER} = Pregnancy Cost per cow per Estrus detection rate, Sum PC1st to PC4th = sum of pregnancy cost 1st, 2nd, 3th and 4th

$TPC4^{th}$ = #PC1st + #PC2nd + #PC3th + #PC4th

where $TPC4^{th}$ = Total number of cows pregnant after 4th services, #PC1st = Number of cows pregnant after the 1st service, #PC2nd = Number of cows pregnant after the 2nd service, #PC3th = Number of cows pregnant after the 3th service, #PC4th = Number of cows pregnant after the 3th service (see example in Table 7.4)

PC_{PDER} = *(((((Hz * %P1,2/100) * PC_{ER} + (((Hz * %P3,4/100) * PC_{IR}) + (((Hz * %P>4)/100 * PC_{IR}))*

where P_{CPDER} = Pregnancy Cost per lactation distribution per *ER*, *Hz* = Herd size, %P1,2 = Percentage of cows in Parity 1-2, PC_{ER} = Pregnancy Cost per cow per *ER*, %P3,4 = Percentage of cows in Parity 3-4, %P>4 = Percentage of cows in Parity >4.

7.3.3. Feeding Costs Per Estrus Detection Method Depending on The Number of AI Services

Feed rations in the modelled farm were divided into three groups; High protein content and energy density (High Ration), Medium protein content and energy density (Mid Ration), and Low protein content and energy density (Low Ration). The allocation of cows in the diet groups depended on the days in milk (DIM), as such, cows were estimated to have 135 days in High Ration diet, 170 days in Mid Ration diet, and 60 days in Low Ration diet as a dry period (C. Felton, personal communication). To estimate the variation in Feeding Cost per ER, the calving interval (i.e. period between calving events within a cow) was calculated based upon the optimum calving interval (12 months; Pelissier, 1972). Every extra AI service after the first AI service represents an extended calving interval of 30 days, which is the waiting time between AI service and pregnancy diagnosis (additional days on calving intervals increase Feeding Costs per day). The Feed Costs per day per lactation phase (e.g. High-Mid-Low) were calculated using prices in Alberta Canada for diet ingredients: Barley silage, Alfalfa hay, Rolled barley, Crude protein %, Fat %, and Net energy (Mcal/kg) following the NRC 2001 guidelines (C. Felton, personal communication). In

addition, historical Feeding Costs (2009 – 2017) for the three diets were estimated using the commercial feed index for Alberta (Statistics Canada, 2019), in which the year 0 was identified as 2012.

For the current study, the different ERs were associated with extended feeding days in the Low Ration diet (dry off period) due to the extended calving interval associated with feeding a Low Ration if cows do not get pregnant every AI service. Of particular note, the IRT and VO methods increase non-lactation (dry off period) feeding days by 21 days (duration of the estrous cycle), while the OVS method only increases the dry off feeding days by 10 days (duration of the OVS protocol; Lucy et al., 1986). The reduced days resulting from the OVS protocol (10 days duration) was due to the re-started protocol after a negative pregnancy diagnosis (30-60 after AI service). Feeding Costs were calculated only for four AI services for the three methods (see Table 7.5).

$TFC_{cow1st} = (HC_{day} * 135d) + (MC_{day} * 170d) + (LC_{day} * 60d)$

Where TFC_{cow1st} = Total Feeding Cost per cow if pregnant during 1st service, HC_{day} = High Ration Cost per day, 135d = expected days in High ration, MC_{day} = Mid Ration Cost per day, 170d = expected days in Mid Ration, LC_{day} = Low Ration Cost per day, 60d = dry-off days.

$TFC_{cow2nd} = (HC_{day} * 135d) + (MC_{day} * 170d) + (LC_{day} * 60d + 51d_{VO\text{-}IRT}$ or $40d_{OVS})$

Where TFC_{cow2nd} = Total Feeding Cost per cow if pregnant during 2nd service, HC_{day} = High Ration Cost per day, 135d = expected days in High Ration, MC_{day} = Mid Ration Cost per day, 170d = expected days in Mid Ration, LC_{day} = Low Ration cost per day, 60d = dry-off days, $51d_{VO\text{-}IRT}$ = 51 days service interval if VO or IRT is used, $40d_{OVS}$ = 40 days service interval if OVS is used.

$TFC_{cow3th} = (HC_{day} * 135d) + (MC_{day} * 170d) + (LC_{day} * 60d + 102d_{VO\text{-}IRT}$ or $80d_{OVS})$

Where TFC_{cow3nd} = Total Feeding Cost per cow if pregnant during 3th service, HC_{day} = High Ration Cost per day, 135d = expected days in High Ration, MC_{day} = Mid Ration Cost per day, 170d = expected days in Mid Ration, LC_{day} = Low Ration Cost per day, 60d = dry-off days, $102d_{VO\text{-}IRT}$ = 102 days service interval if IRT or VO is used, $80d_{OVS}$ = 80 days service interval if OVS is used.

$TFC_{cow4th} = (HC_{day} * 135d) + (MC_{day} * 170d) + (LC_{day} * 60d + 153d_{VO\text{-}IRT}$ *or* $120d_{OVS})$

Where TFC_{cow3nd} = Total Feeding Cost per cow if pregnant during 4th service, HC_{day} = High Ration Cost per day, 135d = expected days in High Ration, MC_{day} = Mid Ration Cost per day, 170d = expected days in Mid Ration, LC_{day} = Low Ration Cost per day, 60d = dry-off days, $153d_{VO\text{-}IRT}$ = 153 days service interval if IRT or VO is used, $120d_{OVS}$ = 120 days service interval if OVS is used.

7.3.4. Return to Equity

To calculate the Return to Equity (i.e. net dairy income; Economics of Milk Production in Alberta), the Total Operation Costs were calculated as the expenses associated with maintenance of the dairy enterprise, such as Feeding Costs, Total Other Costs, Labour Costs, and Pregnancy Costs per lactation distribution and ER.

Total Operation Costs = TFC + *Total Other Costs* + *Labour Costs* + PC_{PDER}

where Total Operation Costs = Sum of Dairy enterprise activities, *TFC* = Total Feeding Costs, *Total Other Costs* = Sum of Bedding and supplies, Veterinary service and medicines, Milk hauling, Producer's Fees, Utilities, Fuels, Machinery repairs, and Miscellaneous, *Labour Costs* = Hired labour, and Family Labour, PC_{PDER} = Pregnancy Cost per Parity group and per ER

The Total Production Costs represent the expenses incurred by a dairy enterprise to produce milk, calculated as the sum of the Total Operation Costs and the Total Capital Costs (Rent, Taxes & insurance, Depreciation, Interest of capital debt). Total Capital Costs were taken from the Economics of Milk Production in Alberta for 2009-20017 (Net dairy income; Economics of Milk Production in Alberta), where depreciation estimates were based on the original cost of farm facilities and equipment for the years used from Alberta dairy herds surveyed.

Total Production Costs = Total Operation Cost + Total Capital Cost

As a result, the Return to Equity was calculated by subtracting the Total Production Cost from the Gross Income. Further, the Return to Equity per cow was estimated for each cow in lactation by dividing the Return to Equity by the Herd size.

Return to Equity = Gross Income – Total Production Costs

Return to Equity$_{cow}$*= Return to Equity / Hz*

where *Return to Equity*$_{cow}$ = Return to Equity per individual cow*, Return to Equity* = Return to Equity of the herd*, Hz = Herd size.*

7.3.5. Culling Risk

Culling Risk in this experiment represented the percentage of cows that failed to get pregnant after 4 consecutive AI services which reduce their permanence in the herd (cows likely to be culled). Note: Culling decisions are not entirely due to low estrus detection rates and reproductive performance. Additional parameters such as lameness, aggressive behaviour, disease (i.e. mastitis), low milk yield, and old age are also factors (Canadian Dairy Information Centre, 2019). The Culling Risk was calculated by dividing the number of cows that failed to get pregnant after 4 breed attempts divided by the Herd number.

Culling Risk = #PC$_{fail}$ */ Hz * 100*

where *Culling Risk* = Percentage of cows that fail to get pregnant after 4 services, *#PC*$_{fail}$ = Number of cows that failed to get pregnant after the 4th service, *Hz* = Herd size

7.3.6. Financial Effects of AI Indication at Different Sensitivity and Specificity Levels

To evaluate economic gain depending on estrus detection accuracy (Financial Effect), estrus detection was examined at different Se (probability of testing positive when estrus occurred) and Sp (probability of testing negative in the absence of estrus) levels (100 – 30%). The pregnancy gain (Pregnancy Gain) was identified as the economic gain of getting a cow pregnant at 1st service (desirable to maintain optimum calving interval) compared to getting a cow pregnant at 4th service (the last service is given to maintain cows lactating) per lactation group (1-2, 3-4, and >4). The economic loss due to failing to detect estrus (Pregnancy Loss) was calculated as reducing in equity by extending the calving interval for each Se Level. Additionally, the cost of AI service, missed AI, and missed-timed AI was calculated as the total breeding cost (Total Breeding Cost) per Se-Sp combination (Se 30 – 100% and Sp 30 – 100%).

Pregnancy Gain$_{1\text{-}2,\ 3\text{-}4,\ \&\ >4}$ = *Return to Equity*$_{1st}$ *- Return to Equity*$_{4th}$

Where *Pregnancy Gain*$_{1\text{-}2,\ 3\text{-}4,\ \&\ >4}$ = Pregnancy gain per Parity group if a cow gets pregnant at the 1st service, *Return to Equity*$_{1st}$ = Return to Equity if a cow gets pregnant at the 1st service, *Return to Equity*$_{4th}$ = Return to Equity if a cow gets pregnant until the 4th service

Pregnancy Loss$_{1\text{-}2,\ 3\text{-}4,\ \&\ >4}$ = *#FN* * *Return to Equity*$_{1st}$ *- Return to Equity4th*
Where *Pregnancy Loss*$_{1\text{-}2,\ 3\text{-}4,\ \&\ >4}$ = Pregnancy Loss per Parity group if a cow did not gets pregnant at the 1st service, *#FN* = number of False Negatives estrus alerts, *Return to Equity*$_{1st}$ = *Return to Equity if a cow gets pregnant at the 1st service, Return to Equity*$_{4th}$ = Return to Equity if a cow gets pregnant until the 4th service

Total Breeding Cost = *#TP* * *Breeding Cost*$_{VO,\ OVS,\ IRT}$ + *#FP* * *Breeding Cost*
where *Total Breeding Cost* = Breeding Cost that accounts for the Se and Sp level, *#TP* = Number of True Positive estrus alerts, *Breeding Cost*$_{VO,\ OVS,\ IRT}$ = Breeding Cost per service if IRT, VO, or OVS are used, *#FP* = Number of False Positive Estrus alerts (IRT and VO) or Number Missed AI Service (False Positive for OVS), *Breeding Cost* = Breeding Cost per estrus detection method

Financial Effect = Pregnancy Gain - Pregnancy Loss - Total Breeding Cost

7.4. Results

The Breeding Costs from 2009 to 2017 were pooled and calculated per cow/year (CAD Mean ± SD). The Breeding Costs differed along with estrus detection methodologies (IRT, VO, and OVS), however, the greatest difference was between VO (104.40 ± 7.13) and OVS (125.32 ± 8.56) and however, VO also differed with IRT (115.14 ± 7.86). Although, the estrus detection methodology with the shortest service interval was the OVS (40 d) compared to IRT and VO (51 d).

Pregnancy Cost directly affected Breeding Cost per AI indication method as expected (Table 7.6). Nonetheless, greater variation in Pregnancy Costs were observed per ERs (30% - 100%) and the Parity group (1-2, 3 -4, and > 4) compared to Breeding Costs per AI indication method. Pregnancy Costs resulted in a high price, as ERs were low and vice versa in all Parity groups, as shown in Table 7.6. In addition, the Pregnancy Cost were highest in the Parity > 4 with an ER 30% (IRT; 322.74 ± 22.04, VO; 292.63 ± 19.98, and OVS; 351.29 ± 23.99) because of the

lowest PR (31%) reported in older cows (Parity >4) compared to younger cows (Parity <4) in Alberta dairies. The most cost-efficient Pregnancy Cost was found in cows with a Parity 1-2 with an ER 100% or 100% Concepcion Rate for OVS (IRT; 102.70 ± 7.01, VO; 100.53 ± 6.86, and OVS; 120.68 ± 8.24).

Breeding Costs and Pregnancy Costs added to the Total Operating Costs and further added to the Total Production Costs (Table 7.7a). As such, the lower ER (30%), the higher number of breeding services (4th services), and higher Pregnancy Costs resulted in the lowest average Return to Equity (2009-2017) per cow (IRT; 1505.04 ± 481.93, VO; 1376.22 ± 381.64, and OVS; 1697.11 ± 629.07 CAD cow/year). However, when the ER was perfect (100%), and cows got pregnant at the 1st service, the average Return to Equity (2009-2017) was the highest (IRT; $2321.25 ± 600.04, VO; 2185.85 ± 344.96, and OVS; 2310.39 ± 600.64 CAD cow/year). Notice that even when OVS had the less cost-effective Breeding Cost and higher Pregnancy Costs compared to VO, the Return to Equity of OVS was higher due to reduced feeding days in an OVS shorter service interval.

The Culling Risk was reduced as ER increased for each of the three methods. The Culling Risk was negative in Parity group 1-2 starting at ER 60% (-13.79%), Parity group 3-4 at ER 70% (-14.12%) and Parity group > 4 ER 90% (-6.65%). The overall Culling Risk was reduced at ER 70% (-10.24%) with the higher Culling Risk at ER 30% (62.96%) Figure 7.1 shows. The Culling Risk was estimated equally for the three methods since no economic effect was associated with the Culling Risk per AI indicator method.

The accuracy of AI indicator methods influenced the economics of variables such as Breeding Costs as tables 7.4, 7.5, and 7.6 shows. Accuracy level increase as Se (True positive estrus) and Sp (True negative estrus) get closer to 100%. The Financial Effect was found to change depending on the Se detected and the Sp (e.g. the number of False Positives increase as Sp is reduced and vice versa). The highest number of False Positive (low Sp level) estrus alerts possible in dairy cows per estrous cycle (21 days long) was stated as 20 (i.e. only 1 True positive) and the minimum number of False Positives were 0. The positive Financial Effect was observed with 75% Sp and 100% Se for IRT (Parity 1-2; 54.89, Parity 3-4; 621.79, & Parity > 4; 73.32 CAD cow/year) and VO (Parity 1-2; 57.78, Parity 3-4; 61.01, & Parity > 4; 96.36 CAD cow/year). However, the positive Financial Effect for OVS was observed at 85% Sp with 100% Se (Parity 1-2; 80.02, Parity 3-4; 88.13, & Parity > 4; 101.68 CAD cow/year) and 95% Se (Parity 1-2; 17.08, Parity 3-4; 24.28, & Parity > 4; 36.33 CAD cow/year). The minimum AI indication accuracy with a positive Financial Effect for IRT was 60% Sp (Parity 1-2; 19.24, Parity 3-4; 20.01, & Parity > 4; 21.29

CAD cow/year) similar to the VO (Parity 1-2; 18.95, Parity 3-4; 19.64, & Parity > 4; 22.46 CAD cow/year) with 100% Se. On the other hand, the minimum Se level required to have a positive Financial Effect for OVS was 65% Se (Parity 1-2; 56.39, Parity 3-4; 58.19, & Parity > 4; 61.20 CAD cow/year) with 100% Sp. The highest Financial Effect was achieved by the IRT (Parity 1-2; 646.30, Parity 3-4; 653.19, & Parity > 4; 664.73 CAD cow/year), VO (Parity 1-2; 633.68, Parity 3-4; 639.91, & Parity > 4; 665.26 CAD cow/year), and OVS (Parity 1-2; 469.99, Parity 3-4; 505.09, & Parity > 4; 518.65 CAD cow/year) at 100% Sp and 100% Sp.

The Parity group differed in Financial Effect depending on Se and Sp level per estrus detection method (Table 7.4 to 7.6). The highest Financial Effect per Parity group was for Parity >4 at Se-Sp of 100%. However, Parity group did not influence the minimum positive Financial Effect per Se (100%) and Sp level (75%; IRT, 60%; VO, and 65%; OVS).

7.5. Discussion

The first objective of this study was to compare the costs associated with three different breeding strategies, IRT, visual observation and Ovsynch protocols at different estrus detection rates (conception rate in the case for Ovsynch) and their impact on net Return to Equity. The changes observed in the Return to Equity were not merely due to the Breeding Cost but influenced by the estrus detection rate. These results are consistent with other studies, which have also found estrus detection rates influence yearly net returns (De Vries and Conlin, 2003; Rutten et al., 2014; Pfeiffer et al., 2020).

From Breeding Cost results, the most economically efficient method to detect estrus independent of accuracy level was visual observation followed by IRT then Ovsynch. Visual observation represents the most economical method to indicate when to inseminate (e.g. estrus detection) and is often performed by the dairy operation owner or by the herds person. The reason why VO is chosen by producers is because it requires little in the way of capital costs upfront and the labour used in estrus observation is already employed on the farm (i.e. no additional personnel required). However, the increasing size of dairy farms in Western Canada (242%, Murray et al., 2013) has become a challenge for those farms which rely on VO for AI indication since larger herds require increased labour input. As a result, visual observation is expected to become more economically inefficient as herd sizes continue to grow. Estrus detection using IRT resulted in breeding costs that were closer to visual observation than Ovsynch, which indicates a potential use of IRT technology in growing and large commercial dairy operations in the near future. The

economic efficiency of IRT would increase as herd size increases in Western Canadian dairy farms, the IRT technology continues to become cheaper, and implementation of IRT in Organic milking systems where Ovsynch is not permitted. The tradeoffs that generate IRT breeding cost results are the fact that reducing insemination costs per cow offset the up-front capital costs. The authors further note that the IRT estrus detection method was not commercially available during the current study and was still used only under research conditions. As such, the production costs for the IRT method used in the current analysis may have been overestimated since the research version of the IRT platform would be substantially more expensive compared with a commercially available version. Additionally, as an automated technology, the cost of maintenance and software support should be included in future economic models and estimations due to the potential increase in the capital and services costs.

The Ovsynch protocol resulted in the least economically efficient (138.99 CAD per service/cow) breeding method. However, Ovsynch was compared in this study as a reproductive method to induce the cow's ovulation to conceive but it can be used to treat anestrus and silent estrus (e.g. cows failing to exhibit standing to be mounted behaviour). For example, the incidence of anestrus cows at 60 DIM has been reported as ~20% (Moreira et al., 2001, Gümen et al., 2003, López et al., 2005) and 7 to 19% follicular cysts (Garverick, 1997) requiring hormone-based protocols in conjunction with AI service. Therefore, it can be expected that Ovsynch protocols, regardless of its high costs, will continue to be used by a segment of dairy producers with anestrus and silent estrus cows.

Pregnancy Cost per AI indication rate and service time can be used to identify reproductive efficiency (e.g. optimum economic calving interval) and economic effects of estrus detection. In this study, Pregnancy Cost was calculated by multiplying the Breeding Cost per the predicted number of cows getting pregnant at a given AI indication rate and pregnancy rate in a specific parity group. The overall pregnancy cost range for the different estrus detection methods was in agreement with previous studies (253 - 274 USD; Stevenson, 2001, 278 USD; 2003, De Vries, 2006). The Lactation group and estrus detection rate were the main variables influencing the pregnancy cost. However, the economic effect of pregnancy is not visible until the dry-off period. The most considerable economic loss of pregnancy was the increased number of non-milking feeding days in the dry off period (> 2 months). The standard lactation length duration (305 d) maintains the calving interval and provides the cow with two months to recover to the following lactation (Syrstad, 1993). Some alternatives to reduce pregnancy costs are extending the lactation

beyond 305 d and eliminating the dry-off period (van Amburgh et al., 1997, Knight, 2005). However, some studies report decreased milk yield during the following lactation (~20%) and a higher risk of udder infections (i.e. mastitis) if the lactation period is extended (Hortet et al., 1999, Hagnestam-Nielsen et al., 2009). To reduce pregnancy cost, one route is to improve estrus detection and increase the overall pregnancy rates (in Canada 13%; LeBlanc, 2005 and Alberta 41.3%) by lactation number (1 to 2; 50%, 3 to 4; 43%, and >4; 31%, Ambrose and Colazo, 2007). Further research should account for the percentage of pregnancy loss, conception rates, and other reproductive techniques such as the economic effects of embryo transfer and in-vitro fertilization in Alberta herds.

Another negative consequence of low estrus detection rates is the increase in culls due to cows failing to get pregnant during lactation. The major culling decision in dairy cattle is due to reduced fertility (i.e. failing to produce a calf), poor health (i.e. mastitis, lameness etc.), and injuries (Roxström and Strandberg, 2002, Canadian Dairy Information Centre, 2019). However, culling rates for fertility problems includes physiological abnormalities (e.g. anovulation, anestrus, and cysts), low estrus detection, pregnancy loss (i.e. abortions), and stillbirth (i.e. dystocia). The current study defined the term 'culling risk' as the risk of cows leaving the farm due to low estrus detection accuracy since other reproductive issues can also have an influence on culling decisions. Low estrus detection resulted in higher pregnancy costs regardless of the parity group, nevertheless, the higher the parity (> 2), the higher the culling risk. A potential explanation for increased culling in older parity cows is lower pregnancy rates (Lucy 2001). Culling risk was mitigated (0%; less chance to leave the herd) by parity group 1 - 2 if estrus detection was around 50% compared with parity group > 4 (Estrus detection 80% needed to avoid culling cows due to low estrus). However, late parities (> 2) are susceptible to lameness and high somatic cell counts, thereby increasing the culling risk and reducing longevity. Unfortunately, many dairy producers fail to recognize that improved longevity is associated with an increased profit due to the increased number of 'milking' days per cost of rearing the heifer (2100 to 2400 CAD; Murray, 2013).

The second objective of this study was to identify the financial effect (gain or loss) at different estrus detection accuracy (Se and Sp level) of IRT, visual observation, and Ovsynch and as reproductive strategies. The estrus detection accuracy of visual observation and Ovsynch (conception rate) are well established in the literature, as such comparisons between traditional estrus detection methods and IRT were possible. Overall, the break-even (net return equal to 0) was identified to occur at 60% estrus detection rate with no false positive estrus alerts (Sp; 100%

- Se; 60%) or 100% estrus detection rate with up to 5 false positive alerts (Sp; 75% - Se; 100%) for IRT and visual observation. Although, the Ovsynch was at a minimum of 65% conception rate with no false positives (miss-timed inseminations; Sp; 100% - Se; 65%) or 100% conception rate with up to 3 miss-timed inseminations (Sp; 85% - Se; 100%).

Visual observation as an estrus detection strategy had been reported from 35% (Se; Peter and Bosu, 1986) to 91% (Se; Kamphuis et al., 2012) of accuracy depending on the methodology applied (e.g. chalk, vasectomized bull, standing to be mounted by a herd mate, etc.) and the time dedicated to visually observe estrus (e.g. 30 min per day, 1 h per day, multiple observations during the day). However, in Canada, the overall estrus detection rate is 33% (LeBlanc, 2005), and in Alberta is 42% (Ambrose and Colazo, 2007), which based on the economic effect with perfect Se and Sp (100%), visual observation had a negative financial effect (-211.57 CAD/cow). In Canada, visual observation remains the most used AI indication method (56%; Denis-Robichaud et al., 2016). One of the reasons for the popularity of using visual observations is that producers only consider that visual observation does not require an initial investment in equipment. Nevertheless, most producers do not understand the importance of the Se and Sp relationship of estrus detection and how this influences visual observation costs and contributes to overall breeding costs.

The use of hormone-based treatments to induce estrus and ovulation were developed in the 1970's (Lauderdale et al., 1974, Hafs and Manns, 1975). The application of injection (GnRH – $PGF_2\alpha$) protocols (Ovsynch is the most commonly used) has partially addressed lower AI indication rates in Canada since artificial insemination timing can be controlled. One of the main reasons hormone-based protocols have been used so frequently in Canada (61%, Denis-Robichaud et al., 2016) is the large number of tie-stall barns in which the visual observation of mounting behaviour is not possible or requires extra labour input. However, the accuracy of Ovsynch has been reported as only 65 to 72% conception rate, which is the minimum Se level to have a positive financial effect ($61.20 CAD/cow, with Se; 65% - Sp; 100%). The primary advantage of Ovsynch protocols is that no estrus indication is necessary, and ovulation can be adjusted to dairy producer practices (Lucy et al., 1986). However, lower pregnancy rates have been found when using protocols that include PGF2α due to ovulation variation in dairy cows (i.e. ovulation of premature follicles; Momont and Seguin, 1983).

Infrared thermography technology is one of many efforts to address low estrus detection in the dairy industry in a non-invasive way. The use of IRT as an estrus alert is based on measuring increases in thermal radiation at the vulva prior to ovulation and reported in several studies (e.g.

Hoffmann et al., 2013, Talukder et al., 2014 and Perez Marquez et al., 2019). Based on changes in thermal radiation, threshold values are set to predict when ovulation will occur 24 to 48 h in advance (Perez Marquez et al., 2019). Combinations of thermal radiation with behaviour biometrics have been demonstrated to further increase the statistical accuracy (Perez Marquez et al., 2020) of IRT as an estrus detection alternative. The present study was interested in comparing the statistical accuracy with the financial effect and discussing the potential use of IRT commercially. In Chapter 4 of this thesis, IRT had higher efficiency levels (> 70%) when combining behaviour and radiated temperature biometrics. However, the Se were around 30% and Sp above 90%, and in the current study, these levels of Se and Sp were found to have a negative financial effect (-2776.77 CAD/cow) due to a lack of cows available to be inseminated (30%). Other combinations of IRT parameters at the vulva were found to increase the Se and Sp level to 65 to 70%. However, to have a positive financial effect the IRT Se level should be 60% with a Sp of 100% to eliminate the false positives estrus alerts. If the Se is 100% the Sp level can be 75% (6 false positives) to have a positive financial effect. The use of IRT technology is still under development and additional prototypes of the real-time, automated technology platform are expected to increase the accuracy of estrus detection by adapting machine-learning methods that can self-improve over time and consider individual cow differences. Despite not reaching a positive financial effect based on estrus detection accuracy, the IRT method was economically similar to visual observation while closer in accuracy to the Ovsynch method. The negative financial effect should be reduced regardless of the estrus detection or synchronization method to maintain sustainable dairy production in Western Canada.

7.6. Conclusions

The objective of this study was to compare the costs of IRT, with breeding strategies such as visual observation and Ovsynch at different estrus detection rates and pregnancy rates. Breeding costs varied depending upon the estrus detection method used, however, estrus detection rate, pregnancy rate, and parity had a more significant economic impact on the return to equity. The IRT breeding costs were close to visual observation and more cost-efficient than Ovsynch, however, visual observation remained as the most cost-efficient estrus detection method for Alberta dairies. Ovsynch was the most expensive in terms of supplies needed despite the short service interval after pregnancy diagnosis compared to visual observations and IRT estrus detection.

Despite the breeding cost per estrus detection strategy, the accuracy is just as important to assess the feasibility of each method's application. In order to have positive financial outcomes, a minimum of 60% of estrus detection rate is required with no false positives for IRT and visual observation. However, the Ovsynch method requires 65% Se to offset the high breeding cost associated with Ovsynch. The Sp level was found to have an economic impact since lower Sp levels resulted in higher incidences of false positives, resulting in miss-timed inseminations. The current estrus detection accuracy of visual observations does not comply with the minimum accuracy of positive financial effects. Ovsynch accuracy could comply with the minimum required (65% sensitivity); however, the variation in ovulation timing could decrease the Sp level and have adverse economic effects. The IRT satisfied the minimum Se level of accuracy (60%), but the Sp was lower than the minimum required (100%) to have positive financial inputs. However, this study proved IRT to be economically competitive with VO estrus detection methods in Alberta and Canada.

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Table 7.1. Data of dairy average enterprise activities (CAD cow/year) kept as default for the years 2009 to 2017 in Alberta, Canada.

	Avg[1]	SD[2]
Income		
Milk sales	7268.86	635.20
Pool Adjustments/Levies (=/-)	24.17	29.15
Miscellaneous Receipts	44.48	6.86
Net Cattle Sales (+/-)	331.47	151.17
Net Inventory Changes	103.33	55.23
Gross Income	7543.18	448.06
Expenses		
Bedding & supplies	240.46	26.458
Vet. & medicine	162.45	15.370
Milk Hauling	268.16	35.770
Producer's Fees	177.01	18.749
Utilities	147.89	8.902
Fuel, Oil, lube	99.76	36.060
Bldg. & Mach. Repairs	213.04	18.151
Miscellaneous	277.53	24.322
Total Other Cost	1676.29	137.081
Labour		
Hired Labour	306.82	21.35
Family Labour	768.34	56.27
Total labour costs	1075.16	51.94

Abbreviations: [1]Avg = Average taken from 2009 to 2017 values; [2]SD = Standard deviation from 2009 to 2017 values.
Note: Data were taking from 39 (n = 39) dairy farms across Alberta.

Table 7.2. Semen Costs over a nine-year period (CAD/semen-straw) in Alberta Canada

	Max[1]	Med[2]	Min[3]	SD[4]
Semen Costs 2009	32.40	24.30	16.20	6.61
Semen Costs 2010	33.26	24.95	16.63	6.79
Semen Costs 2011	34.15	25.61	17.08	6.97
Semen Costs 2012	35.06	26.30	17.53	7.16
Semen Costs 2013	36.00	27.00	18.00	7.35
Semen Costs 2014	36.96	27.72	18.48	7.54
Semen Costs 2015	37.95	28.46	18.97	7.75
Semen Costs 2016	38.96	29.22	19.48	7.95
Semen Costs 2017	40.00	30.00	20.00	8.16

Abbreviations: [1]Max = Maximum price (CAD/semen-straw); [2]Med = Medium price (CAD/semen-straw); [3]Min = Minimum price (CAD/semen-straw); [4]SD = Standard deviation price.

Table 7.3. Example of Breeding Costs for the year 2017 (CAD) per breeding method (IRT, VO, and OVS).

	Breeding Method		
	IRT[1]	VO[2]	OVS[3]
Costs per cow	127.69	115.78	138.99
Costs per herd (166 cows)	21196.54	19219.47	23072.06

Abbreviations: [1]IRT = Infrared thermography platform costs plus breeding supplies; [2]VO = Visual observation method costs plus breeding supplies; [3]OVS = Ovsynch protocol costs plus breeding supplies.

Table 7.4. Example of Pregnancy Costs at different ER (%) using the Breeding Costs provided in the Economics of Milk Production (Alberta Agriculture and Forestry 2017; 92.06 CAD)

ER[1]	PC1[st2]	#PC1[st3]	PC2[nd4]	#PC2[nd5]	PC3[th6]	#PC3[th7]	PC4[th8]	#PC4[th9]	PCER[10]	TPC4[th11]
30%	4584.59	20.58	4016.14	18.03	3518.18	20.27	2958.48	22.78	184.64	81.66
40%	6112.78	27.44	5102.22	22.91	4258.72	26.69	3275.77	31.11	173.37	108.15
50%	7640.98	34.30	6061.97	27.21	4809.27	32.84	3297.69	39.63	162.78	133.98
60%	9169.18	41.16	6895.40	30.96	5185.48	38.63	3051.53	48.21	152.87	158.97
70%	10697.37	48.03	7602.52	34.13	5403.03	44.01	2567.22	56.74	143.63	182.90
80%	12225.57	54.89	8183.31	36.74	5477.58	48.89	1877.23	65.05	135.06	205.56
90%	13753.76	61.75	8637.78	38.78	5424.78	53.20	1016.65	72.99	127.17	226.72
100%	15281.96	68.61	8965.93	40.25	5260.31	56.89	23.16	80.40	119.97	246.15

Abbreviations: [1]ER = Estrus detection rate (IRT-VO), conception rate for OVS; [2]PC1[st] = Pregnancy Cost at 1st service in a herd size of 166 and a given ER; [3]#PC1[st] = number of cows pregnant at 1[st] service with a PR of 41.33% (Ambroze and Colazo, 2007); [4]PC2[nd] = Pregnancy Cost at 2nd service in a herd size of 166 and a given ER; [5]#PC2[nd] = number of cows pregnant at 2nd service with a PR of 41.33% (Ambroze and Colazo, 2007); [6]PC3[th] = Pregnancy Cost at 3th service in a herd size of 166 and a given ER; [7]#PC3[th] = number of cows pregnant at 3th service with a PR of 41.33% (Ambroze and Colazo, 2007); [8]PC4th = Pregnancy Cost at 4th service in a herd size of 166 and a given ER; [9]#PC4[th] = number of cows pregnant at 4th service with a PR of 41.33% (Ambroze and Colazo, 2007); [10]PC_{ER} = Pregnancy Cost per cow per ER; [11]TPC4[th] = Total number of cows pregnant after 4th services at a given ER.

Table 7.5. Feeding Costs (CAD/cow) depending on pregnancy achieved in the 1st to 4th service in a 21-day estrous cycle breeding strategy (eg. IRT and VO)

1st[1]	Days feeding	Cost/day	Total cost
High Ration[2]	135	9.06	1223.1
Mid Ration[3]	170	6.74	1145.8
Low Ration[4]	60	5.00	300
Total	365		2668.9
2nd[5]			
High Ration	135	9.06	1223.1
Mid Ration	170	6.74	1145.8
Low Ration	111	5.00	555
Total	416		2923.9
3rd[6]			
High Ration	135	9.06	1223.1
Mid Ration	170	6.74	1145.8
Low Ration	162	5.00	810
Total	467		3178.9
4th[7]			
High Ration	135	9.06	1223.1
Mid Ration	170	6.74	1145.8
Low Ration	213	5.00	1055
Total	518		3423.9

Abbreviations: [1]1st = Feeding schedule if pregnancy achieved during the first service; [2]High Ration = Feeding ration containing high protein and carbohydrates; [3]Midium Ration = Feeding ration containing medium protein and carbohydrates; [4]Low Ration = Feed ration containing low protein and carbohydrates; [5]2nd = Feeding schedule if pregnancy achieved during the second service; [6]3rd = Feeding schedule if pregnancy achieved during the third service; [7]4th = Feeding schedule if pregnancy achieved during the fourth service.

Table 7.6. Pregnancy Costs per ER and Breeding Costs of IRT, VO, and OVS for each Parity group (1-2, 3-4, and >4) and Parity distribution average in Alberta dairies.

	IRT[1]		VO[2]		OVS[3]	
Parity 1-2	AVG[4]	SD[5]	AVG	SD	AVG	SD
PC[6] with ER[7] 30 %	183.46	12.53	166.35	11.36	199.70	13.63
PC with ER 40 %	170.00	11.61	154.14	10.52	185.04	12.63
PC with ER 50 %	157.55	10.76	142.86	9.75	171.49	11.71
PC with ER 60 %	146.13	9.98	132.50	9.05	159.06	10.86
PC with ER 70 %	135.72	9.27	123.06	8.40	147.73	10.09
PC with ER 80 %	126.34	8.63	114.56	7.82	137.52	9.39
PC with ER 90 %	118.04	8.06	107.03	7.31	128.48	8.77
PC with ER 100 %	110.87	7.57	100.53	6.86	120.68	8.24
Parity 3-4						
PC with ER 30 %	220.27	15.04	199.72	13.64	239.76	16.37
PC with ER 40 %	206.29	14.08	187.05	12.77	224.54	15.33
PC with ER 50 %	193.20	13.19	175.18	11.96	210.30	14.36
PC with ER 60 %	181.00	12.36	164.11	11.21	197.01	13.45
PC with ER 70 %	169.66	11.58	153.84	10.50	184.67	12.61
PC with ER 80 %	159.20	10.87	144.35	9.86	173.29	11.83
PC with ER 90 %	149.63	10.22	135.67	9.26	162.87	11.12
PC with ER 100 %	140.96	9.62	127.82	8.73	153.44	10.48
Parity > 4						
PC with ER 30 %	322.74	22.04	292.63	19.98	351.29	23.99
PC with ER 40 %	307.87	21.02	279.15	19.06	335.11	22.88
PC with ER 50 %	293.66	20.05	266.27	18.18	319.65	21.82
PC with ER 60 %	280.10	19.12	253.97	17.34	304.88	20.82
PC with ER 70 %	267.18	18.24	242.26	16.54	290.82	19.86
PC with ER 80 %	254.89	17.40	231.12	15.78	277.44	18.94
PC with ER 90 %	243.23	16.61	220.54	15.06	264.75	18.08
PC with ER 100 %	232.20	15.85	210.54	14.38	252.75	17.26
Parity distribution[8]						
PC with ER 30 %	204.78	13.98	185.68	12.68	222.90	15.22
PC with ER 40 %	191.04	13.04	173.23	11.83	207.95	14.20
PC with ER 50 %	178.26	12.17	161.64	11.04	194.04	13.25
PC with ER 60 %	166.43	11.36	150.90	10.30	181.15	12.37
PC with ER 70 %	155.53	10.62	141.02	9.63	169.29	11.56
PC with ER 80 %	145.59	9.94	132.01	9.01	158.47	10.82
PC with ER 90 %	136.62	9.33	123.88	8.46	148.71	10.15
PC with ER 100 %	128.67	8.79	116.67	7.97	140.06	9.56

Abbreviations: [1]IRT = AI indication using Infrared thermography; [2]VO = AI indication using Visual Observation; [3]OVS = Ovulation synchronization using Ovsynch protocol; [4]Avg = Average taken from 2009 to 2017 values; [5]SD = Standard deviation from 2009 to 2017 values; [6]PC = Pregnancy Cost (CAD cow/year); [7]ER = AI indication rate; [8]Parity distribution AVG = Parity distribution average in Alberta dairies (1-2; 56%, 3-4; 39%, & > 4; 5%).

Table 7.7.1. Dairy Enterprise final costs per ER (30% - 40%) across breeding methods (IRT, VO, and OVS).

	IRT[1]		VO[2]		OVS[3]	
AI indication costs if 30%	AVG[4]	SD[5]	AVG	SD	AVG	SD
Total Operating Costs 1st service	5167.76	660.14	5296.59	401.21	5185.88	661.02
Total Operating Costs 2nd service	5393.20	678.73	5522.02	424.33	5362.69	675.57
Total Operating Costs 3rd service	5618.63	697.68	5747.46	447.58	5539.51	690.35
Total Operating Costs 4th service	5908.39	570.33	6037.21	454.51	5716.32	705.34
Total Production Costs 1st service	5297.51	806.72	5426.34	581.34	5315.63	807.75
Total Production Costs 2nd service	5522.95	823.18	5651.77	599.14	5492.44	820.63
Total Production Costs 3rd service	5748.38	840.02	5877.21	617.39	5669.26	833.75
Total Production Costs 4th service	6038.14	726.26	6166.96	608.87	5846.07	847.10
Return to Equity if 1st service	2245.66	602.51	2116.84	346.83	2227.54	603.04
Return to Equity if 2nd service	2020.23	613.02	1891.41	356.74	2050.73	611.24
Return to Equity if 3rd service	1794.80	624.32	1665.97	368.01	1873.92	619.92
Return to Equity if 4th service	1505.04	481.93	1376.22	381.64	1697.11	629.07
AI indication costs if 40%						
Total Operating Costs 1st service	5154.03	659.48	5284.13	400.48	5170.93	660.29
Total Operating Costs 2nd service	5379.46	678.06	5509.57	423.59	5347.74	674.84
Total Operating Costs 3rd service	5604.90	697.01	5735.00	446.85	5524.55	689.62
Total Operating Costs 4th service	5894.65	569.57	6024.76	453.87	5701.36	704.61
Total Production Costs 1st service	5283.78	805.95	5413.88	580.53	5300.68	806.90
Total Production Costs 2nd service	5509.21	822.40	5639.32	598.33	5477.49	819.78
Total Production Costs 3rd service	5734.65	839.24	5864.75	616.58	5654.30	832.89
Total Production Costs 4th service	6024.40	725.41	6154.51	608.11	5831.11	846.24
Return to Equity if 1st service	2259.40	602.11	2129.30	346.49	2242.50	602.60
Return to Equity if 2nd service	2033.97	612.60	1903.86	356.37	2065.69	610.78
Return to Equity if 3rd service	1808.53	623.88	1678.43	367.60	1888.88	619.45
Return to Equity if 4th service	1518.78	481.44	1388.67	381.34	1712.06	628.58

Abbreviations: [1]IRT = AI indication using Infrared thermography; [2]VO = AI indication using Visual Observation; [3]OVS = Ovulation synchronization using Ovsynch protocol; [4]Avg = Average taken from 2009 to 2017 values; [5]SD = Standard deviation from 2009 to 2017 values.

Table 7.7.2. Dairy Enterprise final costs per ER (50% - 60%) across AI indication methods (IRT, VO, and OVS).

	IRT[1]		VO[2]		OVS[3]	
AI indication costs if 50%	AVG[4]	SD[5]	AVG	SD	AVG	SD
Total Operating Costs 1st service	5141.25	658.86	5272.54	399.79	5157.02	659.62
Total Operating Costs 2nd service	4582.89	778.95	4566.26	778.42	4550.04	779.35
Total Operating Costs 3rd service	4970.89	658.93	4954.26	658.29	4889.42	657.93
Total Operating Costs 4th service	5545.04	429.98	5528.41	429.09	5350.62	496.95
Total Production Costs 1st service	5271.00	805.22	5402.29	579.78	5286.77	806.12
Total Production Costs 2nd service	4712.64	1010.78	4696.01	1010.15	4679.79	1011.10
Total Production Costs 3rd service	5100.64	899.91	5084.01	899.18	5019.17	898.95
Total Production Costs 4th service	5674.79	686.82	5658.16	685.93	5480.37	742.70
Return to equity if 1st service	2272.18	601.74	2140.89	346.17	2256.41	602.20
Return to equity if 2nd service	2830.54	965.96	2847.17	965.79	2863.39	967.40
Return to equity if 3rd service	2442.54	828.08	2459.17	827.85	2524.01	830.16
Return to equity if 4th service	1868.39	580.58	1885.02	580.34	2062.81	624.87
AI indication costs if 60%						
Total Operating Costs 1st service	5118.51	657.77	5261.81	399.15	5144.13	659.00
Total Operating Costs 2nd service	5343.95	676.34	5487.24	422.28	5320.95	673.54
Total Operating Costs 3rd service	5569.38	695.27	5712.68	445.55	5497.76	688.31
Total Operating Costs 4th service	5859.14	567.61	6002.43	452.72	5674.57	703.29
Total Production Costs 1st service	5248.26	803.94	5391.56	579.09	5273.88	805.39
Total Production Costs 2nd service	5473.70	820.38	5616.99	596.88	5450.70	818.25
Total Production Costs 3rd service	5699.13	837.20	5842.43	615.14	5627.51	831.36
Total Production Costs 4th service	5988.89	723.19	6132.18	606.74	5804.32	844.70
Return to Equity if 1st service	2294.92	601.08	2151.62	345.88	2269.29	601.82
Return to Equity if 2nd service	2069.48	611.52	1926.19	355.70	2092.48	609.97
Return to Equity if 3rd service	1844.05	622.75	1700.75	366.88	1915.67	618.61
Return to Equity if 4th service	1554.29	480.18	1410.99	380.81	1738.86	627.72

Abbreviations: [1]IRT = AI indication using Infrared thermography; [2]VO = AI indication using Visual Observation; [3]OVS = Ovulation synchronization using Ovsynch protocol; [4]Avg = Average taken from 2009 to 2017 values; [5]SD = Standard deviation from 2009 to 2017 values.

Table 7.7.3. Dairy Enterprise final costs per ER (70% - 80%) across AI indication methods (VO, OVS, and IRT).

	VO[1]		OVS[2]		IRT[3]	
Dairy Enterprise Costs if ER 70%	AVG[4]	SD[5]	AVG	SD	AVG	SD
Total Operating Costs 1st service	5251.93	398.57	5132.27	658.43	5107.05	657.22
Total Operating Costs 2nd service	5477.36	421.70	5309.09	672.97	5332.49	675.78
Total Operating Costs 3rd service	5702.80	444.97	5485.90	687.73	5557.92	694.70
Total Operating Costs 4th service	5992.55	452.21	5662.71	702.70	5847.68	566.98
Total Production Costs 1st service	5381.68	578.45	5262.02	804.72	5236.80	803.29
Total Production Costs 2nd service	5607.11	596.24	5438.84	817.58	5462.24	819.72
Total Production Costs 3rd service	5832.55	614.49	5615.65	830.68	5687.67	836.55
Total Production Costs 4th service	6122.30	606.14	5792.46	844.02	5977.43	722.48
Return to Equity if 1st service	2161.50	345.62	2281.15	601.48	2306.38	600.75
Return to Equity if 2nd service	1936.07	355.41	2104.34	609.62	2080.94	611.17
Return to Equity if 3rd service	1710.63	366.57	1927.53	618.24	1855.51	622.38
Return to Equity if 4th service	1420.87	380.57	1750.72	627.33	1565.75	479.77
Dairy Enterprise Costs if ER 30%						
Total Operating Costs 1st service	5242.91	398.03	5121.45	657.91	5097.84	656.78
Total Operating Costs 2nd service	5468.35	421.17	5298.26	672.44	5323.27	675.33
Total Operating Costs 3rd service	5693.78	444.44	5475.07	687.20	5548.71	694.25
Total Operating Costs 4th service	5983.54	451.75	5651.88	702.17	5838.47	566.48
Total Production Costs 1st service	5372.66	577.87	5251.20	804.10	5227.59	802.77
Total Production Costs 2nd service	5598.10	595.66	5428.01	816.96	5453.02	819.20
Total Production Costs 3rd service	5823.53	613.91	5604.82	830.07	5678.46	836.02
Total Production Costs 4th service	6113.29	605.59	5781.63	843.40	5968.22	721.90
Return to Equity if 1st service	2170.52	345.37	2291.98	601.17	2315.59	600.49
Return to Equity if 2nd service	1945.08	355.14	2115.17	609.29	2090.15	610.90
Return to Equity if 3rd service	1719.65	366.28	1938.36	617.90	1864.72	622.09
Return to Equity if 4th service	1429.89	380.36	1761.55	626.98	1574.96	479.45

Abbreviations: [1]VO = AI indication using Visual Observation; [2]OVS = Ovulation synchronization using Ovsynch protocol; [3]IRT = AI indication using Infrared thermography; [4]Avg = Average taken from 2009 to 2017 values; [5]SD = Standard deviation from 2009 to 2017 values.

Table 7.7.4. Dairy Enterprise final costs per ER (90% - 100%) across AI indication methods (IRT, VO, and OVS).

	IRT[1]		VO[2]		OVS[3]	
AI indication costs if 70%	AVG[4]	SD[5]	AVG	SD	AVG	SD
Total Operating Costs 1st service	5118.51	657.77	5251.93	398.57	5132.27	658.43
Total Operating Costs 2nd service	5343.95	676.34	5477.36	421.70	5309.09	672.97
Total Operating Costs 3rd service	5569.38	695.27	5702.80	444.97	5485.90	687.73
Total Operating Costs 4th service	5859.14	567.61	5992.55	452.21	5662.71	702.70
Total Production Costs 1st service	5248.26	803.94	5381.68	578.45	5262.02	804.72
Total Production Costs 2nd service	5473.70	820.38	5607.11	596.24	5438.84	817.58
Total Production Costs 3rd service	5699.13	837.20	5832.55	614.49	5615.65	830.68
Total Production Costs 4th service	5988.89	723.19	6122.30	606.14	5792.46	844.02
Return to Equity if 1st service	2294.92	601.08	2161.50	345.62	2281.15	601.48
Return to Equity if 2nd service	2069.48	611.52	1936.07	355.41	2104.34	609.62
Return to Equity if 3rd service	1844.05	622.75	1710.63	366.57	1927.53	618.24
Return to Equity if 4th service	1554.29	480.18	1420.87	380.57	1750.72	627.33
AI indication costs if 80%						
Total Operating Costs 1st service	5108.57	657.29	5242.91	398.03	5121.45	657.91
Total Operating Costs 2nd service	5334.00	675.85	5468.35	421.17	5298.26	672.44
Total Operating Costs 3rd service	5559.44	694.78	5693.78	444.44	5475.07	687.20
Total Operating Costs 4th service	5849.19	567.07	5983.54	451.75	5651.88	702.17
Total Production Costs 1st service	5238.32	803.38	5372.66	577.87	5251.20	804.10
Total Production Costs 2nd service	5463.75	819.81	5598.10	595.66	5428.01	816.96
Total Production Costs 3rd service	5689.19	836.64	5823.53	613.91	5604.82	830.07
Total Production Costs 4th service	5978.94	722.57	6113.29	605.59	5781.63	843.40
Return to Equity if 1st service	2304.86	600.79	2170.52	345.37	2291.98	601.17
Return to Equity if 2nd service	2079.43	611.22	1945.08	355.14	2115.17	609.29
Return to Equity if 3rd service	1853.99	622.43	1719.65	366.28	1938.36	617.90
Return to Equity if 4th service	1564.24	479.83	1429.89	380.36	1761.55	626.98

Abbreviations: [1]VO = AI indication using Visual Observation; [2]OVS = Ovulation synchronization using Ovsynch protocol; [3]IRT = AI indication using Infrared thermography; [4]Avg = Average taken from 2009 to 2017 values; [5]SD = Standard deviation from 2009 to 2017 values.

Table 7.8. Infrared thermography Financial Effect at different accuracy level (Sensitivity – Specificity) per individual cow (CAD cow/year)

	75% Sp[1]			80% Sp			85% Sp			90% Sp			95% Sp			100% Sp		
Se[2]	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4
100%	54.8	61.7	73.3	173.1	180	191.6	291.4	298.3	309.8	409.7	416.6	428.1	528	534.9	546.4	646.3	653.1	664.7
95%	-23.4	-17.3	-7.1	94.7	100.9	111.1	213	219.2	229.4	331.3	337.4	347.7	449.6	455.7	466	567.9	574	584.3
90%	-101.8	-96.5	-87.5	16.4	21.7	30.7	134.6	140	149	252.9	258.3	267.3	371.2	376.6	385.5	489.5	494.9	503.8
85%	-180.2	-175.6	-167.9	-61.9	-57.3	-49.6	56.3	60.9	68.6	174.5	179.1	186.8	292.8	297.4	305.1	411.1	415.7	423.4
80%	-258.6	-254.8	-248.4	-140.3	-136.5	-130.1	-22	-18.2	-11.8	96.2	100	106.4	214.4	218.3	224.7	332.7	336.6	343
75%	-337	-333.9	-328.8	-218.7	-215.6	-210.5	-100.4	-97.3	-92.2	17.8	20.8	26	136.1	139.1	144.3	254.3	257.4	262.5
70%	-415.4	-413.1	-409.2	-297.1	-294.8	-290.9	-178.8	-176.5	-172.6	-60.5	-58.2	-54.4	57.7	60	63.8	176	178.3	182.1
65%	-493.7	-492.2	-489.6	-375.5	-373.9	-371.4	-257.2	-255.6	-253.1	-138.9	-137.4	-134.8	-20.6	-19.1	-16.5	97.6	99.1	101.7
60%	-572.1	-571.4	-570.1	-453.8	-453.1	-451.8	-335.6	-334.8	-333.5	-217.3	-216.5	-215.2	-99	-98.2	-96.9	19.2	20	21.2
55%	-650.5	-650.5	-650.5	-532.2	-532.2	-532.2	-413.9	-413.9	-413.9	-295.7	-295.7	-295.7	-177.4	-177.4	-177.4	-59.1	-59.1	-59.1
50%	-728.9	-729.6	-730.9	-610.6	-611.4	-612.6	-492.3	-493.1	-494.4	-374	-374.8	-376.1	-255.8	-256.5	-257.8	-137.5	-138.2	-139.5

Abbreviations: [1]Sp = Specificity level (%); [2]Se = Sensitivity level (%).

Table 7.9. Visual observation Financial Effect at different accuracy level (Sensitivity – Specificity) per individual cow (CAD cow/year)

	75% Sp[1]			80% Sp			85% Sp			90% Sp			95% Sp			100% SP		
Se[2]	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4
100%	54.7	61	86.3	170.5	176.7	202.1	286.3	292.5	317.9	402.1	408.3	433.7	517.9	524.1	549.4	633.6	639.9	665.2
95%	-22	-16.5	6	93.7	99.2	121.7	209.5	215	237.5	325.2	330.8	353.3	441	446.6	469.1	556.8	562.3	584.9
90%	-98.9	-94	-74.3	16.8	21.7	41.4	132.6	137.5	157.2	248.4	253.2	273	364.2	369	388.7	480	484.8	504.5
85%	-175.7	-171.5	-154.6	-59.9	-55.8	-38.9	55.8	59.9	76.8	171.5	175.7	192.6	287.3	291.5	308.4	403.1	407.3	424.2
80%	-252.5	-249.1	-235	-136.8	-133.3	-119.2	-21	-17.5	-3.4	94.7	98.2	112.3	210.5	214	228	326.3	329.7	343.8
75%	-329.4	-326.6	-315.3	-213.6	-210.8	-199.6	-97.8	-95	-83.8	17.9	20.6	31.9	133.6	136.4	147.7	249.4	252.2	263.5
70%	-406.2	-404.1	-395.7	-290.4	-288.4	-279.9	-174.7	-172.6	-164.1	-58.9	-56.8	-48.4	56.8	58.9	67.3	172.6	174.7	183.1
65%	-483.1	-481.7	-476	-367.3	-365.9	-360.3	-251.5	-250.1	-244.5	-135.7	-134.3	-128.7	-19.9	-18.6	-12.9	95.7	97.1	102.8
60%	-559.9	-559.2	-556.4	-444.1	-443.4	-440.6	-328.3	-327.7	-324.8	-212.6	-211.9	-209.1	-96.8	-96.1	-93.3	18.9	19.6	22.4
55%	-636.7	-636.7	-636.7	-521	-521	-521	-405.2	-405.2	-405.2	-289.4	-289.4	-289.4	-173.6	-173.6	-173.6	-57.8	-57.8	-57.8
50%	-713.6	-714.3	-717.1	-597.8	-598.5	-601.3	-482	-482.7	-485.5	-366.2	-366.9	-369.8	-250.5	-251.2	-254	-134.7	-135.4	-138.2

Abbreviations: [1]Sp = Specificity level (%); [2]Se = Sensitivity level (%).

Table 7.10. Ovsynch Financial Effect at different accuracy level (Sensitivity – Specificity) per individual cow (CAD cow/year)

	75% Sp[1]			80% Sp			85% Sp			90% Sp			95% Sp			100% SP		
Se[2]	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4	1 to 2	3 to 4	> 4
100%	-197.9	-189.8	-176.2	-58.9	-50.8	-37.3	80	88.	101.6	21	227.1	240.6	35	366.1	379.6	496.9	505	518.6
95%	-260.9	-253.6	-241.6	-121.9	-114.7	-102.6	17	24.2	36.3	156	163.2	175.3	295.0	302.2	314.3	434	441.2	453.3
90%	-323.8	-317.5	-306.9	-184.8	-178.5	-168	-45.8	-39.5	-29	93.1	99.4	109.9	232.1	238.4	248.9	371.1	377.4	387.9
85%	-386.7	-381.3	-372.3	-247.7	-242.	-233.3	-108.8	-103.4	-94.3	30.1	35.5	44.6	169.1	174.5	183.6	308.1	313.5	322
80%	-449.7	-445.2	-437.6	-310.7	-306.2	-298.7	-171.7	-167.2	-159.7	-32.7	-28.2	-20.7	106.2	110.7	118.2	245.2	249.7	257.2
75%	-512.6	-509.0	-503.	-373.6	-370	-364.0	-234.6	-231.0	-225.0	-95.7	-92.1	-86	43.2	46.8	52.9	182.2	185.8	191.9
70%	-575.6	-572.9	-568.3	-436.6	-433.9	-429.4	-297.6	-294.9	-290.4	-158.6	-155.9	-151.4	-19.6	-16.9	-12.4	119.3	122	126.5
65%	-638.5	-636.7	-633.7	-499.5	-497.7	-494.7	-360.5	-358.7	-355.7	-221.5	-219.7	-216.7	-82.	-80.8	-77.7	56.3	58.1	61.2
60%	-701.4	-700.5	-699	-562.5	-561.6	-560.1	-423.5	-422.6	-421.1	-284.5	-283.6	-282.1	-145.5	-144.6	-143.1	-6.5	-5.6	-4.1
55%	-764.4	-764.4	-764.4	-625.4	-625.5	-625.4	-486.4	-486.4	-486.4	-347.4	-347.4	-347.4	-208.4	-208.4	-208.4	-69.4	-69.4	-69.4
50%	-827.3	-828.2	-829.7	-688.3	-689.2	-690.8	-549.4	-550.3	-551.8	-410.4	-411.3	-412.8	-271.4	-272.3	-273.8	-132.4	-133.3	-134.8

Abbreviations: [1]Sp = Specificity level (%); [2]Se = Sensitivity level (%).

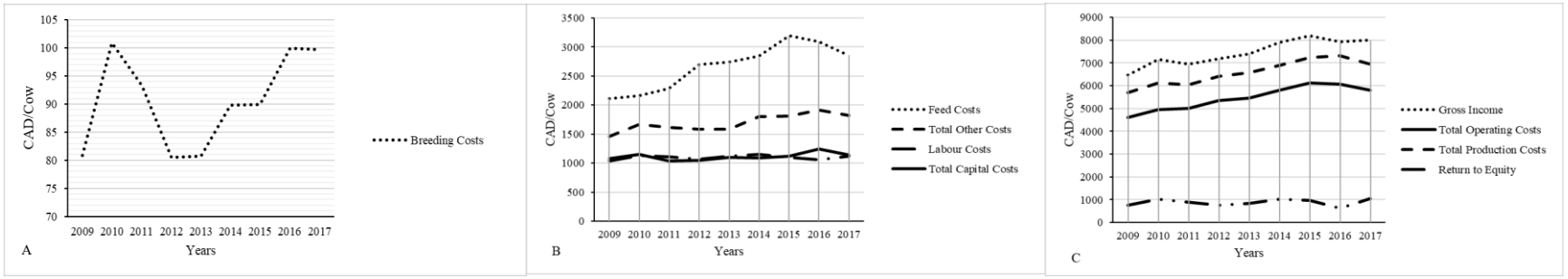

Figure 7.1. A) Breeding Costs average data from the Economics of Milk Production in Alberta (Economics and Competitiveness Branch, Economics Section, Alberta Agriculture and Forestry) edition 2009 to 2017. B) Feed Cost average yearly changes (increase 2009 to 2015 and decrease during 2016 and 2017) with similar pattern in Total Other Costs. Labour Costs and Total Capital Costs were consistent during the same period. C) Gross Income shows a steady increase over time. However, Total Operation Costs and Total Production Costs also increased which resulted in a consistent Return to Equity.

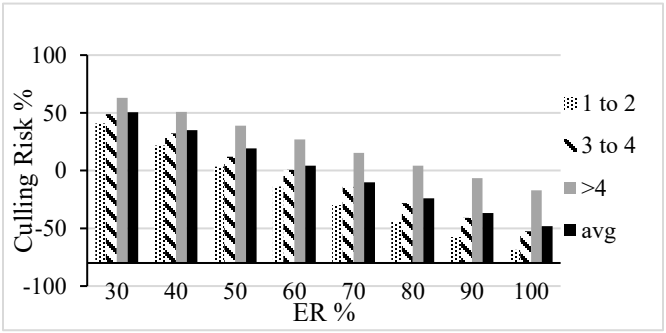

Figure 7.2. Culling Risk per ER at different Parity groups (1 -2, 3-4, and > 4) and Parity distribution average. Negative Culling Risk for Parity 1 -2 was ER 60%, Parity 3-4 was ER 70%, Parity > 4 was ER 90% and ER 70% in a Parity distribution average for Alberta dairies.

Chapter 8. General Discussion and Conclusions

The implementation of new technologies in dairy reproduction programs aims to optimize estrus detection accuracy, increase financial profits by reducing labour costs, be useful in different housing types (e.g. tie-stalls and free-stalls), and collect data consistently. As such, this thesis work intended to use infrared thermography to measure skin temperature and behaviour biometrics under different environment conditions (e.g. different housing types, seasons, and ambient temperature). Additionally, this research aimed to characterize micro-behaviour biometrics using 3D-kinematics assessment, the evaluation of IRT based estrus detection, and finally developed an automated IRT platform capable of detecting estrus in a commercial dairy herd.

8.1. Main Findings

To study the use of IRT as a metric technology to detect estrus in dairy cows, three initial projects characterized the changes in skin temperature and behaviour biometrics associated with the estrus period using the disappearance of dominant follicles as confirmation of ovulation and estimation of estrus retrospectively. In the first study (Chapter 3), seven different anatomical locations (eye, cheek, neck, rump, flank, vulva area and withers) exhibited increased skin temperature (IRT; +0.3 – 1.2°C) at 48 to 24 h before ovulation which was confirmed in 18 multiparous lactating dairy cows induced into estrus using hormone-based protocols (Figure 3.1). During the same study, estrus behaviours were observed (stepping, laying, shifting, neighbour-interactions and tail movement) in dairy cows housed in tie-stalls to identify changes in behaviour frequencies (Events/5min) before ovulation was confirmed. In that study (Chapter 3), tail movements were the only behaviour parameter that increased in frequency as ovulation approached.

Based on these results, the second experiment (Chapter 4) focused on changes in behaviour biometric frequencies in the rump region (vulva, tail and hip areas) which could be measured using an infrared camera in naturally cycling (non-synchronized), primiparous cows. This second experiment found increases in skin temperature at the vulva area (+ 0.80°C) 48 h before the disappearance of the dominant follicle, similar to the previous experiment (Chapter 3). However, tail movements were not different when using IRT pictures and tail movement events scored visually during milking (Events/5min) compared to the tail movement events 5 min before milking in the previous study (Chapter 3). Estrus alerts were created using the most optimum reference

value identified using ROC curve analysis of vulva skin temperature, hip, and tail frequency of events. The greatest accuracy achieved for the estrus alerts that combined vulva skin temperature and hip movements (DOR; 26.62) compared to vulva skin temperature (DOR; 4.97) or tail and hip movements (DOR; 6.13) evaluated individually.

The third study (Chapter 5) aimed to characterize the micro-behaviour biometrics and locomotion associated with the estrus period using 3D-kinematic motion capture in dairy cows continuously housed in a tie-stall environment. Overall, the use of 3D kinematics demonstrated that even when dairy cows are standing seemingly stationary (e.g. in a stall), subtle behaviour movements are both detectable using specialized software (e.g. Vicon Nexus 2) and the patterns of these movements characteristically change leading up to the estrus period. Results from this study confirmed previous findings (Chapter 3 and 4) that tail movements could be an indicator of estrus (48-24 h before ovulation). Kinematic assessment was able to differentiate tail events at different movement resolutions such as micro-tail movements and large tail movements. Thus, these findings suggest that higher resolution measures of estrus using micro-behaviour biometrics are an area in which more exploration is warranted and may be useful in detecting other biologically important states in livestock.

The fourth and fifth studies (Chapter 6 and 7) focused on the feasibility of IRT as an estrus detection method in the dairy industry. In the fourth study (Chapter 6), the objectives were to develop a fully automated IRT platform (Estrus Benchmark^{TM}) that can record and analyze skin temperature and behaviour biometrics at every milking event in a free-stall barn. Additionally, the accuracy of EBM was compared with a 3-axis accelerometer-based system (CowManager SensOor^{TM} tags system) and to in-line milk P_4 analysis (Herd Navigator^{TM}) as current industry standards. The Estrus Benchmark recorded 20 IRT frames each time a cow left the robotic milking area. Furthermore, the IRT software was able to discriminate between frames to identify which frame provided the most accurate vulvar skin temperature and to identify the presence or absence of vulva exposure (i.e. due to lateral tail movement).

Increases in skin temperature were found during the estrus day, similar to the first and second study involving 46 cows in free-stall housing in Chapter 6. The vulva exposure was identified via software that recognized changes in each frame's total temperature distribution and a fixed area of interest (i.e. rectangle at the vulva area). The vulva exposure was present in a higher percentage of cows during the estrus day than pre and post estrus period. The accuracy of estrus

detection via IRT was greater than the CowManager sensor tag system, however, the IRT system did not achieve the totality of estrus alerts created by Herd Navigator (58% sensitivity).

The fifth study (Chapter 7) aimed to identify the financial implications of estrus detection using IRT compared to visual observation of estrus and a hormone-based synchronization protocol (i.e. Ovsynch). The secondary objective was to identify the implications of Se and Sp levels in profit returns. This study demonstrated that the breeding costs associated with the use of IRT as an estrus method were comparable (127.69 CAD) to visual observation of estrus behaviours (115.78 CAD) and more cost-efficient than Ovsynch protocols (138.99 CAD).

Additionally, profit returns were positive when the IRT estrus detection rate (sensitivity) was at 60% ($20.13 CAD) and a Sp (percentage of non-mistimed AI service) of 100%. Further estimations found that if 100% of the estrus detection rate is achieved the profit return is expected to be 654.7 CAD based on feed cost savings during the dry period.

8.2. Discussions, Limitations and Implications

The vulva skin temperature patterns observed in this thesis (Chapters 3, 4 and 6) during the estrus period can be associated with the concentration of steroid hormones, specifically with estrogens (i.e. E_2). Steroid hormones are highly involved in protein synthesis via nuclear transcription factors and the activation of hydrolysis of circulating triglycerides, creating a negative energy balance which is not observed with P_4 concentrations in rodents (i.e. adiposities movement; Toth et al., 2001). Estradiol-lipid breakdown may be associated with increased radiant energy detected and transformed into temperature measurements detectable using IRT cameras. Another explanation for increases in skin temperature could be the presence of E_2 receptors ($E_2\alpha$) at the vasculature of smooth muscular and endothelial cells, which regulate peripheral arterial function (i.e. vasodilatation; Miller et al., 2008).

Estradiol has been associated with an increase in body temperature during estrus in dairy cattle (+ 0.9 ± 0.3°C; Kyle et al., 1998) measured using vaginal and rectal data loggers. Other authors hypothesized that this increase in temperature is due to the LH surge, ovulation (e.g. Redden et al., 1993, Talukder et al., 2014) and increased physical activity (i.e. sexual receptivity behaviours; Walton and King, 1986). In this thesis, the measurement of steroid hormones P_4 and E_2 were restricted to associated physiological changes in the hormone profiles expected during the estrus period rather than identify the sources of radiated energy during the expected estrus. Further

studies should measure endocrine parameters as ovulation approaches (i.e. E_2, P_4, LH, GnRH, Cortisol, $PGF_{2\alpha}$ etc.) with hourly sample collection (note: the sample intervals in Chapter 3 and 4 were 24 h).

Other explanations for the increases in skin temperature could be attributed to vasodilatation of the skin in the presence of E_2 and P_4. Radiated heat could result from changes in the integumentary system influenced by activation of warm-sensitive neurons at the preoptic area of the hypothalamus, creating vasodilatation already demonstrated in in-vitro tissue slices treated with E_2 (Silva and Boulant, 1986). Knowledge in integumentary physiology could explain the effects of ambient temperature, relative humidity, and metabolic processes experienced by the cows on a normal basis that can be screened via data discriminations (e.g. using machine learning algorithms) and, as such, eliminate potential false-positive alerts in estrus detection using IRT. Further research should associate changes in blood perfusion (i.e. volumetric flow rate per volume of vulva skin) with vulva skin temperature during estrus and its interaction with different ambient temperatures. The associations described above can provide valuable information to understand the variation in vulva skin temperature intensity during estrus (0.3 to 1.2°C) observed in Chapter 3 and 4 and its implications on conception rate, pregnancy rate and calving rate.

Behaviour results varied across the studies included in this dissertation (Chapter 3, 4, 5, and 6). In the first study (Chapter 3), tail movement was observed to increase 48 h before ovulation and then suddenly decreased 24 h later (when observed 5 min before milking). However, Tail movements were not found to differ significantly during the second study (Chapter 4). A potential explanation for these differing results may be that sample collection and recording methods differed in these studies. In the first study (Chapter 3), tail movements were measured using digital cameras (at 30 frames/sec) 5 min before milking when the cows were also observed exhibiting increased anticipatory activity as the milking machine approached (i.e. as an unconditioned autonomic response). Another explanation for the increase in activity is that the approaching milking machine may have served as an antecedent cue as part of operant conditioning via negative reinforcement (e.g. udder pressure relief). Nevertheless, the increase in fidget movements may not be merely associated with pre-milking but likely to change frequency during the estrus period due to hormonal changes and appearance of the dominant follicle as the first study reported. In the second study (Chapter 4), tail movements were measured using IRT frames (4 frames/sec) during milking (5 min) when the unconditioned autonomic or operant conditioning responses would not

be expected to be present, and the frame rate was not able to identify smaller tail movements similar to behaviour observations in Payne et al. (2017).

Frame rate is important when behaviours such as tail movement are relatively short (within seconds) and behaviour scoring is performed visually. Limitations associated with behaviour observations were finding a suitable location (> 2m) to record behaviour with digital cameras during milking time without interfering with worker movement. Also, the distance, angle, lack of contrast (e.g. white cows), and a large number of videos influenced results by affecting the visibility and eye strain of observers while behaviour scoring in the Chapter 3 experiment. In the same study, re-training observers, re-scoring, and elimination of an outlier observer was required and the inter-observer reliability was 85% (Kappa coefficient). Future experiments requiring intensive behaviour scoring of tail and leg movements in a tie-stall during milking and non-milking times should implement high-resolution cameras (1600 x 1200 pixels), use wireless connections, and colour the tail and legs (e.g. red, orange, or green) to identify movements easily via visual behaviour scoring.

In the third study (Chapter 5), tail movements increased significantly at 48 h before ovulation, a finding that was in line with the results described in Chapter 3. However, tail movements were analyzed at 300 frames/sec using kinematic assessment at a non-milking time, which showed that tail movements were not entirely associated with the pre-milking period. Regardless of the objective and precision of kinematics assessments, the practical implementation on-farm is a difficult task due to the extensive amount of cable required, the need for calibrated areas, and the use of passive markers. Additionally, the sample collection was limited to 7 cows per day due to the time required to place markers without interfering with milking times (i.e. 0300 and 1500), large data storage space (e.g. 1.5. TB in 14 cows), and a limited number of markers. Further investigations using kinematics in dairy cows housed in tie-stalls should reduce the number of required markers (note: markers can be model based on other marker information), use wireless connection system-to-cameras, and simplify image sample collection to reduce memory needs. Furthermore, tail movement function and causation (e.g. motivation, restless, affective state, operant condition, etc.) needs to be investigated further to characterize the different types of tail locomotion using kinematics and its associations with estrus. The characterization of tail movements could be helpful as an indicator of mental states in dairy cattle that could contribute to improving the cow's well being.

The overall increase in activity observed during the onset of estrus is generally followed by the standing to be mounted behaviour in dairy cattle (Sveberg et al., 2011). The gradual increase in behaviour (i.e. walking, social interactions, and female-to-female mounting) from 80 to 16 h before ovulation has also been reported in previously published studies (e.g. Arney et al., 1994, Roelofs et al., 2005a, Silper et al., 2015). The explanation for the increase in cow activity at the beginning of estrus is to attract the attention of males (bulls) in order to facilitate mating (proceptive behaviour; Beach, 1976). However, increases in activity, social interactions, and female-to-female mounting require large locomotory movements that are not possible in restricted spaces such as tie-stalls. One of the objectives of this thesis work was to identify movements that can be measured in restricted spaces and provide biometrical information useful in detecting changes during estrus. Previously, Guesgen and Bench (2018) characterized hip movements using 3D-kinematics as cows approached ovulation in tie-stall housing. The frequency of hip-movements was found to increase one day before ovulation, however, hip movements were significant only when the resolution of such movements (i.e. movement size) was reduced to 10 mm (e.g. hip shifts side-to-side and backwards-forward; Guesgen and Bench, 2018) associated with lordosis during the estrus period. However, in the third experiment (Chapter 5), the kinematic assessment of tail movements were found to increase 48 h before ovulation and then suddenly decrease 24 h before ovulation and at ovulation day. The potential explanation for the biometric differences between hip-movements and tail movements 24 h before ovulation may be related to vulva exposure in cattle (*Bos Taurus and Bos Indicus*; Price, 2008). In natural service (i.e. bull mating), bulls tend to sniff the anogenital area to confirm the sexual receptivity of cows in estrus by detecting chemical cues present in urine and vaginal secretions during the estrus period resulting in a flehmen response by the male. Cows that are not sexually receptive tend to walk away, stop urinating and do not expose their vulva (Price, 2008). This type of vulva exposure during estrus day was also found in experiment five (Chapter 6), in which the majority of cows in estrus (~60%) exposed their vulva as they walked out of the milking robot.

Limitations regarding the measurement of behaviour biometrics during estrus included the absence of estrus behaviours normally seen during the estrus period. The occurrence of standing to be mounted in cows has historically been is largely regarded to be the most reliable sign of sexual receptivity since many other behaviours associated with estrus (e.g. restlessness) can also be present during non-estrus periods (Hafez et al., 1969, Roelofs et al., 2005a). However, in this

doctoral research, the estrus period was identified using associations with ovarian dynamic changes in Chapters 3, 4 and 5 (i.e. development of dominant follicles, the disappearance of dominant follicles, and increase of corpus luteum diameters; Perry et al., 2017) and P_4 declined as expected during the estrus period (Holman et al., 2011). The limitations of using ovarian dynamics and hormone profiles as a standard to confirm estrus are the negative effect of silent estrus incidence. Future validation of vulva exposure via the IRT platform should be performed to confirm and replicate the Chapter 6 findings and associate vulva exposure with the presence of chemical cues and standing to be mounted during the estrus period.

Furthermore, silent estrus (silent ovulation) is present mostly in the first and second ovulation during the puberty stage of dairy heifers (1^{st}; 40%, 2^{nd}; 35%, and 3^{rd}; 0% cycles; Del Vecchio et al., 1992) but is also present in mature cows (1^{st}; 83%, 2^{nd}; 46%, and 3^{rd}; 13 cycles after parturition; Isobe et al., 2004). As such, our behaviour biometrics could have been affected by the intensity of estrus behaviour (low frequency of sexual receptivity behaviours and low duration of sexual receptivity) and silent estrus. A possible explanation for silent estrus is the role of kisspeptin regulation of the estrous cycle in pubertal and seasonal anestrus (i.e. sheep; Caraty et al., 2007). The concentration of kisspeptin and the number of kisspeptin positive cells present during the breeding season are higher compared to the non-breeding season. Similarly, kisspeptin contact with GnRH neurons at the hypothalamus increases after puberty in ovariectomized ewes (i.e. no direct relationship between kisspeptin with LH and ovulation; Smith et al., 2011). Further studies to identify the relationship between behaviour biometrics and kisspeptin-hypothalamus contact in dairy cows at the estrus period would help provide a better understanding of any potential causal mechanism in this regard. Additionally, the incidence of silent estrus affects mounting and secondary estrus behaviours displayed but no scientific literature has reported an influence of olfactory cues on vulva exposure. However, we hypothsize that olfactory cues and vulva exposure could be present in silent estrus and alleviate low reproductive outcomes by using these parameters as estrus detectors.

This thesis work gave importance to the lactation stage (45 to 120 DIM) when reproductive management and financial benefits are remarkable for producers but physiologically hard to achieve. However, multiple studies have encountered abnormal estrus cycles (i.e. short cycles; Sartori et al., 2017, anovulatory periods; Bamber et al., 2009, and prolonged inter-ovulatory intervals; Ball and McEwan, 1998) during the first 70 DIM. The physiological explanation for the

reduction or absence of sexual receptivity behaviours in pubertal and post-calving cows challenges the hypothesis that E_2 is the hormone responsible for estrus behaviour since pubertal heifers and first ovulation cows with E_2 concentration levels capable of producing an LH surge to suddenly ovulate. As such, the expression of estrus behaviours could be related to the presence of P_4 and the quality of a CL from previous estrous cycles. However, cows with abnormal P_4 concentrations due to prolonged estrous cycles (>25 days) could be related to reduced $PGF_2\alpha$ concentrations present in uterine abnormalities (i.e. infections and uterine involution; Sartori et al., 2017). Abnormal interactions between a CL and a dominant follicle could explain the absence of sexual receptivity however, CL and dominant follicle interaction does not explain the absence of sexual receptivity for all silent estrus (only ~20% and heifers do not have previous CL and P_4 concentrations). As such, further applications of vulva skin temperature could provide information regarding reproductive tract health (e.g. metritis and retained placenta) for its proximity with the vulva-vagina region and increases in radiated heat observed during infectious diseases previously reported (Schaefer et al., 2012). It is expected that IRT platforms would be able to track the behaviour biometrics and vulva skin temperature during the complete lactation period. As such, associations between uterus health, estrus cycle length, and reproductive outcomes are important to identify the most viable AI service and to diagnose reproductive tract diseases opportunely.

The second objective addressed in Chapter 6 was to develop an automated estrus detection platform that can be feasibly used in different housing systems (i.e. to collect skin temperature and behaviour biometrics) and accurately alert that estrus is occurring. The reason for developing an automated IRT platform was to collect reliable data without the need for labour input. Most previous IRT studies conclude that a fixed angle, distance, focus, and use of data analysis that accounts for environmental challenges are required to obtain accurate IRT data (Loughmiller et al., 2001, Talukder et al., 2014, Cook et al., 2016). The accuracy of measuring skin temperature relies on the quality of acquisition of IRT frames (e.g. angle, distance, debris presence, ambient temperature and relative air humidity), which could influence IRT data and create false positive estrus alerts. In Chapter 6, all the parameters mentioned above were mitigated by placing the IRT camera at the exit of the milking robot with a fixed angle, distance, and focus for every milking event.

The main limitations of implementing the EBM system in VMS housing were the placement of the IRT camera and the hardware required. Robotic milking systems (e.g. DeLaval

VMS, DeLaval Delpro^{TM}, International, Tumba, Sweden) require concrete and steel made facilities that make it challenging to adapt hardware to the existing structures, reduces wireless connections due to the thickness of building materials and radio frequency interference (e.g. RFID scanners and antennas). In addition, the DLC Lakeland barn is located in rural Alberta (Vermilion River County No. 24) where the internet connection is via fixed wireless access (i.e. radio links between fixed points), which was unable to provide the speed needed (e.g. 1 G per second; high-speed internet) to create real-time analysis and it was highly sensitive to environmental changes (e.g. snow, rain, wind, etc; Rysavy, 1998). Further studies implementing automated systems should consider that all rural Alberta Canada is limited to fixed wireless access and real-time analysis would require on-farm analysis using software that can communicate only at low network congestion, use broadband fixed wireless, or satellite internet runner-up.

In addition to the technical application of the IRT, another limitation was the creation of estrus alerts retrospectively. The IRT platform's greatest accuracy was confirmed at ± 48 h window compared to the decline in P_4 measured with the HN system and ± 72 h window compared to the increase of activity measured with the CowManager SensOor tag system. Estrus BenchMark estrus alerts are limited to create an estimation of the most effective time to inseminate since the ovulation interval from the drop of P_4 can be within four days (Bruinjé et al., 2017) and 28.7 h from the increase of activity using accelerometers (Valenza et al., 2012). In addition, the IRT platform error rate measured was 2% of the actual temperature measured (e.g. 2° C in 100° C). The temperature range of the IRT frames used in this thesis was 13.5°C to 36.5°C with an error rate of 0.45°C (i.e. 2%) which could create false positive estrus alerts since skin temperature increases during estrus are +0.3 to 1.2°C in this thesis work. As such, further studies should consider including additional controls for factors such as the ambient temperature affect per individual cow and the emissivity of skin temperature with different lengths of cow hair. Further, research in this area needs to standardize the size of the IRT area of interest, location and add a reference point with a known temperature (i.e. black body) to reduce the margin of error rate to less than 1% of the measured temperature (FLIR, 2016) in real-time analysis. Additionally, investigate AI service outputs (e.g. conception rate, pregnancy rate, and calving rate) to identify the most accurate estrus alerts using different machine learning algorithms (e.g. decision tree and random forest). Finally, simplify the use of memory, IRT camera resolution, number of frames needed to identify vulva exposure, and

identify suitable locations (e.g. parlour milking) to identify cows using pattern recognition (e.g. face recognition) to avoid radio frequency interference with the equipment on-farm.

The accuracy of estrus detection using IRT and behaviour biometrics in the first 4 studies was comparable with the estrus detection rate of visual observation of estrus in other studies (> 50%). As mentioned previously, estrus detection can be affected by multiple factors (e.g. management, environmental, physiological, and health) after the VWP (i.e. >50 DIM). The accuracy observed in other automated estrus detection systems varies in the literature (60-80%; Mayo et al., 2019, Burnett et al., 2018), which indicates that experimental methodologies can influence estrus detection rates. In the first three studies (Chapters 3, 4, and 5), the threshold values used to discriminate cows in estrus from non-estrus stages were identified as the most optimum reference value using a ROC curve analysis, which balances the Se and Sp at their highest balanced point. The estrus detection accuracy resulted in the ROC curve analysis was higher (70%: Chapter 3) to the average estrus detection rates in Canadian herds (<40% LeBlanc, 2005) using a balanced Se and Sp. However, decision making at the herd management level often relies upon assessing the financial benefit of an estrus detection method under consideration. In the 4th study (Chapter 7), a positive profit return related to estrus detection was achieved at a 60% Se with a 100% Sp using production and operation costs of Alberta dairy operations with estrus detection rates similar to those found in U.S. herds (65% sensitivity; De Vries and Colin, 2003). However, IRT, visual observation and hormone-based protocols (Ovsynch; using conception rates as accuracy level) achieved positive profit returns using accuracy reported in scientific literature. The standard of evaluation for this study (Chapter 4) was the disappearance of the dominant follicle retrospectively. The limitation of using transrectal ultrasonography as a standard was considering silent ovulations as true estrus when performing the evaluation of accuracy, which affected the Se level.

Including all cyclic cows after the voluntary waiting period as eligible cows for breeding can result in low estrus detection rates, poor pregnancy outcomes (19%) and indirectly lower conception rates at first service in Canadian dairies (<40%; LeBlanc, 2005). Further studies should evaluate estrus detection relative to occurrences of standing to be mounted and the ovarian quality to be inseminated (e.g. quality of CL, dominant follicles, and estrous cycle duration) in eligible cyclic cows for breeding and combine an economic analysis, likelihood to achieve pregnancy and accuracy of estrus detection in the model. In addition, the return to equity (i.e. net gain) of using

accelerometers, in-line milk P_4 analysis, visual observation of standing to be mounted, and the Estrus Benchmark should be tested using dairy operation costs across Canada.

8.3. Conclusions

Identification of cows in estrus is not an easy task. This thesis concludes that the practical implementation of infrared thermography and behaviour biometrics to detect estrus in dairy cows is possible. The development of an infrared thermography platform provides valuable fundamental information to commercialize a novel method of estrus detection and to provide producers with additional decision-making tools to optimize reproductive management in the context of estrus detection. Nevertheless, the infrared thermography platform studied in this doctoral research only partially addresses the challenges of estrus detection by developing a consistent and systematic manner to create estrus alerts under housing and labour challenges. The infrared thermography platform used in the current research was not programmed to evaluate abnormal estrus cycles, metabolic challenges affecting cows in the transition period, diseases, and extreme ambient temperatures. This thesis accepts the hypothesis that combining IRT and behaviour biometrics in an automated platform optimizes the accuracy levels of estrus detection in a non-invasive platform suitable in tie-stalls and free-stalls housing systems.

The contributions of the current thesis to the scientific literature, companies, dairy production advisors, and dairy producers are fundamental answers regarding estrus detection using IRT and behaviour biometrics, financial outcomes, and their application in a commercial farm. Additionally, this thesis proved the ability of IRT cameras to measure skin temperature and behaviuor biometrics combined to detect estrus, which, the same principle could be adopted in other dairy production challenges, other livestock species and other scientific disciplines. Furthermore, this thesis is an example of fundamental and applied research, which, adds to the scientific findings combined with the on-farm application of results that are required by the dairy industry. However, further research should continue adding essential elements to adequate commercialization and implementation of the IRT platform as a reproductive management alternative. This thesis work is also part of the newest dairy science discipline, “precision dairy” which encourages the discovery and development of new technologies such as IRT to optimize overall dairy management in a non-invasive manner. The development of estrus alerts using IRT outputs, the adjustment of external factors such as ambient temperature and the physiological

associations that validated the occurrence of estrus in dairy cows encourages the application and replication of these thesis findings to new research projects.

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