Assessing the impact of lameness on fertility and oestrus behaviour of dairy cattle: A comparison of oestrus detection methods with the evaluation of a modern detection method

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Abstract

It is well documented that lameness is a painful and debilitating condition that can have adverse effects on welfare and reproductive performance, including oestrus expression and intensity. Despite extensive research, lameness continues to affect dairy cow populations. This study assessed the impact of lameness on fertility and oestrus behaviour of dairy cattle. The objectives of this research were: 1) to assess dairy producers' perception of reproductive efficiency between lame and non-lame cattle, and to determine how they manage oestrus detection for lame cows; 2) to evaluate herd fertility parameters (number of days from calving to first service, number of days from calving to conception and the number of inseminations to conception) in lame and non-lame cows, to calculate lameness prevalence for the duration of the study, and to determine if access to pasture improves locomotion scores (LCS) and oestrus activity; 3) to compare common oestrus detection methods (Kamar®, Estrotect^{TM} scratch cards, chalk, activity monitors (NeDap, IceQube®) in lame and non-lame cows, from pastured and housed conditions; and 4) to evaluate a new oestrus detection technology (Infrared Thermography (IRT)).

Through an online questionnaire, it was determined that 85% of respondents noticed behavioural changes associated with lameness (reduced oestrus expression, less mounting activity, increased lying times), and that lame cows required more inseminations to conception (3.1 (±0.1) v 2.1 (±0.1); $p<0.001$). The majority of respondents use the same oestrus detection methods for all cows, despite behavioural differences between lame and non-lame cows stated by the respondents.

Fertility assessment of dairy cows from Rodwell farm showed that lame cows had more days from calving to first AI (n=94 (n=25 lame; n=69 non-lame)) (63.8 v 53.5; $p<0.01$) and from calving to conception (n=69 (n=22 lame; n=47 non-lame)) (113.5 v 84.2; $p<0.01$). Evaluation of LCS showed that lameness prevalence decreased during the month's cows had pasture access. Furthermore, there was significant improvement in LCS (by 0.21 units/week) after pasture access for all of study cows (both lame and non-lame) ($p<0.001$). Results showed that as the study cows LCS improved, subsequent oestrus activity (step counts, motion index) also increased thus indicating improved oestrus expression. Assessing multiple oestrus events from the study cows determined that there was a significant difference in the motion index ($p<0.001$), and the number of steps before, during and after oestrus from different LCS ($p<0.001$). Cows with a LCS of 1 at the time of oestrus had significantly more steps than a LCS of >2.5 during an oestrus event. Cows with a LCS of 1 at the time of oestrus had a significantly higher motion index than a LCS of >2 during an oestrus event. Housing affected activity, with housed cows having lower step counts ($p<0.05$), and reduced motion index ($p<0.001$). The mean LCS for oestrus events in housed conditions was higher than oestrus events occurring at pasture (2.5 v 1.9) ($p<0.001$).

Comparison of oestrus detection methods between lame and non-lame cows revealed no significant difference ($p>0.05$). Estrotect™ scratch cards were more efficient at pasture than in housed conditions ($p<0.05$). Lame cows at pasture had fewer step counts ($p<0.05$), motion index ($p<0.01$), and fewer lying bouts when compared to non-lame cows ($p<0.05$) before, during or after oestrus. Housed lame cows had fewer step counts ($p<0.05$), motion index

($p<0.05$), fewer lying bouts ($p<0.05$), and increased lying bout lengths ($p<0.05$) when compared to non-lame cows before, during or after oestrus. Overall lame cows had lower progesterone values at 7 days (difference of 1.2 ±0.2 ng/ml; $p<0.05$) and 10-days (difference of 1.7±0.2ng/ml; $p<0.001$).

Ongoing challenges in oestrus detection has led to advances in oestrus detection aids. Evaluation of a novel oestrus detection method (IRT) for lame and non-lame cows will determine its practicality for all cows. Based on the coefficient of variation the most reliable point to take IRT temperature measurements from is the pocket (under tail) (CV%; 0.6), and the eye (CV%; 1.3). However, the eye is more practical and its moderate positive correlation relationship (R^2=0.45) to the core body temperature makes it appealing. Comparison of baseline temperatures with temperatures recorded when the cow is in oestrus revealed a significant increase in the core (+0.59°C; $p<0.001$), eye (+0.58°C; $p<0.001$), ear (+0.48°C; $p<0.05$), and pocket (+0.66°C; $p<0.001$) temperatures. Lame cows had significantly reduced temperatures (baseline & oestrus) from the core ($p<0.01$), eye ($p<0.001$), pocket ($p<0.001$), and pin ($p<0.05$) locations compared to non-lame cows. IRT can be implemented to identify oestrus in lame and non-lame cows, in addition to potentially detecting lameness based on temperature readings.

These studies demonstrate that lameness affects fertility and physiological parameters. Activity during oestrus can be increased if locomotion scores can be improved. Reducing lameness will enhance animal welfare and productivity. IRT can accurately identify cows in oestrus, and has the potential to identify lame cows, as they had reduced temperatures when compared to non-lame

cows. This study provides an insight for the potential of IRT for increasing oestrus detection, and for automated lameness detection.

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List of Abbreviations

AI	Artificial insemination
ANOVA	Analysis of Variance
BCS	Body condition score
CL	Corpus luteum
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EEC	European economic community
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
GnRH	Gonadotropin-releasing hormone
GPS	Global positioning systems
IRT	Infrared thermography
LCS	Locomotion Score
LH	Luteinising hormone
NEB	Negative energy balance
NR	Numerical rating
NZD	New Zealand Dollars
P^4	Progesterone
PDD	Papillomatous digital dermatitis
PGF	Prostaglandin $F_{2\alpha}$
PLP	Prolonged luteal phase
R_2	Coefficient of determination
S.E	Standard error
s.e.d	Standard error of the differences
s.e.m	Standard error of the mean
TMR	Total mixed ration
U. K.	United Kingdom
USA	United States of America
UWB	Ultra-wideband radio technology
VAS	Visual analogue scales
VEI	Vaginal electric impedance

Chapter 1: Introduction

Throughout the years, dairy producers have selectively bred their cows based on genetic parameters such as conformation, (Gillespie and Flanders, 2010), to maximise milk production (Søndergaard *et al.,* 2002; Shook, 2006). The use of artificial insemination (AI) has been described as the most valuable technique implemented for the genetic improvement of dairy cattle (Vishwanath, 2003; Parkinson, 2004; Howley *et al.,* 2012). The use of AI has enabled producers to maximise milk yield through the pairing of elite cows and bulls. A study by Miglior *et al.* (2005) reported that when comparing selection indices in 15 countries, emphasis on production was 59.5%, whereas durability-health and reproduction were 28%, and 12.5% respectively. Milk yield also depends on breed of cow. United Kingdom breed performance statistics reported by the Centre for Dairy information (2015) reported that based on a 305-day lactation Jerseys and Guernsey breeds produce approximately 6000 kg, Ayrshire and British Friesians approximately 7000 kg, and Holsteins produced just under 10,000 kg. Additionally, it has been reported from several countries that despite an annual increase in milk yield, herd sizes are decreasing. For example, In the UK the average dairy herd size was 2229 (thousand head) in the year 2001/02, whereas in 2016 it declined to 1906 (thousand head) despite an increased average yield (kg per cow per annum) from 6449 to 7912 respectively (DairyCo, 2013a; AHDB, 2017a). In 1959, Canadian dairy cows were producing on average 5211 kg of milk annually, whereas in 2008 the yield increased to 9836 kg, a difference of 4625 kg (Greenough, 2009). Additional records from the Government of Canada (Canadian Dairy Information Centre, 2018) listed that in 2002 the total number of dairy cows (not including heifers) was 1083.9 (thousand head) with an

average annual production of 75,455,180 hectolitres (hl), whereas in 2017 the dairy cow population decreased to 945 (thousand head) with a yield of 89, 841, 872 (hl). Although the dairy cow population decreased, there was an increase in the number of higher yielding Holstein dairy cows (8,496 cows) registered within Canada from 2013 to 2017 when compared to lower yielding breeds (Canadian Dairy Information Centre, 2018). In 1970, the United States dairy population was 12 million with an average yield per cow of 5,085 kg. In 2005, the population reduced to 9 million with an average milk yield of 9,945 kg. Dillon *et al.* (2006) also reported that between 1985 and 2003 an annual gain in milk production per cow was 131 kg for the Netherlands, 193 kg for the United States, 46 kg for Ireland and 35 kg for New Zealand. In the U.K., the average annual milk yield per cow increased by 0.9% (68kg/cow) in 2015/16 (AHDB, Dairy, 2017a).

Although selection based on genetic parameters estimated from phenotypes has been highly successful in maximising milk yield, several studies have determined agonistic relationships between high milk production, lameness (Buitenhuis *et al.,* 2007; Mattiello *et al.,* 2011), fertility (Laben *et al.,* 1982; Lucy, 2001; Nebel and McGillard, 1993; Roxström *et al*., 2001; Stevenson *et al*., 1983; Windig *et al*., 2006), and increased susceptibility to disease (Carlén *et al*., 2004; König *et al.*, 2008). In the past 25 years reproduction efficiency has reduced in the modern Holstein-Friesian dairy cow (Aungier *et al*., 2012), which may be a result of poor oestrus detection (Homer *et al.,* 2013). For example, studies have documented that high yielding dairy cows (10,814 kg/305 days) displayed less intense signs of oestrus, in addition to shorter oestrus periods (5.5 v 11.1 hours) when compared to low yielding cows (6912 kg/305days)

(Harrison *et al.,* 1990; Lopez *et al.,* 2004a). Additionally, it was found that the interval from parturition to first oestrus was shorter for low yielding cows than for high yielding cows (43 v 66 days respectively). Therefore, detecting oestrus in high yielding cows is becoming increasingly difficult, especially in modern commercial dairy farms as herd sizes grow, and available numbers of farm workers decrease.

Additionally, increased metabolic stress occurs (Nielsen,1999), which can result in disorders such as subacute ruminal acidosis, ketosis, and hypocalcaemia (Oetzel, 2004), leading to negative effects on longevity, soundness, and increased incidence of health disorders (Pryce *et al.,* 1997; Pryce *et al.,* 1998; Dillon *et al.,* 2006; Oltenacu and Algers, 2005; Flint, 2006; Heringstad *et al.,* 2007; Berry *et al.,* 2011; Walsh *et al.,* 2011), which decrease conception rates due to a compromised immune system, extended anovulatory periods, decreased oestrus intensity, poor oestrus detection, and an increase in embryonic mortality (Thatcher *et al.* 2006; Boer *et al.* 2009). Subsequently due to the decline in fertility, within the last decade utilisation of hormone manipulation therapies to control the oestrus cycle has become more common (Bruno *et al.,* 2013).

Lameness is a multifactorial condition and several cow level factors such as milk yield (Bichalo *et al*., 2008) and low body condition score (Green *et al*., 2014; Randall *et al*., 2015) have been associated with an increased incidence of lameness. It is well documented that lameness is one of the most concerning diseases of the modern dairy cow (Alawneh *et al.,* 2011). Twenty to thirty percent of lactating dairy cows in the UK and North America are clinically lame

at any given time (Cook, 2003; Espejo *et al.,* 2006; Defra, 2008a). Additionally, European countries reported prevalence estimates from 19% on organic farms in Germany (March *et al.,* 2008), to 31% in Austrian Simmental herds (Dippel *et al.,* 2009). Research indicates that lameness is ever increasing in the UK, with figures of 36%, and over 70% from 2006 to 2007 (Barker *et al.,* 2010). A reduction in overall fitness affects reproductive function, which contributes to decreased animal welfare through increased incidence of lameness and premature culling, consequently leading to severe economic losses (Heringstad *et al.,* 2007; Ettema *et al.,* 2010). It is vital to evaluate current oestrus detection methods in lame cows to determine if lameness affects the type of oestrus detection method used.

This thesis aims to provide insight into what oestrus detection methods are used by dairy farmers, and if they use the same detection methods for lame cows. This study examined the reproductive performance for both lame and non-lame cows, while assessing the effect of pasture on locomotion scores and oestrus expression in lame and non-lame dairy cattle. The main aim of this thesis was to assess different oestrus detection methods that are currently utilised in dairy production systems on lame and non-lame cows. With a final investigation evaluating the use of a new oestrus detection technology (Infrared Thermography) on lame and non-lame cows.

Chapter 2: Literature Review

2.1 Oestrous Cycle

The oestrous cycle is the cyclical pattern of ovarian activity that enables female animals to alter from a period of reproductive non-receptivity, to a period of reproductive receptivity, potentially leading to mating, and pregnancy (Forde *et al.,* 2011). A dairy cow's oestrous cycle can average 18-24 days in length, and includes distinct phases, which are regulated by cascades of hormonal fluctuations (Forde *et al.,* 2011; Phillips, 2010). Two distinct phases that occur are the luteal phase (14-18 days) and the follicular stage (4-6 days) (Forde *et al.,* 2011). The luteal phase is the period following ovulation when the corpus luteum (CL) is formed, the luteal phase is commonly further categorised as met-oestrus and di-oestrus (Forde *et al.,* 2011).

The follicular phase is the time period following the regression of the CL (luteolysis) until ovulation occurs (commonly designated as pro-oestrus and oestrus) (Forde *et al.,* 2011). The development of follicles is controlled by a hormonal feedback system including gonadotropin releasing hormone (GnRH), Follicle stimulating hormone (FSH), luteinising hormone (LH), oestrogens androgens, progestins and proteins (Pryce *et al.,* 2004). Follicle development occurs in waves lasting approximately 7-10 days, with between 2-4 waves in an oestrus cycle of 21 days in length (Pryce *et al.,* 2004). Each wave results in the recruitment of 5-7 primordial follicles, whereby one will become larger (dominant follicle) and the others regress (Pryce *et al.,* 2004). Final maturation and ovulation of the ovulatory follicle occurs during the follicular phase,

enabling the release of the oocyte into the oviduct for potential fertilisation (Forde *et al.,* 2011). (see Plate 2-1)

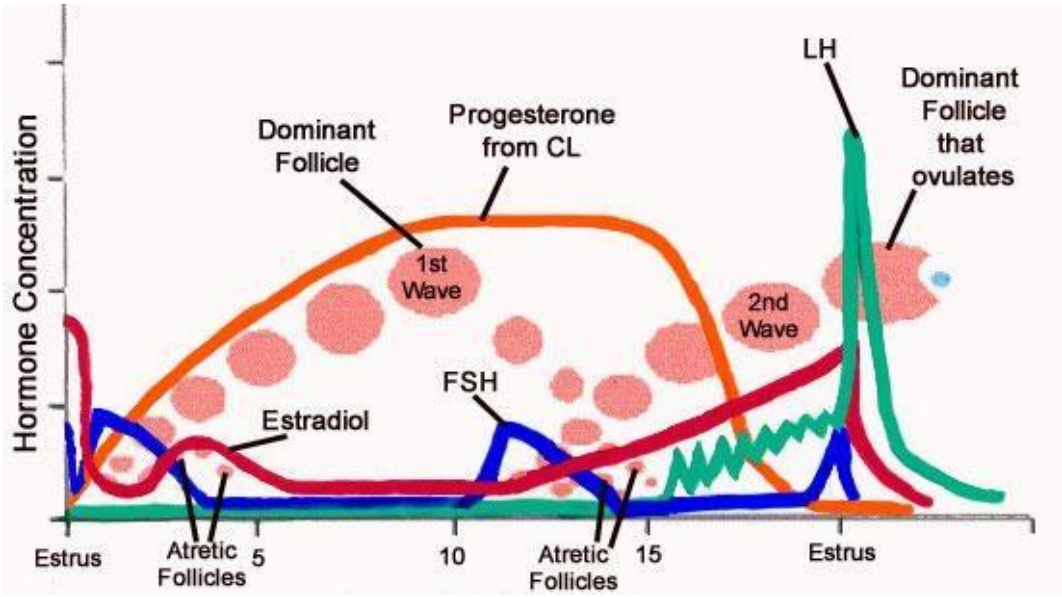

Plate 2- 1 Oestrous cycle in cattle (Source: Penn Veterinary Medicine Computer Aided Learning, 2017).

2.1.1 Progesterone

Progesterone is a crucial hormone responsible for follicle, embryo and foetal development (Brooks *et al*., 2014). Progesterone profiles may be analysed to monitor ovarian activity, and to determine pregnancy in dairy cattle (Petersson *et al.,* 2006a; Simersky *et al.,* 2007; Oku *et al*., 2011; Squires, 2010). Sufficient circulating concentrations of progesterone are vital for the normal oestrous cycle, the maintenance of a pregnancy (Stevenson *et al.,* 2008; Yan *et al*., 2016), and is crucial for the full expression of oestrus behaviours (Walker *et al.,* 2008a). Progesterone increases the number of oestradiol receptors in the medio basal hypothalamus during the luteal phase, resulting in an enhanced sensitivity to oestradiol (Blache *et al.,* 1991; Blache *et al.,* 1994). An indication of the onset of the follicular phase and subsequent ovulation in cyclic cows is

identified by a decline of progesterone concentrations at day 18-19 due to luteolysis (Roelofs *et al*., 2006; Squires, 2010). Döcke (1994) reported a decline of milk progesterone from >10ng/ml to <3 ng/ml when pro-oestrus begins. During oestrus, and ovulation progesterone levels drop to <0.5 ng/ml (Wiltbank *et al*., 2014). During the first 4-6 days following ovulation progesterone levels slowly rise and reach a maximum concentration at 10-17 days (Squires, 2010).

2.2 Oestrus Behaviours

The term oestrus is a Latin adapted Greek word “Oistros”, which was used to describe a “period of sexual desire in a female” (Rao *et al.,* 2013). Oestrus behaviour is a period of attractiveness, proceptivity and receptivity (Rao *et al.,* 2013). During this time, the cow is sexually receptive. Increasing concentrations of oestrogens directly affect the cow’s reproductive tract and influence behavioural changes. Behaviours associated with oestrus include primary and secondary behavioural signs.

2.2.1. Primary Oestrus Behaviour

A distinctive primary sign of true oestrus, is the standing heat period which is still considered to be the most reliable behaviour to indicate oestrus (Allrich, 1994; Van Vilet and Van Eerdenburg, 1996; Cutullic *et al.,* 2009; Palmer *et al.* 2010). During standing heat, the cow will stand to be mounted by herd mates with no attempt to escape (Hurnik *et al.,* 1975; Ball and Peters, 2004). Twenty percent of mounts received by an animal in standing oestrus, are typically carried out by animals that are not in oestrus themselves (Hurnik *et al.,* 1975). This has been described as an ‘altruistic act’, performed to attract the attention

of the bull to the animal that is standing (Phillips, 2002; Roelofs *et al.,* 2010). It is documented that mounting behaviour increases when more cows are in oestrus simultaneously (Hurnik *et al.,* 1975; Roelofs *et al.,* 2005a; Sveberg *et al.,* 2011; Zebari *et al.,* 2019).

Standing heat is considered to be day 0 of a new cycle (Palmer *et al.,* 2010; Rao *et al.,* 2013). Dairy cows typically have oestrous behaviours approximately every 21 days (Forde *et al.,* 2011). Approximately 30 hrs after the onset of oestrus, once behavioural indications have ceased, the dominant follicle reaches maturity and ovulates (Divers, 2008). The duration of the oestrus period depends on factors such as breed, feed intake, environment and health status (Firk *et al.,* 2002). For example, some oestrus periods may last from 10-18 hours, whereas in modern high yielding cows it may be shortened to 8 hours (Divers and Peek, 2008). It has been reported that oestrus duration in river and swamp buffaloes' can range anywhere from 5-27 hours, with ovulation occurring from about 24-48 hours after the onset of oestrus (Kanai *et al.,* 1990).

2.2.2. Secondary Oestrus Behaviours

Assessment of secondary oestrus characteristics can also be evaluated and included in the criteria for the determination if a cow is in oestrus. Secondary oestrus behaviour(s) include increased locomotion and restlessness (Kiddy *et al*., 1977; Roelofs *et al.,* 2010), increased body temperature (Firk *et al*., 2002), increased physical interactions (sniffing, head butting, chin resting, playful behaviours) (van Eerdenburg *et al*., 1996; Roelofs *et al*., 2005; Sveberg *et al.,* 2011), ano-genital licking and sniffing (Hurnik *et al*., 1975; Phillips and Schofield, 1990), observable mucus flow (Layek *et al.,* 2013), bellowing (van

Eerdenburg *et al*., 1996; Sveberg *et al.,* 2011), and the flehmen response (Sveberg *et al.,* 2011). Detection of oestrus is achieved through evaluation of primary and secondary behaviours. Many oestrus detection methods have been developed and will be discussed below.

2.3 Oestrus Detection

Oestrus detection is vital in order to sustain dairy herd productivity. Dairies often set a target to achieve a 365-day production cycle whereby the cow has a lactation length of 305 days, allowing for a 12-month calving interval, with 10 months producing milk, and a two-month drying off period (Butler *et al.,* 2010). In order to achieve a 365-day production, cycle the dairy cow must become pregnant within 85 days after calving (Ball and Peters, 2004). Therefore, accurate determination of oestrus is crucial in order to achieve this target. Oestrus detection rates directly influence milk production and calving interval (Fischer-Tenhagen *et al.,* 2011). It has been reported that the average oestrus detection rates on commercial dairy farms range from 30-50% (Becker *et al.,* 2005; Fetrow, 2006). Various technical aids have been developed and implemented to improve oestrus detection rates, in an attempt to maximise reproductive performance and milk production in high yielding dairy cows. Technical aids such as activity measuring devices, mount detectors and video cameras have improved oestrus detection rates to 90%, in particular if more than one method is utilised (Peralta *et al*., 2005). The implementation of some aids may be preferred over others due to the complexity of use and/or financial costs to use such technology (Fischer-Tenhagen *et al.,* 2011). However, detecting oestrus continues to challenge producers worldwide despite the development of many aids.

2.4 Oestrus Detection Methods

2.4.1 Visual Observation

In previous years, oestrus detection relied heavily on continuous visual, or video observations made by skilled stockmen in order to record oestrus duration, and behaviours associated with oestrus, particularly the number of mounts a cow receives (Esslemont and Bryant, 1976; Esslemont *et al*., 1980; Hurnik *et al*., 1975). Some farmers continue to use the observation of standing to be mounted as the only criteria to determine whether a cow is in oestrus or not (Palmer *et al.,* 2010). However, as the expression of oestrus behaviours has changed over the years, increasing emphasis has been made to include secondary oestrus behaviours which may be measured on an oestrus intensity point scale. The scale was developed by Van Eerdenburg *et al.* (1996) to determine the oestrus intensity for a specific animal. Table 3-10 (Chapter 3, section 3.4) describes the intensity points scoring scale where a specific behaviour is allocated points, and as the cow increasingly expresses these behaviours, the cow receives more points. The higher the points, the greater the oestrus intensity. Therefore, herdsmen could decide if a cow was in oestrus based on the intensity points. This is beneficial as standing to be mounted period has been significantly reduced or may be absent all together. Oestrus intensity varies between breeds. Studies comparing Senepol, Angus and Brahman cows reported that oestrus was more intensely expressed during the initial three periods (9h) after the onset of oestrus occurred (Landaeta-Hernández et al., 2002; Landaeta-Hernández et al., 2004a). Whereas Zebu cattle had increased mounting activity during the first and last periods of oestrus (Mattoni *et al.,* 1988).

Cows that are in oestrus may initiate social interactions as well as cows that are not in oestrus themselves. Chin resting may indicate that a cow is in oestrus as the cow is motivated to engage in oestrus behaviours. Similarly cows not in oestrus may rest their chins on the backs of cows that are in oestrus as they are stimulated by pheromones produced by the cow in oestrus (Sveberg *et al.,* 2011). Chin resting and anogenital sniffing can be performed by cows that are not in oestrus, therefore these behaviours may be less predictive of true oestrus than mounting (Phillips and Schofield, 1990), and should be carefully considered. However increased frequency of these signs around predicted oestrus may be used to gauge oestrus intensity, in addition to identifying cows in oestrus from those that are not (Sveberg *et al.,* 2011).

Natural chemicals such as pheromones play an important role in the reproductive behaviour in both male and female animals (Fischer-Tenhagen *et al.,* 2011). Flehmen is an olfactory response required for the perception of biochemical communication (Sankar and Archunan, 2004). In cows, the urine or genital area may be sniffed which can stimulate the flehmen response in either bulls or cows. This enables the cow/bull to determine if an individual is in oestrus. Therefore, this behaviour may be used as an indicator to identify cows in oestrus.

Playful social interactions such as head-butting/rubbing and grooming can also be an indicator of oestrus (van Eerdenburg *et al*., 1996; Roelofs *et al*., 2005; Sveberg *et al.,* 2011), As some cows in oestrus seek out social interactions, they may engage in the behaviours described above (Kerbrat and Disenhaus, 2004). However, headbutting can also be aggressive in nature, whereby cows

establish and maintain social order in the herd (Rousing and Wemelsfelder, 2006). Competition for resources such as food, water, and stall space may also occur, particularly in housed conditions (Bouissou *et al.,* 2001). Therefore, an inexperienced observer may misinterpret these interactions as oestrus behaviours.

Observable vaginal mucus discharge may indicate the reproductive status of cow and may also be an indicator of the onset of oestrus, different stages of oestrus and ovulation time (Alena *et al.,* 2008). Increased concentrations of oestrogen during the periestrual period influences the reproductive tract making it highly secretory and tonic, leading to an increased thick mucus flow (Layek *et al.,* 2013). Therefore, the presence of thick mucus may be used as an indicator for oestrus detection. Properties of the discharge should be checked as anything but clear may indicate a reproductive disorder (Sheldon *et al.,* 2008). Additionally, it is quite common for cows to have mucus discharge when they are not in heat, therefore observation of mucus should be carefully assessed along with other indicators of oestrus. All cows produce cervical mucus during oestrus, however it may not be visible as the mucus may not be expelled by all cows during heat (Lim *et al*., 2014).

The efficiency of visually detecting oestrus in cattle varies greatly among studies. It has been reported that standing heat was observed from 90% (Hall *et al*., 1959) to less than 50% (Van Vilet and Van Eerdenburg, 1996; Van Eerdenburg *et al*., 2002; Roelofs *et al*., 2006). However, accurately determining if a cow is ready for insemination based solely on visual observation requires a highly skilled stockperson making frequent observations throughout the day.

Roth *et al.* (1987) suggested at least five, 20-30-minute observations are required in order to effectively detect oestrus. Additionally, Yoshida and Nakao (2005) reported a shorter oestrus duration when using visual observation. Roelofs *et al.* (2010) reported a detection rate of 90% detection with three daily observations of 30 minutes. Whereas Looper (2005), reported that detection rates of 75-80%, 85%, and 90 % may be achieved through 2x30 minute, 3x30 minute and 4x30 minute observations per day respectively. Esslemont and Kossaibati (1997) suggested making observations between 6-8am, 12-2pm, and 9-11pm. Diskin and Sreenan (2000) reported a detection rate of at least 70% when cows were carefully observed in the early morning and late evening, the rate increased to 90% when three additional checks were made in around 4-5-hour intervals. However, staff members may not be able to observe the herd at multiple times due to other responsibilities, and large herd sizes. Some may carry out observations at convenient times such as during feeding and milking. Although these times are convenient, they should be avoided (Gillespie and Flanders, 2010) as observing for oestrus during these times are not ideal, as cows may be crowded, thereby limiting the space to perform oestrus behaviours.

Effectiveness of visual observations may also be limited as the time the cows display oestrus behaviour may not be during the time the staff are making their observations. Many studies have suggested to observe cows at particular time periods during the day, as cows are more likely to display at these times. A study by Diskin and Sreenan (2000) reported that cows have a distinct pattern of oestrus expression, with the most activity occurring early in the morning and late at night. Other studies suggest dairy cows have increased oestrus

behaviours in the evening and throughout the night (Hall et al*.,* 1959; Orihuela *et al.,* 1983; Mattoni *et al*., 1988; Homer *et al.,* 2013), or during early hours of the morning or daytime (Hurnik *et al.,* 1975; Amyot and Hurnik, 1987; Gwazdauskas *et al.,* 1990; Van Vilet and Van Eerdenburg, 1996; Walker *et al.,* 2008a). These differences may depend on varying managerial implementations between establishments. Contrastingly some studies suggest there is no difference in the time of day oestrus is expressed (Esslemont and Bryant, 1976; Esslemont *et al.,* 1980; Alexander *et al.,* 1984; De Silva *et al.,* 1981; Xu *et al.,* 1998). Although recommendations are in place, they may not be feasible for all herds, as the herdsmen may not be present to observe the cows. Additionally, lame cows have been identified to display oestrus behaviours less frequently in the early morning when compared to non-lame cows (Walker *et al*., 2008a). This could be a result from an altered behavioural repertoire due to lameness. Lame cows are typically lower ranking and can alter their time budgets to avoid higher ranking cows (Galindo and Broom, 2000). For example, the cow might be lying down for much of the night, and therefore may spend the morning eating when other cows may be engaging in oestrus behaviour. Therefore, if staff members designate their time to visually observe the herd early in the morning, lame cows may go undetected by visual observation alone.

Climate of the surrounding environment may also play an important role in dairy cow oestrus expression. Studies report that climate may affect both oestrus duration and intensity. For example, Lamothe-Zavaleta *et al*. (1991) reported that when temperatures were below 27°C the duration of oestrus was 12.4 hours whereas at temperatures above 27°C duration decreased to 9.3 hours.

Contrastingly it has been documented that oestrus behaviour in zebu type cows increased during the hottest months of the year (Galina *el al*., 1996). Alves *et al.* (2009) reported that Guzera cows were not affected by heat stress during the summer months, and that they had a natural oestrus length of 13.18h (±1.17), and 11.64 (±0.98) in the winter. Identification of heat-tolerant cows within high producing breeds has assisted in the genetic selection towards thermotolerant dairy cattle (Das *et al*., 2016). For example the '*SLICK'* haplotype has been introduced into Holstein cattle, resulting in lactating Holsteins with slick hair resulting in superior thermoregulatory ability compared with wild-type Holsteins (Dikmen *et al*., 2008).

However, as the demand for dairy products increases, an intensification of dairy production has occurred. Increasing animal numbers within dairy herds means that the amount of time farm staff can devote to visual observation alone is increasingly difficult. Not only do they have reduced time to accurately observe the herd, they may be incapable of observing so many animals at any given time. Therefore, the development and use of other techniques to complement visual observations include tail or chalk painting, pressure activated heat mount detectors or scratch cards, pedometry, radiotelemetry, hormone treated or vasectomised bulls, vulvar and intravaginal electrical impedance, and even scent dogs trained to detect oestrus specific odours (Firk *et al*., 2002; Cavalieri *et al*., 2003; Fischer-Tenhagen *et al.,* 2011).

2.4.2 Teaser Animals

The use of surgically altered bulls, bulls across a fence line, and hormone treated cows have been used for oestrus detection. The presence of a male

animal has been reported to have a positive bio-stimulatory effect on the onset of oestrus in sheep and goats (Rekwot *et al.,* 2001), to increase oestrus expression in sows (Langendijk *et al.,* 2000), and to have a positive effect on bovine fertility parameters (Custer *et al*., 1990, Fernandez *et al*., 1993; Fike *et al*., 1996).

2.4.2.1 Altered Bulls

Surgically altered bulls may be used to detect cows in oestrus without the risk of conception. Surgical alterations are achieved either through vasectomising, or penile alterations (Hendrickson and Baird, 2013). Bulls can undergo penile surgery to prevent contact with the cow either through a plastic device attached to the sheath preventing contact, or so that the pathway of his penis is deviated so it exits to the side of the bull, making natural service impossible (Christenson, 1974). However, this surgery is quite invasive and can cause ethical implications. Vasectomising is less invasive, and fertilisation will be inhibited as the vas deferens are cut, thereby not enabling spermatozoa to be released into the cow (Hendrickson and Baird, 2013). A teaser bull may be fitted with a chin ball marker, so that after mounting a cow, the back will be marked with paint when the bull dismounts (Halley and Soffe, 1988).

However, housing dairy bulls can be difficult, as they are known to be highly aggressive (Price, 2002). Therefore, some producers may use vasectomised beef bulls for this reason, as they are usually slightly smaller in stature which enables safer handling for the farmer, and easier management in free stall housing (Hopper, 2014). When choosing a teaser bull, considerations must be made in terms of selection. Bulls selected should have a mild temperament, be

a moderate adult size, and have a strong libido (Morgan and Dawson, 2008). Determining if a young bull has a strong libido is difficult to predict (Morgan and Dawson, 2008). If a teaser bull is purchased it is difficult to ascertain if the bull carries venereal diseases.

If a producer chooses to use a homebred bull, there is no guarantee that the bull will be an effective teaser animal (Morgan and Dawson, 2008). Additional costs of surgery for the homebred bull may also limit application in the industry. Furthermore, post-surgery complications may arise rendering the bull ineffective, or may require post-surgical care costing additional money. Additional costs are required for maintenance of the bull (feed, veterinary care etc.), and depending on herd size more than one bull may be required to cover all of the cows in the herd. During a study assessing teaser bulls, it has been recommended that 1:100 ratio is effective (Norton, 2008). It should however be noted that in the study by Norton the teaser bull was rotated with a fresh bull every 2 days. Introducing a fresh bull every 2 days reduces the chances of fatigue, and lameness for that animal thereby potentially maintaining a high level of oestrus detection continuously. However, this is not very practical for most dairy farms.

2.4.2.2 Hormone treated animals

In the past cows, steers or freemartin heifers could be treated with testosterone and oestrogen, or just testosterone so that they may be used as a teaser animal (Varner, 1914; Nix *et al*., 1998). Although treated steers or cows may be less aggressive than a bull, there was still an increased risk of aggression when dealing with hormone treated cows. Particularly as they are required to be

treated with the hormone(s) every 2-4 weeks (Varner, 1914). There were added costs for housing the animal, cost of treatment, and a withdrawal period if the animal was intended for slaughter. However, this practice is a violation of the Animal Medicinal Drug Use Clarification Act, and is now illegal (Morgan and Dawson, 2008).

2.4.3 Electronic Vaginal Probes

During the period of oestrus, there is an increased amount of mucous and ionic content within the vagina, thus making the environment more able to conduct electricity (Gupta and Purohit, 2001; Rorie *et al*., 2002). Some probes have been designed to detect a fall in the electrical resistance within the vagina (vaginal electric impedance (VEI), which typically lasts for no more than 24 hours (Ball and Peters, 2004), and therefore indicating oestrus. VEI has been shown to have a marked decrease in the period prior to ovulation in many species such as; pigs (Ko *et al*., 1989; Řezáč, P. and Olič, 2006), horses (Larsen and Normal, 2008), cattle (Peters, 1989), sheep (Bartlewski *et al*., 1999), buffalo (Gupta and Purohit, 2001), blue fox (Moller et al., 1984), and rhesus monkeys (Fischer *et al*., 1990). VEI has been successful in detecting oestrus in the mare (1-3 readings per day) (Larsen and Norman, 2008). However, there has been a large variation of readings reported between cattle, additionally this method has proved to be labour intensive. It is suggested that readings be taken at least twice a day, a few days prior to the suspected time of oestrus (Rorie *et al.,* 2002). There is also a vast range of readings between cows that may be due to variables such as position of the probe within the vagina (Foote *et al.,* 1979; Heckman *et al.,* 1979), depth inserted into the vagina

(Aboul-Ela *et al.,* 1983), pressure exerted against the mucous membranes and possible pathological conditions (Leidl and Stolla, 1976). A study by Kitwood *et al.* (1993) reported that when readings were taken at 15 and 20 cm from the vulva the recordings were significantly lower than at other fixed depths, however there were no changes in readings during oestrus at that point. Therefore, if readings were taken at 15 and 20 cm from the vulva when a cow is in oestrus, the cow may go un-detected as the electrical resistance would not be reduced. Research has concluded that an electric probe measuring the electrical resistance could be used to predict ovarian status, and the stage of the oestrous cycle in order to determine the time of onset of oestrus and insemination (Tadesse *et al*., 2011; Malakar *et al*., 2017), but does not greatly increase oestrus detection rate alone (Kitwood *et al*., 1993). Gupta and Purohit (2001) reported that inseminating buffaloes at a low vaginal electrical resistance improves the conception rate by almost 65%. This method may be less invasive than rectal palpations, however it is not practical, and multiple readings can stress the cows. This process is time consuming as the external labia of the cow should be cleansed prior to insertion, in addition to the disinfection of the probe between each cow to reduce contamination and possible risk of infections. The cost (USD) of probes range from $540 (Farm Tech Solutions, 2017), $1695 (Animark, 2019), to $2000 (Rorie *et al.,* 2002). Implantable electrodes have been proposed, however the implants also have drawbacks and commercial systems have not yet been introduced (Andersson *et al*., 2016).

2.4.4 Mount Recording Systems

2.4.4.1 Tail painting

Tail painting involves the application of paint or chalk to the coccygeal vertebrae of the cows' tail head prior to oestrus (Boyd, 1984). As the paint dries it hardens, therefore the disappearance of the paint/chalk is taken as a signal of mounting activity (Xu *et al.,* 1998; Firk *et al.,* 2002. However, it has been reported that the paint/chalk may not always be removed during mounting activity (Firk *et al.,* 2002). The practice of tail painting combined with visual observation has been researched. Results vary among authors. For example, Xu *et al.* (2002) reported high oestrus detection rates (98.4%) when a combination of mounting recording and visual observations were implemented. However, a study by Palmer *et al*. (2010) reported that the accuracy and efficiency of tail paint was not enhanced significantly with the combination of visual observation. Varying results among studies depend on the frequency of visual observations and herd size. For example, using between 5-12 visual observations per day was reported to be 13% more efficient than using tail paint alone (O'Farrell, 1984).

False positives can occur with tail paint and chalks. Macmillan and Curnow (1977) reported a false positive rate of 5% (Macmillan and Curnow, 1977). Another study by Trombello and Shanks (2008) reported a false positive rate of 17%. It has been reported that paint/chalk may be removed from licking, rubbing on brushes or stall bars (AHDB, 2017c). Brushes are often installed in cattle sheds to provide the cows with enrichment through grooming, rubbing, and or scratching. However, brushes may cause the removal of the paint/chalk thereby indicating a false heat. Paint/chalk may also be removed from general cow activity, grooming, and interactions that may not necessarily be stimulated from

oestrus. Although direct paint licking is reported on dairy farms, the behaviour is rare compared to other types of social licking (Skenandore and Cardoso, 2014). For example, a study by Skenandore and Cardoso (2014) reported that heifers received more than one paint lick in less than 2% of all observations. Paint licking may also be mistaken for anogenital sniffing, which is a more frequent behaviour (Skenandore and Cardoso, 2014; Skenandore and Cardoso, 2017). When cows are being brought in for milking, some animals may get bumped by others, or chin resting may occur in confined spaces, which can also remove the paint. Additionally, cows that are in oestrus may attempt to mount cows that are not in oestrus themselves, thereby removing chalk on cows that are not in heat. In indoor housing systems, some cows may get trapped/cornered in stalls by cows in oestrus trying to mount them, thereby indicating a false heat.

2.4.4.2 Pressure Activated detectors

Pressure activated detectors are small apparatuses that are applied to the tail head of the cow with an adhesive, and are used to identify ‘true oestrus events’, that is a cow that was mounted during standing heat. Once the cow has been mounted, the pressure will activate the detector, which will (depending on the design) initiate a colour change or send a radio message to a receiver to record the number of times a cow has been mounted (Sheldon *et al*., 2006). Thus, indicating a cow is in standing heat. At-Taras and Spahr (2001) detected 86.8 and 71.1% of oestrus in two separate trials by use of pressure mount detectors. Some pressure mount detectors available are Kamar®, Estrotect™, Bovine Beacon®, and the HeatWatch® System.

2.4.4.3 Detectors with colour change

Brands of mount detectors that are commercially available that indicate a colour change after mounting are Kamar®, Estrotect™, and the Bovine Beacon®. The Kamar® mount detector requires a cow to be mounted for a minimum of three seconds to activate the capsule (van den Berg, 2014). Following adequate mounting, the capsule ruptures and releases red ink into the surrounding area (Foote, 1975, Sheldon *et al*., 2006, Holman *et al*., 2015).

Bovine Beacon® mount detectors are similar to Kamar®, however with these the pressure during mounting ruptures a capsule, which emits a chemiluminescent indicator/glow (Herriott, 1994). The glow emitted from the Bovine Beacon® is due to a peroxyoxalate chemiluminescent reaction that takes place in response to the pressure received from mounting (Herriott, 1994). Bovine Beacon® detectors activate after a single mount, and also produce a glow that lasts for approximately 12-18 hours and is visible up to 1/8 of a mile (Bovine Beacon®, 2013).

Estrotect™ breeding indicators are a small sticker (8x3 cm) that have a self-adhesive design and are coated with rub off silver, and black ink. The design resembles that of a scratch card, when the cow stands to be mounted, the silver and black surface is removed due to the friction of mounting (van den Berg, 2014). Therefore, the more mounts a cow receives results in a more obvious colour change (Holman *et al*., 2015).

Efficiencies of mount detector vary greatly between studies. A study by Perry (2005) reported that Estrotect™ mount detectors correctly identified 91% of oestrus events. However, a study by van den Berg (2014) reported that only 36% of Estrotect™ detectors were correctly activated on the day of oestrus. A study by Holman (2011) identified that scratch cards were less efficient at detecting mounting behaviour when compared to the Kamar® mount detectors. However, the Kamar® gave a higher incidence of false positives, thus causing misinterpretation of mounting behaviour. Another study reported a rate of false positives of 26% (AHDB Dairy, 2017b). The detectors may be falsely triggered/and or removed from the surrounding environment such as stall bars, other cows, cow brushes in the house (Borsberry, 2011), and trees, bushes etc. if they are at pasture. A study by Gwazdauskak *et al*. (1990) reported a loss of rump mounted detectors (including Kamar®) exceeded 40%. A study using by Rich (1972) reported that 13 heifers were visually observed standing to be mounted for as many as 4 mounts, and the Kamar® mount detectors failed to indicate any colour change. This study is quite dated, and the detectors may have been improved since that study.

Detectors can also be removed by other cows through curiosity. Cows have been observed to lick, and sniff detectors applied to their herd mates, thus increasing the risk of false positives and or removal of the detectors all together. False positives are more frequent in crowded pens such as collecting yards (Noakes *et al*., 2009), and can occur if a cow is trapped when being mounted by a cow that is perhaps coming into heat (Noakes *et al*., 2009). The detectors can become detached due to moulting, or if the detectors are placed on dirty or wet fur (Noakes *et al*., 2009). The mount detector adhesive may be affected by

temperature, which can cause them to fall off. Estrotect™ for example, requires the detectors adhesive to reach body temperature before application to clean dry hair (van den Berg, 2014). This may be more difficult in colder climates.

Visibility of the detectors can also be reduced. For example, during the winter some locations have only 6-8 hours of day light. With shorter days, one or both of the observable times for oestrus detection (time of milking) will be dark. Another factor that makes mount detectors difficult to see is that the colour of the cows' body can be in low contrast to the mount detector (Herriott, 1994).

Applying detectors take time, in addition to the reapplication of any that fall off. If the cows are housed indoors with cubicles, reapplying can be easier, and the cows can be secured in a cubicle while a new detector is applied. However, if the cows are at pasture reapplying detectors can be more difficult as they may not be willing to stand. If the detector goes missing overnight, this can greatly hinder oestrus detection, as the herdsman cannot definitively say whether or not the detector was removed from mounting activity, or in fact if the oestrus was missed. Similarly, if the detector is removed after milking (a time when detectors are typically checked), if a cow stands to be mounted, this will not be indicated clearly. The staff can make an educated decision whether or not the cow is indeed bulling. However, these considerations must be made when using aids such as detectors. Coupled with visual observation they can improve detection rates.

2.4.4.4 Radiotelemetry

Radiotelemetric devices utilise a pressure sensitive transmitter attached to the tail head, which records the number of times a cow is mounted (Xu *et al.,* 1998; Dransfield *et al.,* 1998). Palmer *et al.* (2010) utilised visual observations combined with HeatWatch® II System transmitters to record mounts made to each individual cow. Overall during visual observations HeatWatch® II System recorded fewer occasions when cows displayed standing oestrus than the observer. Another study also found that HeatWatch® II System missed a mean of 42 percent of mounts per standing oestrus and that the duration was 24 percent shorter than by continual visual observations (Cavalieri *et al.,* 2003). Possible detachment of the transmitters could have caused the inefficiency of HeatWatch® II System in the study done by Cavalieri *et al.* (2003). Palmer *et al*. (2010) did not report slippages, however they did identify a lag period in HeatWatch's operation. When a cow is mounted, the signal is sent from the transmitter to the receiver, which is thought to have a 30-60 second lag time before the next signal can be processed (Cavalieri *et al.,* 2003). Therefore, during times of intense oestrus activity (mounting) there may be an increase in the number of signals that cannot be processed. Palmer *et al.* (2010) also reported lower accuracy of oestrus detection in cubicle housed cattle opposed to cows at pasture. This was possibly due to the transmitters being activated by pressure from side bars in a cubicle when cows were lying down. Additionally, they also recorded false mounts as some cows were trapped in cubicles, thus unable to escape.

As previously discussed, the fertility of the modern dairy cow is declining with increasing milk yields (Royal *et al*., 2000, Butler, 2003, Dobson *et al*, 2008).

Alongside the decline of fertility, the occurrence of standing to be mounted has decreased over the years (Lyimo *et al*., 2000, Van Eerdenburg *et al*., 2002, Roelofs *et al*., 2005b, Walker *et al*., 2008a). Therefore, the use of pressure activated mount detectors may not be as effective as they once were 50 years ago. The implementation of other detection aids has been developed to compensate for the reduction in standing to be mounted periods.

2.4.5 Activity Monitors

2.4.5.1 Pedometer & Accelerometer

Utilising electronic locomotion devices can assist in recording a cows' activity levels. Walking, standing rest period and lying periods may be quantified using a pedometer and/or an accelerometer. Typically, older pedometers simply measured step count, whereas accelerometers work in 3D, which can measure the time spent lying down and also alterations in gait (Chapinal *et al.,* 2010). Both a neck version and leg version have been developed that identifies increased physical activity (Aungier *et al.,* 2012). They may be attached around the neck with a collar, or to the hind leg by a plastic or Velcro band (López-Gratius *et al*., 2005). Cows typically increase their physical activity during the oestrus period (López-Gratius *et al*., 2005; Roelofs *et al*., 2017), and are approximately 2 to 4 times more active (Kiddy, 1977). Pedometers identify when a cow's activity level increases, potentially indicating that she will be coming into oestrus. Schofield *et al.* (1991) reported significantly higher activity rates on the day of oestrus than on other days. Additionally, it has been documented that the number of steps taken each hour by a cow in oestrus is approximately two to four times higher than during di-oestrus (Kiddy, 1977). Although no significant differences were found between nocturnal and daily

activity (Lewis and Newman, 1984; Schofield *et al.,* 1991; Eradus *et al.,* 1992), Arney *et al.* (1994) reported higher locomotion rates in the afternoon and evening. This may depend on management and environmental conditions, for example if the weather is hot during the morning and midday, the cows may reduce their movement due to the ambient temperature.

Efficiency and accuracy differences between the neck and leg versions are apparent. These differences may be a result of how the pedometers work, attachment point, or housing conditions. For example, a study by Sakaguchi *et al.* (2007) determined that attachment of a pedometer to the neck was not as accurate at detecting oestrus under grazing conditions in dairy heifers, when compared to pedometers attached to a leg, and it was therefore suggested this was an impractical tool for grazing multiparous cows. Eradus *et al.* (1992) reported higher false positives with neck pedometers when compared to pedometers attached to the foreleg. Differences between front and hind leg placement have been reported to not be significantly different (Schofiled *et al.,* 1991 and Eradus, *et al.,* 1992). A study by Roelofs *et al*. (2017) compared two activity monitors (neck version and leg version) in cows housed indoors and at pasture. They reported no significant differences in the performance of both types of monitors to detect oestrus in different housing conditions.

Previous limitations of activity monitors included unit failure over extended time periods, lack of technical user control of settings for thresholds, failure of blinking lights as flags due to manure covering the tag, and lack of automated recording of data (Moore and Spahr, 1991). Additionally, Borsberry (2011) determined that cows with a body condition score of less than 2.0 (using

Edmonson *et al.* (1989) method), and a lameness score of 2.0 (using the Sprecher *et al.* (1997) method) or more reduces the efficiency of the pedometers. They described that out of 77 false positives, 42% were pedometer related. It has been documented that lame cows have a shorter stride length than non-lame cows (Telezhenko *et al*., 2004; Blackie *et al*., 2013), and therefore require more strides to reach the same distance as a non-lame cow (Rushen and de Passille, 2006; Beer *et al*., 2016). Therefore, if step counts are used the lame cow may be interpreted as bulling if the number of steps exceeds other herd mates. However modern pedometers typically have a threshold for each cow to minimise false positives. Contrastingly lame cows may reduce the time spent walking and may not exhibit typical restless behaviour (Walker *et al*., 2008a), and the lame cow may go undetected.

The method of data transfer can limit the effectiveness of activity monitors. For example, some devices store the data in block periods, and download the information when the cow is within close proximity of an antenna/downloading system, usually at milking time. Therefore, the ability to compare individual cow activity against the herd is limited until milking is complete (Morton, 2011). Additionally, if the cow is within the proximity of the system every 12 hours, it could go undetected if the cow is in heat for a short period of time around the time of download. Therefore, the increase in activity would be hindered by the fact that the cow is being milked, and the increase in activity would go undetected.

Management procedures can also induce false positives in the activity monitors. If the devices measure step counts, or activity thresholds, and on a

particular day the cows were turned out to pasture the system may falsely indicate an oestrus (Roelofs *et al*., 2017). Systems may transmit a heat alert, or show a marked increase in activity, leading to a potential false heat. The increase in activity would be correct, however to determine if it was due to an actual oestrus event would be difficult unless the animal was observed to be displaying oestrus behaviours, or if other measures such as mount detectors or progesterone testing were used. The cow may have indeed been in oestrus at the time of turn out, however this may be masked by pasture turnout (Roelofs *et al*., 2017). Additionally, if cows are rotated between pastures, there will be an increase in activity, particularly if pasture sizes are different (Hart *et al*., 2013). Therefore, on days that the herd is subjected to alterations in routine management procedures, careful attention must be made to account for potential missed heats.

The presence of external factors such as pedestrians, dogs, weather, new herd mates, going out to pasture etc. may have the potential to increase herd activity. For example, a study by Nakanishi *et al*. (1993) reported that introducing a new cow into a herd showed large increases locomotion at the expense of lying and eating time. Lying and eating time returned to normal on the 8th day after moving cows (Krohn and Konggaard, 1980; Nakanishi *et al*., 1993). Perhaps around the time a new cow is introduced extra observations could be made to ensure oestrus is not missed.

Battery life has been shown to vary between systems. Current activity monitor batteries can last from to 5 (Cow Alert, 2017) to 10 years (Martinez, 2012).

Faults may occur such as batteries failing prematurely, which could cause disruption to heat detection.

As cows can participate in oestrus activity regardless of whether they are in oestrus themselves, activity monitors may produce false positives for some cows. If a cow that is either pregnant, or not in oestrus participates in oestrus activity (increased movement, standing to be mounted etc.), it may be misinterpreted as oestrus. This can be catastrophic if the cow is pregnant, as re-insemination can cause abortion of the foetus (Sturman *et al*. 2000). Struman *et al*. (2000) reported approximately 7% of pregnant cows being reported as in oestrus, with 3% standing to be mounted. Use of additional measures may assist in detecting pregnant cows that engage in standing behaviours. Milk progesterone tests can identify if a cow is pregnant, depending on how long ago she was inseminated. Some tests can detect pregnancy at from 18-25 days' post insemination (Faustini *et al*., 2007). Using milk progesterone tests would be beneficial to test cows further along in pregnancy that may be showing signs of heat.

The use of activity monitors could also indicate other health issues, which may also indicate a false heat. For example, it has been reported that cows with acute mastitis increase their step counts on the day they were affected (Siivonen *et al*., 2011). Although this may indicate a heat falsely, the use of activity monitors may assist in preventing debilitating diseases and or lameness. Therefore, it may be useful to examine cows flagging up as in heat as she may have an underlying illness.

2.4.6 Progesterone Assays

Monitoring progesterone concentrations in blood plasma or milk have been widely utilised to determine oestrus and ovulation by observing the period of low progesterone followed by the subsequent increase (Plotka *et al.,* 1967; King *et al.,* 1976; Walton and King, 1986; Darwash *et al.,* 1999; Petersson *et al.,* 2006a; Simersky *et al.,* 2007). Pairing the measurement of progesterone levels with monitoring behaviour has been successful in oestrus detection (Friggens *et al.,* 2008; O'Connell *et al.,* 2011). However, collecting blood plasma samples is an invasive, time consuming procedure and is not a viable option for most dairy enterprises. Utilising milk as progesterone source is more convenient as milk samples may be readily collected daily at milking time (Oku *et al.,* 2011). Milk progesterone measurements are subject to a large variability, partly caused by sampling technique, or the fat content in the milk (Pennington *et al*., 1981; Friggens *et al*., 2008), calibration method and measurement technique (Adriaens *et al*., 2017). Despite these variations, milk progesterone has been successfully used to obtain a clear image of a cow's reproduction status (Friggens and Chagunda, 2005; Martin *et al*., 2013). A study by McLeod *et al.* (1991) determined that milk progesterone testing accurately predicted 99% of ovulations in their study group (n=88).

Using cow-side progesterone analysis can provide farmers with the ability to use milk progesterone for heat detection more readily than in previous years (Ingenhoff *et al*., 2016). Some milk progesterone kits require the samples to be prepared in stages, with the use of standards, which can be time consuming and not ideal for the producer, especially with large herds. The development of the P^4 rapid kit is a dip stick principal, where milk is collected and the strip is

placed in the sample for >5 minutes to determine the concentration (Ingenhoff *et al*., 2016). Utilising a quicker method such a P^4 rapid may increase the uptake of using milk progesterone to detect oestrus (Ingenhoff *et al*., 2016), which would be beneficial as modern dairy cows may not display oestrus in the conventional way (standing to be mounted). Some pregnant cows may stand to be mounted, and if this is interpreted as true oestrus, insemination can cause abortion of the foetus. Therefore, milk progesterone can also help identify if a cow is falsely in heat, to confirm pregnancy, to determine if she is cycling normally, and if there are potential problems such as cysts (Yu and Maeda, 2017). If cows are fitted with an additional detection method (mount detector, pedometer, etc.) milk progesterone can assist the farmer in accurately identifying cows that are in true oestrus. As mount detectors can give false readings from external influences (trees, stall bars, etc.) (Borsberry, 2011), utilising milk progesterone alongside other oestrus aids may assist in clarifying if the cow is cycling, or if it is a false positive (Yu and Maeda, 2017). Although results can be read at >5 minutes, the uptake may be limited due to the perception of additional responsibilities during milking. Additionally, if the results are not readable by the time the cow is milked, the herdsman will have to suspend milking the next batch of cows until the results are clear. Alternatively, the cows being tested could be moved to a holding pen, however this may be time consuming and not feasible for all establishments. Integrating a milking system that has an automatic in-line milk progesterone analysis would eliminate the additional responsibilities required for the dipstick tests (Yu and Maeda, 2017). A management program called Herd Navigator has been developed during the 2000s and has been installed on farms since 2009 (Birgersson, 2013). The system analyses milk parameters from individual cows

to monitor reproductive health (Adriaens *et al*., 2017; Bruinje *et al*., 2018), mastitis, and energy protein balance (Mazeris, 2010). In an e-mail on the 25th of April 2017 K. Fitzgerald quoted the cost of installing the Herd Navigator for a 300-cow herd milking with a 32/32 (16 per side) will be £123 per cow/year. Although expensive, K. Fitzgerald also stated that an expected gross benefit from using this system to improve the management of reproduction, udder health, and nutrition of £200-£250 per cow per year, equating to a net gain of £77-£127 per cow/year. Mazeris (2010) reported that Herd Navigator can bring profit improvement potentials for farmers from 250-350 euros per cow per year. Another system called the Milkalyser has been developed in the U.K., this can be retro-fitted to existing robotic milking machines (BBSRC, 2016).

2.4.7 Body Temperature

A healthy dairy cow has an internal body temperature range between 38-39.3°C (Divers and Peek, 2008; Ortiz *et al.,* 2015; Salles *et al.,* 2016). It has been reported that sexually mature cows have a consistent rhythm of body temperature, showing a marked rise of approximately 1.3°C every 21 days on the day of oestrus (Kadzere *et al.,* 2002). However, other studies report a lower increase of approximately 0.2-0.6°C (Fordham *et al.,* 1988; Kyle *et al.,* 1998). There are different methods to measure animal body temperature in animals and these include; rectal thermometers (mercury, digital); tympanic infrared thermometer; thermal microchips; and infrared thermal imaging (Goodwin, 1998; Chen and White, 2006; Greer *et al.,* 2007; Robinson *et al.,* 2008; Johnson *et al.,* 2011).

2.4.7.1 Internal temperature measurements

Accurate representation of core body temperature is obtained internally. This is the most effective measurement for body temperature. Determining if an animal has an elevated body temperature can be carried out effectively with minimal restraint. However, measuring an entire herd's internal temperatures manually is labour intensive and is not practical in most situations. The use of implanted thermal microchips eliminates the need to manually take rectal or vulva temperatures (Lee *et al*., 2016). Commercially available implantable thermo-transponders are very reliable, but the communication distance between a transponder and a reader is very short (<5 cm) and temperature readings occur only when a reader transmits energy and signal to the transponder (Lee *et al*., 2016). The accuracy of measurements from different thermometers changes with animal species. For example, Chen and White (2006) reported that an implanted microchip correlated most strongly with rectal temperature in rabbits than with an environmental non-contact infrared thermometer, and tympanic infrared thermometers. Similarly, rectal temperatures and thermal microchip readings were not significantly different in goats (Goodwin, 1998). Contrastingly, Goodwin (1998) also reported that rectal temperatures were significantly different from microchip readings in horses and sheep. They also determined that tympanic infrared thermometry correlated well with traditional rectal thermometry in goats and sheep, and implantable microchip transponders in goats could be used, as those temperatures also correlated well with rectal temperatures obtained. However, they reported poor correlation with rectal temperatures and the tympanic probe in horses. In cattle, other studies reported that rectal temperatures and tympanic temperatures are more accurate or consistent than subcutaneous locations under dynamic

environmental conditions (Hahn *et al*., 1997; Carroll *et al*., 2009). Additionally, a study by Myers *et al*. (1996) reported that tympanic temperatures were lower than rectal temperatures in swine, and dairy calves and cows, but were not statistically significant.

Rumen temperature bolus systems utilise an indwelling temperature sensing device to automatically monitor individual animal rumen temperatures over time (Knauer *et al*., 2016). Rumen temperature bolus devices are commercially available (Knauer *et al*., 2016), and are closely correlated with rectal temperatures (Bewley *et al*., 2008a; Knauer *et al*., 2016). The cost of these devices ranges from 60-100£ per animal, and can have a battery life of up to 4 years (AgriSmart, 2017). A study by Dolecheck *et al*. (2015) evaluated the use of a rumen bolus for oestrus detection in dairy cattle. They reported that the reticulorumen temperature increased by 0.43 °C during oestrus ($P < 0.01$). However, in their study the bolus recorded reticulorumen temperature twice daily, at the time the cow entered the milking parlour. Therefore, reticulorumen temperature readings at those times most likely did not accurately represent the entire 12-h period between milking's (Dolecheck *et al*., 2015). Temperatures obtained with rumen devices are influenced by season, milking, housing system, and parity (Bewley *et al*., 2008a). Additionally, the rumen environment can be influenced by factors related to feed and water intake, which can affect temperature recordings. It is reported that water intake temporarily, but dramatically, decreases rumen or reticular temperatures (Simmons *et al*., 1965; Yamada *et al*., 2001; Bewley *et al*., 2008b). Following water intake it can take 20 to 120 min for temperatures to return to predrinking

levels (Simmons *et al*., 1965; Brod *et al.*, 1982; Yamada *et al*., 2001). If the system only records temperatures when the cow passes a reader (milking time), false readings can be obtained if the cow drinks water before the reading is done. Other systems are available that can read temperatures continuously and should be considered if the devices will be used for oestrus detection. Use for these devices as an oestrus detection method may be limited due to the impact of many factors that influence rumen temperatures.

2.4.7.2 Infrared Thermography

Infrared thermography (IRT) is a diagnostic tool used to measure the surface temperature of an object. The body surface temperature in animals is a function of blood circulation and tissue metabolism rate (Berry *et al.,* 2003). Changes in surface temperature may be associated with infectious diseases, inflammation, or other physiological processes. Therefore, the physiological state of the underlying cells may be assessed by measuring skin temperature using IRT (Berry *et al.,* 2003). IRT has been used to monitor the welfare of animals in relation to pain and stress in rabbits (Ludwig *et al.,* 2007) cattle (Stewart *et al.,* 2008a ; Schaefer *et al.,* 2012; Stewart *et al*., 2017), and horses (Burton *et al.,* 2010; Hall *et al.,* 2010; McGreevy *et al.,* 2012). Ludwig *et al*. (2007) reported that an optimal area for thermographic measurement of stress in animals is the eye. An increase in eye temperature was positively correlated to cortisol concentrations in response to pain (Stewart *et al*., 2008a; Stewart *et al*., 2008b), stress (Ludwig *et al*., 2007; Stewart *et al*., 2007), and fear (Stewart *et al*., 2008c). Infrared thermography is also a promising non-invasive diagnostic tool in the dairy industry. For example, the early detection of foot conditions

(Nikkhah *et al.,* 2005, Alaasood *et al.,* 2014); mastitis (Polat *et al.,* 2010); oestrus detection and ovulation (Hurnik *et al.,* 1985; Jones *et al.,* 2005; Talukder *et al.,* 2014) have been identified through the use of IRT.

As previously mentioned sexually mature dairy cows have an increase in body temperature during oestrus. IRT measurements for oestrus detection in dairy cows has been explored, and temperatures have been recorded from the flank (Hurnik *et al.,* 1985), and measurements for ovulation have been recorded from the vulva and muzzle (Talukder *et al.,* 2014). Research has determined that measurements taken from the eye can accurately represent core body temperatures as eye temperatures are approximately 1°C lower than core temperatures. Several studies report that the hottest area of the eye called the lacrimal caruncle is closely representative of the core body temperature (Stewart *et al.,* 2005; Stewart *et al.,* 2008a; Gloster *et al.,* 2011; Valera *et al.,* 2012), due to its dense capillary beds innervated by the sympathetic nervous system (Stewart *et al.,* 2009; McGreevy *et al.,* 2012). The use of eye IRT measurements for oestrus detection is of great interest.

2.4.7.3 Implications of using body temperature methods

Utilising body temperature as a method to detect oestrus has limitations. For example, under increased temperatures (15 to>25 °C) cows gain heat from solar radiation in addition to normal metabolic processes (Finch, 1986). If the heat gained from solar radiation surpasses the heat lost, evaporation, convection and conduction, the heat will be stored in the body, thus increasing the internal body temperature resulting in heat-stress (Finch, 1986), and potentially indicating a false oestrus. Furthermore, high producing dairy cows

also generate more heat when compared to lower yielding or dry cows (Purwanto *et al*., 1990). This may cause a slight increase in overall body temperature which may indicate false oestrus if body temperature is used as a detection method. Sickness may also cause body temperature to increase. For example, Siivonen *et al.* (2011) reported that cows suffering from mastitis had a marked increase in rectal body temperatures remaining above 39.2°C, and with peaks reaching 41°C. The time of day may also influence body temperature.

Handling animals while obtaining either core or eye temperatures may increase body temperature as handling is reported to affect physiological parameters (Grandin, 1997). Obtaining body temperature measurements through non-invasive ways would be more practical to avoid temperature changes cause through handling. IRT has the potential to become an effective way to obtain body temperature, as it is non-invasive. However, more research is required to develop this technology into an automatic detection method, perhaps measuring each individual cow as they enter the parlour, or during milking. Measurements can then be stored and compared for each cow, thereby detecting any fluctuations for each individual in addition to monitoring the entire herd as environmental conditions can change body temperatures. For example, on a hot day the cows may be heat stressed, therefore they would all be flagged as being in oestrus. However, if a system were developed to take other parameters into account such as ambient temperature this would reduce the potential errors. This technology would also have the benefit of monitoring herd heath as infrared temperatures have also been linked to various ailments such as foot and mouth disease (Rainwater-Lovett *et al*., 2009; Gloster *et al*., 2011),

bovine viral diarrhoea (Schaefer *et al*., 2004), lameness (Nikkhah *et al*., 2005; Alsaaod *et al*., 2012; Alsaaod *et al*., 2014 ; Wilhelm *et al*., 2014), and mastitis (Siivonen *et al.*, 2011). However, infrared temperatures recorded for mastitis (udder) and foot conditions (feet) are localised to that particular body part. Therefore, the relationship between eye temperatures, and other body tissues under stress should be examined.

2.4.8 Automated Pheromone System

Pheromones are chemical substances, that are excreted from the body, and of which one individual can smell the scent of the second individual of the same species, resulting in a specific reaction (Pkra, 2016). The composition of the pheromones may be saturated carbonic acid, steroids, aldehydes, ketones, alcohols or other compounds (Pkra, 2016). Sex pheromones play an important role in cow reproduction and sexual behaviour (Rekwot *et al*., 2001). During oestrus, cows excrete sex pheromones in their urine or faeces indicating the stage of their cycle and stimulating sexual behaviour and functions of bulls (Rekwot *et al*., 2001; Wiegerinck *et al.,* 2011). Devices such as an electronic nose (eNose, or BOVINOSE) are of increasing interest to monitor fertility and disease in dairy cows (Sanderink *et al*., 2017). Several studies have investigated the potential for electronic noses to detect oestrus in dairy cows (Lane and Wathes, 1998; Mohamed *et al*., 2009; Mottram *et al*., 2000; Wiegerinck *et al.,* 2011; Sanderink *et al*., 2017). The theory behind the system is that the specific sex pheromones (acetic acid, propionic acid) (Sankar and Archunan, 2008) secreted to signal that cows are in oestrus will be picked up by the pheromone system, and will trigger an alarm, thus identifying cows in oestrus (Lane and Wathes, 1998). The device is composed of numerous

chemical sensors designed to analyse chemical compounds in gaseous phase electronically, and a pattern recognition model that takes the electronic signal as an input and provides an output signal that is interpretable by the user (Wiegerinck *et al.,* 2011). The sensors are optimised to be more sensitive to pheromones, and sensitivities to other gasses usually present in a cow shed (ammonia, ethanol, water vapor etc.) have been reduced (Wiegerinck *et al.,* 2011). However, Wiegerinck *et al.* (2011) reported that the sensitivity and selectivity of each element was too low for reliable oestrus detection. Cows that are lame, or have untypical, or silent oestrus's may do undetected by this system if they excrete reduced concentrations of pheromones Wiegerinck *et al.,* 2011. Furthermore, lame cows can emit stress related pheromones in addition to excreting reduced quality of sexual pheromones (Walker *et al*., 2008a), which would limit the use of this type of oestrus detection method. Additionally, due to limited understanding of the biological aspects of the results there were no interests in further developing the BOVINOSE system (Personal communication, Dr Arunas Setkus). However, Sanderink *et al*. (2017) researched a similar device using breath sampling rather than faeces sampling, as a new method for detecting oestrus. They obtained a diagnosis performance of 83% sensitivity and 86% specificity with their automated detection of oestrus via breath sampling. Further evaluation is needed to determine the feasibility of using this method on farm.

2.4.9 Milk Production

Previous studies have reported reduced milk production during the oestrus period (Horrell *et al*., 1985; Lopez *et al*., 2004a; Akdag *et al*., 2010). The reduction may be caused by a decrease in feed intake (Maltz *et al.,*1997) and

rumination time (Reith and Hoy, 2012) due to increased restlessness and more time displaying oestrus behaviours (Roelofs *et al*., 2005). As cows in oestrus are typically more active, their feed intake may decrease, which subsequently reduces rumination time, thus affecting milk yield (Maltz *et al.,*1997; Reith and Hoy, 2012). If the reduction of milk production is significant enough, this can be used as a tool to detect oestrus (Britt *et al*., 1986; Rao *et al*., 2013). In smaller herds, observant herdsmen may be able to detect the decrease in milk production in specific animals. However, in larger herds milk yield drops may go unnoticed if the reduction is not obvious. Monitoring milk yield may indicate oestrus, however there are many confounding factors that can affect milk yield, and therefore cannot be accurately used to determine if a cow is in oestrus. For example, as cows that are not in oestrus themselves may participate in oestrus activity, using milk yield as an indicator of oestrus may be invalid as they may also have a decrease in yield due to increased activity. Additionally, during hot ambient temperatures cows may also reduce feed intake, which subsequently may cause a reduction in milk yield. Health conditions such as mastitis may also cause milk production to decrease (Bobbo *et al*., 2017). Other studies suggest that the milk reduction during oestrus was due to a decrease in milk ejection rather than secretion (Horrell *et al*., 1985; Schofield *et al*., 1991). However, this oestrus detection method might not be suitable for high yielding dairy cows, as they have been reported to have decreased expression of oestrus due to reduced circulating concentrations of oestradiol (Lopez *et al*., 2005). Therefore, a reduction in milk production might not be significant enough to indicate oestrus in these cows. Whereas a greater reduction of milk production in low yielding cows could be due to more intense oestrus expression due to greater circulating concentrations of oestradiol (Lopez *et al*.,

2005). As many factors affect milk yield, using this parameter as an oestrus detection method is not adequate without close monitoring of each cow.

2.4.10 Local Positioning Systems

Ubiquitous positioning systems are focused on integrating global navigation satellite systems, including the global positioning system (GPS), which are capable of 3-dimensional positioning with other location technologies (Homer *et al.,* 2013). Accuracy of these positioning systems ranges from a few millimetres to tens of meters, depending on the techniques and algorithms used (Homer *et al.,* 2013). However, GPS has poor accuracy and reliability indoors or in obstructed environments (Meng *et al*., 2007), as metal structures and other obstacles can cause reflections of signal and artefacts (Gygax *et al*., 2007), therefore have limited use in barn environments. Data logging intervals can also influence the accuracy of the behaviours recorded. For example, inaccuracies in speed and distance travelled can occur due to longer fixed intervals, which can create uncertainty about cow location (Pepin *et al*., 2004; Swain *et al*., 2008). Power consumption is a weakness of GPS receivers which limits practical application in the dairy industry (Williams *et al*., 2016; Minnaert *et al*., 2018). In order to overcome GPS battery issues, wireless charging at designated points such as feed troughs, or in the milking parlour have been designed (Minnaert *et al*., 2018). Minnaert *et al*. (2018) determined that wireless power transfer is viable, however extended field testing is required to evaluate the reliability and robustness of the system. Ultra-wideband radio technology (UWB) has been developed for the application of oestrus detection in dairy herds and may help overcome the issues related to GPS. It has been reported that UWB technology can be accurate in environments with many obstructions

(Ingram, 2006; Harmer *et al.,* 2008), can achieve accurate positioning within centimetres in the horizontal and vertical dimension (Ingram, 2006), and have a battery life ranging from months to years depending on sampling rate and battery size (Pastell *et al*., 2018). However, Pastell *et al*. (2018) reported a large number of missing data when cows were lying in the stalls. They attributed signal loss due to metal tubes and plywood in the stall structures, concrete posts, and possibly to cows standing in neighbouring stalls (Meunier *et al*., 2017). Although they were able to correct the data using interpolation, this technology needs further evaluation to reduce the number of missing data. UWB may be used to detect the positioning of cows by monitoring their behaviour, including oestrus behaviours such as standing and mounting (Homer *et al.,* 2013; Arcidiacono *et al*., 2018). Fluctuations in height indicates that a cow is mounting another one, and therefore the cow that is standing to be mounted is most likely in heat. Homer *et al.* (2013) reported that the UWB technology accurately detected 9 out of 9 cows in oestrus, in addition to accurate confirmation of 6 cows that were not in oestrus. Although this research used a relatively small sample size, it is promising for future oestrus detection. However, this technology is not yet available commercially, and a new prototype is being developed in order to make the devices practical to fit on the cows. The units used in this study were rucksack sized, which is not ideal for use in the dairy industry. Smaller devices such as a neck, or leg unit would be more feasible. It is unknown how much this system will cost farmers. Therefore, the integration of this technology is limited if it is excessively expensive.

2.4.11 Saliva Ferning

Fern-like crystallisation patterns can be observed in saliva, tears, cerviovaginal fluid, and nasal mucus during the oestrus period (Ravinder *et al.*, 2016). Salivary ferning is a technique using dried saliva on a glass slide to observe a fern-like pattern. Salivary glands are one of the targets for oestradiol (Ozono *et al*., 1992), the levels of which are typically high during the oestrus stage to trigger the LH surge for ovulation (Terzano *et al*., 2012). Salivary ferning during the fertile period is primarily attributable to high salt and mucus in saliva as a response to increased oestrogen levels prior to ovulation (Ravinder *et al*., 2016). This method has been used for ovulation prediction in women (Guida *et al*., 1993; Fehring and Gaska, 1998), determining pregnancy in cattle (Skalova *et al*., 2013) determining optimal mating time in the dog (Pardo-Carmona *et al*., 2010), and for detecting oestrus in buffaloes (Ravinder *et al*., 2016) and Umblachery cattle (Gnanamuthu and Rameshkumar, 2015). This method is of interest for water buffaloes, as typical oestrus behaviour is not overt in this species, especially during summer months (Ravinder *et al*., 2016). Therefore, detecting oestrus based on behavioural cues is difficult (Warriach *et al*., 2015). Saliva ferning may be useful for modern dairy herds, as detecting oestrus is increasingly difficult. However, the process is labour intensive, as the animals need to be restrained in order to collect the saliva, then the samples have to be evaluated with a microscope. To the knowledge of the author, no studies have investigated the use of saliva ferning to detect oestrus in dairy cows. Therefore, more research is needed.

2.5 Factors Affecting Oestrus Expression and Detection

Dairy cow reproductive performance is a multifactorial variable, whereby cow genetics, nutrition, uterine and systemic health, management and temperature control, bull fertility and artificial insemination technique appear to play a role (Ball and Peters, 2004). Increasing infertility and reproductive disorders has been associated with increasing milk yield (Lopez-Gatius, 2003). Reduced oestrus expression, and detection directly affects conception rates, and continues to be an issue in the dairy industry worldwide (Ranasinghe *et al.,* 2010). A reduction in typical oestrus behaviours, and the presence of abnormal oestrus behaviour, can affect the oestrus detection rates. Over the past 30-50 years the incidence of cows that stand to be mounted has decreased from 80% to 50% (Dobson *et al.,* 2008). Oestrus duration has also been documented to have shortened over the years from up to 18 hours to as little as two hours (Esslemont and Bryant, 1976; Roelofs *et al.,* 2005b; Dobson *et al.,* 2008; Johnson *et al.,* 2012; Homer *et al.,* 2013).

Abnormal behaviours during standing heat may include increased lying behaviour, a reduction in locomotion, and failure to stand for mounting during the oestrus period. For example, research has shown that in more than 50% of all oestrous periods, standing heat is not displayed (Van Eerdenburg *et al.,* 1996; Heres *et al.,* 2000; Roelofs *et al.,* 2006). Sveberg *et al.* (2011) also reported reduced mounting activity during standing oestrus, making heat detection based on mounting behaviours difficult, thus influencing detection rates. Additionally, oestrus may not be displayed at all (sub-oestrus or silent heat), or for a very short time making it difficult to identify cows for insemination

(Roelofs *et al.,* 2005b).

Oestrus expression, and therefore detection is influenced by many factors such as lameness, nutritional state, environment, genetics, body condition score, oocyte quality, housing type, number of cows simultaneously in oestrus, and floor surfaces (Hurnik *et al.,* 1975; Hackett *et al.,* 1984; Vailes and Britt, 1990; Ball and Peters, 2004; Dobson *et al.,* 2008; Cutullic *et al.,* 2009; Sood and Nanda, 2006; Berry *et al.,* 2011).

2.5.1 Housing and Oestrus Behaviour

Cows require sufficient space to adequately display mounting behaviour, alongside sturdy, soft footing (Squires, 2010). It has been reported that housing conditions may also affect the duration standing heat. For example, mounting activity may rise to 3-15 times greater on soil surfaces, whereas there is a sharp drop in mounting activity on slippery surfaces (Rao *et al.,* 2013) such as concrete. Therefore, cows housed on concrete, uneven, or slippery flooring can reduce their oestrus intensity, which in turn reduces mounting behaviour (Squires, 2010; Rao *et al.,* 2013). Palmer *et al.* (2010) determined that cows at pasture were detected more efficiently than cows housed indoors. They also observed that housed cows were slipping and falling when attempting mounting behaviour, however this did not occur in the pasture-based system. This study also supports previous research that oestrus expression is reduced on concrete flooring when compared to dirt surfaces (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996). Mounting behaviour of cattle is presumably based on the ratio of costs and benefits to the cow in that particular time (Kerr *et al.,* 2004). Therefore, cows that are housed have an increased

risk of falling and slipping when attempting to mount, which can reduce this oestrus behaviour (Palmer *et al.,* 2010). This reinforces the requirement to interpret oestrus behaviour of individual cows based on their level of soundness, and environment.

2.5.2 Herd Dynamics and Oestrus Behaviour

Sexual behaviour, and both oestrus expression and duration are influenced by social structures among dairy cattle (Galina *et al.,* 1996; Chenoweth and Landaeta-Hernández, 1998; von Boreli *et al.,* 2007). Additionally, the number of cows simultaneously in oestrus affects behavioural displays. For example, as the number of cows in oestrus increases, the greater the opportunity there is to engage in sexual behaviours (Roelofs *et al.,* 2005; Sveberg *et al.,* 2011; Zebari *et al*., 2019). Social dominance decreases both the duration of oestrus, and the number of cows in oestrus at any one time (Orihuela, 2000). Dominant cows have been shown to come into oestrus earlier (16h vs 30-34h) following the decline in progesterone values, and express oestrus for longer (20 vs 12) (Landaeta-Hernández et al., 2004). Contrastingly a study by Kabuga *et al.* (1992) reported neither dominance value, nor antagonistic interaction, is associated with the number of mounts received. Young cows seem to be more susceptible to abnormally short ovarian cycles, possibly because they are often dominated by mature cows that exhibit aggression towards them (Moberg, 1985). Cows that have a higher social ranking have been reported to be more fertile with a calving to conception interval of 97 days versus 143 days, and a lower number of inseminations per pregnancy (1.6 vs 2.2) (Dobson and Smith, 2000). A study by Landaeta- Hernández *et al.* (2002) identified that in dominant beef cows it took longer to identify oestrus than in subordinate cows, possibly

due to a reluctance to allow subordinate cows to mount them. Dominant cows tend to form associations together to exhibit oestrus behaviour (Gutierrez *et al.,* 1993) however, as the number of dominant cows in a herd is typically less than the number of subordinates (Chenoweth *et al.,* 1998), dominant cows may have difficulty identifying others to display behaviour with (Galina *et al.,* 1996).

2.5.2.1 Presence of a bull

Cows display oestrus behaviours to draw the attention of a bull to the cows in oestrus (altruistic act). The presence of a bull is known to have a bio-stimulatory effect on cows, positively affecting reproductive cycles (Baruah and Kanchev, 1993; Berardinelli and Joshi, 2005; Roelofs *et al*., 2007). For example, having a bull on-site decreased the calving to first oestrus interval and the number of primiparous females cycling prior to the start of the breeding season (Custer *et al*., 1990, Fernandez *et al*., 1993 and Fike *et al*., 1996). Additionally, it has been reported that conception rates increased for cows exposed to bulls prior to the breeding season when compared to cows not exposed to bulls (Fernandez *et al.,* 1993), and overall AI to pregnancy rate of primiparous cows was enhanced by the exposure of bulls prior to, during, and following an oestrus synchronisation protocol (Berardinelli *et al*., 2007).

Lack of such stimulus may cause a reduction in oestrus intensity, and/or motivation for the cows to display oestrus behaviours, thereby reducing overall oestrus detection rates. Cows that are in oestrus actively seek interaction with a bull if present (Roelofs *et al.,* 2008), therefore lack of a bull may reduce the frequency, or range of oestrus behaviours expressed. A study by Roelofs *et al*. (2007) reported subtle increases in basal and average LH-concentration and

LH pulse frequency in postpartum, anoestrous dairy cows after exposure to a fence line bull. Baruah and Kanchev (1993) found an increase in LH and FSH concentration 80 min after exposure to bull urine and this increase was seen until 4–5 h after treatment. Berardinelli and Joshi (2005) found a shorter interval between calving and resumption of luteal activity in beef cows that were exposed to bull excretory products, suggesting that the bio stimulatory role of a bull is mediated by pheromones present in their excretory products. Further investigations by Roelofs *et al*. (2008) showed that cows, given the opportunity, will visit a contact area and interact with a bull more during oestrus than when they are not in oestrus. Interestingly they did not report an increase in oestrus expression with a bull present. Landaeta-Hernandez *et al*. (2006) found no effect on duration of oestrus and on total mounts received with a bull present in the herd. Therefore, even when full contact is possible between bulls and cows in oestrus, it does not appear to increase behavioural expression of oestrus. Based on the literature it appears that bulls have a stimulating effect on reproductive cyclicity of dairy cows. As the development of AI has removed the need to have a bull onsite, perhaps a reduction in reproductive cyclicity can also be attributed to lack of their male counterpart.

2.5.2.2 Prolonged Luteal Phase (PLP)

PLP has increasingly been identified in many herds, and has been reported as a common abnormality during the period from calving to 90 days postpartum (Lamming and Darwash, 1998; Opsomer *et al.,* 2000; Royal *et al.,* 2000; Shrestha *et al.,* 2004a). Peter and Bosu (1986) reported that oestrus detection efficiency depended on the number of postpartum ovulations. For example, in the first, second and third ovulations postpartum oestrus detection rates using

pedometers were 57, 91, and 93% respectively. Similarly, visual oestrus detection was 19, 37 and 79% respectively. Studies have identified that cows with PLP had significantly reduced first insemination conception rates, more services per conception, and more days open compared with cows with normal recommencement of ovarian activity (Lamming and Darwash, 1998; *Royal et al*., 2000; Shrestha *et al*., 2004b; Hommeida *et al*., 2005). However other studies have reported that occurrence of PLP did not affect the abovementioned fertility conditions (Taylor *et al*., 2003; Samarütel *et al.,* 2008; Gautam *et al*., 2010). Postpartum complications such as dystocia, retained foetal membranes, abnormal vaginal discharge, metritis, endometritis, or pyometra have been associated with PLP (Opsomer *et al.,* 2000; Petersson *et al.,* 2006b). For example, a study by Ranasinghe *et al.* (2011) found that cows that suffered from one or more of these conditions were 5 times more likely to experience PLP.

2.5.2.3 Silent Ovulation

Silent ovulations are described as when cycling cows are unable to express oestrus behaviour(s) (Ranasinghe *et al.,* 2010), potentially resulting in the oestrus period going unnoticed. This has been reported to be one of the most predominant fertility dysfunctions causing suboestrus (Shipka, 2000; Yániz *et al.,* 2008). Ranasinghe *et al.* (2010) reported a silent first ovulation caused a 28% decrease in pregnancy rates. Silent ovulations have been associated with poor reproductive performance, and have been linked to high milk production (Shipka, 2000; Yaniz *et al*., 2008), and warm temperatures (Labhsetwar *et al.,* 1963). A study carried out by Ranasinghe *et al.* (2010) investigated 769 ovulations in 277 lactations. This study identified that of the 277 lactations, up

to four ovulations occurred within 90 d postpartum. Additionally, the occurrence of silent ovulations at the first, second, third and fourth postpartum ovulations were 55.2%, 23.8%, 21.3%, and 10.5% respectively. Overall, 33.6% of 769 ovulations were silent. Another study by Johnson *et al.* (2012) reported that 88.4% (38/43) of the first ovulations postpartum were not accompanied with behavioural signs of oestrus. A significant risk factor for silent ovulations at the second, third, and/or fourth ovulations postpartum has been linked to high milk production (Ranasinghe *et al.,* 2010). Typically, high milk producing postpartum dairy cows are in a negative energy balance throughout early lactation, which may reduce oestradiol production in the preovulatory follicle thus reducing the sensitivity of the hypothalamus to oestradiol, subsequently resulting in an increased incidence of silent ovulations (Isobe *et al.,* 2004; Ranasinghe *et al.,* 2010). As oestrogen is responsible for initiating the behavioural expression of oestrus, a reduction in the sensitivity/concentration of this hormone may result in reduced oestrus behaviour. A study by Lopez *et al.* (2004b) reported that cows producing more than 39.5 kg of milk per day had reduced serum oestradiol concentration on the day of oestrus, and that the expression of oestrus was greatly reduced when compared to lower yielding cows (>39.5 kg/d). It should be noted that in high yielding cows' metabolic clearance of steroid hormones is elevated with increased feed intake (Sangsritavong *et al.,* 2002). Therefore, both the concentration of oestradiol, and increased metabolic clearance of oestrogen associated with high producing cows may account for decreased oestrus expression.

2.5.2.4 Heat Stress and Fertility

Stress has been defined as an animal's inability to cope with its environment (Broom and Johnson, 1993). Stress has been shown to reduce fertility by interfering with physiological mechanisms responsible for controlling the intensity of oestrous behaviour and fertile oocyte production (Dobson and Smith, 2000). Heat stress is an environmental issue that negatively affects many production parameters, including milk production and composition, growth, and reproductive performance (Sharma *et al.,* 2010; Baumgard and Rhoads, 2013; Schuller *et al*., 2017). Heat stress occurs when environmental conditions (temperature and humidity) (West, 2003), combined with internal thermogenesis, create a heat load that exceeds thermolytic capacity (Bernabucci *et al*., 2010). Dairy cows are more susceptible to heat stress than most farm animals due to their high metabolic heat production and low surface area: mass ratio (West, 2003; Liu et al., 2014). Warm temperatures influence ovarian function (Yániz *et al*., 2008) and can alter lying and standing behaviour of cattle (Allen *et al*., 2015), which can directly affect fertility (De Rensis and Scaramuzzi, 2003; Cook *et al.,* 2007; Yániz *et al*., 2008). Heat stress on the day of oestrus significantly reduces the duration, and intensity of oestrus expression, including a reduction in mounting activity (Pennington *et al*., 1985; Gwazdauskas *et al*., 1983; Orihuela, 2000; Schuller *et al*., 2017). Therefore, oestrus detection methods based on mounting activity would be less accurate. Cows suffering from heat stress have been reported to have an increased incidence of silent ovulation and anoestrus (De Rensis and Scaramuzzi, 2003), with pregnancy rates as low as 10% (Hansen and Arechiga, 1999). It has been documented that failure to ovulate following insemination to a given oestrus can

range between 6 and 16% (López-Gatius *et al*., 2005; Demetrio *et al*., 2007) and is 3.9 times more likely to manifest during high (>25 °C) ambient temperatures (López-Gatius *et al.,* 2005). Additionally, silent ovulations have been reported in hot climates. For example, a study in Florida determined the percent of possible silent ovulations was 60% for October-May, and 80% for June-September (Thatcher and Collier, 1986). During warmer temperatures cows have been observed to increase their standing time (Cook *et al.,* 2007). As prolonged standing has been linked to increased incidence of lesions (Galindo and Broom, 2000), ambient temperature plays an important role in altering cow behaviour, thus increasing susceptibility to lameness, and reducing fertility. Precautions such as the use of cooling systems have been beneficial on fertility (Yániz *et al*., 2008), however these alone do not fully restore normal reproductive function (De Rensis and Scaramuzzi, 2003).

2.5.3 Effects of Lameness on Behaviour and Fertility

It is widely accepted that lameness is a painful condition negatively affecting production, and animal welfare (Green *et al.,* 2002; Whay, 2002; Ettema and Østergaard, 2006; Amory *et al.,* 2008) in the modern dairy industry (Ito *et al.,* 2010; Chapinal *et al.,* 2013). Lameness may be defined as a response to pain that causes an animal to alter its natural behaviour, as a way to alleviate the pain (Scott, 1976). As lame dairy cows have been observed to alter the way in which they stand and walk (Sprecher *et al.,* 1997; Leach *et al.,* 2010a; DairyCo, 2011d; Van Nuffel *et al*., 2015), the term lameness in dairy herds is often used to describe whether a cow has impaired movement as a result from pain caused by disease, leg and hoof injuries (Flower and Weary, 2009). Other behavioural and physiological alterations may include reduced curiosity and vocalisations,

reluctance to move, changes in facial expression (Molony and Kent, 1997; Underwood, 2002; Gregory, 2004), reduced feed intake, changes in heart rate, blood pressure, temperature, pupil dilation, and respiratory rate (Molony and Kent, 1997; Lee, 2002). The aetiology of lameness is multifactorial and factors such as genetics (Jones *et al.,* 1994; Buitenhuis *et al.,* 2007; Howard *et al*., 2017), milk yield (Bichalo *et al*., 2008), low body condition score (Green *et al*., 2014; Randall *et al*., 2015), and nutrition (Boettcher and Dekkers, 1997) have been associated with an increased incidence of lameness.

2.5.4 Housing and Lameness

Research has shown that facility design and management systems can affect lameness (Espejo and Endres, 2007; Bernardi *et al*., 2009), which directly affects dairy cow welfare (Whay *et al*., 2003; Bicalho *et al*., 2007b). Dairy production systems may use indoor (freestall or tie stall) or pasture-based systems. Pasture based systems such as those in New Zealand have a lower incidence of lameness when compared to indoor housing systems (Alawneh *et al*. 2011). Pasture systems require a great deal of space and are less commonly used in the Northern Hemisphere (Laven and Holmes, 2008). When compared to freestall cubicles, tie stalls are less common (Chapinal *et al.,* 2013). However, over half of United States dairy producers utilise tie stall systems (Tucker *et al.,* 2009), as does a proportion of Canada and Europe (Tucker *et al.,* 2009). Reports show that 88% of Norwegian dairy cattle (Sogstad *et al.,* 2005), 74% of Ontario dairy cattle (CanWestDHI, 2007), 75% of all Swedish dairy herds (Loberg *et al.,* 2004) are kept in tie stalls. Research suggests that the risk of lameness increases when freestall housing is implemented over other housing systems such as tie stalls, and straw yards (Cook, 2003; Sogstad

et al., 2005). Despite this, indoor freestall systems are more commonly used to house lactating dairy cows as this system enables producers to easily manage groups of cows to promote efficient cleaning and feeding (Stefanowska *et al.,* 2001).

The term freestall derives from the fact that cows are able to freely move throughout the barn when they are not being milked in the parlour. In a freestall system the cows are able to move around to carry out behaviours such as resting, feeding and social interactions (Gomez and Cook, 2010). The amount of time devoted to these behaviours is known as a 'time budget' (Pollard and Blumstein, 2008). Situations out of the animals' control (milking) is not included in the animal's time budget. Sufficient rest is an important component of overall dairy cow well-being (Munksgaard and Simonsen, 1996). Dairy cows have been reported to be highly motivated to lie down for approximately 12 to 13 hours/day in indoor housing systems (Jensen *et al.,* 2005; Munksgaard *et al.,* 2005). When cows are prevented from lying down, they begin to exhibit stress induced reactions within a few hours such as increased levels of plasma cortisol concentrations (Fisher *et al.,* 2002; Tucker *et al.,* 2007), and decreased levels of plasma of growth hormone (Munksgaard and Lovendahl, 1993). As growth hormone is positively linked to milk production (Munksgaard and Lovendahl, 1993), prolonged stress could lead to a reduction in milk production. Factors that may affect resting behaviour which in turn can affect incidence of lameness will be discussed below.

2.5.4.1 Walking and Lying Surface and lameness

Typically, the flooring inside the houses are fully slatted concrete or solid concrete floors with cubicles that may contain bedding such as straw, shavings, sand, mats and mattresses (Boyle *et al.,* 2007; Bernardi *et al*., 2009; O'Driscoll *et al.,* 2009). Concrete has shown to increase the development of lameness (Cook *et al.,* 2005; Vanegas *et al.,* 2006), as it may not provide sufficient friction that is essential for cows to walk and display behaviours naturally (van der Tol *et al.,* 2005). It has been reported in a study from North America that the highest rates of lameness were observed in herds housed in a freestall system (Cook, 2003; Haskell *et al.,* 2006; Cook and Norlund, 2009). Additionally, it has been documented that cows prefer to walk, and stand on soft flooring such as rubber matting, or bedding when compared to concrete (Boyle *et al.,* 2007; Telezhenko *et al.,* 2004; Telezhenko *et al.,* 2007; Platz *et al.,* 2008).

Producers bedding choice may be influenced by cost, availability, cleanliness, maintenance, and possibly health concerns. Research has highlighted that the lying surface provided for cows is a vital component that influences incidence of lameness and injuries in intensively housed dairy cattle (Fregonesi *et al*., 2007a**;** Bernardi *et al*., 2009). Dairies that provided mattresses and minimal bedding had increased severe hock lesions in addition to higher clinical lameness (24% v 11%) (Cook *et al*., 2004b), than cows at dairies that provided deep bedded stalls (Weary and Taszkun, 2000; Fulwider *et al*., 2007). Cook (2003) reported that prevalence of lameness in herds that used sand bedding was reduced when compared to facilities with other surface types. Additionally, hock lesions and hoof health improve on sand bedding (Espejo *et al.,* 2006; Norring *et al.,* 2008). The lying surface type (Tucker *et al.,* 2003; Norring *et al.,*

2008), amount (Tucker and Weary, 2004; Drissler *et al.,* 2005; Norring *et al.,* 2010), quality and dryness (Fregonesi *et al.,* 2007a; Reich *et al.,* 2010) can all dictate how much time cows spend lying down (Gomez and Cook, 2010).

2.5.4.2 Housing design and lameness

Housing design is an important variable when considering impaired locomotion, and increased lameness rates. Stall size is an important variable for cow comfort, as this is where the cow may rest (standing, or lying) (Bernardi *et al.,* 2009). Providing cows with wider free stalls improved lying times, which may be due to decreased contact with partitions (Tucker *et al*., 2004). Wider stalls enabled cows to stand in the stalls with all four feet inside, rather than perching on 2 front feet in the stalls, or elsewhere in the dairy on concrete flooring (Tucker *et al*., 2004). Stalls typically have a neck rail situated in them to prevent cows from fully standing within them, with the intention of preventing soiling from faeces and urine (Bernardi *et al*., 2009). Although the presence of neck rails has been shown to increase hygiene, they have been reported to affect both standing and lying behaviour. Bernardi *et al*. (2009) reported that cows spent more time standing with all four feet in the stall when the neck rail was positioned in a less restrictive manner.

Passage widths can also affect cow locomotion scores. Barker *et al.* (2007) reported that freestall houses with wider passage widths had decreased locomotion scores. Providing ample room in houses allows cows to move more freely, which is especially important when new cows enter the herd. Social pecking orders are established when new members enter the herd, providing

adequate room enables lower ranking cows to retreat more readily if displaced by a dominant cow.

To ensure animals are managed under hygienic conditions, animal waste (slurry) must be removed regularly. Slurry removal for indoor housing systems may be carried out manually or through the use of automatic scrapers. Automatic scrapers are popular in commercial dairy herds (Buck *et al*., 2013), as frequent scraping means increased hygiene, and reduced manual labour. Stalls are typically designed to ensure waste accumulates/drains into the alley. This is achieved through the use of brisket boards, and neck rails. These prevent the animal from lying too far forward in the stall, which may result in waste accumulating in the stall rather than the alley. The automatic scraper then sweeps periodically throughout the day pooling animal waste into a collecting pit. Although automatic scrapers may improve hygiene in the barn, they have been associated with impaired locomotion, reduced resting and feeding times, and increased digital dermatitis (Wells *et al*., 1999; Barker *et al.,* 2007). As scrapers sweep along the alley, cows must move out of its way. If cows do not see the scraper it may come into contact with their feet, causing minor injuries, or panic, thereby causing stumbling or slippages (Stefanowska *et al*., 2001).

2.5.4.3 Stocking Density, Hierarchy and Resting Behaviour

Stocking density, and cattle grouping affects the time budget, and normal resting behaviour of dairy cows. The optimal stocking density for free housed dairy cows is 1 cow per stall (Rushen *et al*., 2008). However, a study by Wagner-Storch *et al.* (2003) reported an average stall occupancy of 74% when

the stocking density for the herd was 100%. Therefore, dairy producers often increase stocking densities above 100% (>1 cow per stall) to maximise financial productivity, without compromising the cow's ability to express natural behaviours such as resting (Bewley *et al*., 2001; Spinka, 2006). However, it has been reported that if stocking densities are increased, daily management procedures such as milking will inevitably take longer, which may result in cows spending more time away from their housing, resulting in a reduction in resting time (Matzke *et al.,* 2002). Numerous studies have determined that stocking densities above 100% decreased time spent lying or standing in the stalls, and increased total time spent standing idly in an alleyway (Fregonesi *et al.,* 2007b; Krawczel *et al.,* 2008; Hill *et al.,* 2009). As increased standing has been linked to a higher risk of developing a type of lameness, increased stocking density may be directly related to increased prevalence of lameness in a dairy herd. Additionally, as stocking densities increase the number of aggressive interactions also increase (Val-Laillet *et al.,* 2008).

Compared to older cows, heifers are generally smaller bodied, have a smaller bite rate and spend more time feeding. Mature cows are generally more dominant and can push younger, subordinate heifers away from prime feeding, and resting areas. Therefore, it is recommended that first lactation heifers should be housed separately from multiparous cows to ensure they have sufficient feeding and ruminating time throughout the day. Bach *et al.* (2006) reported that heifers housed separately have increased efficiency of fat correlated milk production and reduced overall body weight loss during the first month of lactation. However not all farms can house first lactation heifers separately. This can increase standing times of less dominant cows and heifers

(Cook, 2004c), possibly increasing the risk of lameness. Cows that are already lame, may consistently worsen as they have reduced feed intake and prolonged standing periods in less desirable resting locations. Overall resting periods decrease for the herd when stocking densities are increased. For example, when primi and multiparous cows are mixed, resting time is typically reduced for heifers by approximately 4.2h/day, whereas older cows resting times were reduced by 2.6h/day (Matzke, 2003).

2.5.4.4 Effects of lameness on resting and oestrus behaviour

Lying down is a crucial, basic component of a dairy cows' natural behaviour repertoire, and if hindered it can induce stress, which can greatly compromise health and welfare (Andreasen and Forkman, 2012). During this time the cow will ruminate, socially interact, and simply rest (Metz, 1985; Cook *et al.,* 2004a). Lying behaviour may be influenced by the resting environment provided for the cows. Several studies have reported that mattress stalls reduce lying time, particularly if no additional bedding is provided (Tucker *et al.,* 2003; Tucker and Weary, 2004; Cook *et al.,* 2010). If the stall environment is uncomfortable for the cow it will increase the standing time rather than lying down (Tucker *et al.,* 2005).

Reducing lying times through daily managerial methods may consequently predispose cows to an increased risk of developing lameness, which may lead to a negative relationship between lying time, and increased locomotion scores. It has been documented that a dairy cow prefers to lie down for approximately 12h/d, and it is generally accepted that when compared to non-lame cows, lame cows spend more time lying down, have decreased oestrus intensity (Singh *et*

al., 1993a; Juarez *et al.,* 2003; Walker *et al.,* 2008a; Chapinal *et al.,* 2009), perform fewer aggressive interactions (Galindo and Broom, 2002), spend less time feeding (Hassall *et al.,* 2003; González *et al.,* 2008; Gomez and Cook, 2010). have a lower bite rate (Juarez *et al.,* 2003), have a reduced milk production (Juarez *et al.,* 2003; Amory *et al.,* 2008), are hesitant to interact with other cows (Tadich *et al.,* 2013), and are less active (O'Callaghan *et al.,* 2003).

However, there are discrepancies whether lame cows rest for longer than non-lame cows. For example, some studies claim longer resting times for lame cows (Ito *et al*., 2010) and some report shorter resting times (Chaplin *et al*., 2000; Cook *et al*., 2004b; Cook *et al*., 2008), or no difference at all (Hassall *et al.,* 1993). Hassell *et al.* (1993) observed both oestrus periods and non-oestrus periods, whereas Walker *et al*. (2008a) only observed oestrus periods. This may indicate that although lame cows may walk and stand as much as non-lame cows, during oestrus, when non-lame cows increase walking, lame cows suppress this behaviour (Walker *et al.,* 2008a). Additionally, lame cows may travel with cows that are in standing heat, but may require more rest, thus lay down more and dedicate less time to expressing oestrus behaviours. Walker *et al.* (2009) reported an overall reduction in oestrus intensity in lame cows of approximately 37%.

Some studies suggest that cows alter their circadian rhythm, displaying oestrus behaviour more frequently at night (Hall *et al.,* 1959; Orihuela *et al.,* 1983; Mattoni *et al.,* 1988), or early hours of the morning (Hurnik *et al.,* 1975; Amyot and Hurnik, 1987; Gwasdauskas *et al.,* 1990; Van Vilet and van Eerdenburg 1996). Whereas, other studies found no variation in oestrus behaviour

(Esslemont and Bryant, 1976; Esslemont *et al.,* 1980; Alexander *et al.,* 1984; Xu *et al.,* 1998). Variations may result from managerial differences between establishments. However, it has been documented in mammals that altering the circadian rhythm increases reproductive health issues (Miyauchi *et al.,* 1992; Ahlborg *et al.,* 1996; Bisanti *et al.,* 1996; Labyak *et al.,* 2002; Shechter *et al.,* 2008; Mahoney, 2010) alongside reducing luteinising hormone (LH) pulsatility. For example, women who are night workers have altered reproductive function associated with changes in the follicular phase and the concentration of follicle stimulating hormone secretion (Kloss *et al*., 2014). Desynchrony of hypothalamic-pituitary gonadal oscillators caused by shift work alters the amplitude and timing of pituitary secretion. Based on human research, it may be that lame cows that are more active at night have the added negative side effect that humans encounter when altering their circadian clock. Not only does lameness reduce fertility, but increasing nocturnal activity may also hinder their reproductive cycle.

2.5.4.5 Lameness, fertility and oestrus behaviour

It is well documented that lameness can have a detrimental effect on reproductive performance, oestrus behaviour and intensity (Sprecher *et al.,* 1997; Whay *et al.,* 1997; Hernandez *et al.,* 2001; Garbarino *et al.,* 2004; Walker et al., 2008a; Olmos *et al.,* 2009), leading to decreased frequency of primary and secondary oestrus behaviours (Walker *et al.,* 2008a). However, a study by (Walker *et al.,* 2008b) reported that lame cows were just as restless when compared to non-lame cows. Thus, suggesting that reduced oestrus intensity is not the result from impaired physical movement, but that lame cows dedicate a smaller proportion of their daily activities to expressing oestrus behaviour.

Severe lameness may lead to occasional oestrus periods to be classified as sub oestrus rather than standing oestrus (Palmer *et al.,* 2010). Garbarino *et al*. (2004) reported that lameness is associated with delayed ovarian activity and that lame cows were 3.5 times more likely to have a delayed cyclicity than non-lame cows. Sprecher *et al.* (1997) reported an increase in days to first service from 80 to 90, and number of days from calving to conception 115 to 125.

Walker *et al*. (2010) examined the effects of lameness on oestrus by measuring the duration, and frequency of oestrus behaviour in relation to milk progesterone levels. The authors reported that lame cows had lower progesterone concentrations during the 6 days prior to oestrus, in addition to a decreased intensity of oestrus, and reduced periods whereby herd-mates mounted the lame cows. More interestingly perhaps is the finding that the incidence of oestrous remained unaffected. Additionally, a study by Morris *et al.* (2011) investigated the influence of lameness on follicular development, and they identified that: 29% of all lame cows in their study were completely unresponsive to a synchronization regime; 21% of all lame cows that did respond to synchronization failed to ovulate; and 50% of all lame cows responded to synchronization and ovulated.

Current oestrus detection techniques may not factor lameness as a variable in interpreting oestrus behaviour. That is, a lame cow in oestrus may not display behaviours in a conventional manner, and therefore the lame cow remains undetected. For example, Blackie *et al.* (2008) reported that lame or sick cows preferred to lie longer than healthy animals. Therefore, it may be a possibility that lame cows are not able to display mounting behaviour as they have

increased lying times. It has also been shown that cows with foot problems are more reluctant to express oestrus behaviours such as mounting (Boyle *et al.,* 2007). If a cow has relatively short oestrus duration, it may easily go undetected. Although it has been reported that similar lameness rates occur for both pasture-based (Tranter and Morris 1991; Clark *et al*., 2007), and housed cubicle production systems (Logue *et al*., 1993; Clarkson *et al*., 1996; Nordlund *et al*., 2004), it has been identified that the incidence of lameness is higher in housed cows when compared to pasture-based systems (Olmos *et al.,* 2009). In particular cows subjected to prolonged standing periods on concrete surfaces are more predisposed to developing lameness (Bergsten and Frank, 1996).

2.6 Nutritional Influences on Lameness and Fertility

2.6.1 Nutritional Influences on Lameness

Dairy cow nutrition is an important variable to maintain health, and productivity. Higher yielding cows, and cows in early lactation may be fed separately from lower yielding cows in order to sustain their high milk production. Many establishments feed a high concentrate (Lechartier and Peyraud, 2011), low roughage diet to accommodate for the increased milk production. Corn silage is often fed, as it provides a high source of energy, it can encourage voluntary feed intake, and enhance milk yield and milk protein content (Phipps *et al.,* 1995; O'Mara *et al.,* 1998; Phipps *et al.,* 2000). However, feeding high starch diets to ruminants has been linked to an increased risk of rumen acidosis, which can lead to clinical lameness and laminitis (Boettcher and Dekkers, 1997). Acidosis is a metabolic disorder caused from vast quantities of rapidly fermentable carbohydrates, which exceeds the rumens buffering capacity

(Chiquette, 2009). Rumen acidosis may be acute, or subacute (SARA), and is well recognised among well-managed dairy herds (Enemark, 2008). The difference between acute and SARA are that during acute ruminal acidosis, the pH depression is more severe, the concentration of lactic acid in the rumen digesta is higher, and the clinical signs more prominent (Kleen *et al*., 2003; Plaizier *et al*., 2014). Typically, the normal ruminal pH for cows can range between 6-7 (Krause and Oetzel, 2006), which is considered to be the optimum for cellulolytic bacteria (Abdela, 2016). Ruminal pH may decline periodically below 6 when dietary grain content increases (Abdela, 2016). During acute acidosis, the rumen pH can drop to below 5.0, and during SARA the rumen pH is depressed for several hours per day due to accumulation of volatile fatty acids, and inadequate rumen buffering (Plaizier *et al*., 2008). Generally, SARA occurs when ruminal pH stays in the range of 5.2 and 6 for a prolonged period of time (Li *et al*., 2013). This decrease in pH can cause the digestive system to stop working effectively, making the rumen atonic. This leads to depressed appetite and production. The fluctuation in pH alters the microbial populations of the rumen, disrupting the balance between lactate-producing bacteria and lactate-utilising bacteria (Nagaraja and Titgemeyer, 2007). Diets lower in forage are consumed faster (DeVries *et al*., 2007) and are ruminated less, resulting in reduced saliva production, which can decrease the buffering capacity in the rumen (Maekawa *et al*., 2002; Beauchemin *et al*., 2008).

Cows that are at higher risk of developing SARA are cows in early lactation, and cows that reach their peak dry matter intake (DMI) (Norlund *et al.,* 2005). Cows early in their lactation are often introduced to a high grain diet following parturition, and their rumen may not be accustomed to such a high starch diet.

Cows at their peak DMI are particularly sensitive to abrupt feed changes and are at risk due to the volatile fatty acids produced by microbial fermentation, which supersedes the buffering and absorptive capacity of the rumen (O'Grady *et al.,* 2008). The prevalence of SARA in the United States has been reported to be up to 19% for early lactation cows, and 26% of cows in their mid-lactation have SARA (Garret *et al.,* 1997). A study by Kleen (2004) reported in a German/Dutch study cases of SARA for cows in their early lactation to be 11%, and 18% for mid lactation. There are no clinical signs of SARA in affected cows (Krause and Oetzel, 2005). However, many disorders are associated with SARA, which may include ruminitis, metabolic acidosis, decreased feed intake, abomasal displacement and ulcers, laminitis, hoof overgrowth, sole ulcers, sole abscesses, bloat, and fertility (Enemark, 2008; Abdela, 2016). Many researchers have indicated that laminitis is a major source of lameness, and is the most significant disorder arising from SARA (Nocek, 1997; Cooke *et al.,* 2004a; Abdela, 2016).

Laminitis is an aseptic inflammation of the hoof dermal layers, and results in the loss of normal mechanisms that control normal distal phalanx function in hoofed animals (Orsini, 2011). Main causal agents for the development of bovine laminitis are: histamine, rumen endotoxin, and metalloproteinases activated by gastrointestinal *Streptococcus bovis* (Bergsten, 2003). Diets high in grain play a significant role in the development of endotoxemia through the depression of ruminal pH, which eventually leads to the lysis of numerous ruminal microbiota, subsequently increasing the concentration of free endotoxins in the rumen fluid (Mao *et al*., 2013). Endotoxins (lipopolysaccharides) are membrane components of both gram-negative and

gram-positive bacteria that strongly elicit an immune response when present in circulation (Draing *et al*., 2008; Knirel and Valvano, 2011). Excess production of histamines from protein sources or endotoxins from gram-negative bacteria occur following feeding the high starch diets as previously described (Hudson *et al*., 2010). Histamine and endotoxin are thought to be the primary vasoactive substances that are absorbed and cause vascular changes within the dermal capillary beds of the corium (Donovan *et al*., 2004). These vascular changes cause pooling of blood in the corium that leads to ischemia, inflammation, and necrosis of the corium-epidermal junction (Donovan *et al*., 2004). Ultimately these changes lead to haemorrhage and impaired function of keratin producing cells resulting in sole haemorrhages', discoloration, and reduced quality of horn in the sole (Donovan *et al*., 2004; Hudson *et al*., 2010). This can also weaken tissue in the claws, directly affecting suspensory tissue designed to support the distal phalanx (pedal bone) (Danscher *et al.,* 2010). Weakening of the supportive tissue combined with pressure from the body weight of the cow may result in the pedal bone being forced downwards, damaging the soft tissue between the bone and the horn capsule (Danscher *et al.,* 2010). Sinking of the pedal bone within the hoof, can cause haemorrhages, and severe damage to the corium (Hudson *et al*., 2010). Disruption of normal horn production caused by laminitis may also lead to further damage through managerial mechanisms (slurry). Therefore, rumen acidosis can lead to inflammation within the hoof, which can interfere with horn production (Mulling *et al*., 1999).

Animals suffering from laminitis have been reported to have reduced milk fat composition, and increased connective tissue in the hoof, which increases the risk of damage to the corium (Hudson *et al*., 2010). Laminitis is a painful

condition, that can make dairy cows severely lame, directly affecting their health and welfare in addition to negative economic consequences. This highlights the importance of how the diet may affect lameness among dairy cows, and how high yielding herds may be at higher risk of lameness when compared to low yielding herds (Barker *et al.,* 2007), as the dietary requirements are different based on their milk production. Reproductive performance is also directly linked to nutrition and milk production.

2.6.2 Nutritional Influences on Reproductive Function

High producing cows in early lactation increase their food intake and have a greater metabolism rate than lower producing cows (Huntington, 1990; Butler, 2000). Therefore, as milk production and feed intake increase, there is an increase in liver blood flow, which increases the clearance of steroid hormones (Sangsritavong *et al.,* 2002; Wiltbank *et al.,* 2006). Resulting in lower circulating oestradiol and progesterone concentrations, which may interfere with luteolysis (Rabiee *et al.,* 2002; Sangsritavong *et al.,* 2002; Wiltbank *et al.,* 2006). This may cause a prolonged luteal phase, which can restrict the producers' ability to predict when cyclic cows will return to oestrus. High producing cows have been documented to exhibit reduced oestrus behaviour and also have a shorter duration of oestrus (Lopez *et al.,* 2004b). Oestrus expression may be affected by numerous physiological events (Walsh *et al.,* 2011). Compared to low yielding dairy cows, high yielding cows (≥39.5 kg/day) have a shortened oestrus period (6.2 h vs 10.9 h), reduced standing time (21.7 s vs 28.2 s) and decreased serum oestradiol concentrations (6.8 pg/ml vs 8.6 pg/ml) (Lyimo *et al.,* 2000; Lopez *et al.,* 2004b). Contrastingly, cows with high milk yields in early lactation may not be able to physically consume the amount of food required to sustain

the milk production. If energy requirements for milk production are not met through feed intake, the cows body begins to mobilise body tissues (Bauman and Currie, 1980; Bastin and Gengler, 2013) thereby reducing its body condition score (BCS) (Dobson *et al.,* 2007). This is most common in early lactation, when milk production is at its highest. Cows with a high milk yield, and a low body condition score during the early postpartum period take >10days longer to conceive (Lopez-Gatius *et al.,* 2003; Garnsworthy, 2006). Additionally, cows with a reduced BCS during early lactation can take an additional 30 days to display the first post-partum oestrus (Butler, 2003), and once the oestrus cycle resumes, the oestrus periods are shortened in higher producing cows (Walker *et al.,* 2005). Interactions between low BCS and high milk production also affect oocyte quality. *In vitro* cleavage rates are lower in high producing cows with low BCS when compared to cows with a higher BCS (Snijders *et al.,* 2000). Singh *et al.* (2009) determined that high producing cows in early lactation mobilise more body condition score when compared to lower producing cows. However, McGuire *et al.* (2004) reported that in early lactation high-production itself does not relate to body condition score, or use of body reserves. However, cows in a negative energy balance (NEB) have been reported to have decreased pulsatile LH secretion and IGF-I concentrations (Diskin *et al.,* 2003). Both IGF-I and LH are synergistic, and encourage follicular development (Lucy, 2000) therefore follicular efficiency is hindered in NEB cows which leads to decreased oestradiol concentrations resulting in reduced oestrus expression (Butler, 2000; Walsh *et al*., 2011). Additionally, cows that enter a NEB may be ay an increased risk of developing lameness, or becoming increasingly susceptible to disease (Esposito *et al*., 2014). Lame cows have

been reported to decrease their voluntary feed intake, which has a direct effect on their body condition. However, a study by Cutullic *et al.* (2009) reported that reduced oestrus expression in high yielding dairy cows was apparent even when body condition loss was moderate and not severe.

2.7 Economic Losses Due to Lameness and Reduced Fertility

The presence of several diseases in the UK, including lameness has caused significant direct and indirect costs. These are caused from increased Veterinary treatments, discarded milk, reduced milk yield, increased labour, reduced fertility, premature culling, increased calving interval, decreased carcass weight, fat cover class, and conformation class thus reducing carcass value of culled cows (Kossaibati and Esslemont, 1997; Booth *et al.,* 2004; Fjeldaas *et al.,* 2007; Blowey and Edmonson 2010; Poursaberi *et al.,* 2010; Raboisson *et al.,* 2011). Willshire and Bell (2009) reported that lameness in the UK accounted for losses of up to £127.8 million in 2009. Based on a 305-day lactation with a normal milk production of 20kg/day, the total mean reduction in milk yield was approximately 360 kg (1.2 kg/day) for lame cows (Poursaberi *et al.,* 2010). Additionally, an estimated annual direct cost of disease (lameness, mastitis, vulval discharge, retained foetal membranes, milk fever, treatments for oestrus-not-observed, twinning, calf mortality and aid at calving) in an average English dairy herd (152 cows) was £6300 per 100 cows (Kossaibati and Esslemont 1997; Morris, 2007). They reported the main losses were caused from mastitis (38%) and lameness (27%). CAFRE (2006) reported that in a 100-cow herd there is on average 22 cases of lameness annually.

However, incidence of lameness can vary greatly between farms, for example DEFRA (2008a) reported that an average herd may have between 20-70 new cases of lameness annually per 100 cows costing on average £180 per case, and 20-30% of the herd may be lame at any one time. North American estimates suggest 21-55% of cows in freestall housing with a are lame (Espejo et al., 2006; Ito *et a*l., 2010; von Keyserlingk *et al*., 2012; Solano *et al*., 2015), Although the incidence of lameness varies greatly, there is an average of 55 cases per 100 cows/yr., with 22% of the cows being lame at any given time (DEFRA, 2008a). Investigating both direct and indirect losses due to lameness alone, based on Bristol's average 55 cases per 100 cows costing £180 per case, would equate to approximately £9900. Kobbaibati *et al.* (1999) reported that specific losses from horn diseases (e.g. white line abscess) was put at £151.50, single limb-case of sole ulcer at £246.30, skin disease (e.g. digital dermatitis) was £58.90, and the total cost of an average limb-case of lameness was estimated at £136.20, while the total cost per affected cow was £152.80. Cha *et al.* (2010) reported the average cost per case (U.S.D) of digital dermatitis, sole ulcer, and foot rot were 132.96, 216.07 and 120.70 respectively. According to DEFRA (2008b) the population of female dairy cows on permanent agricultural holdings or on common land over 2 years of age that have calved was 1,567,492. An additional 352,057 heifers (not calved) also reside on permanent agricultural holdings or on common land. Therefore, based on the cows over 2 years of age (assuming they are lactating) the total annual economic loss due to lameness is £9900 per 100 cows would equate to £155,181,708.

Lameness is associated with an increased interval between calving and first service (delay of resumption of normal ovarian cyclicity), as well as an increased interval from first service to conception, therefore increasing the time between calving and conception (Lucey *et al*., 1986; Collick *et al*., 1989; Barkema *et al*., 1994; Sprecher *et al.,* 1997; Hernandez *et al*., 2001; Dobson *et al.,* 2010). When comparing healthy cows to lame cows, both the interval between calving and first service was 4 days longer, and the calving to conception interval is documented to range from 14 to 50 days longer for lame cows, even after treatment (Collick *et al*., 1989; Melendez *et al.,* 2003; Hernandez *et al*., 2005b). For example, it has been reported that services per conception are increased for lame cows versus non-lame cows, from 1.72 to 2.14 respectively (Hernandez *et al.,* 2001; Melendez *et al.,* 2003). A study by Esslemont *et al.* (2001) determined that each day a cow failed to become pregnant resulted in a net loss of £1.74 to £6.52 depending on the cow's level of yield, quota costs and also at what point in the postpartum period the delay in conception occurred. Additionally, a single dose of semen from a bull with high genetic merit can cost up to $22 USD (Lima *et al.,* 2010), £29 (Mastergen, 2017a), and if sexed semen is used costs can range from £25-45 (The Dairy Site, 2010). However, some companies offer sexed semen for under £20 (Mastergen, 2017b). If insemination occurs at an incorrect time, this will also add to financial losses.

Failure to correctly identify oestrus equates to a 21-day loss of production (Rao *et al.*, 2013). Accurate and efficient oestrus detection is vital to maintain profitability and reproductive performance in modern dairy herds (Van Vilet and Van Eerdenburg, 1996; Kinsel and Etherington, 1998; Palmer *et al.,* 2010).

Misdiagnosis or failure to detect oestrus is often the greatest limitation to high reproductive efficiency (Liu and Spahr, 1993). Inaccurate oestrus observations can lead to severe economic losses (Senger, 1994) through increased calving intervals, increased number of inseminations to conception, milk production losses, increased culling rates, and decreased birth rates (Liu and Spahr, 1993).

2.8 Culling Due to Reduced Productivity

Culling may be described as being either voluntary, or involuntary (Ahlman *et al.,* 2011). Voluntary culling is mainly carried out due to decreased milk productivity, an increase in herd size, or if a cow is sold to another farm (Hadley *et al.,* 2006). Whereas involuntary culling is done due to presence of disease, lameness, reduced fertility (Esslemont and Kossaibati, 1997; Whitaker *et al.,* 2000; Langford and Stott, 2012), nutrition related issues and higher metabolic stress levels (DairyCo, 2011b). Although involuntary culling is done to reduce suffering, high involuntary culling rates may indicate poor herd welfare (Ahlman *et al.,* 2011). Herds with increased disease levels have been linked to higher culling rates, where in the UK culling rates range from under 18% to over 35% (Bell *et al.,* 2010; DairyCo, 2011b; AHDB Dairy, 2017d). Reasons for culling vary, however lameness, infertility and mastitis are common reasons in many dairy herds (Bascom and Young, 1998; Pinedo *et al*., 2010; Pinedo *et al*., 2014). In 2007, a national survey was carried out in the U.S.A. to determine the reasons cows were culled included. The reasons included; reproductive failure (approximately 26.3% of culled cows), udder problems including mastitis (23%), injury or lameness (16%), other diseases (3.7%), decreased milk production

(16%), and miscellaneous reasons (8%) (The Dairy Site, 2011). Research by Bell *et al.* (2010) demonstrated that 59% of cows were culled before their fourth parity. In the United States, the average lifetime parity number has decreased from 3.4 in 1989 to 2.8 in 2004 (Nieuwhof *et al*., 1989; Hare *et al.,* 2006). Increased culling rates of Holstein cows is reported in Portugal, with as little as 15% of first-time calving cows reaching their fourth parity (Rocha *et al.,* 2010). In a study by Pritchard *et al.* (2013) they reported that in 2009 the average number of calving's for a dairy cow was 3.6, and the average productive lifespan (calving till death) was 4.3 years. Variation in productive lifespans may be affected by breed, management practices, seasonal effects, and replacement heifer rearing strategies. For example, a study by Hultgren and Svensson (2009) reported that heifer rearing conditions can affect the length of their productive life. Replacement heifers reared in large groups on slatted floors had an increased risk of culling when compared to heifers reared in litter pens.

In addition to reduced fertility, it has been reported that lame cows are 8.4 times more likely to be culled than non-lame cows suffering from reduced fertility (Sprecher *et al.,* 1997). In the UK conception rates have declined approximately 1% every 3 years, and is reported to be at around 40% (Royal *et al.,* 2000). Cows culled prematurely leads to an increased number of replacement heifers required, and if a heifer is reared until three years old it will cost an average of £1,150 per head (DairyCo, 2011c).

2.9 Objective Summary

Although significant advances in animal welfare have been made, lameness continues to affect dairy populations. Lame cows have a reduction in overall fitness affecting reproductive function, which contributes to decreased animal welfare through increased incidence of lameness and premature culling, consequently leading to economic losses (Heringstad *et al.,* 2007; Ettema *et al.,* 2010). It is accepted that lame cows do not display oestrus as overtly as non-lame cows, however there is no evidence suggesting if there are specific oestrus detection methods that are more efficient for lame cows. This thesis aims to provide insight to dairy producers' perception of reproductive efficiency between lame and non-lame cows, and to determine how they manage oestrus detection for lame cows.

Research has shown pasture access can improve locomotion scores, however determining if oestrus expression/activity also improves alongside locomotion scores has yet to be investigated. This study examined multiple oestrus events from cows with varying locomotion scores in different housing conditions (pasture v housed).

Methods of oestrus detection have been greatly evaluated. However, determining if a particular oestrus detection method is more efficient for lame cows has not been researched. Therefore, this study aimed to compare common oestrus detection methods (Kamar®, Estrotect^{TM} scratch cards, chalk, activity monitors (NeDap, IceQube®) in lame and non-lame cows, from pastured and housed conditions.

There has been a large range of temperature based oestrus detection methods evaluated. Recent advances include the use of Infrared thermography (IRT). Oestrus detection and ovulation (Hurnik *et al.,* 1985; Jones *et al.,* 2005; Talukder *et al.,* 2014; Perez Marques *et al*., 2019) have been identified through the use of IRT. However, the evaluation of IRT for lame and non-lame cows has not been assessed. Determining optimal body locations for IRT is under development. Therefore, this study evaluated IRT from different body locations, assessed the use of IRT for lame and non-lame cows, and investigated if lame cows had different baseline and oestrus temperatures from non-lame cows.

Chapter 3: Materials and Methods

3.1 Animals and farm management descriptions

3.1.1 Rodwell Dairy farm

Three of the experimental studies (those detailed in Chapters 5, 6 and 7) were conducted at Rodwell Dairy farm, located in Ipswich, Suffolk. The farm was chosen as it was within travelling distance for the researcher, and the farm owner agreed for the research to be carried out. The dairy herd consisted of 130 Holstein Friesian cows with approximately 100 milking at any one time, with average milk production over 11,000 litres per lactation. Cows were milked twice daily at 0500 and 1500. Average daily yield was 35.2 kg/day with an average milk fat of 3.92% and average protein of 3.15%. The cows calved year-round, and parity ranged from 1 to 9. Replacement heifers were bred naturally by a red angus bull to reduce the risk of dystocia for their first calving. All multiparous cows were managed by Genus Reproductive Management Systems, and all cows were artificially inseminated. Inseminations were performed by two AI technicians on an alternating roster. Fertility parameters (from a 12-month rolling average in 2012) number of services to conception 2.39 (215 services), % conceived to 1st service 34 (94 services), number of days to conception 113 (n=90), calving index 397 (n=80).

The cows were milked through a Delaval herringbone 8/8 parlour, with concrete flooring. Milk recordings were carried out monthly through National milk records.

Lactating cows were housed during the winter months in one barn which included freestall cubicles. The cubicles measured 3.0m x 1.15m (outer row), and 2.6m x 1.15m (inner row). Cubicles were cleaned and raked twice daily and sand bedding (6 inches deep) was added once a week. The cows were stocked to 95.2% (105 stalls to approximately 100 cows). The alleyways were scraped twice daily using a tractor and scraper. The yards, alleyways and collecting yards had grooved concrete floors. Dry cows were housed in cubicles, a straw yard, or at pasture depending on the time of year. During the summer months, dry cows were kept at pasture until their predicted calving date. Approximately three weeks before expected parturition the dry cows were brought into a straw yard. During winter months, all dry cows were housed either in a straw yard, or cubicles. After parturition, the cows were moved to a maternity pen, and remained there for three days. The freshly calved cows then joined the lactating herd providing their health was satisfactory. All lactating cows were managed in one group.

During the summer months, the cows were fully pastured. Prior to the cows being fully pastured, they were initially given access to pasture for three hours a day. This was then gradually increased until they were fully pastured (approximately 2 months' transition period). In 2012, the cows were fully housed from October and were given access to pasture starting on May 25, 2013, and were fully pastured on June 26, 2013. The cows were then fully housed from September 21, 2013 to March 27 2014. From March 27 2014, the cows were given limited access to pasture until June 7, 2014 when they were fully pastured. Lactating cows were fed a total mixed ration (TMR), and provided with water ad lib. TMR ration is as follows (Table 3-1).

Table 3- 1 Typical lactating dairy cow diet at Rodwell dairy farm

Item	
Maize silage, kg DM	10.24
Baled grass DM	1.03
Techpromaixe 45	1.05
Double rate yeast	0.19
Protein Blend	7.08
Wheat	1.28
BF-Nutrilac	0.43
Straw mean qual	0.42
Prem high spec min	0.16
Baled wholecrop	1.9
Brewers grains	1.92
DMI (kg/day)	25.7
Diet DM %	42.9
Forage intake (kg DM, % total)	13.6
ME (MJ/day)	301
M/D	11.7
FME (MJ/day)	240
Total NDF (%)	35.8
Starch (kg/day)	4.9
Sugar (kg/day)	2
Oil (g/day)	1245
Crude Protein (%)	17.3

Dry cows were provided with water and straw ad lib, and were also fed a mixed ration detailed in Table 3-2. Diets were manged under the guidance of a commercial nutritionist and were monitored regularly.

Table 3- 2 Typical diet fed to dry cows at Rodwell dairy farm

Item	kg fresh
Maize silage	18
Techmol premium	0.50
Protein blend	1.25
Straw-wheat	3.50
Advanced DCAB	1.50
Total intakes	24.8
Energy required	
Diet energy (% of req.)	117
Protein required (g)	99
Diet protein (%of req.)	643
Forage intake (% of total DM)	103
	76

The cows routinely had their hooved trimmed at the end of lactation, and when required throughout lactation. Cows were foot-bathed twice weekly post milking in a formaldehyde solution. If the cows' feet were exceptionally dirty, they were cleaned with a jet hose before the cows exited the parlour to walk through the footbath in the exit alley.

3.1.2 Lucky Hill Dairy Farm

The experiment described in Chapter 7 included Lucky hill dairy farm, located in Lacombe, Alberta, Canada. The herd consisted of 215 milking cows with average milk production over 10,000 litres per lactation. Average daily yield was 43.6 kg/day with an average milk fat of 3.8% and average protein of 3.1%. Fresh cows were housed in a separate pen temporarily post calving, and later added to the main milking herd. Lactating cows were housed in a freestall barn with stalls equipped with gel mats and were topped with sawdust. The barn had an automatic scraper, as well as water misters and fans to keep the cows cool

during the summer months. The cows were milked three times daily (05:00, 13:00 and 20:00), had access to water adlib, and were fed a total mixed ration (Table 3-3).

Table 3- 3 Typical diet fed to lactating cows at Lucky Hill Farm

Item	% of
Maize silage, % of forage DM	56
Lucerne silage, % of forage DM	29
Legume/grass forage, % of forage DM	15
Ration starch, % DM	26
Ration crude protein, % DM	18.3
Ration NDF, % DM	32.7
Forage NDF, % bodyweight	1
Forage, % of ration DM	62

The method used for locomotion scoring was that of Flower and Weary (2006b) and criteria used are shown in Table 3-5. Cows that scored 3 and above were considered lame. Prevalence of lameness was calculated as the proportion of cows scoring 3 or above out of the cows scored. The average lameness prevalence was 33%, inseminations to conception was 3.0. The heat detection method used at farm 3 was the Heatime program (Heatime®, SCR Engineers Ltd., Netanya, Israel), which monitors activity. The Heatime system consists of animal tags, a small control terminal and an identification (ID) transceiver. The animal tags monitor individual cow activity levels and 24 hour cumulated activity. Every animal movement and movement intensity are provided using a three-dimensional accelerometer. The data is analysed and filtered by using an algorithm in an on-board processing unit. The neck collar positions the logger on the left side of the neck. 14 d after calving, all cows are fitted with an activity

monitoring tag. The ID transceiver unit was positioned at the entrance of the milking parlour. This scans all the devices as the cows pass by. The data is then transferred to the activity monitoring system herd management software (Heatime for PC, SCR Engineering Ltd., Netanya, Israel) installed on the on-farm computer. When increased activity is detected, the monitoring system will send an alert (flashing light), which is visible when entering the barn.

3.1.3 Thornspyc Dairy Farm

The experiment described in Chapter 7 included Thornspyc dairy farm, located in Lacombe, Alberta, Canada. This farm consisted of 167 milking cows with average milk production over 10,000 litres per lactation. Average daily yield was 42.5 kg/day with an average milk fat of 3.6% and average protein of 2.9%. Lactating cows were housed in free-stall cubicles equipped with rubber matting and topped with chopped straw. Cows were milked twice daily (03:30 and 15:30) had access to water adlib, and were fed a total mixed ration (Table 3-4).

Table 3- 4 Composition of typical diet fed to lactating cows at Thornspyc Farm

Item	% of
Maize silage, % of forage DM	56
Leucerne silage, % of forage DM	40
Grass silage, % of forage DM	4
Ration starch, % DM	24
Ration crude protein, % DM	17.2
Ration NDF, % DM	32
Forage NDF, % bodyweight	1
Forage, % of ration DM	62

The barn had an automatic scraper, and fans to cool the cows during the summer months. The method used for locomotion scoring was that of Flower

and Weary (2006b) and criteria used are shown in Table 3-5. Cows that scored 3 and above were considered lame. Prevalence of lameness was calculated as the proportion of cows scoring 3 or above out of the cows scored. The average lameness prevalence and number of inseminations to conception were 24%, and 2.8 respectively. Cows were seen every two weeks by a veterinarian, and they were managed on a PreSynch, OvSynch, synchronisation protocol for timed artificial insemination (Plate 3-1). PreSynch is the use of two Prostaglandin $F_{2\alpha}$ (PGF) injections, given at 14 days apart, with the last injection given 14 days before initiation of the OvSynch protocol (Pursley *et al*., 1995). OvSynch is the injection of Gonadotropin-releasing hormone (GnRH) 14 days after the PGF injection, followed by second GnRH injection 48 hours after the PGF injection (Pursley *et al*., 1995). Cows were given the final injection on 15/7/14.

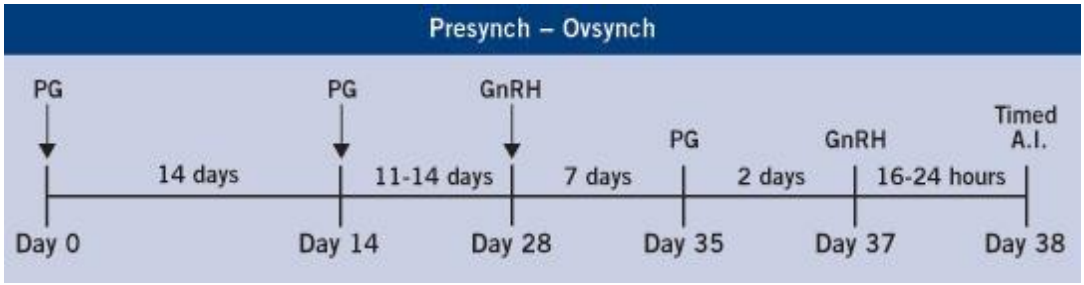

Plate 3- 1: Synchronisation protocol (Source: ABS Dairy, 2017)

3.2 Locomotion scoring

The method used for locomotion scoring was that described by Flower and Weary (2006b). This method was based on a 5-point scale, in which a score of 1 represented a sound animal and 5 represented a severely lame animal (Table 3-5). If a cow exceeded the requirements of a particular score, but did not meet all the requirements of the next successive score, a half-integer score was allocated. This method was chosen following a review of the other methods

available. This method was favoured because cows can be scored while walking. Additionally, cows only have to be scored once unlike the method of Sprecher *et al.* (1997) where cows have to be scored while standing and walking. There was also no requirement to score cow rising behaviour as in the method of Manson and Leaver (1988). Cows were always scored following afternoon milking (15:00 to 18:00) while walking along on a flat concrete alley that led to a 90-degree left turn. This enabled observation of the hind leg abduction/adduction, to assess symmetry of the cows' gait. Additionally, all milking cows were filmed (Samsung HD 200 camcorder, Samsung Electronics Co., Samsung GEC, 26, Sangil-ro 6-gil, Gangdong-gu, Seoul, Korea) to enable careful studying of each individual cow's locomotion, and to assess intraobserver reliability of locomotion scoring. The camera was positioned approximately 5 metres away to capture at least 3 full strides. Cows were recorded from the right side upon their return from the milking parlour. The cows were habituated to the presence of the camera, and to the act of being moved down the alleyway by the researcher, four times before official locomotion scores were given. The mean number of cows' locomotion scored monthly were n=101.3 (±2.5) equating to 6707 locomotion scores given from February 2013-September 2014.

Table 3- 5 Locomotion scoring criteria

Score	Description	Behavioural Criteria
1	Smooth and fluid movement	Flat back Steady head carriage Hind hooves land on or in front of forehooves (track up) Joints flex freely Symmetrical gait All legs bear weight equally
2	Imperfect locomotion but ability to move freely is compromised	Flat or mildly arched back, Steady head carriage, Hind hooves do not track up perfectly, Joints slightly stiff, slightly asymmetric gait, all legs bear weight equally
3	Capable of locomotion but ability to move freely is compromised	Arched back, Steady head carriage, Hind hooves do not track up perfectly Joints show signs of stiffness, Asymmetric gait, Slight limp can be discerned
4	Ability to move freely is obviously diminished	Obvious arched back Head bobs slightly Hind hooves do not track up Joints are stiff and strides hesitant Asymmetric gait Reluctant to bear weight on at least one limb but still uses that limb in locomotion
5	Ability to move freely is severely restricted and must be vigorously encouraged to move	Extremely arched back Obvious head bob Poor-tracking up with short strides Obvious joint stiffness characterised by lack of joint flexion with very hesitant and deliberate strides Asymmetric gait Inability to bear weight on one or more limbs

(Flower and Weary, 2006b)

3.2.1 Reliability of locomotion scoring method

The locomotion scoring sessions were recorded on video from February 2013 to September 2014. Six videos were randomly selected (Excel random number generator) and were reviewed to re-score the cows to calculate the reliability of the scoring method (Table 3-6). To determine how closely the scores were to one another the correlation coefficient was calculated using Excel.

Most cows passed the camera in a single file, and the 90-degree left turn at the end of the alley enabled the freeze brand to be read. From the videos 96-100% of the cows were identified and re-scored. Videos were scored by the same observer (AW) and were compared with the live locomotion scores given to the cows. Percentage agreement was calculated from the number of cases in which the original locomotion score and new locomotion score matched (Table 3-6).

$$\text{Percentage agreement} = \frac{\text{total number of LCS in agreement}}{\text{total number of LCS}} * 100$$

Cows were given a score from 1-5, where 1 and 2 are non-lame, and scores 3 and above are lame. Agreement with lame/non-lame category was where the scores were within one point but the overall classification of the animal was still correct i.e. score 1-2 are categorised as non-lame and score 3-5 are categorised as lame.

$$\text{Percentage agreement} = \frac{\text{total number of category agreement}}{\text{total number of cows}} * 100$$

Videos were then rescored to assess the agreement between scoring from videos.

Table 3- 6 Reliability of Locomotion scoring

	March 2013	July 2013	October 2013	April 2014	June 2014	August 2014
Proportion of cows re-scored	96%	97%	100%	98%	100%	100%
Proportion agreement	79%	81%	75%	81%	80%	88%
Proportion agreement within lame non-lame	100%	94%	100%	100%	80%	100%
R^2 stat	0.88	0.83	0.76	0.81	0.74	0.99

Cows re-scored from June 10 2014, were scored again to assess the agreement between scoring from video. The first scoring took place on 12th September 2016 and the second scoring was undertaken on the 4th of November 2016, which had an agreement of 82%, with 100% within one score (R^2=0.72) These results are within the range of other studies such as Kaler *et al*. (2009) who reported within observer agreement of 76% (range 73-77%).

3.3 Oestrus Detection methods

3.3.1 IceQube® activity monitors

IceQube® activity monitors (IceQube®, Ice Robotics Ltd, Roslin, BioCentre, UK) were used in Chapters 5 and 6 to measure to activity in dairy cattle. IceQube®, is a logger with accelerometric sensors that measures animal activity with sampling rate 4 Hz, and summarises data into 15-minute blocks. The logger is programmed to record the g-force in three dimensions. The activities recorded were step count, lying time, standing time, number of lying bouts, lying bout length, and motion index. Motion index is the sum of the measured net acceleration in the three dimensions minus an offset for gravity,

and as such an expression of leg activity. The waterproof loggers are typically attached to the lateral side of the cow's hind leg above the metatarsophalangeal joint with a special strap (Plate 3-2).

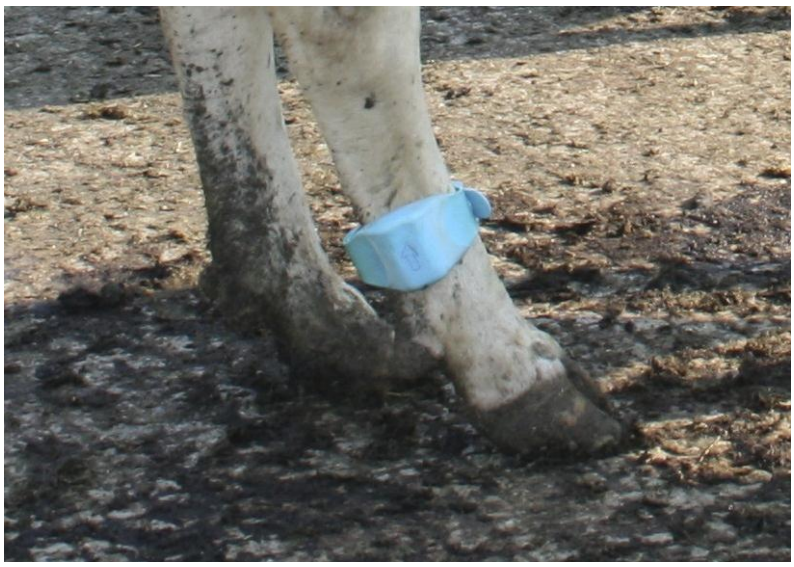

Plate 3- 2 Study cow fitted with an IceQube® activity monitor

Individual cow data were downloaded wirelessly using an IceQube® reader connected to a laptop. The data were processed using IceManager2010 software (IceRobotics Ltd), and were stored as CSV files before being converted to Excel files for statistical analysis.

Each download recorded the following information;

1) the time the cow spent lying and standing, determined by the sensor passing a specific threshold between horizontal/vertical position;

2) lying bouts count determined by start and end time of each lying bout;

3) step count determined on the number of times the cow lifts her tagged leg, based on the acceleration of the animal leg;

4) the motion index which reflects the average magnitude of acceleration on each of the 3 axes (IceRobotics Ltd, Product Guide 2010).

3.3.1.1 Validation of IceQube® activity monitors for cows at pasture

Similar activity monitors (IceTag) were validated by Munksgaard *et al.* (2006), and Blackie (2009). Direct observations were made for walking, standing and lying. These observations were highly correlated with the data from the IceTags (Table 3-7).

Table 3- 7 Validation of IceTag activity monitors from previous studies

Behaviour	Munksgaard *et al.* (2006)	Blackie *et al.* 2009
Walking	0.95 0.97	0.84-0.95
Standing	0.99	0.84-0.95
Lying		

The IceQube® sensors were validated by Elischer *et al.* (2013); activity reported by the IceQube® and live observations were strongly correlated (R^2=0.91), as were live observations of lying (R^2=0.97) and the number of steps reported by the IceQube and live observations of walking (R^2=0.90).

The author (AW) undertook further validation of the IceQube® sensors which consisted of two small studies at Rodwell Dairy farm. The first involved analysing lying bout length and frequency for cows under simulated pasture conditions. For this study 10 high yielding lactating Holstein-Friesian dairy cattle were used. The mean parity of the cows was 3.3 (±0.6). Each cow was fitted with one IceQube® Sensor to their right hind leg above the fetlock. Before observations were collected, the cows were habituated to wearing the IceQube® (minimum 7 days). Cows were identified by their freeze brand, and were marked with animal marking paint (Richey sprayline coloured stock

marker). Each cow was painted with a different number/letter on both the right and left side. Validation took place over two days. The 10 cows were divided into two groups of 5, and were observed continuously for 24 hours via CCTV. Video footage was recoded onto a digital video recorder (Inspire Silver DVR, COP Security, Delph New Road, Dobcross), from two waterproof wired cameras (SecurityIng®, Longhua, Shenzhen city, Guangdong province, China, 518131) with up to 20-meter night vision range were installed and positioned to ensure all cows were visible at all times. Day one recorded 24 hr footage from n=5 cows, day two recorded the other group (n=5) for 24 hours.

To represent pastured conditions cows were housed in a straw pen for a minimum of 24 hours in order for continuous video observation to take place. The bedded area measured 7mx10m, and the loafing area measured 3mx10m (see Figure, 3-1).

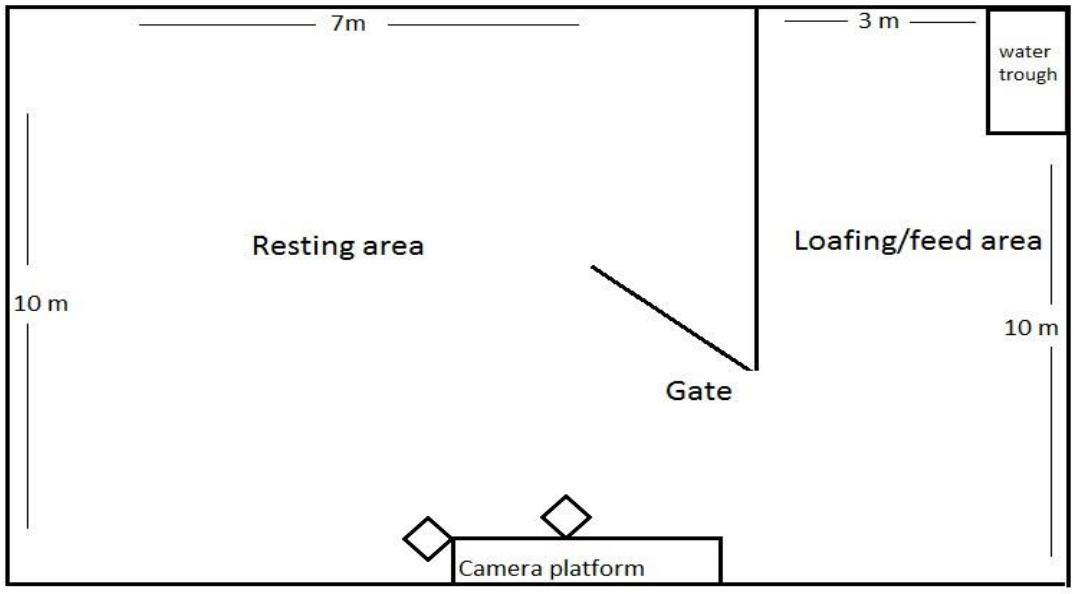

Figure 3- 1 Floor plan and CCTV positioning of straw barn

The footage was then analysed by one observer (AW). The time each cow lied down, and stood up was recorded. The time from lying down to standing is called a lying bout. The start of a lying bout was determined when the cows flank touched the ground, and the end of a lying bout was when both hind legs were vertical to the ground. Notes were made if unusual lying postures were observed i.e. flat on side, hind leg extended etc. IceQube® data were downloaded wirelessly, then transferred to an Excel file, and were then compared to the observed lying bout lengths. Lying bout lengths were measured in seconds, and the correlation coefficient was calculated in Excel.

Data was then plotted on Excel graphs. The data show high correlations (R^2=0.99) between observed lying bout length and lying bout lengths obtained from the IceQube®'s, and are presented in Table 3-8.

Table 3- 8 Comparison of lying bout length calculated from IceQube® activity monitors and visual observation from video footage in 10 lactating Holstein-Friesian dairy cows over 24hrs

Cow ID	Lying bout length observed (s)	Lying bout length IceQube (s)	Correlation R^2
36	4108	4107	0.99
43	3161	3158	0.99
47	3319	3316	0.99
62	2794	2791	0.99
76	2732	2729	0.99
140	3157	3154	0.99
145	3071	3063	0.99
164	1273	1271	0.99
186	2763	2759	0.99

The data from one IceQube® and the corresponding direct observation is presented in Figure 3-2 to demonstrate the relationship between lying bouts calculated from the IceQube®'s and those determined from CCTV. The other 9 graphs can be found in Appendix 1 on page 330. There was a very high correlation between the direct observation and correlating IceQube®. On two occasions two separate cows lied down on their side, extending their hind legs (Plate 3-3 and Plate 3-4). These positions did not affect the activity recordings from the IceQube® monitors.

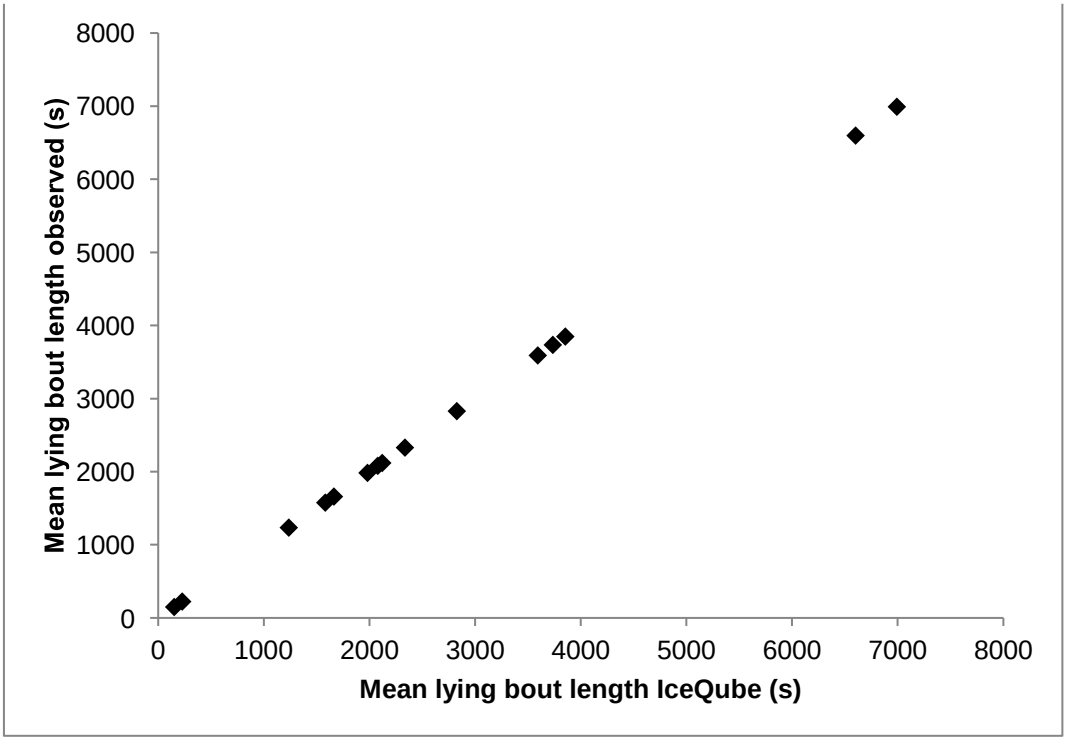

Figure 3- 2 The relationship between lying bout length determined from IceQube® and CCTV footage from cow 76

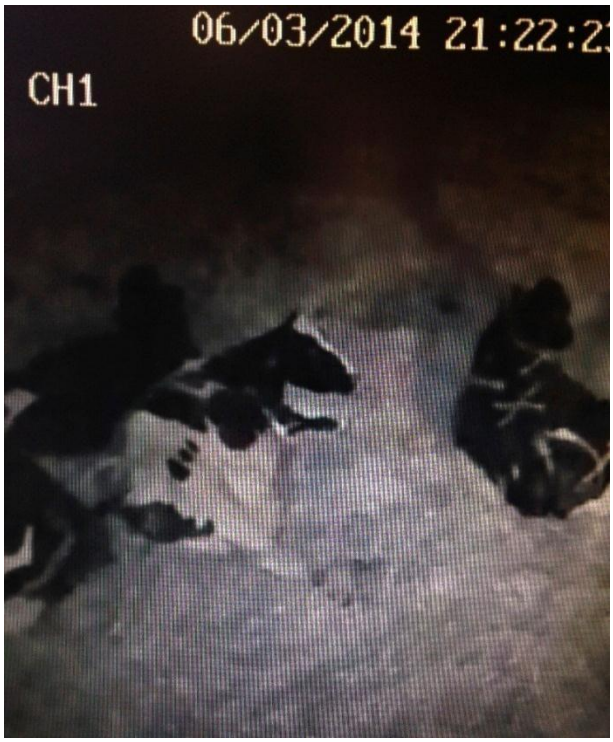

Plate 3 3 Study cow laying with right hind leg extended while wearing an IceQube® monitor

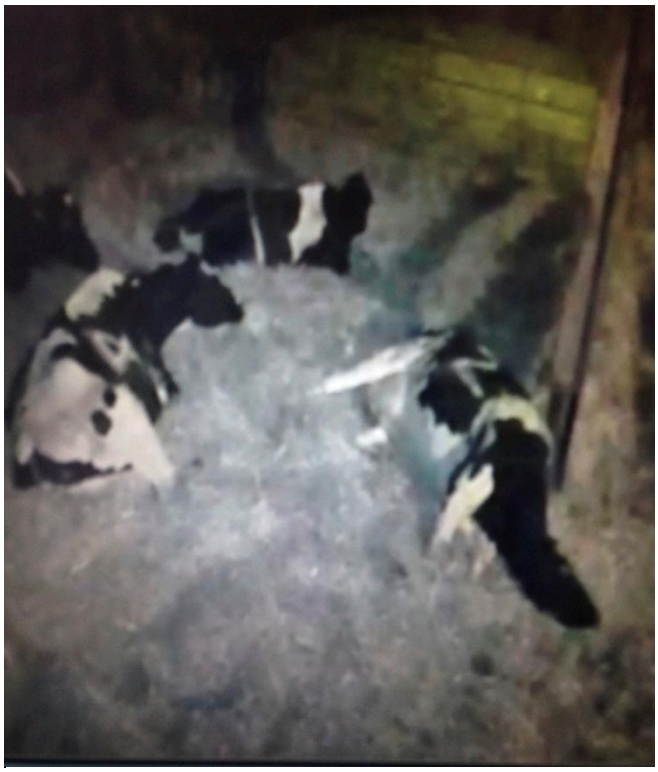

Plate 3- 4 Study cow lying flat with hind leg extended while wearing an IceQube® monitor

3.3.1.2 Validation of IceQube® activity monitors for housed cows

The second validation study of lying bout length and frequency used 10 high yielding lactating Holstein-Friesian dairy cattle housed in a freestall barn. The mean parity of the cows was 4 (±0.3). Each cow was fitted with one IceQube® Sensor to their right hind leg above the fetlock. Before observations were collected, the cows were habituated to wearing the IceQube® (minimum 7 days). Cows were identified by their freeze brand, and were marked with animal marking paint (Richey sprayline coloured stock marker). Each cow was painted with a different number/letter on both her right and left side. Validation took place over 24 hours. The 10 cows were observed continuously for 24 hours via CCTV Video footage was recorded with a digital video recorder (Inspire Silver DVR, COP Security, Delph New Road, Dobcross), and four cameras. Three were night vision waterproof wired cameras (SecurityIng®, Longhua, Shenzhen city, Guangdong province, China, 518131) with up to 20meter night vision range. One non-night vision camera (Sanyo VCC6695P, Moriguchi, Osaka, Japan) with a range of 25 meters was also installed. Cameras were positioned to maximise coverage of the shed (see Figure 3-3) The files were transferred to an external memory source for analysis.

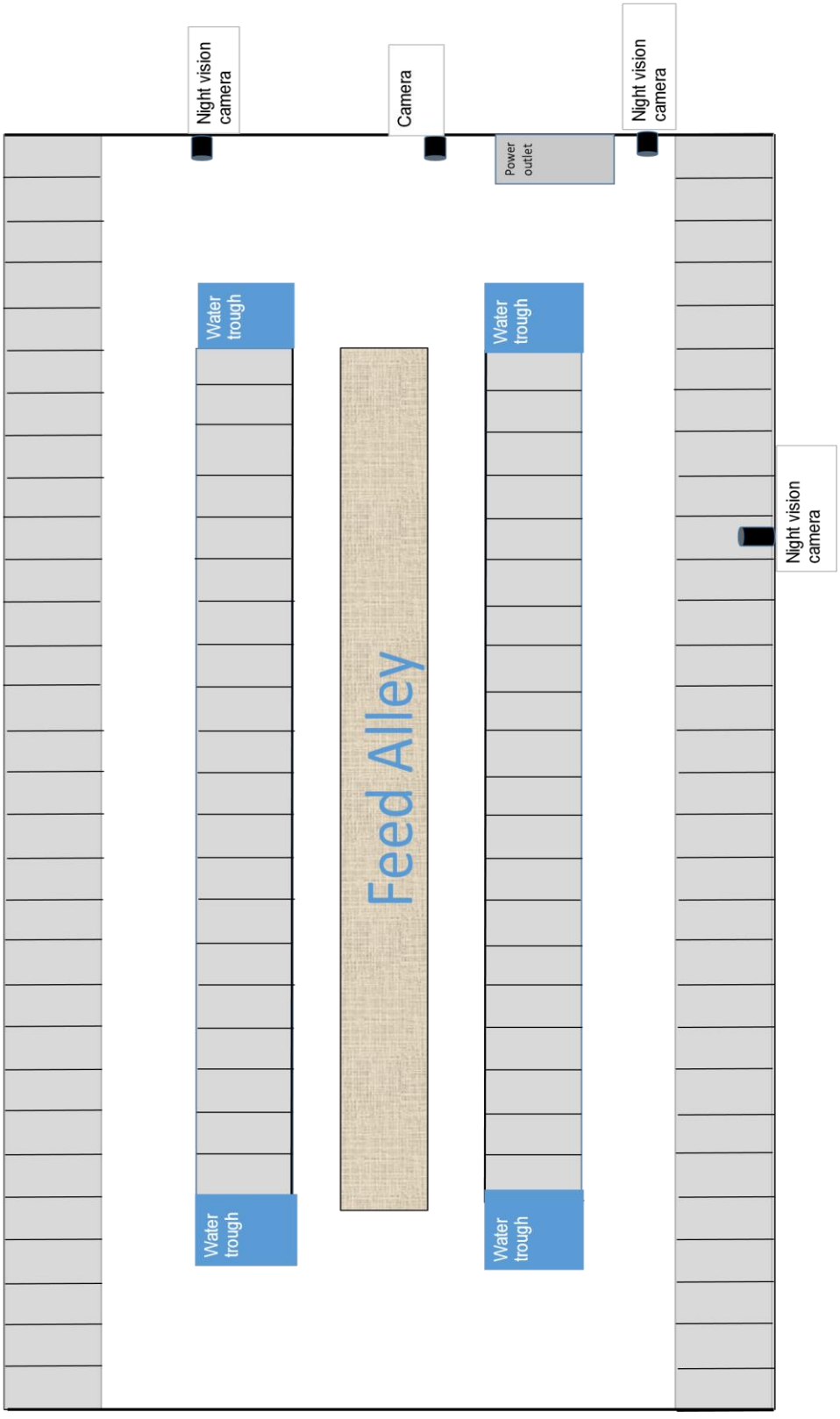

Figure 3- 3 Floor plan of the dairy cow accommodation at Rodwell Farm. CCTV positioning also included

The footage was analysed by one observer (AW). The time each cow lied down, and stood up was recorded. The time from lying down to standing is called a lying bout. The start of a lying bout was determined when her flank touched the ground, and the end of a lying bout was when both hind legs were vertical to the ground. Notes were made if unusual lying postures were observed i.e. hind leg extended into alleyway etc. Data from the IceQube®'s were downloaded wirelessly, then transferred to an Excel file, and were compared to the observed lying bout lengths. Lying bout lengths were measured in seconds, and the correlation coefficient was calculated in Excel. Data was then plotted on Excel graphs. The data show high correlations (R^2=0.99) between observed lying bout length and lying bout lengths obtained from the IceQube® sensors and are presented in Table 3-9. Two of the cows (99, 138) were not continuously visible. Cow 99 for 2 hours, and cow 138 for 3 hours. Therefore, additional observations from the CCTV were made after the 24-hour period to make up for the missing time. No unusual lying patterns were identified.

Table 3- 9 Comparison of lying bout length calculated from IceQube® activity monitors and visual observation from video footage in 10 lactating Holstein-Friesian dairy cows over 24 hrs.

Cow ID	Lying bout length observed (s)	Lying bout length IceQube (s)	Correlation R^2
7	4114	4540	0.99
51	5485	5490	0.99
59	3223	3228	0.99
88	3150	3152	0.99
99	4461	4462	0.99
106	2652	2656	0.99
138	3094	3097	0.99
145	2533	2534	0.99
165	4959	4962	0.99
166	4143	4130	0.99

The data from one IceQube® and the corresponding direct observation is presented in Figure 3-4. to demonstrate the relationship between lying bouts calculated from the IceQube®'s and those determined from CCTV. The other 9 charts can be found in Appendix 2 on page 335. There was a very high correlation between the direct observation and correlating IceQube®'s for all cows.

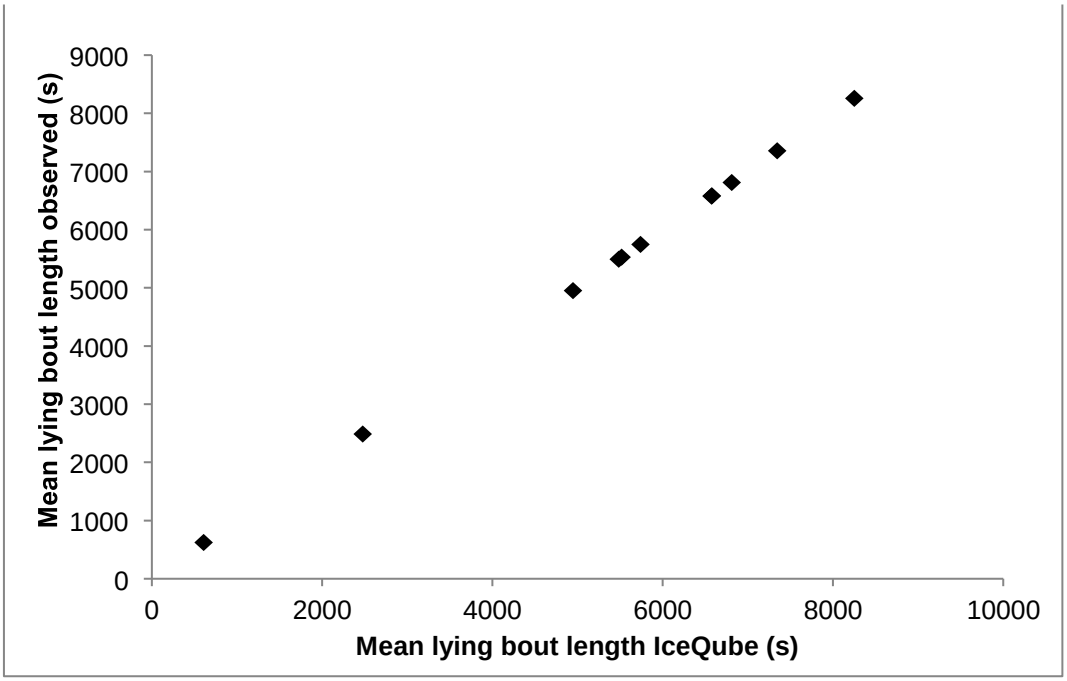

Figure 3- 4 The relationship between lying bout length determined from IceQube and CCTV footage from cow 51

3.3.2 NeDap Activity Monitors

Experiments in chapters 5 and 6 used a NeDap heat detection system. This system monitors dairy cow activity by continuously recording cow behaviour and movements. Activity is recorded on either a neck, or leg transponder. The behaviour and movements (leg or neck movements) of each cow are measured and recorded in two-hour periods. The activity in one period is compared with the activity in the same period over the preceding days. If the cow's activity

either increased, or decreased significantly over several consecutive time periods, attention alerts are possible at several levels. Alerts can be sent for; a suspected heat, a heat with optimum insemination time, reduced leg activity, and reduced eating behaviour (neck version). The information is available in RealTime, and records from 75 metres around the antenna in the barn, or up to 1,000 metres around the Long-Range antenna for grazing. An ID controller collects all the data received by the antenna and sends it to the process controller. The process controller analyses the individual data for each cow and send the results to a PC (Plate 3-5). The cost of a NeDap heat detection system for a farm with 120 cows is 13,000 Euros (11196.76 GBP) on January 19, 2017

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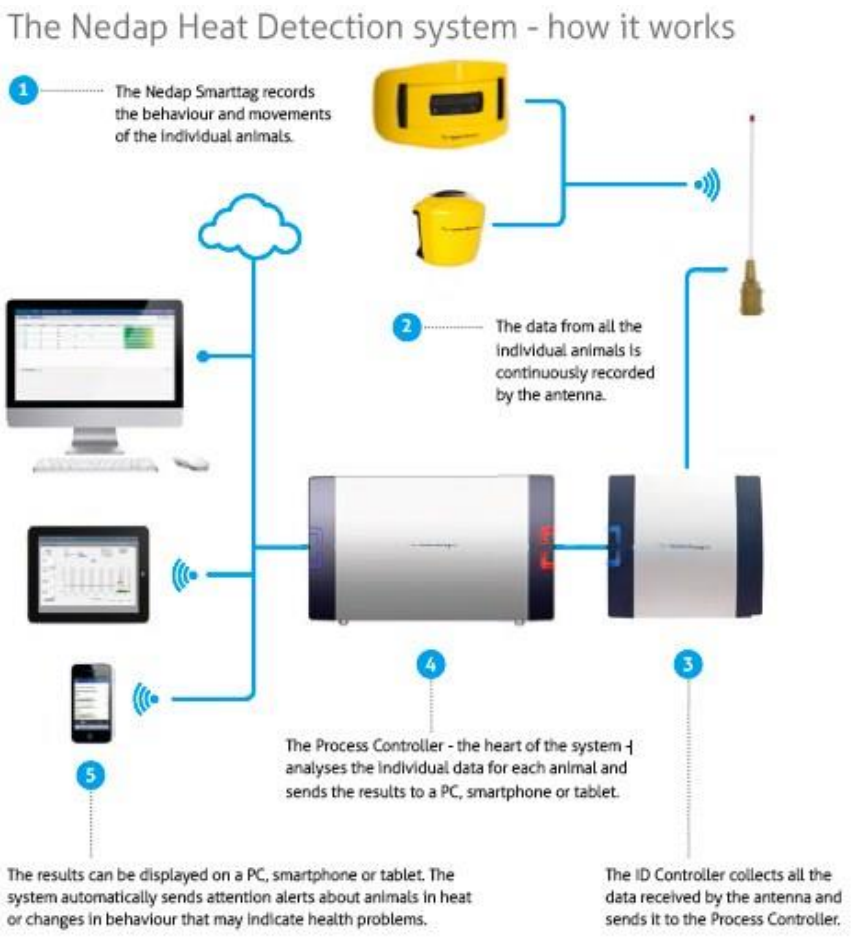

Plate 3- 5 NeDap activity system (Source: NeDap Agri, 2017)

3.3.3 Mount detectors

Three different colour indicative mount detectors were used for chapters 5 and 6.

3.3.3.1 Kamar® Heatmount detectors

Kamar® Heatmount detectors are a pressure sensitive device with a built-in timing mechanism designed to be activated by standing heat behaviour. The detector requires a cow to be mounted for a minimum of three seconds to activate the capsule (van den Berg, 2014). This timing mechanism aims to distinguish between true standing heat versus false mounting activity. Following adequate mounting, the capsule ruptures and releases red ink into the surrounding area (Foote, 1975, Sheldon *et al*., 2006, Holman *et al*., 2015). The detectors are glued onto the sacrum (tail head) with a strong adhesive (Plate 3-6). These detectors are readily available to purchase online, costing £30.79 ex VAT for a 25 pack as of May 4, 2019, (Farmacy, 2019a).

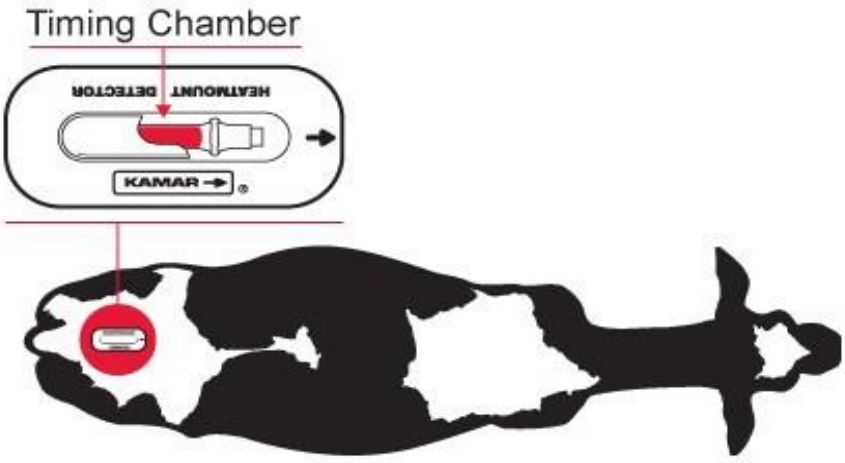

Plate 3- 6 Kamar® mount detector (Source: Kamar, 2017)

3.3.3.2 Estrotect™ scratch cards

Estrotect™ scratch cards are a heat detection aid, which works on a scratch card principle. They are self-adhesive and come in peel off packs, and the more times that a cow is mounted, the more of the silver foil that is removed and the more of the Day-Glo colour shines through and can be seen from a distance (DairyMac, 2017). They are attached between the hip and the tail head and placed perpendicular over the spine (Plate 3-7). These detectors are readily available to purchase online, costing £50.48 ex VAT for a 50 pack as of May 4, 2019 (Farmacy, 2019b).

Plate 3- 7 Estrotect™ scratch cards (Source: DairyMac, 2017)

3.3.3.3 Tail chalk

Tail chalking involves placing a mark on the cow's tail head (Plate 3-8), so that when the cow stands to be mounted, this mark will be erased, or at least changed. Therefore, oestrus can be diagnosed based on the absence or change to the mark, in combination with secondary signs of heat and farm

records (SelectSires, 2017). Tail chalk sticks are readily available to purchase online, costing price for one stick is £2.70 +VAT as of May 4, 2019 (Fanevalleystores, 2019).

Plate 3- 8 Tail chalk (Source: Genus ABS, 2012)

3.4 Visual Observations

A closed-circuit television system (CCTV) was installed to assist in visually identifying oestrus behaviours in housed cows without the presence of a human, and to record observations frequently throughout the day. A digital video recorder (Inspire Silver DVR, COP Security, Delph New Road, Dobcross) and four cameras were used. Three were night vision waterproof wired cameras (SecurityIng®, Longhua, Shenzhen city, Guangdong province, China, 518131) with up to 20-meter night vision range. One non-night vision camera (Sanyo VCC6695P, Moriguchi, Osaka, Japan) with a range of 25 meters was also installed. Cameras were positioned to maximise coverage of the shed (Figure 3-3). The files were transferred to an external memory source for analysis. Live

observations were carried out by the researcher (AW). The main behaviour expected for oestrus detection was when a cow stands to be mounted. However secondary behavioural signs were also recorded using the Dutch points scale (Table 3-10).

Table 3- 10 Point scoring scale for behaviour signs of oestrus

Behaviour	Points
Mucous vaginal discharge	3
Flehmen	3
Restlessness	5
Sniffing the vulva of another cow	10
Mounted but did not stand	10
Resting chin on the back of another cow	15
Mounting the rear of another cow	35
Mounting the head of another cow	45
Stood-to-be-mounted (STBM)	100

(van Vliet and van Eerdenburg, 1996)

3.5 Milk progesterone analysis

Experiments in chapter 5 and 6 used milk progesterone analysis to determine a true oestrus event. This was done using progesterone enzyme linked immunosorbent assay (ELISA) test kits (Ridgeway Science Ltd., Rodmore Mill Farm, Alvington, Gloucestershire, UK), which is an accepted technique for P^4 analysis (Gillis *et al*., 2002; Roelofs *et al*., 2006; Gorzecka *et al*., 2011; Nyman *et al*., 2014; Blavy *et al*., 2016; Adriaens *et al*., 2017; Daems *et al*., 2017). Milk sample handling analysis was carried out following the manufactures protocol, except for incubation period which is described in (Section 3.5.4).

3.5.1 Biochemical principals of ELISA

An ELISA test, is an immunological assay used to measure antibodies, antigens, proteins and glycoproteins in biological samples (Horlock, 2014). These can be used to determine the level of progesterone in a sample of milk, plasma or serum. Techniques for assaying progesterone in milk are commonly used to determine the stage of the bovine oestrus cycle (Nebel, 1988; Isobe, *et al*., 2004; Rioux and Rajotte, 2004; Gorzecka *et al*., 2011). The principals for this assay are based on competition. A sample containing milk progesterone is added to a conjugate solution which contains molecules of progesterone linked to an enzyme (Rioux and Rajotte, 2004). The mixture is further inoculated with an antibody. The antibody links with both the sample and the progesterone linked to the enzyme (Rioux and Rajotte, 2004). The quantity of the antibody should be small, but plentiful enough that all antibody binding sites will be occupied (Rioux and Rajotte, 2004). The progesterone from the sample and the conjugate compete for these sites. The quantity of enzyme-labelled progesterone binding to the antibody is therefore inversely proportional to the concentration of the sample (Rioux and Rajotte, 2004). Post incubation, the surplus of non-linked progesterone and conjugate will be removed. Only molecules linked to the antibody remain in the wells. A chromogen solution (substrate of the enzyme) is then added which reacts with the conjugates' enzyme (linked to antibody) to give a colouration (Rioux and Rajotte, 2004). Colouration intensity is inversely proportional to the concentration of progesterone in milk (Nebel, 1988; Rioux and Rajotte, 2004). The stronger the colour changes indicate low progesterone, suggesting oestrus (Rioux and Rajotte, 2004).

3.5.2 Milk Sampling method

Milk sampling started when a cow entered the study 25.3 (±0.7) DIM, and stopped 10 days after oestrus was observed. This would enable individual analysis of progesterone profiles prior to, during, and after oestrus. Each cow had 11 samples analysed, 5 samples prior to oestrus, the day closest to oestrus, and 5 samples post oestrus. Chapter 5 analysed n=187 milk samples, and Chapter 6 analysed n=737 milk samples. Milk samples were collected three times weekly for use in milk progesterone (P^4) analyses (Monday, Wednesday, Friday), at afternoon milking from the right rear quarter, with one exception (cow 185) as her right rear quarter was dry, therefore the sample was taken from her left rear quarter. Samples were collected from one quarter to reduce interference with the herdsmen's milking routine. Teats were dipped prior to sapling using iodine teat disinfectant followed by wiping to ensure hygienic conditions for animal welfare and milking purposes. Gloves were worn for sampling, and initial stripping's (foremilk) were discarded before samples were obtained. Milk samples were collected into 25ml test tubes containing a preservative (Lactab Mark III; Thompson and Cooper Ltd., Runcorn, UK). Cow number and date were written on the side of the test tubes, and were placed into a test tube stand. Milk samples were placed into a cool bag, and were chilled immediately in the farm fridge (4°C), until freezing (within 3.5 hours). Frozen milk samples were stored at Writtle University College laboratory until P^4 analysis could be carried out.

3.5.3 Progesterone ELISA Kit

Progesterone analysis was done using an enzyme immunoassay (Ridgeway Science Ltd., Rodmore Mill Farm, Alvington, Gloucestershire, UK) Each progesterone ELISA test kit came with a;

- 5x96-well ELISA microplates coated with an antibody with a high specificity for progesterone
- 130ml of Progesterone-Enzyme conjugate (Ingredients: Gelatine, Sodium Chloride, Sodium Phosphate monobasic monohydrate, Sodium phosphate, dibasic dodecahydrate, Thiomersal, water)
- 10 pots of Substrate Powder (Sigma P5758) (Ingredients: Phenolphthalein monophosphate bis (cyclohexyl ammonium) salt,
- 1 bottle of DEM Substrate buffer (Ingredients: Water, Diethanolamine, Thiomersal, Magnesium Chloride Hexahydrate, PMP Substrate)
- Full set of progesterone standards (0, 1, 2, 5, 10, 20, 50ng/ml).

3.5.4 Protocol optimisation

Milk samples were handled as directed by the manufacturer, except for incubation period, which is described below. Both the milk standards and the ELISA plates were brought to room temperature. Milk samples were thawed in a 40°C water bath for 1h and were inverted immediately before analysis to generate a homogenous emulsion. Milk samples, standards and enzyme conjugate were pipetted, and distributed into the ELISA wells using a Biomek 2000 © (Laboratory Automation workstation, Beckman Coulter, Fullerton, CA, USA). 10 µl of distilled water was pipetted into the first 2 wells. 10 µl of each concentration of progesterone standard were pipetted into the next 14 wells (7

standards, replicated twice). 10 µl of milk samples were pipetted into the wells (each day analysed was replicated twice). After all samples were pipetted into the wells, the enzyme conjugate label.

In order to optimise the results obtained from the ELISA kits, further steps were taken. Alterations included 1) the ELISA plates were placed on a plate shaker (KS125 basic, IKA Labortechnik) at 400 Mot 1/min for 1hr 30 mins after the addition of the standards, samples, and progesterone enzyme label 1 to maximise interaction between the ELISA well walls and the reagents. 2) After washing the wells with cold distilled water three times, the substrate II buffer was added, and the plate was then placed back onto the shaker for 30 minutes to maximise adhesion within the wells.

Plates were read using a spectrophotometer/fluorometer at 570nm (Molecular Devices, E Max, precision microplate reader) to determine the absorbance values of the samples. The subsequent reaction produced a colour change which is inversely proportional to the amount of progesterone in the sample i.e. a strong colour means low progesterone. Values were then obtained and recorded into Excel for statistical analysis.

Chapter 4: A survey of dairy cow fertility- Evaluation of oestrus detection methods in different housing systems and determination of reproductive performance of lame and non-lame dairy cattle

4.1 Introduction

Poor reproductive performance and increasing rates of lameness are major issues in modern dairy herds (Chapinal *et al*., 2013), resulting in reduced animal welfare (Ettema *et al.,* 2010; Heringstad *et al.,* 2007; Vermunt, 2007), and profitability among dairy farmers worldwide (Maatje *et al*., 1997; Shearer and Amstel, 2000). Although many individuals in the dairy industry are concerned about lameness, the prevalence remains high (Higgins Cutler *et al*., 2017). The continued high prevalence and the large variation in lameness prevalence among herds (Solano *et al*., 2015) indicate that producers have difficulty successfully reducing lameness in their herds (Higgins Cutler *et al*., 2017), or that they don't consider it an important issue (Leach *et al*., 2010a). Leach *et al*. (2010a) reported that dairy producers in the United Kingdom described that time, labour, and financial constraints limit their ability to reduce lameness in their herds. Other difficulties in lameness control may include a lack of awareness of the problem, ignoring the cause, or even underestimating the severity of the issue (Bell *et al*., 2009; Leach *et al*., 2010a; Bran *et al*., 2018). It has been reported that US and UK producers substantially underestimate the prevalence of lameness in their herds by 26 to 40% compared with trained assessors (Wells *et al*., 1993; Whay *et al.,* 2003; Espejo *et al*., 2006). A study by Higgins Cutler *et al*. (2017) reported that trained

researchers reported lameness to be 3.6 times higher than the producers did. Furthermore, in their study they reported that more than 50% of producers they surveyed claimed lameness was not or was only a minor issue on their farm. This perception that lameness is not an issue likely results from the underestimation of lameness prevalence in their herds (Higgins Cutler *et al*., 2017).

Lameness affects dairy cow fertility at all reproductive stages (Alawneh *et al.,* 2011). Lameness is associated with an increased interval between calving and first service (delay of resumption of normal ovarian cyclicity), as well as an increased interval from first service to conception, therefore increasing the time between calving and conception (Barkema *et al*., 1994; Collick *et al*., 1989; Dobson and Smith, 2000; Garbarino *et al*., 2004; Hernandez *et al*., 2001; Lucey *et al*. 1986; Sprecher *et al.,* 1997). When comparing non-lame cows to lame cows, both the interval between calving and first service was 4 days longer, and the calving to conception interval is documented to range from 14 to 50 days longer for lame cows, even after treatment (Collick *et al*., 1989; Melendez *et al.,* 2003; Hernandez *et al*., 2005). For example, it has been reported that services per conception are increased for lame cows versus non-lame cows, from 1.72 to 2.14 respectively (Hernandez *et al.,* 2001; Melendez *et al*., 2003). Overall poor fertility costs related to a loss in milk production, reduced calving, increased culling, and additional veterinary treatment(s) have been estimated at £25,000 per year for the average 100 cow herd (DairyCo, 2011b).

Lameness also has a detrimental effect on oestrus behaviour (Walker *et al*. 2008a), including reduced oestrus intensity (Walker *et al*., 2010), and

shortened periods whereby herd-mates mount the lame cows (Walker *et al*., 2010). A key contributor to low reproductive performance is poor oestrus detection (Maatje *et al*., 1997). This poses a crucial problem within the farming community, as the accurate interpretation of signs of oestrus behaviour is fundamental to overall conception rates and productivity. As oestrus intensity, and primary oestrus behaviours are reduced in lame cows (Dobson *et al*., 2008; Walker *et al*., 2008a), common oestrus detection methods employed may not accurately identify lame cows in oestrus. Oestrus expression, and thereby detection are current issues in modern cows with no lameness (Denis-Robichaud *et al*., 2018), therefore these factors may be further exacerbated in lame cows, as lame cows display different behaviours from non-lame cows (Navarro *et al*., 2013). As the perception of lameness prevalence is often underestimated in dairy herds, the impact lameness has on fertility may also be underestimated.

The aims of this study were to assess dairy producers' perception of reproductive efficiency between lame and non-lame cattle, how they manage oestrus detection in their lame cattle, if they use different methods for lame and non-lame cows, and what influences their choice of oestrus detection method.

4.2 Materials and Methods

An online questionnaire (Appendix 3, page 340) was designed, and hosted by Thesis Tools (www.thesistools.com), which collected all data and converted it into a Microsoft Excel spreadsheet. The questionnaire was approved by the Writtle University College Ethics Committee, and complied with the UK Data

Protection Act 1998. The questionnaire was comprised of 25 questions, of which 10 were multiple choice, and 15 were open-ended questions (Appendix 3). The questionnaire addressed areas related to general herd information (n=11); reproductive management strategies for lame and non-lame cows (n=7); lameness and fertility (n=7). From September 2012 to December 2012, the questionnaire was accessible online. Dairy farmers and breeders were found through breeder directories, search engines, public forums and a social networking website. Dairy farmers and breeders were sent private e-mails with details of the study accompanied with a link to the questionnaire. Alberta Holstein association hosted a link to the questionnaire on their website. Three public dairy forums (DairyForums.com; Udderly Fantastic; The Dairy Site) had an introductory message with details of the study also accompanied with a link to the questionnaire. Countries included in data analysis were; The United States of America (U.S.A), Australia, Canada, European Countries, and New Zealand. These countries were selected for analysis as they have comparable farm conditions, and regulations governing how animals must be cared for on farms (Cardoso *et al*., 2017). Developed countries have the knowledge and resources to be able to offer the best management systems for their farm animals (Rushen *et al*., 2008). In developing countries, human survival can be a challenge on a daily basis, so animal welfare must be balanced against human welfare (Rushen *et al*., 2008). Therefore, respondents from developing countries were excluded from data analysis.

4.2.1 Data handling and analysis

The results of the questionnaire survey were subjected to descriptive statistical analyses and presented as proportion of answers, means and ranges. To determine if there was a significant difference in the number of inseminations to conception required for lame and non-lame cattle, data were checked for normality using the Pearson's Skewness test, and an unpaired Two-sample t-test was performed. Farm data that had complete information (both lame and non-lame inseminations to conception information) was analysed.

4.3 Results

4.3.1 Response Rate

The response rate was 18%, (189 out of 1025 questionnaires had one or more question completed). The responding countries were; the United States of America (U.S.A) (n=74), Australia (n=31), Canada (n=30), European Countries (n=49), and New Zealand (n=5).

4.3.2 Housing Method and herd size

The most common housing method reported from all respondents was free stall housing (n=112), followed by partially pastured (n=75), two of those respondents replied using both methods of housing. One hundred seventy respondents described their housing method further. Categories were; having zero access to pasture (n=56), fully pastured (n=41), and tie stall (n=43). One hundred eighty-six respondents provided their herd size. The mean herd size from all respondents was 316 (±50.1).

4.3.3 Oestrus Detection Methods

One hundred sixty-four respondents answered the question regarding what oestrus detection method(s) they use. Some respondents reported using up to 3 different types of oestrus detection methods. Eighty-four respondents used 2 oestrus detection methods, n=57 used 1 oestrus detection method, and n=25 used 3 oestrus detection methods. The type of oestrus detection method used by the respondents is detailed in table 4.1. Table 4.2 and Table 4.3 details the combinations of 2, and 3 oestrus detection methods used respectively.

Table 4- 1 The number of, and type of oestrus detection method(s) used by the respondents

		Number of respondents that use specific oestrus detection methods (%)						
# of respondent (%)	**# of Oestrus det. methods**	**V**	**P**	**M**	**T**	**S**	**A**	**P^4**
57 (35)	1	51 (89.5)	3 (5.3)	0	1 (1.8)	1 (1.8)	1 (1.8)	0
84 (51)	2	81 (96.4)	31 (36.9)	22 (26.2)	3 (3.6)	7 (8.3)	24 (28.6)	0
23 (14)	3	23 (100)	21 (91.3)	13 (56.5)	5 (21.7)	4 (17.4)	4 (17.4)	1 (4.3)

Abbreviations of oestrus detection methods: det. (Detection), V (Visual observation), P (Tail paint), M (Mount detector), T(Teaser), S(Synchronise), A (Activity monitors), P^4 (milk progesterone)

Table 4- 2 Combinations of two different oestrus detection methods used by the respondents

V+P	**V +A**	**V+M**	**V+ S**	**V+T**
31	24	20	6	3

Abbreviations of oestrus detection methods: V (Visual observation), P (Tail paint), M (Mount detector), T (Teaser), S (Synchronise), A (Activity monitors)

Table 4- 3 Combinations of three different oestrus detection methods used by the respondents

V+P+M	V+P+A	V+P+T	V+ P+S	V+P+ P^4
14	3	3	2	1

Abbreviations of oestrus detection methods: V (Visual observation), P (Tail paint), M (Mount detector), T (Teaser), S (Synchronise), A (Activity monitors), P^4 (milk progesterone)

As the mean herd size increased, the more oestrus detection methods were used. Table 4.4 details the number of oestrus detection methods used depending on herd size.

Table 4- 4 Mean herd size and the number of oestrus detection methods used

mean herd size	# of detection methods used
218 (±55.3)	1
326 (±76.9)	2
497 (±159.9)	3

One hundred sixty-two respondents answered the question regarding the reason why they use their chosen detection method(s). Table 4.5 illustrates the respondent's reason(s) for choosing their oestrus detection method.

Table 4- 5 Reasoning for oestrus detection method used

Reason method chosen	C+E	C+E+A	E	C	A	C+A	A+E
Number of respondents	69	38	25	16	15	11	11

Abbreviations for the reasons oestrus detection methods were chosen; C(Cost), E(Ease), and A(Accuracy)

4.3.4 Reproductive Management and Fertility

Data were normally distributed. One hundred fifty-three respondents attempted to answer fertility questions regarding number of inseminations to conception. Of these respondents n=150 knew the average number of inseminations required for the cows in their herd. Fifty reported that they did not differentiate this data between lame and non-lame cows. The number of respondents that reported knowing the number of inseminations to conception for lame cows was n=59. There was a significant difference in the number of inseminations to conception for lame and non-lame cows (t test: $t_{58} = 2.37$, $P<0.01$) (Table 4.6), as reported by the respondents (farms that did not differentiate were not included).

Table 4- 6 The number of inseminations to conception for lame and non-lame cows reported by the respondents

	Lame	Non-lame	SED	p-value
Mean number of inseminations to conception	3.1	2.1	0.14	<0.001

The mean number of inseminations to conception reported by respondents that did not differentiate between lame and non-lame cows was 2.1 (±0.1) (total of n=34 respondents). Comparison of the number of inseminations to conception between lame and non-lame cows revealed that lame cows required significantly more inseminations to conception than non-lame cows (3.1 (±0.1) v 2.1 (±0.1) (t-test: $t_{58}=7.11$, $p<0.001$). (farms that did not differentiate were not included). Seven respondents reported they do not breed lame cows. One reported they bred lame cows with no success (requiring infinite amount of straws).

4.3.5 Lameness and Fertility

Data were checked for normality using the Pearson's Skewness test. Data were normally distributed. One hundred thirty-nine respondents answered the question regarding the approximate percent of lameness in their herd. Responses ranged from 0-40%, with the mean being 6.6% (±0.6). One hundred thirty respondents answered the question regarding who locomotion scored their cows. Of these n=67 said themselves (mean lameness prevalence 5.6% (±0.8), n=23 responded that no locomotion scoring is carried out (mean lameness prevalence 8.1% (±2.3), n=13 reported themselves and a professional (vet, trimmer, etc.) (mean lameness prevalence 6.9% (±2.1), n=10 reported herdsman (mean lameness prevalence 8.3% (±2.3), n=8 reported vet (mean lameness prevalence 7.3% (±3.4), n=5 reported nutritionist (mean lameness prevalence 6.2% (±2.7), n=4 reported other (hoof trimmer, consultant, milk recorder, and technician).

One hundred thirty-eight respondents answered the question regarding the oestrus detection methods they use for lame cows and non-lame cows. Of these respondents, n=129 of them use the same oestrus detection methods for both lame and non-lame cattle. One hundred thirty-seven respondents answered the question regarding altered oestrus behaviour in lame cows. Of these, n=117 noticed altered behavioural changes associated with lameness (reduced oestrus expression, increased lying times).

Nine respondents that noticed altered behaviour, also used differing oestrus detection methods for lame cows. For example, the respondents that do alter detection methods stated that they may synchronise lame cows (n=3), use secondary behaviours (n=1), or they either do not breed lame cows at all (n=5).

One hundred seven respondents answered the question asking if any precautionary measures were employed to ensure conception in lame cows. Seventy-four stated yes, n=33 stated no. Sixty seven respondents listed what precautionary measures they took (Table 4.7).

Table 4- 7 Type of precautionary measures taken for lame cows to ensure conception

Precautionary measures taken for lame cows						
Moved to straw pen n (%)	**Treat/Trim n (%)**	**Synch. n (%)**	**Rested-Not bred n (%)**	**Bull n (%)**	**D.A.I n (%)**	**Extra VO n (%)**
22 (32.8)	15 (22.4)	11 (16.4)	8 (11.9)	5 (7.5)	5 (7.5)	1 (1.5)

Abbreviations for precautionary measures taken; Synch. (Synchronised), Bull (a bull is placed with the lame cows for breeding), D.A.I (Double artificial inseminated), Extra VO (Extra visual observations of secondary oestrus signs)

One hundred twenty-three respondents attempted to answer the question regarding what their oestrus detection rates were for lame and non-lame cows. Respondents that knew their oestrus detection rate for lame cows was n=41. Thirty-six respondents did not know the oestrus detection rate for lame cows, n=46 did not differentiate between lame and non-lame. Reported oestrus detection rates for lame cows ranged from 1-100% (mean oestrus detection rate 50% ±4.6).

Ninety-five respondents knew what their oestrus detection rate for non-lame cows was, therefore n=28 did not know the oestrus detection rate for non-lame cows. Reported oestrus detection rates for non- lame cows ranged from 20-100% (mean oestrus detection rate 76% (±1.7)).

4.4 Discussion

With a response rate of 18% this survey falls within previously reported response rates. For example, response rates from surveys in the dairy industry range from 9%-67% (Groover, 1997; Caraviello *et al*., 2006a; Olynk, 2008; Gordon *et al.,* 2012; Denis-Robichaud *et al*., 2016).

Results from this study indicated that regardless of the number of oestrus detection methods employed, visual observation was the most popular method of oestrus detection, followed by tail paint. The number of oestrus detection methods used increased as the mean herd size increased. Herds that used one oestrus detection method were smaller (218 (±55.3)), and largely used visual observations to detect oestrus. Larger herds employed a combination of oestrus detection methods, including technologies such as activity monitors rather than relying on one method. There was a large range in herd sizes that used 3 or more methods (±159.0). Further research would be beneficial to ascertain the number of oestrus detection methods used from a range of farms with different herd sizes. Many dairy regions have reported a reduction in the number of dairy farms, along with an increase in herd size and milk production per herd (AHDB Dairy, 2016; Dairy NZ 2016; USDA, 2017). As dairy herd sizes continue to increase, monitoring and managing cows has become increasingly

more difficult and requires enhanced management ability (Edwards *et al*., 2015; Bewley, 2016). As herd sizes are increasing, using visual observation alone is not adequate to correctly identify oestrus in cows, which is suggested from this study. Gargiulo *et al*. (2018) sought to identify the relationship between herd size, current precision technology adoption, and perception of the future of precision technologies. They determined that most of the precision technology currently installed on-farm is of the type that addresses labour issues, associated with larger herds.

Based on the information provided by the respondents, it was determined that that lame cows required significantly more inseminations to conception than non-lame cows (3.1 (±0.1) v 2.1 (±0.1)). An optimum number of inseminations to conception is considered to range from 1.6 and 1.8 (Borkowska *et al*., 2012). However, according to Mordak (2008) the number of services per conception around 2 is still acceptable, but values exceeding 3 are indicative of considerable reproduction and/or health issues. It was also determined that many farmers noticed altered behaviour in lame cows, however the same oestrus detection methods are used for both lame and non-lame cows, despite observable differences. As lame cows alter their behaviour when in oestrus, it would be beneficial to design an oestrus detection protocol that incorporates lameness as a variable. However, when asked if they take precautionary measures for lame cows to ensure conception, a higher number of respondents answered, stating they did take precautionary measures. Precautionary measures may include but are not limited to; keeping them in a separate straw bedded pen, treating the cow (foot trim, medication if required), synchronising using hormone therapy, resting the cow and not breeding them, letting a bull

breed them, double A.I., and carrying out extra visual observations for oestrus behaviours. Alawneh *et al*. (2011) reported that in New Zealand, lame cows are commonly separated from the herd and milked once a day rather than twice. Despite these precautionary measures, lame cows still require more inseminations to conception. As lame cows may be isolated from the herd, they may not have ample opportunity to express oestrus behaviour even if they are physically able to. It has been reported that the intensity of oestrus expression may depend on the number of cows in oestrus simultaneously (Roelofs *et al.,* 2005; Sveberg *et al.,* 2011; Zebari *et al*., 2019), therefore if a lame cow is isolated, she will not have sufficient physical, and social contact with fellow herd mates to initiate primary or secondary oestrus behaviours. Therefore, methods that rely on mounting behaviour are limited for their use in isolated cows.

Implementing detection methods such as milk progesterone, and/or activity monitors for the isolated animal may be useful to overcome the limitation of visual observations and mount detectors when insufficient animals are available to initiate the expression of typical oestrus behaviours. Increases in activity are detected by activity monitors with varying efficiency (Løvendahl and Chagunda, 2010), and can indicate a cow is in oestrus regardless if it is lame or not. Walker *et al*. (2008a) reports that lame cows had reduced oestrus intensity, but the incidence of oestrus was unaffected. However, the use of activity monitors may not be as efficient in moderate and severely lame cows, as these animals may be reluctant to move. Additionally, if the cow does not increase her activity such as in a silent heat, or if she is reluctant to move, milk progesterone testing may be beneficial to ensure oestrus is not missed.

It may be beneficial to develop an oestrus detection protocol specifically for lame cows, perhaps considering alternative footing (pasture v concrete), milk progesterone and/or activity monitors in addition to close, frequent visual observations alongside the use of secondary behaviours to accurately detect the problem or lame animal. Ensuring that lame cows are properly managed through the inclusion of specific oestrus detection methods could increase their overall welfare, and reduce the risk of premature culling due to reduced fertility parameters caused by lameness. Each lame cow may require alternative oestrus detection methods to successfully be detected, and to conceive in a timely manner to limit financial losses to the farmer. Preventing and treating lameness is key. Allowing cows access to pasture improves locomotion score (Hernandez-Mendo *et al*., 2007; Cook and Norlund, 2009; Olmos *et al*., 2009; Somers *et al*., 2015), and provides secure footing for oestrus behaviour (mounting) (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996; Palmer *et al*., 2010). Therefore, it might be beneficial to provide pasture access, or to have a loafing area with soft footing for housed cows to engage in oestrus behaviours. Ensuring swift conception will reduce losses through increased days open, multiple inseminations, and excessive use of detection methods. The money saved by ensuring prompt conception in the lame cow, can perhaps be incorporated into additional preventative management (specific oestrus detection method/supplies, pasture access, extra bedding, housing separately, additional foot trimming etc.) if not already exercised in practice.

Many respondents either did not know or do not keep a record of average insemination to conception rates for separate groups of cows i.e. lame and non-

lame animals. Producers may have an overall herd average, however identifying differences between lame and non-lame animals may not be common practice especially in large commercial dairy herds, where defining a distinction between the two groups may prove to be very difficult. Additionally, identifying lame cows from non-lame cows can be subjective between individuals, and therefore it is difficult to assess the reliability of the data. An individual's opinion may vary considerably as to what is considered to be a normal gait, moderately lame, or severely lame. Even the same observer may vary their locomotion scoring accuracy each time scoring is carried out. Biased decisions may also be made regarding ones' herd; for example, omitting behaviours relating to lameness, or not scoring a cow as lame because the rest of the herd was largely healthy (Hollenbeck, 1978). Flower and Weary (2009) determined that inconsistency with gait scoring occurred with lower or intermediate scores, whereas agreement was best with higher locomotion scores. Additionally, the reliability of observational research may depend on the individual on a particular day. For example, ones' motivation may be lacking to perform accurately (Cohen *et al.,* 2011), which may directly affect the scores given, and may result in inaccurate gait scores. Variation of the prevalence of lameness may be due to varying scoring systems used within each establishment, in addition to subjective opinions and perception of lameness, time of observation, gait scoring environment, and fluctuating scoring skills among observers (Telezhenko and Bergsten, 2005; Poursaberi *et al.,* 2010).

It has also been reported that farmers underestimate lameness in their herd (Wells *et al*., 1993; Whay *et al.,* 2003; Alawneh *et al*., 2012; Fabian *et al.,* 2014). Whay (2002) reported that farmers underestimated lameness by 16%

and of the farmers in the study 96% underestimated lameness prevalence within their herd in the UK. Espejo *et al.* (2006) reported that lameness prevalence was over three times that estimated by farmers. Additionally, farmers' priorities within the herd may vary between establishments. A study by Leach *et al.* (2010b) determined that 90% of farmers did not perceive lameness as a major issue, despite the prevalence of lameness being 36%. Leach *et al.* (2010b) reported farmers understanding of the implications of lameness for the farm as a business was limited. Additionally, a lack of prompt treatment of lame cows is also associated with increased lameness prevalence (Barker *et al.,* 2010). A cow may be identified as lame; however, it was reported that some farmers prolong treating the cows until the next visit by the routine hoof trimmer, in some cases up to 6 weeks later (Barker *et al.,* 2010). Animal care varies between farms, as general attitude towards the cows can either be positive or negative. Having a poor attitude towards the cattle may result in impaired animal care. Whereas it is likely that a positive attitude will result in a farmer who pays closer attention to indicators of manifesting problems, thereby treating cows more promptly after identifying lameness or disease (Barker *et al.,* 2010). Following the study, they concluded that farmers underestimate the extent of lameness and the implications for the performance of their cows and their business, while restricted time, labour and finance all present obstacles to change.

The very low mean lameness prevalence (6.6%) in the current study could be due to the respondents underestimating lameness in their herd. Therefore, these rates may not be a reliable, accurate representation of lameness reported by the respondents.

The issues with gait scoring often occur with low scores. Cows with high gait scores are typically easily identifiable even by the most inexperienced of individuals. It is unrealistic to expect observers to be in complete agreement when gait scoring cattle. However, despite variability between scoring, it is beneficial to continually score in order to develop these skills required. Accuracy of gait scoring may develop over time, with experience. For example, Brenninkmeyer *et al*. (2007) and March *et al.* (2007) found that with more scoring experience, gait scorers had increased agreement over time. Additionally, in order to score with accuracy, a minimum of 200 to 300 cattle is required to train an observer (March *et al.,* 2007). However, if the observers are trained by individuals that themselves lack experience, this can affect the accuracy of gait scoring, which may lead to an underestimation of gait scored in dairy herds.

Some producers may cull a severely lame animal from the herd before insemination can be carried out, therefore data from these animals may not be available. Additionally, more respondents knew the oestrus detection rate for non-lame cows when compared to their lame counterparts and in some cases, producers do not differentiate lame cow fertility parameters from non-lame cows. It is possible that producers do not routinely locomotion score their cows, or that the locomotion scores may be underestimated. This under-recognition not only delays treatment, but also affects fertility parameters. If farmers do not identify individual lameness cases, they will be unaware of specific fertility parameters. Therefore, failure to separate lame cow fertility data from non-lame cows will negatively affect the overall herd fertility parameters, particularly in

herds with higher than average prevalence of lameness. As lame cows are reported to have decreased reproductive efficiency (Huxley, 2013), this affects fertility parameters such as delayed cyclicity (Garbarino *et al*., 2004a), increased number of services to conception (Sprecher *et al*., 1997), the percentage conceived on first service (Melendez *et al*., 2003) etc. Thus, by adopting a system that evaluates individual cows with impaired locomotion separately, it may be easier to identify the problem cows, resulting in early treatment, or management to improve animal welfare and overall reproductive performance.

Many factors such as cost, ease of use, and/or accuracy will influence a dairy producers' choice in what oestrus detection method to use. It has been previously reported that more than one factor can influence what oestrus detection method is used (Garforth *et al.,* 2006). Sixty six percent of the respondents from this study considered more than one factor when implementing an oestrus detection protocol. Evaluation of why farmers used a particular oestrus detection method(s) revealed that a combination of 'cost and ease', were the most influential factors for choice of oestrus detection method(s), followed by cost, ease and accuracy. When evaluating respondents that consider a single factor when using a detection method, ease and cost were the top factors, followed by accuracy. Additionally, many dairy farms may also carry out arable farming, and have other responsibilities, and therefore have additional commitments in order to sustain their establishment. Using oestrus detection methods that are straightforward can maximise time efficiency, costs, and future uptake.

When evaluating the detection methods in terms of ‘ease’ there may be many issues relating to relatively straightforward methods such as mount detectors for example. These methods are relatively easy to apply and relatively low in cost. However, if for some reason they are falsely activated, or are dislodged before they can be correctly activated, the producer must spend time replacing detectors. Therefore, if this occurs often, the producer will be spending time replacing detectors. Additionally, falsely activated detectors can lead to inseminating the cow when she is not cycling, effectively costing additional money for wasted semen. Although some methods are user friendly, they may in fact require more time and effort. Implementing more expensive methods (activity monitors, in line milk monitoring) may be costly, however they can save time for the producer by eliminating the need to replace missing or falsely activated detectors. When considering the term ‘ease’ in relation to detection methods, this concept may vary greatly among producers. Additionally, the uptake of new methods may be limited due to a lack of knowledge transfer in the industry.

Altering oestrus detection methods for specific groups of cows such as lame and non-lame, is difficult for producers. Declining numbers of dairy employees means there is less time available to monitor oestrus behaviours closely (Blackie *et al.,* 2011). Therefore, implementing different oestrus detection methods for different groups of cows may not be viable due to inconvenience, and labour-intensive costs, particularly in large commercial dairy herds. Therefore, utilising one method across the whole herd is more manageable, and may be more cost effective. Making monetary and non-monetary benefits of the use of automated oestrus detection clear to farmers (Jago *et al*., 2013)

could assist in the uptake of new technologies. It is also vital to be aware that some barriers, such as lack of infrastructure or skills (computer knowledge or management and integration of data), can slow down the uptake of some precision technologies (Jago *et a*l., 2013; Eastwood *et al*., 2017). However, if the benefits of introducing additional and/or precision technologies are made explicit, the uptake of such measures may be readily accepted despite the inconvenience. Bennett *et al*. (2014) reported that farmers value lameness reduction more than reducing inconvenience, and are therefore more willing to incur inconvenience as long as the lameness prevalence is reduced.

Lameness and fertility issues are continual concerns in the dairy industry despite extensive research. Reducing lameness is crucial, and should be a main priority across dairy herds. However, as lameness continues to affect dairy cows, evaluating effective protocols for oestrus detection in the problem and lame animal is fundamental. By refining oestrus detection methods with particular relevance to problem and lame dairy cows, there is an opportunity to enhance animal welfare through reduced risks of premature culling due to fertility problems, in addition to reducing financial losses to the producer through increased conception rates.

4.5 Conclusions

From the data provided by the respondents, lame cows require more inseminations to conception, and some producers are aware that lame cows alter their oestrus behaviour(s). However, the majority of respondents use the same oestrus detection methods for all animals, despite behavioural

differences between lame and non-lame cows. Additionally, producers that take extra measures to ensure conception (e.g. isolation/recovery pen) in lame cows may be faced with oestrus detection limitations when using conventional detection methods. As pasture access can improve locomotion score, and encourage mounting activity it may be beneficial to provide cows with access to soft under footing to reduce lameness while improving oestrus expression. As mounting activity increases on dirt surfaces, pasture access can potentially increase oestrus expression in lame cows. These additional preventative measures can reduce lameness and improve oestrus detection, thereby enhancing reproductive performance and animal welfare. Chapter 5 investigates the effect of pasture access on locomotion score and oestrus activity in lame and non-lame dairy cows.

Chapter 5: Analysis of fertility, LCS, and oestrus expression over time in lame and non-lame dairy cattle with access to pasture

5.1 Introduction

Resumption of normal ovarian cyclicity following parturition is essential for successful productivity in dairy herds. Typically, dairy cows have been reported to resume ovarian activity and ovulation within 15-45 days postpartum, with regular cycles approximately every 18-24 days. Early postpartum ovulation is associated with improved reproductive fertility (Galvão *et al.,* 2010). It is well documented that lameness is a painful and debilitating condition that can have a detrimental effect on reproductive performance, oestrus behaviour and intensity (Sprecher *et al.,* 1997; Dobson and Smith, 2000; Hernandez *et al.,* 2001; Walker *et al.,* 2008a).

Many farms house dairy cows indoors for a significant part of the lactation period (AHDB, Dairy, 2017c). Continuous housing (zero-grazing) is common in regions where the climate is unsuitable, or where grazing the cattle is not cost effective or the most efficient use of the land (Haskell *et al*., 2006). Zero-grazing has been practiced in some parts of North America since the 1960s (Allbright and Alliston, 1971). Zero-grazing figures range from 0% in Sweden (legislation for mandatory grazing), to more than 50% in Alpine regions and in Italy (BSAS, 2011). In the UK, the majority of cows are seasonally grazed in summer periods (Haskell *et al*., 2006; BSAS, 2011), however this tradition may be changing. In 2011, it was reported that approximately 5% of UK dairy herds were continuously housed (BSAS, 2011), however in 2016 this figure was reported

to have increased to 20%. Zero-grazing enables the producer to closely monitor the cow diets, while maximising milk yield as feeding high levels of concentrates is more accessible (Haskell *et al*., 2006). However, it has been reported that cows managed under zero-grazed conditions are more susceptible to knee injuries and lameness (Haskell *et al*., 2006; Ranjbar *et al*., 2016). Previous research shows that lameness prevalence varies considerably across countries, herds, housing types and seasons, ranging from approximately 3% to as high as 60% (Espejo *et al*., 2006; Cramer, 2007; Tadich *et al*., 2010; Hoffman *et a*l., 2014). Each year in the UK approximately 25% of dairy cows are lame (Logue and Mayne, 2014). Lameness is multifactorial, however housing conditions such as concrete flooring, bedding type, etc. can affect the development of lameness. Allowing dairy cows access to pasture improves overall locomotion score (Hernandez Mendo *et al.,* 2007; Cook and Norlund, 2009; Olmos *et al*., 2009; Somers *et al*., 2015), therefore incorporating access to pasture may assist in preventing, and treating lameness in the dairy industry. This may also assist in increasing oestrus expression among dairy cows, as it has been reported that dirt surfaces are preferred to display oestrus expression, as there is less risk of the cow slipping, therefore the cow feels more confident/stable on this surface. Due to land restrictions, this may not always be possible. Providing an area with soft flooring may counteract increased lameness risk, and may also provide an area that cows can display oestrus behaviour without the risk of slipping.

The aims of this study were to evaluate herd fertility parameters (number of days from calving to first service, number of days from calving to conception

and the number of inseminations to conception) in lame and non-lame dairy cattle, to calculate lameness prevalence for the duration of the study, and to determine if access to pasture improves LCS and oestrus activity.

5.2 Materials and Methods

5.2.1 Animals and data collected

The study was conducted from April 2013-December 2013 at Rodwell dairy farm (Ipswich, England). The herd consisted of 130 Holstein Friesian cows with average milk production over 11,000 litres per lactation. Cows were chosen based on their locomotion score, and their current reproductive stage. Cows were grouped as lame and non-lame at the beginning of the study. Twenty-one cows were initially recruited in the study. At the start of the study n=11 cows were classified as lame, and n=10 were non-lame. However, from the non-lame cow group two IceQube® sensors fell off and were not recovered, and batteries in two in IceQube® sensors failed and data could not be recovered. Freshly calved cows with normal reproductive history were enrolled in the study on average at 25.1 DIM (±1.1). Study cows were housed on average for 67 (±6.5) days before access to pasture, and spent an average a total of 112.4 (±4.8) days on pasture. A total of n=51 oestrus events were recorded from n=17 cows (n=11 lame; n=6 non-lame). All study cows had 2 oestrus events, n=10 had 3, and n=7 had 4. Each multiple oestrus event was assigned a corresponding number (e.g. 1,2,3,4), and relevant LCS. Lame cows had a mean locomotion score and parity of 3.2 (±0.2) and 3.6 (±0.3) respectively. Non-lame cows had a mean locomotion score and parity of 1.6 (±0.2) and 3.2 (±0.2) respectively. Lame and non-lame cows were pair matched based on parity. The voluntary

waiting period for the herd was 35 days. Healthy cows expressing oestrus at 35 DIM were served, therefore recruiting cows before this time (24.1 DIM (±0.8) ensured oestrus was observed. The management of these cows is detailed in Chapter 3-section 3.1.1. Briefly, cows were milked twice daily through a Delaval herringbone 8/8 parlour. Lactating cows were housed during the winter months, and fully pastured during the summer months.

5.2.2 Herd fertility parameters

Calving day, parity, days to first service, days to conception, and number of inseminations to conception were obtained from farm records. Number of days from calving to first service was recorded from n=94 cows (non-lame n=69, lame n=25). Number of days from calving to conception was recorded from n=69 cows (non-lame n=47, lame n=22), n=5 cows were culled (marked as barren, and no longer inseminated) before pregnancy could be established, and n=18 cows were not diagnosed pregnant at the time of data analysis. The cows studied had a mean parity of 2.38. (±0.14). Non-lame cows had a mean parity and locomotion score of 1.45 (±0.06) and 1.86 (±0.14) respectively, and lame cows had a mean parity and locomotion score of 3.84 (±0.27) 3.08 (±0.06) respectively.

5.2.3 Oestrus expression

5.2.3.1 Activity Monitors

IceQube® Sensors (IceRobotics Ltd) were fitted 20 days' post calving to the right hind leg as described by the manufacturer. Sensors were left on until the cow was diagnosed as pregnant by a veterinarian.

Study cows were also fitted with the NeDap activity monitors 10 days' post calving to the front right leg as described by the manufacturer. These devices require 10 days to establish the individual locomotion behaviour of each cow so that fluctuations from typical activity could be identified in the future. The right side of the cow was easily accessible when the cows were in the crush, therefore the limbs on the right-hand side were used to attach the activity monitors.

5.2.3.2 Tail chalk

Tail chalk was applied daily to the tail head of each cow by one of two experienced AI Technicians. The AI technician, and herdsmen routinely checked the cows for any signs of oestrus, and this was recorded daily in a designated notebook. Additional notes were made regarding the percentage of chalk removed. These notes were checked by the researcher. A record was made of those cows where their chalk is removed.

5.2.3.3 Milk Sampling and progesterone analysis

Milk samples were collected three times weekly for use in milk progesterone (P^4) analyses (Monday, Wednesday, Friday), at afternoon milking from the right rear quarter, with one exception (cow 185) as her right rear quarter was dry, therefore the sample was taken from her left rear quarter. Milk sampling started when a cow entered the study 25.3 (±0.7) DIM, and stopped 10 days after oestrus was observed. This would enable individual analysis of progesterone profiles prior to, during, and after oestrus. Each cow had 10 samples analysed,

5 samples prior to oestrus, and 5 samples post oestrus. A total of n=170 milk samples were analysed. Samples were collected from one quarter to reduce interference with the herdsmen's milking routine. Teats were dipped prior to sampling using iodine teat disinfectant followed by wiping to ensure hygienic conditions for animal welfare and milking purposes. Gloves were worn for sampling, and initial stripping's (foremilk) were discarded before samples were obtained. Whole milk samples were collected into 25mL test tubes containing a preservative (Lactab Mark III; Thompson and Cooper Ltd., Runcorn, UK). Cow number and date were written on the side of the test tubes, and were inverted to mix until the tablet was dissolved. Milk samples were placed in a test tube stand in a cool bag, and were chilled immediately in the farm fridge (4°C), until freezing (within 3.5 hours). Frozen milk samples were stored at Writtle University College laboratory until P^4 analysis could be carried out. Progesterone was analysed using the Ridgeway ELISA-kit (Ridgeway Science Ltd., Rodmore Mill Farm, Alvington, Gloucestershire, UK), which is an accepted technique for P^4 analysis (Gillis *et al*., 2002; Roelofs *et al*., 2006; Gorzecka *et al*., 2011; Nyman *et al*., 2014; Blavy *et al*., 2016; Adriaens *et al*., 2017; Daems *et al*., 2017). Milk sample handling analysis was carried out following the manufactures protocol, except for incubation period which is described in Chapter 3 (Section 3.5.4).

5.2.4 Locomotion score

All cows in the milking herd with the exception of cows in the hospital pen (through illness, or freshly calved) were locomotion scored weekly using the method of Flower and Weary (2006) shown in Table 3-5 in Chapter 3. The mean number of cows scored each month were 102 (±4.1), equating to a total

of n=2955 locomotion scores during the study period from April 2013-December 2013. The cows were locomotion scored leaving the milking parlour on grooved concrete. The cows were observed walking along the alley way and then turning left into the barn. A detailed description of LCS method is outlined in Chapter 3-section 3.2 All locomotion scoring was carried out by the same observer (AW). Similar to Flower and Weary (2006b), If a cow exceeded the requirements of a particular score, but did not meet all the requirements of the next successive score, a half-integer score was allocated. For example, cows that had improved locomotion scores but still exhibited lame cow characteristics (e.g. arched back) were given a score of 2.5 rather than a 2. Each study cow had a corresponding LCS during an oestrus event.

5.3 Data handling and analysis

5.3.1 Herd fertility Parameters

Calving day, parity, days to first service, days to conception, and number of inseminations to conception were recorded and entered into Excel. Data were checked for normality using the Pearson's Skewness test and were analysed using an unequal variance T-test to assess the effect of lameness on the fertility parameters mentioned above (GenStat 18th edition).

5.3.2 Monthly lameness prevalence and LCS affects

Data were checked for normality using the Pearson's Skewness test. Locomotion scores were entered into Excel files weekly. Prevalence of lameness for the herd was calculated monthly as the proportion of cows scoring 3 or above out of the cows scored.

Eight weekly locomotion scores from each study cow were analysed using repeated measures ANOVA to assess the effect of pasture access on LCS. Hernandez-Mendo *et al*. (2007) reported improved LCS after providing cows with pasture access over a 4-week period. Therefore, four weekly scores were analysed before cows were given pasture access, the week they had pasture access (0), and 4 weekly scores after pasture access.

Study cow LCS from April 2013 to November 2013 were analysed using repeated measures ANOVA. The repeated measure was the month and factors assessed were treatment (lame v not), time, and interaction.

One way-Analysis of variance (unbalanced design) was performed to determine if housing (pasture v housed), month, and heat number had an effect on LCS from the study cows (n=17). All statistical analysis were carried out using GenStat (18th edition).

5.3.3 Oestrus expression

IceQube® were downloaded wirelessly weekly and files were made for each cow in Excel. Days that were analysed for oestrus activity were 10 days prior to oestrus, the day of oestrus, and 10 days' post oestrus. Oestrus was identified through a collective assessment of information available. Records made in research books by the herdsmen/AI technician were checked, as was the NeDap activity system for any alerts made. The cows were examined for tail chalk for removal. The first oestrus event was verified through milk progesterone assay analysis. When pro-oestrus begins milk progesterone will

drop from >10ng/ml to <3 ng/ml (Döcke, 1994). During oestrus, and ovulation progesterone levels drop to <0.5 ng/ml (Wiltbank *et al*., 2014). Therefore, an oestrus was assumed to have occurred if the progesterone concentration decreased from >1 ng/ml to <1 ng/ml over one to three sampling periods.

The effect of different housing conditions (Housed v Pasture), and LCS on activity (IceQube® data) and progesterone concentrations were analysed using repeated measures ANOVA. Repeated measure were days, 10 days prior to oestrus, day of oestrus and 10 days post oestrus. The factors assessed were: treatment (LCS*Housing), time and interaction. This was followed by a Tukey test for post hoc comparison.

5.4 Results

5.4.1 Herd fertility parameters

Data were normally distributed. Lame cows had significantly more days from calving to first service (t test: $t_{92} = 2.66$, P=0.009) (Table 5-1). Lame cows had significantly more days from calving to conception (t test: $t_{67} = 2.69$, P=0.009) (Table 5-1). There was not a significant difference in the number of inseminations to conception for lame and non-lame cows (t test: $t_{69} = 1.22$, P<0.225) (Table 5-1).

Table 5- 1 Fertility parameters for lame and non-lame dairy cows from Rodwell Dairy Farm

	Lame (n)	Not (n)	SED	P-value
Days from calving to first service	63.8 (25)	53.5 (69)	3.88	0.009
Days from calving to conception	113.5 (22)	84.2 (47)	11.4	0.009
Number of inseminations to conception	2.5	2.1	0.329	NS

5.4.2 Monthly lameness prevalence

The data were normally distributed. The prevalence of lameness from February 2013-August 2014 is shown in Figure 5-1. Red diamond data points indicate when cows had access to pasture, and when they were housed. Decreases in lameness prevalence coincides with cows gaining access to pasture during the late spring/summer months. With increased lameness prevalence occurring when the cows start to become fully housed during the winter months.

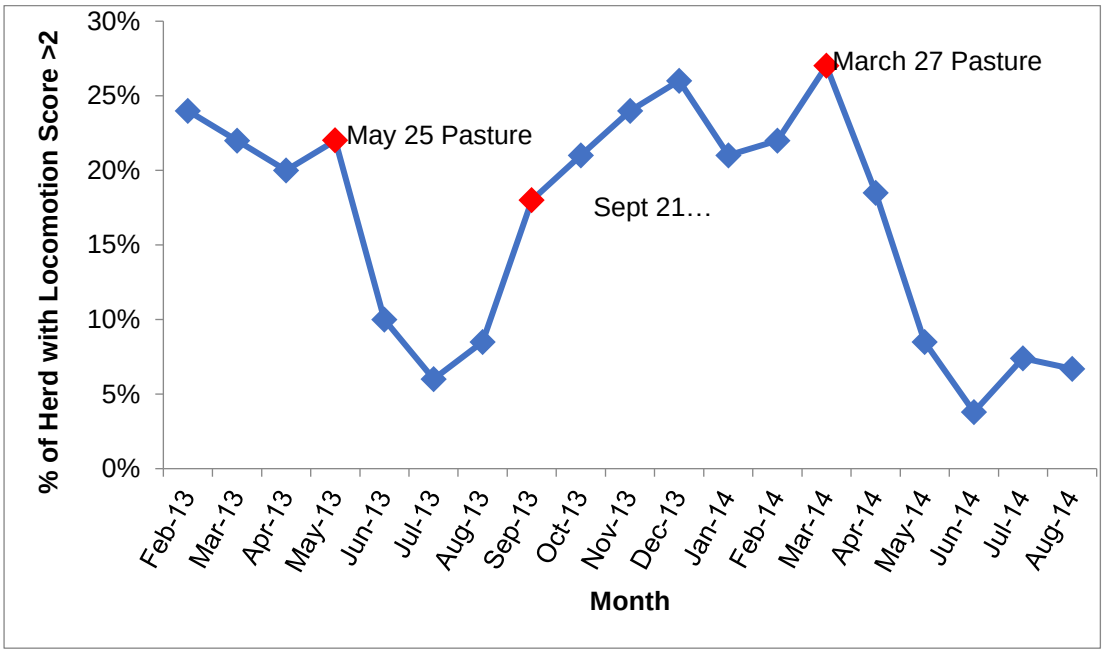

Figure 5- 1 The prevalence of lameness from February 2013- August 2014 at Rodwell dairy farm

The LCS trend for the study cows is shown in Figure 5-2. Both lame and non-lame cow groups have significant LCS improvement after gaining access to pasture. Lame and non-lame cows continually have significantly different LCS from April to November. LCS decline when the cows change to winter housing conditions.

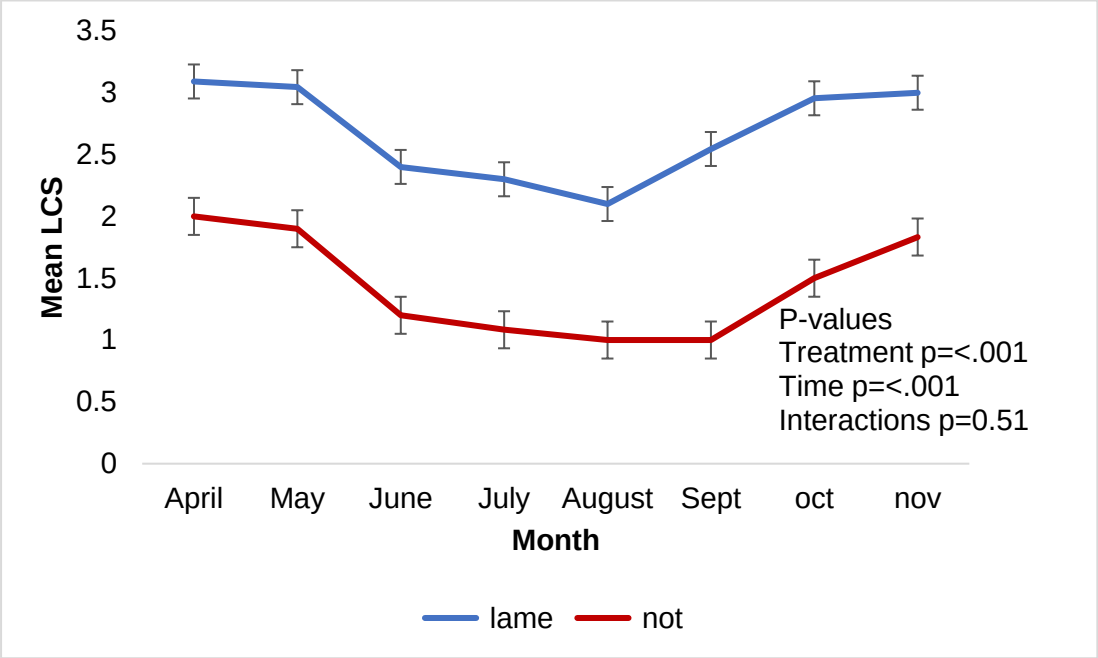

Figure 5- 2 Monthly LCS trend for lame and non-lame study cows

Repeated measures ANOVA determined there was significant improvement in locomotion scores after pasture access (week 0) for all of study cows (Figure 5-3). The LCS for cows after pasture improved by 0.21 units/week.

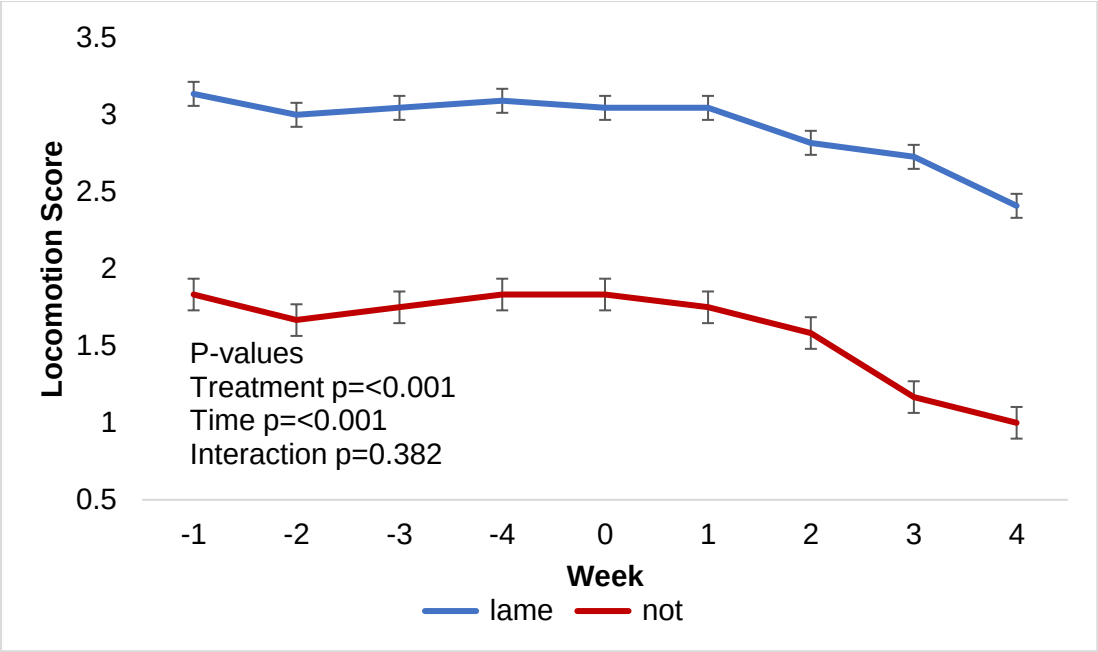

Figure 5- 3 Changes in LCS for dairy cows, 4 weeks before pasture access, the week of pasture access (0) and 4 weeks after pasture

From the study cows it was determined that month did not significantly affect mean LCS (Figure5-4), however Tukey post hoc revealed that months May and July were significantly different from one another.

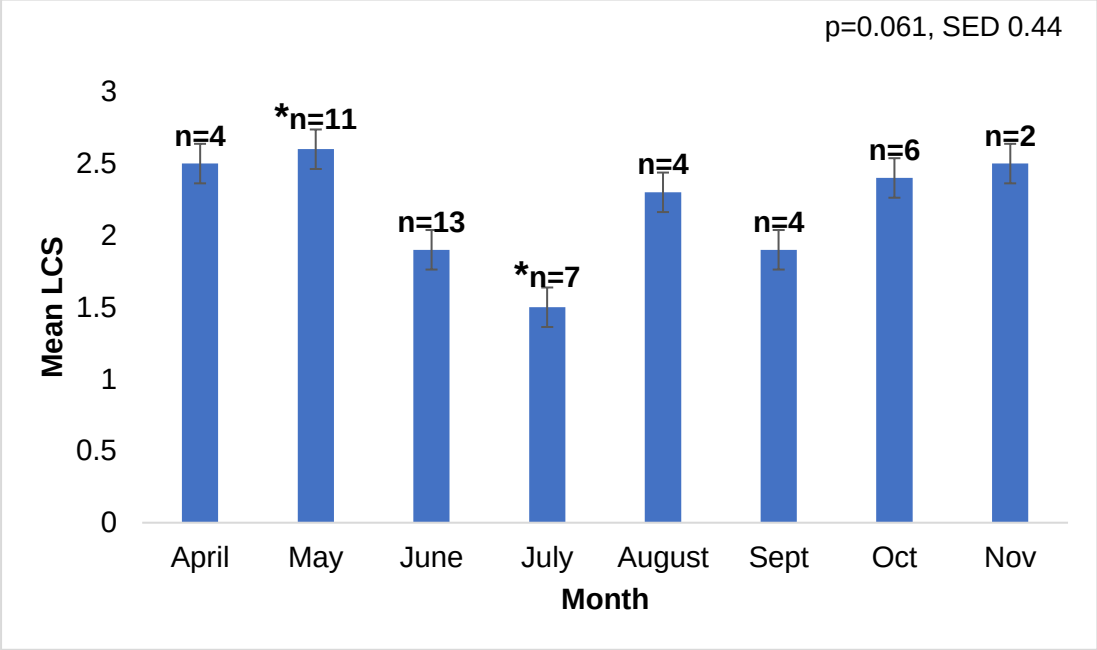

Figure 5- 4 Monthly average LCS for study cows. The number of oestrus events occurring each month is listed above the bar (n=). * indicates Tukey post HOC significance between months

Cows having a housed oestrus events had significantly higher mean LCS compared to oestrus events occurring at pasture (Figure 5-5).

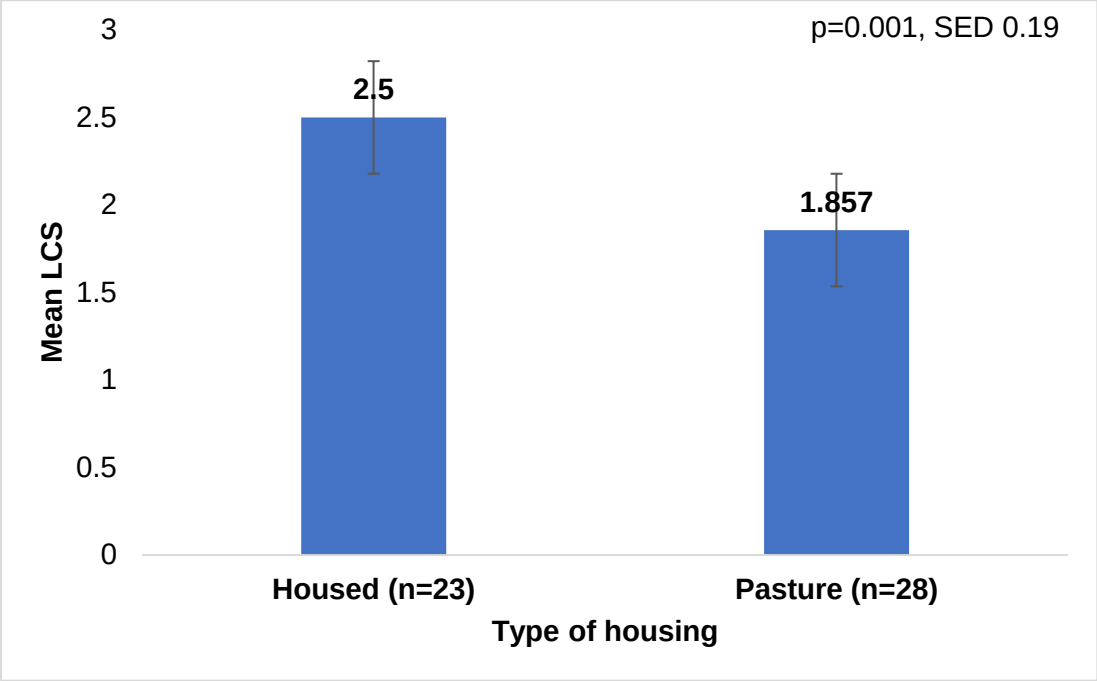

Figure 5- 5 Mean LCS and number of oestrus events in different housing conditions

The first oestrus event had significantly higher mean LCS compared to the third oestrus event (Table 5-6). This would coincide with improved LCS after pasture access. The percentage of multiple heats in different housing conditions is illustrated in Table 5-2.

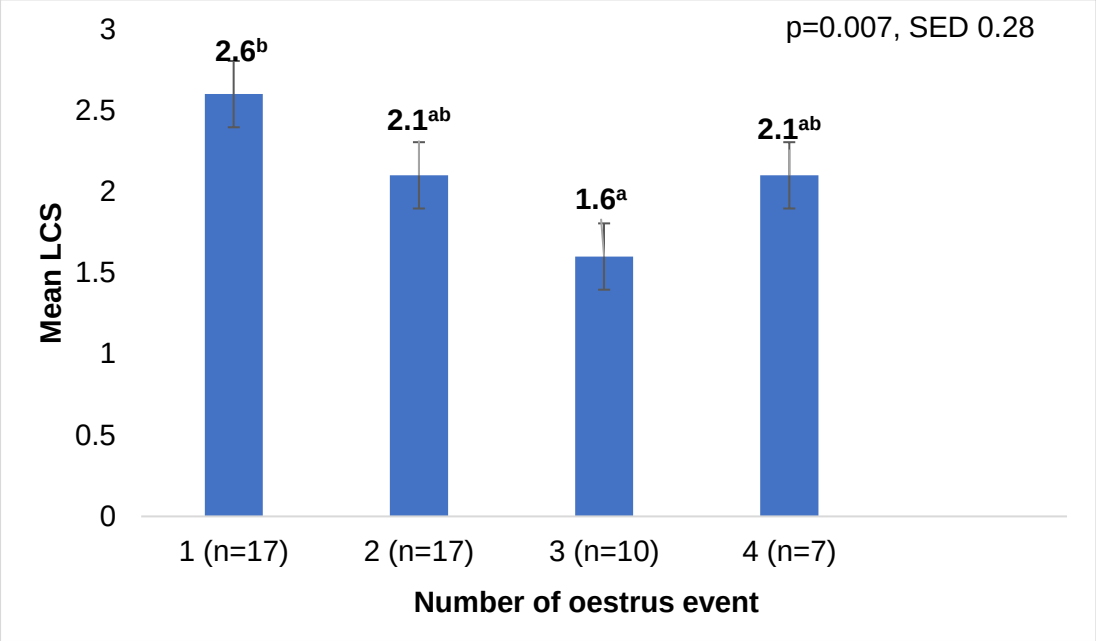

Figure 5- 6 Mean LCS for study cows from multiple oestrus events. [b] different letters next to mean LCS above bars indicate Tukey post hoc test significant difference.

Table 5- 2 The percentage of multiple heats (1,2,3,4) occurring in different housing conditions. [b] different letters in the same row indicate Tukey post hoc test significant difference

Heat number	Mean LCS	% of heats housed	% of heats at pasture
1	2.6[b]	76.5	23.5
2	2.1[ab]	17.6	82.4
3	1.6[a]	30	70
4	2.1[ab]	57.1	42.9

5.4.3 Oestrus activity

Repeated measures ANOVA determined there was significant difference in the number of steps (Figure 5-7) before, during and after oestrus from different LCS. Housing affected the number of steps, with housed cows having significantly lower step counts (Figure 5-8). There was an effect of time, with the day of oestrus (0) having the most steps.

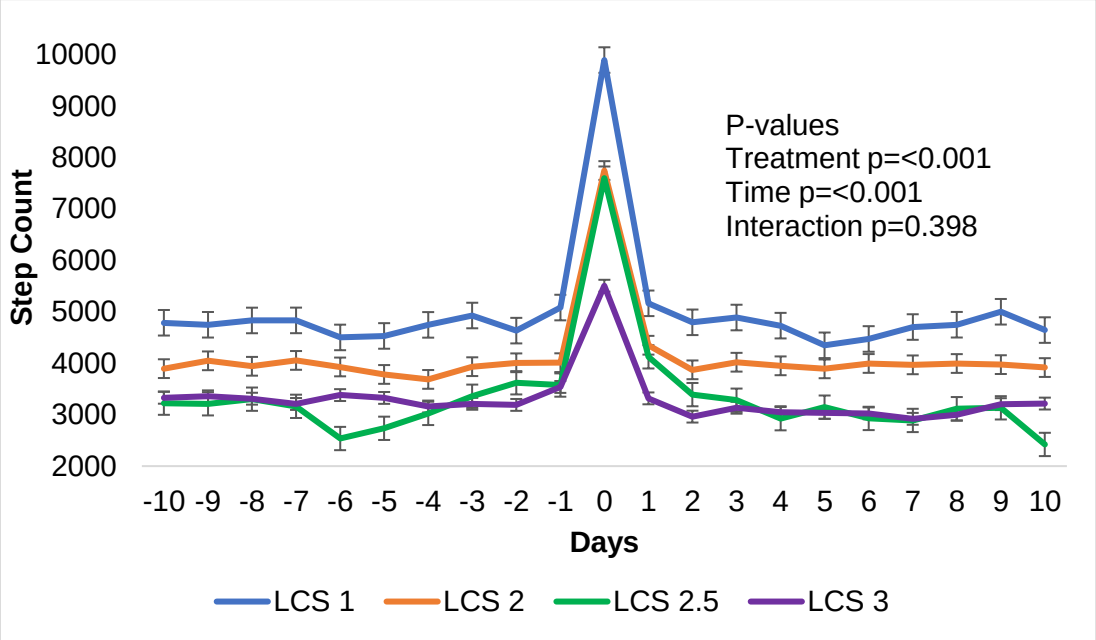

Figure 5- 7 Step counts for different LCS before, during, and after oestrus

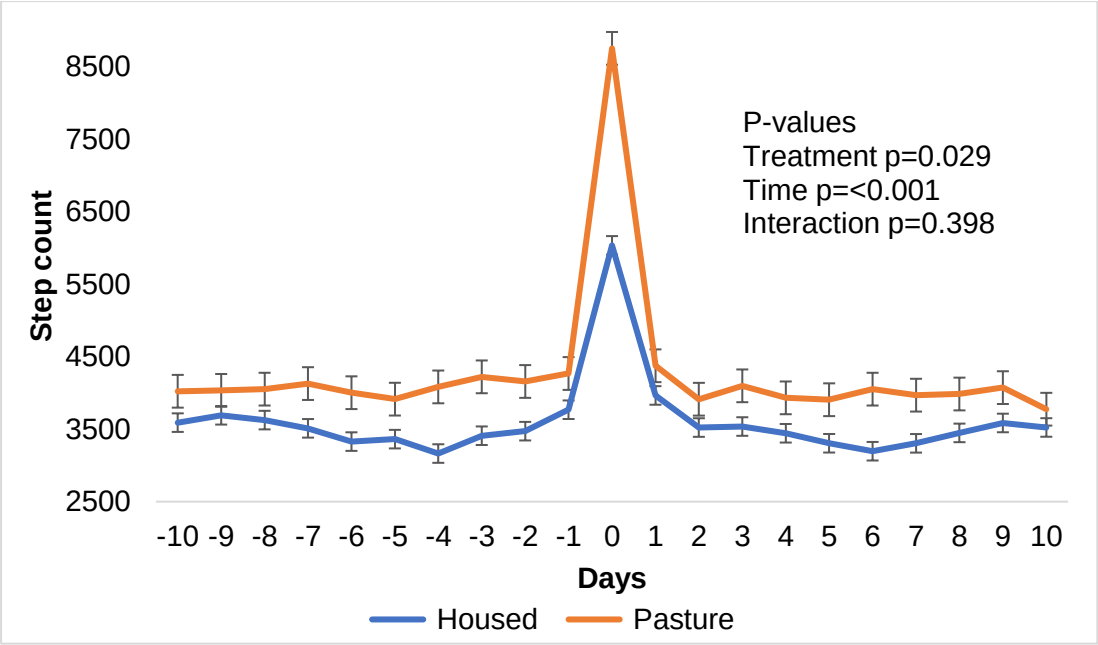

Figure 5- 8 Step counts for dairy cattle in different housing conditions (Housed v pasture), before, during, and after oestrus

Repeated measures ANOVA determined there was significant difference in motion index (Figure 5-9) before, during and after oestrus from different LCS. Housing affected the motion index, with housed cows having a significantly lower motion index (Figure 5-10). There was an effect of time, with the day of oestrus (0) having the most steps.

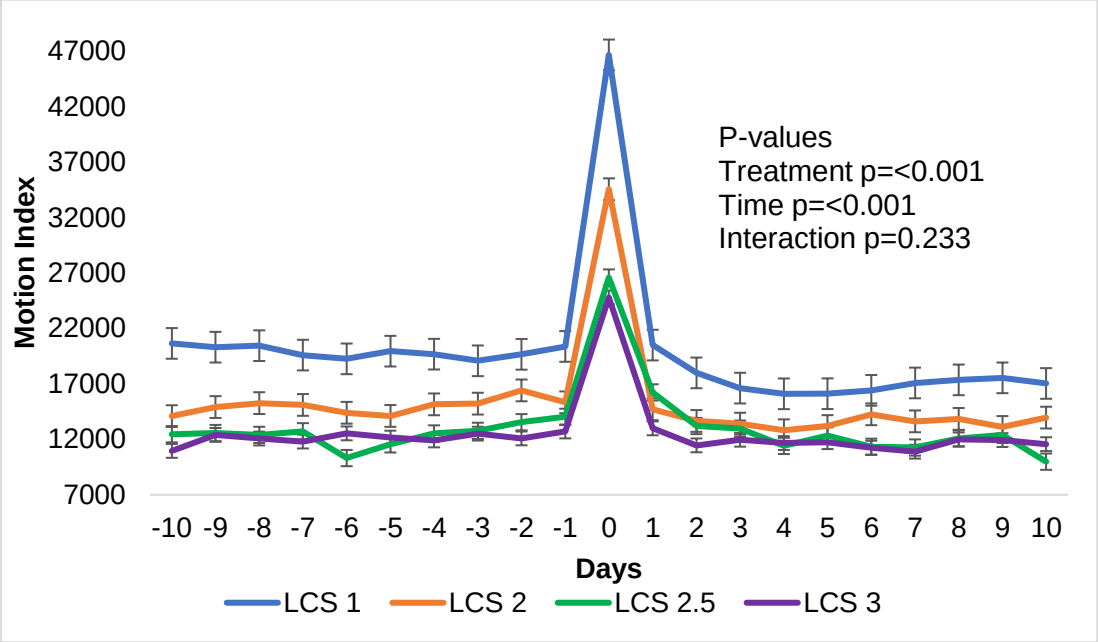

Figure 5- 9 Motion index for different LCS before, during, and after oestrus

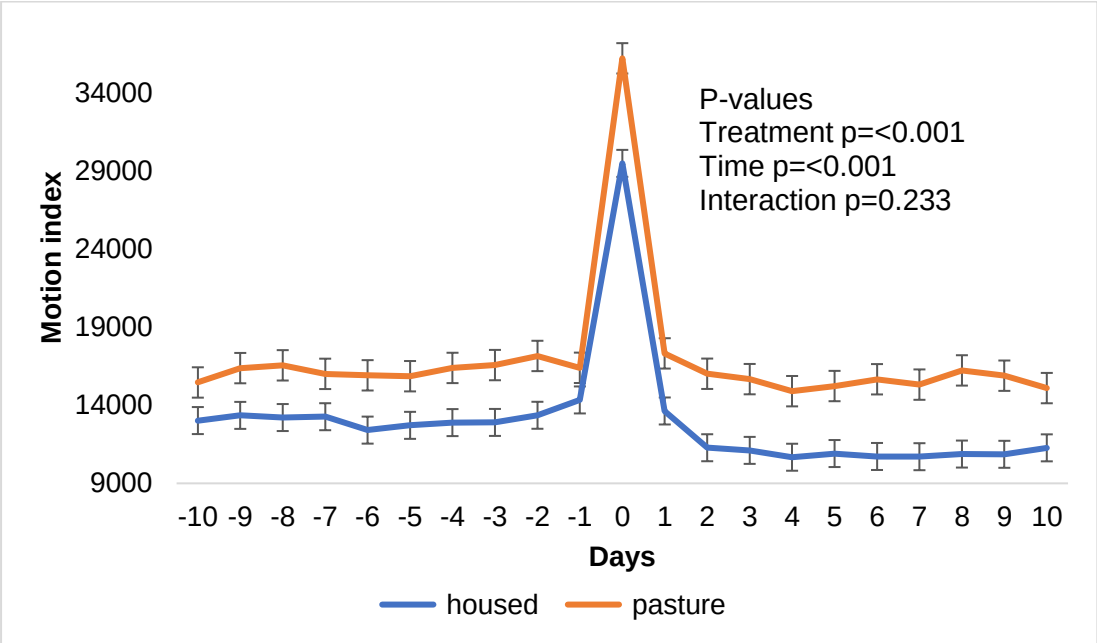

Figure 5- 10 Motion index for dairy cattle in different housing conditions (Housed v pasture), before, during, and after oestrus

Repeated measures ANOVA determined there was no significant difference in standing times (Figure 5-11) before, during and after oestrus from different LCS. Housing had no effect on motion index, (Figure 5-12). There was an effect of time, with the day of oestrus (0) having the highest standing times.

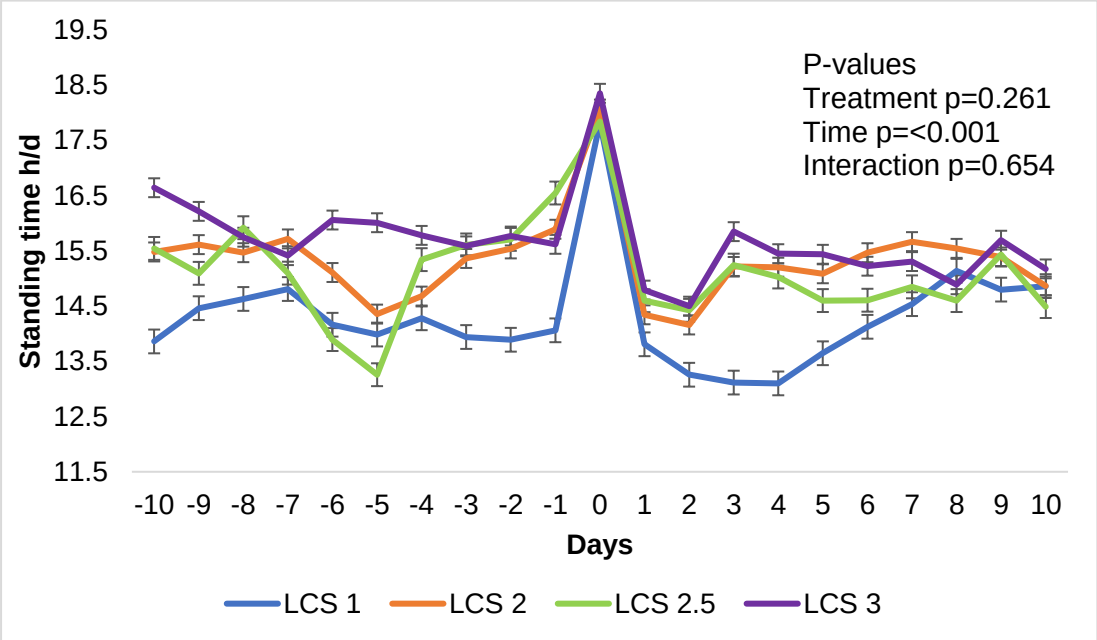

Figure 5- 11 Standing times for different LCS before, during, and after oestrus

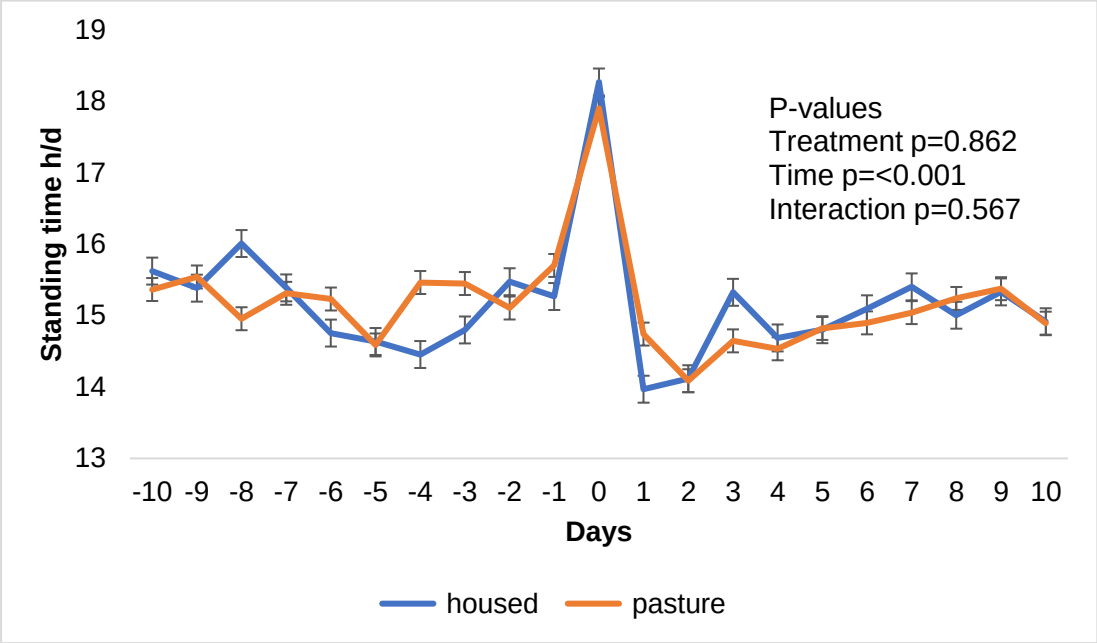

Figure 5- 12 Standing times for dairy cattle in different housing conditions (Housed v pasture), before, during, and after oestrus

Repeated measures ANOVA determined there was no significant difference in lying times (Figure 5-13) before, during and after oestrus from different LCS. Housing had no effect on lying times, (Figure 5-14). There was an effect of time, with the day of oestrus (0) having the lowest lying times.

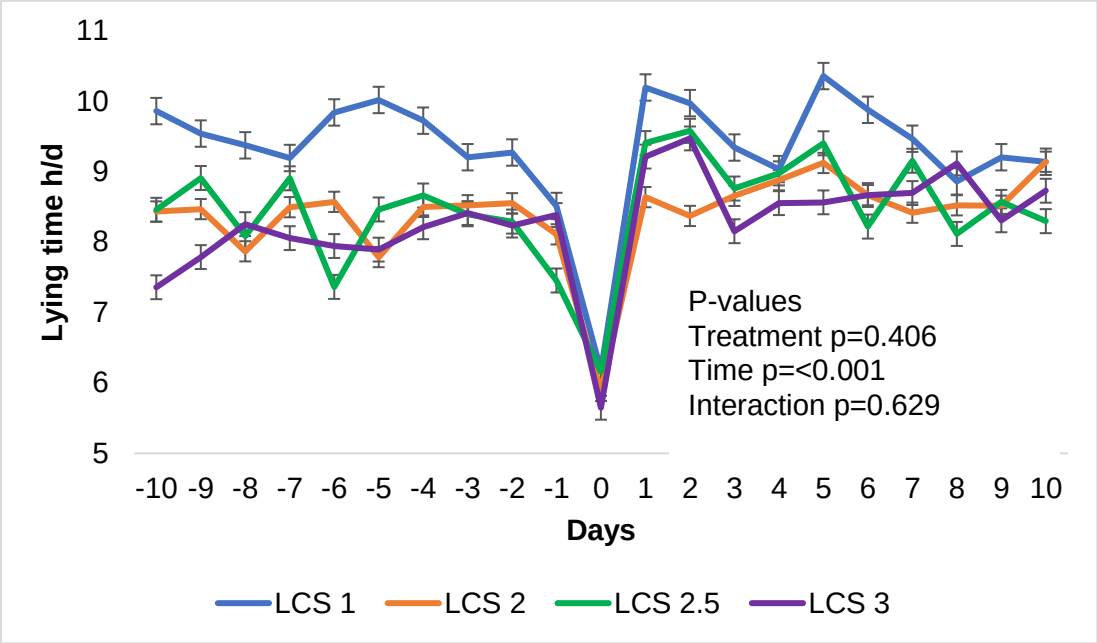

Figure 5- 13 Lying times for different LCS before, during, and after oestrus

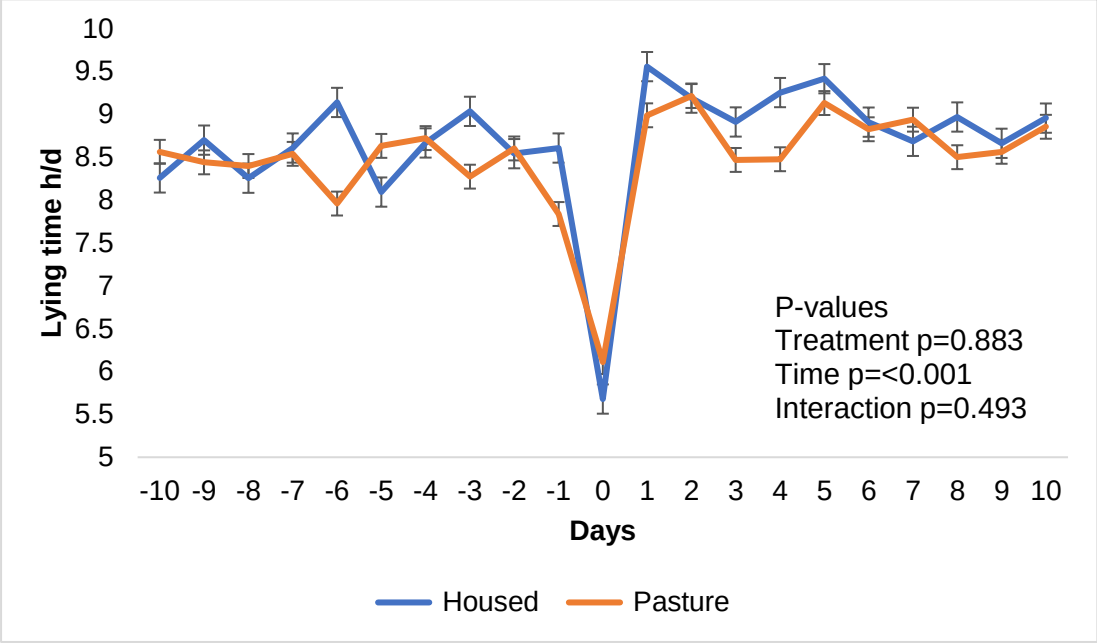

Figure 5- 14 Lying times for dairy cattle in different housing conditions (Housed v pasture), before, during, and after oestrus

Repeated measures ANOVA determined there was no significant difference in the number of lying bouts (Figure 5-15) before, during and after oestrus from different LCS. Housing had no effect on the number of lying bouts, (Figure 5-16). There was an effect of time, with the day of oestrus (0) having the lowest number of lying bouts.

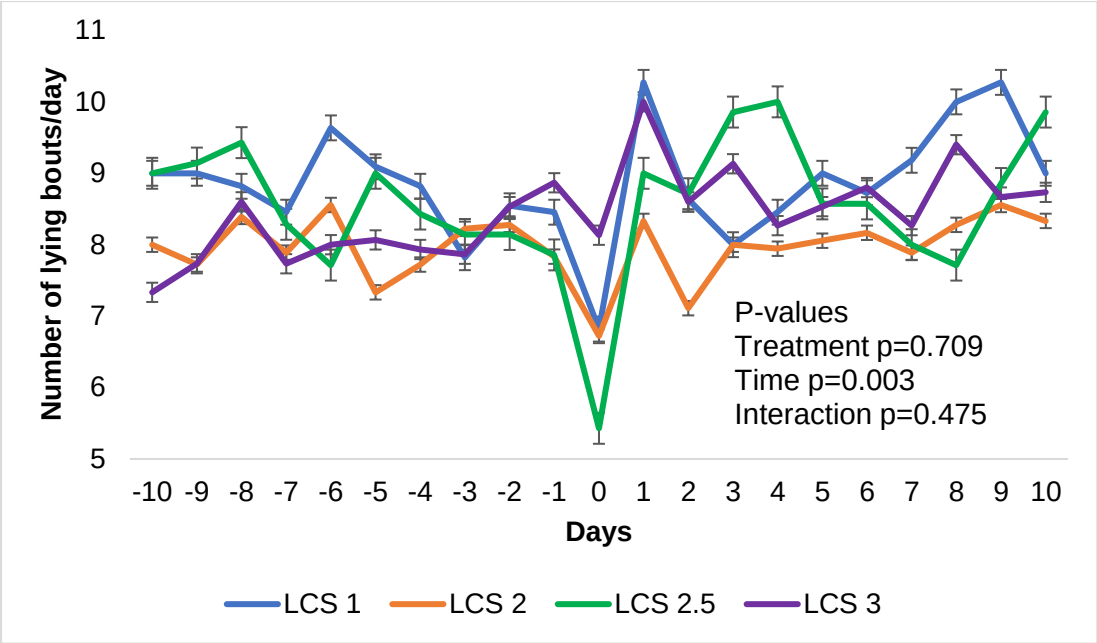

Figure 5- 15 Mean number of lying bouts/day for different LCS before, during, and after oestrus

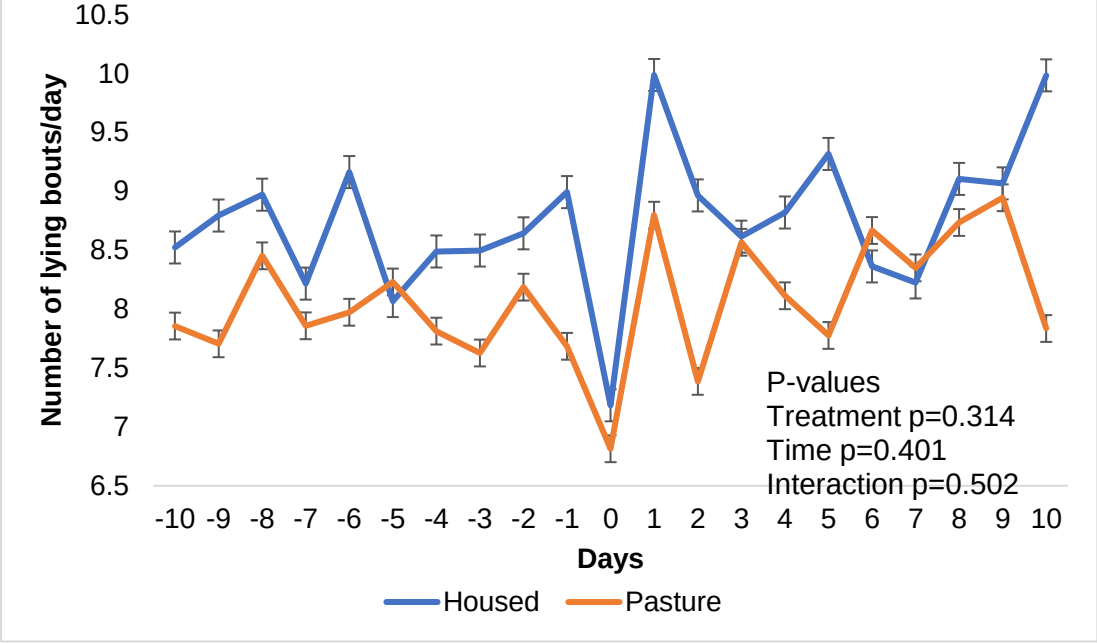

Figure 5- 16 Mean number of lying bouts/day in different housing conditions (Housed v pasture), before, during, and after oestrus

Repeated measures ANOVA determined there was no significant difference in mean lying bout length (Figure 5-17) before, during and after oestrus from

different LCS. Housing had no effect on mean lying bout length, (Figure 5-18). There was an effect of time, with the day of oestrus (0) having the lowest lying bout length.

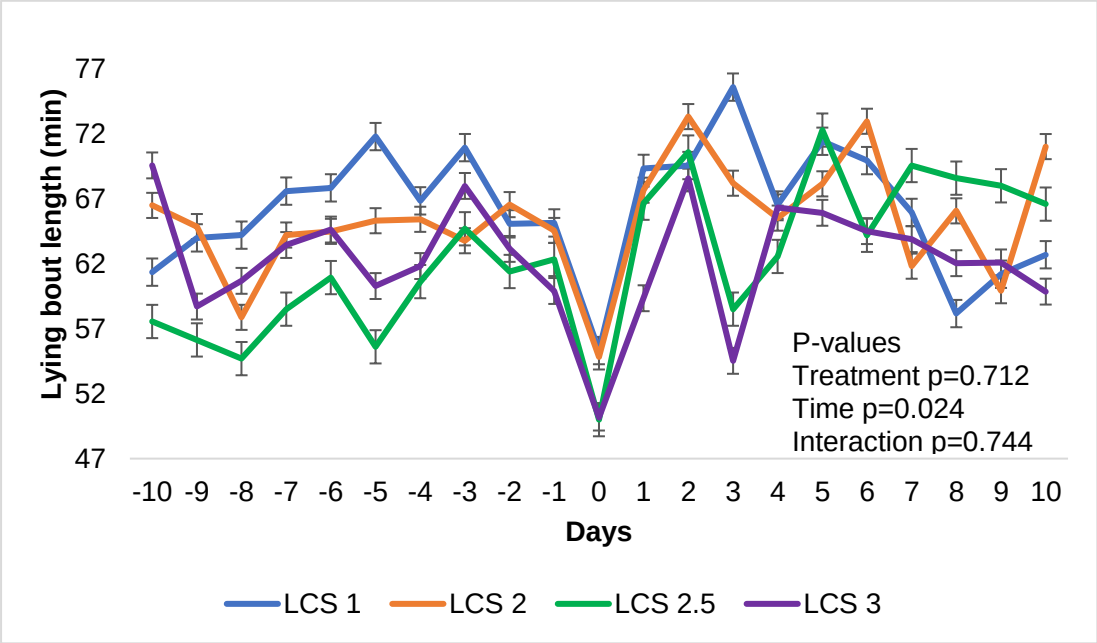

Figure 5- 17 Mean lying bout length (min) for different LCS before, during, and after oestrus

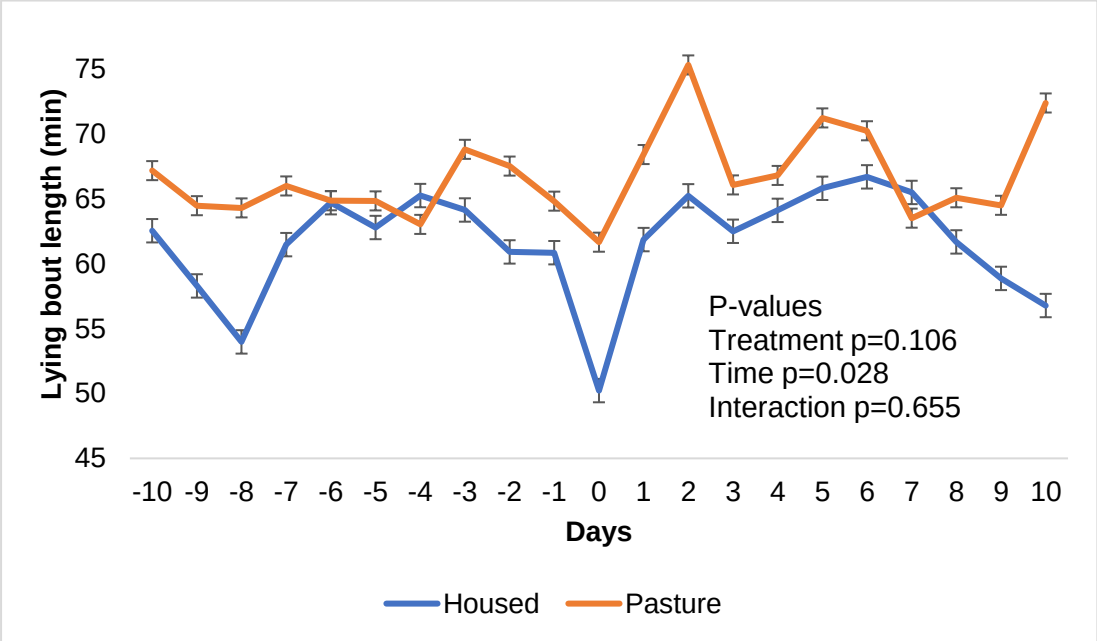

Figure 5- 18 Mean lying bout length (min) in different housing conditions (Housed v pasture), before, during, and after oestrus

There was no significant difference in milk progesterone concentrations (Figure 5-19) before, during and after oestrus from cows with different LCS. However, there was an effect of time, with day (0) having the lowest progesterone concentrations.

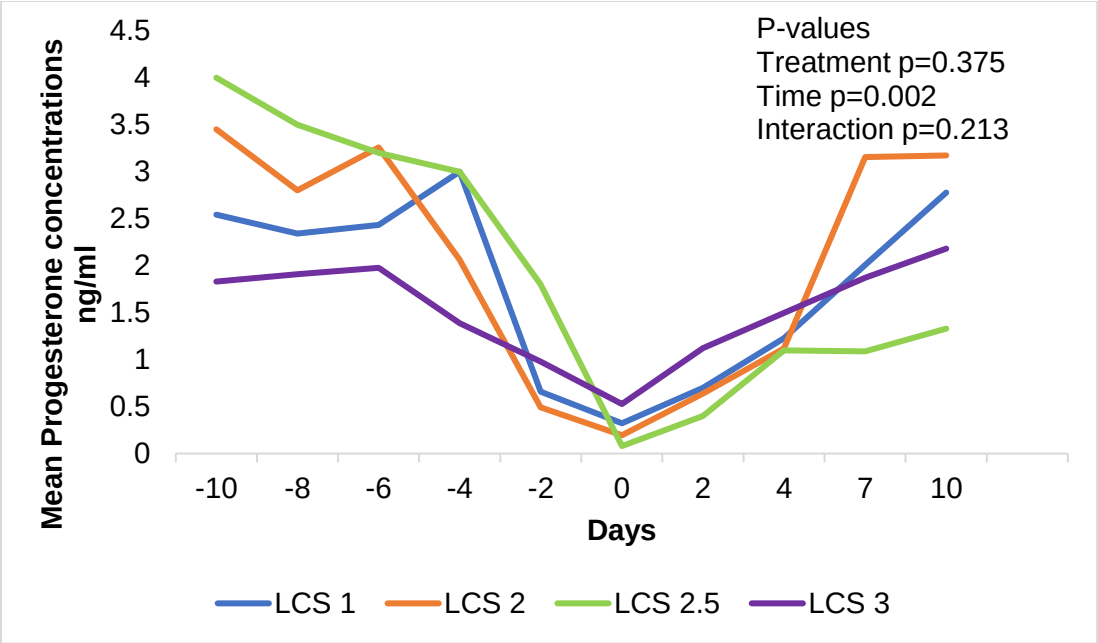

Figure 5- 19 Milk progesterone concentrations (ng/ml) from different LCS before, during, and after oestrus

5.5 Discussion

5.5.1 Herd fertility

Hernandez *et al.* (2005a) reported increased days from calving to conception for cows with higher locomotion scores when compared to cows with low locomotion scores. Lame cows in the present study had increased number of days from calving to conception, and calving to first service. However, the number of inseminations to conception did not differ between lame and non-lame cows. These findings contradict other studies that have reported that lame cows require more inseminations to conception (Sprecher *et al.,* 1997; Alawneh *et al*., 2011). For example, Collick *et al.* (1989) reported that non-lame cows required on average 1.72 inseminations to conception, whereas lame cows required 2.14. The number of inseminations to conception are above 2 for both lame (2.5) and non-lame (2.1) cows from this herd. An optimum value is considered to range from 1.6 and 1.8 (Borkowska *et al*., 2012). It should be noted that the guidelines do not take lameness into account, which might not be reflective of insemination rates at farms that do not separate lame and non-lame cow insemination data. However, according to Mordak (2008) the number of services per conception around 2 is still acceptable, but values exceeding 3 are indicative of considerable reproduction and/or health issues Abdollahi-Arpanahi *et al*. (2013) reported an average number of inseminations to conception of 2.14. However, Abdollahi-Arpanahi *et al*. (2013) did not take lameness into account, which may be an explanation for rates above 2.

Improper detection of oestrus can increase the number of inseminations required for conception. If cows are displaying oestrus behaviours within 4 to

17 days' post service, this can indicate follicular cysts (Statham, 2016), or poor oestrus detection. This may suggest that either improper heat detection, incorrect timing of AI, or poor insemination techniques are employed at this farm. The optimal time for insemination is reported to be approximately 2-14 hours after the onset of standing oestrus (Dransfield *et al.,* 1998; Roefofs *et al.,* 2005). Frozen-thawed spermatozoa are reported to have a life-span of around 12 to 24 hours (Gordon, 2003). The ovum also has a viable lifespan of approximately 6 to 12 hours (Gordon, 2003). Therefore, timing of insemination is vital since the viability of ova and semen is limited (Vartia *et al*., 2017). The AI technician visited the farm once daily, therefore some cows may not be inseminated at the correct time. If a cow comes into standing oestrus shortly after the technician leaves, the cow will not be served until the following day which can be 24hrs after the onset of standing oestrus. If a cow comes into standing oestrus at the time the AI technician arrives, the cow will be inseminated early, which may result in sperm perishing before the egg can be fertilised. This will result in the cow either; being inseminated the following day (if the cow displays oestrus behaviours for an extended period of time), or the cow fails to conceive on that cycle. Additionally, if cows are engaging in oestrus activity with herd mates, secondary oestrus behaviours may be interpreted incorrectly, resulting in cows being incorrectly inseminated. For example, one cow at this farm was identified as being in oestrus by a technician, upon veterinary examination it was determined that she was pregnant when she was inseminated. Inseminating pregnant cows may cause embryonic mortality or abortion (Vandemark *et al.,* 1952). Pregnant cows have been known to display oestrus behaviours indistinguishable from non-pregnant cows (Thomas and Dobson, 1989). Some may even stand to be mounted by another cow or bull

as a non-pregnant cow in oestrus would (Thomas and Dobson, 1989). However, Thomas and Dobson (1989) reported that the physiological changes in the genital tract normally associated with true oestrus were not observed in the pregnant cows showing oestrus behaviours. The cow from this study did not have tail chalk removed, and it was inseminated based on ‘uterine tone’. It could be that this technician was not experienced in assessing differences associated with oestrus and pregnancy.

These factors would lead to an increased number of inseminations to conception at this farm. Having a technician check the herd twice a day would increase oestrus detection rates, however this is not possible due to management and availability of the company.

Another explanation could be that the lame cows had an extended time period whereby their overall condition improved, thereby increasing their chances of conception after service. Either a delay of ovarian activity, or suppression of oestrus behaviours may be the cause of increased number of days to insemination, as the lame cow may not cycle normally. Additional days before insemination may result in improved body condition, which may result in resumption of normal ovarian activity whereby the lame cow has the same conception rate as non-lame cows.

The mean locomotion score for lame cows from this herd was 3.08 (±0.06). Using the Flower and Weary (2006) method these cows are categorised as ‘lame’, whereas locomotion scores of 4, and 5 are categorised as moderately lame and severely lame respectively. As the majority of cows in this study were

lame, this may suggest that their ability to conceive was not greatly affected by their locomotion score. Five cows were culled from the herd before conception could take place. These five cows had LCS of 3 (n=1), 4 (n=2) and 5 (n=2), with a mean LCS of 4.2 (±0.37). Hernandez *et al.* (2005a) found that cows that were classified as severely lame had a reduced chance of conception (58% decreased hazard of pregnancy) when compared to that of mildly lame cows (29% decreased hazard of pregnancy). Had these culled cows been included in the data analysis, the number of inseminations to conception for lame cows may have been influenced. Although the number of inseminations to conception is not affected, other fertility parameters such as days from calving to conception, and days from calving to first service were.

The voluntary waiting period (VWP) is the time period postpartum during which producers refrain from inseminating cows even if they display oestrus (Inchaisri *et al.,* 2010). Implementing a VWP has been reported to be beneficial to freshly calved cows as this allows for uterine involution and resumption of normal ovarian activity to enhance conception rates (Fetrow *et al.,* 2007). The cows from this study had a VWP of 35 days. Recommendations for VWPs' vary. For example, some reports suggest a longer VWP postpartum (<50 days, Foote 1978; <41 days, Caraviello *et al.,* 2006b; 45-60 days, Fetrow *et al.,* 2007; 80 days Schefers *et al.,* 2010) is associated with improved conception rates. As cows may have been inseminated as early as 35 days, it may be that their uterus was not ready for pregnancy, therefore inseminating cows before the cow is ready would increase the number of inseminations to conception. This may be why the lame and non-lame cows did not differ. However, recommendations for what the VWP should be are conflicting. Other studies

suggest that inseminating cows after a long VWP (93 ± 17 d) reduces their fertility (Schneider *et al.,* 1981), and that inseminating from 30 days' postpartum is profitable (Dijkhuizen *et al.,* 1985; Inchaisri *et al.,* 2011). As the average number of days from calving to first service in non-lame cows is 53.5, it is unlikely that early inseminations are the reason for increased inseminations to conception.

Sperm quality can be affected by thawing procedures. Many commercial AI centres suggest warm-water thaw methods for semen processed at their centres (Kaproth *et al.,* 2005). The warm-water thaw procedure features a semen straw being removed from liquid nitrogen and immediately placed in 33–35 °C water for a minimum of 40 seconds prior to preparing the AI gun (Kaproth *et al.,* 2005). In contrast, semen processed with procedures specifically intended to facilitate a flexible-thawing method, including the pocket thaw, is currently widely used in the United States (Kaproth *et al.,* 2005). The pocket thaw method features a straw retrieved from liquid nitrogen being placed immediately in a folded paper towel for protection, and then placed into a thermally protected pocket for 2–3 min to thaw before preparing the AI gun (Kaproth *et al.,* 2005). Previous investigations have reported that warming sperm too quickly, or to above 35°C, can result in permanently damaging the spermatozoa (Senger *et al.,* 1976). However, thawing procedures outlined on the Genus ABS website states that thawing semen should be done in a 35 °C to 37°C water bath for 30 s (Genus Breeding Ltd, 2016). Care must be taken to protect the semen straws from rapid temperature fluctuations, and environmental factors such as air temperature. Exposure to temperatures either too hot or too cold can cause damage to the sperm cells. Reducing the

amount of time the semen straw is exposed to environmental elements (e.g. temperature, wind) will ensure the semen remains viable until deposited in the cow. Thawing procedures at the study farm used the pocket-thaw method. Incorrect handling and thawing of the semen straws will directly affect conception rates. Additionally, the core body temperature of humans is approximately 37°C (Parsons, 2013). However, the temperature of human skin can range from 33.5 °C to 36.9°C (Bierman, 1936). If the semen straws are thawed in a shirt pocket, this temperature may not be warm enough to ensure proper thawing before being deposited in the cow.

The cows from this herd are considered high-yielding with an average milk production of over 11,000 litres per lactation. There is evidence that high yielding cows often enter a state of negative energy balance (NEB) when the energy demand for lactation and maintenance exceeds that of dietary intake (Bauman and Currie, 1980; Chebel *et al*., 2004). Cows in a NEB can have reduced fertility (Lucy, 2001) leading to poor conception rates (Wathes *et al*., 2003; Wathes *et al*., 2007). Research has shown that high yielding cows in a NEB have reduced oocyte quality and inadequate corpus luteum function (associated with reduced progesterone and low insulin-like growth factor concentrations), which can cause a suboptimal uterine environment that is incapable of sustaining early embryonic life (Mann and Lamming, 1999; Leroy *et al*., 2006). This may also be a possibility for slightly higher numbers of inseminations to conception from cows studied in this herd.

5.5.1 Locomotion score

During the study, the prevalence of lameness decreased during the months when the cows had access to pasture. January 2014 has a decrease in lameness prevalence. Four lame cows were removed from the milking group in January, which would cause a decrease in lameness prevalence. Additionally, January 2014 also had n=14 fresh cows entering the milking herd, 100% of the fresh cows had a LCS <2.

The LCS trend for lame and non-lame study cows showed improved LCS from April to September. When winter housing occurred, LCS deteriorated for all study cows. LCS from the study cows for the months May and July were significantly different. All study cows had improved LCS during the 4-week period after pasture access. These findings are consistent with other studies (Hernandez-Mendo *et al*., 2007; Olmos *et al*., 2009; Somers *et al*., 2015; Alsaaod *et al*., 2017). Whether LCS improvements were because of improved hoof health cannot be assessed because hoof health was not measured in the current study. Bergsten (2001) suggested that more than 90% of lameness cases are caused by hoof lesions. Previous research has shown that access to pasture can improve hoof health in lactating dairy cows (Somers *et al*., 2003). The presence of sole ulcers can have a direct effect on cow LCS (Hernandez-Mendo, *et al*, 2007). Some cows have visible injuries but appear to have a normal LCS, and others can be clinically lame but have no visible hoof ailments (Flower and Weary, 2006). This poor correlation may occur partly due to a lag between the time that hoof ailments can be scored and when LCS is affected (Hernandez-Mendo, *et al*, 2007). Changes in LCS may not be associated with hoof health, but perhaps joint stiffness, or physical exercise (Hernandez-

Mendo, *et al*, 2007). Hard surfaces such as concrete do not provide cows with secure footing (Hund *et al*., 2019), this can reduce the range of motion in the joints (Phillips and Morris, 2001), thereby causing the cows to walk with a stiff gait (van der Tol *et al*., 2005). Pasture provides optimal locomotory comfort to cows (Alsaaod *et al*., 2017). Improvements in LCS at pasture could be due to increased exercise, as pastured cows spend more time walking and grazing than housed cows (Hernandez-Mendo, *et al*, 2007).

Results showed that as the study cows LCS improved, subsequent oestrus activity (step counts, motion index) also increased. Cows had up to 4 oestrus events before becoming pregnant. During the first 2 oestrus events the mean LCS was 2.4 (±0.3) and was significantly different from the third oestrus event (LCS 1.6), but not the 4th (2.1). Fifty-seven percent of cows having a 4th oestrus event were in winter housing conditions, and their LCS deteriorated. Whereas 70% of the third oestrus event occurred at pasture (June and July) when mean LCS were the lowest (1.6). The 2nd oestrus event was not significantly different from the first, possibly due to the gradual LCS improvement after pasture access. The increase in activity could be due to footing type. Pastured cow activity was higher than housed activity, which could be a result of more secure footing. As previously mentioned, concrete surfaces do not provide cows with secure footing (Hund *et al*., 2019), whereas pasture provides optimal locomotory comfort to cows (Alsaaod *et al*., 2017). Seven cows became pregnant and did not have third or fourth oestrus events. Therefore, the reduced sample size could affect the mean LCS.

Cows with a LCS of 1 at the time of oestrus had significantly more steps than a LCS of >2.5 during an oestrus event. Cows with a LCS of 1 at the time of oestrus had a significantly higher motion index than a LCS of >2 during an oestrus event. As the LCS from the experimental cows significantly improved after access to pasture, it could be that the increase in oestrus activity is associated with improved LCS, and more secure footing. These results are in agreement with Walker *et al*. (2008a) that cows with impaired mobility have reduced oestrus expression on the day of oestrus when compared to cows with no mobility impairments. Oestrus expression is further reduced on concrete flooring when compared to dirt surfaces (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996). For example, mounting activity may rise to 3-15 times greater on soil surfaces, whereas there is a sharp decline in mounting activity on slippery surfaces (Rao *et al.,* 2013). Therefore, cows housed on concrete, uneven, or slippery flooring may reduce their oestrus behaviour intensity, which in turn reduces mounting behaviour (Squires, 2010; Rao *et al.,* 2013). Step counts from cows with a LCS of 2 were only significantly different from cows with a LCS of 3. One explanation for this could be that lame cows that had an improved LCS score were given a 2, and perhaps the effects of lameness were still restricting the cows' overall activity even though the LCS improved. Even though cows with a LCS of >3 improved, they never achieved a LCS of 1. This may be why the motion index from cows with a LCS >2 were not significantly different from one another. Lame cows improving to a 2.5 or 2 have increased activity than they had when they scored >3, but their locomotion could still be impaired, thereby affecting activity.

Previous research by Walker *et al*. (2008a) determined that lame cows had lower progesterone concentrations during the 6 days prior to oestrus, in addition to a decreased intensity of oestrus, lower intensity of sexual behaviours, and reduced periods whereby herd-mates mounted the lame cows. Due to limited resources, it was not possible to analyse milk progesterone concentrations for each oestrus event. It would be beneficial to monitor progesterone profiles over time to assess if the concentrations were influenced by improved LCS through pasture access. This study would have benefitted from a larger sample size, through monitoring multiple oestrus events over an extended time period. However due to limited resources it was not possible to extend the length of time data were collected.

5.6 Conclusions

In conclusion fertility parameters of cows from this herd were affected by lameness, and improved LCS after pasture access reduced lameness prevalence. Study cows had increased activity at pasture, and had rapid LCS improvement after pasture access. As LCS improved, each subsequent oestrus event had increased oestrus activity (steps/motion index). Therefore, the benefits of allowing cows pasture access can be extrapolated to other dairies which include improved mobility, thereby enhancing oestrus expression and improving fertility parameters. However due to land constraints this is not always possible. Perhaps incorporating a comfortable area within the barn/house for cows to loaf in could assist in reducing lameness, improving LCS, oestrus expression, and oestrus detection. As expression of primary

oestrus behaviours (standing to be mounted) is affected by LCS, it would therefore be beneficial to determine certain detection methods are more efficient in detecting oestrus in lame cows.

Chapter 6: Comparison of different oestrus detection methods (visual, tail chalk, mount detectors, milk progesterone, IceQube®, NeDap Lactivator RealTime) in pastured and zero grazed lame and non-lame lactating Holstein-Friesian dairy cattle.

6.1 Introduction

Several studies have determined negative relationships between high milk production, lameness, fertility (Laben *et al*. 1982; Lucy, 2001; Nebel and McGillard, 1993; Roxström *et al*., 2001; Stevenson *et al*. 1983; Windig *et al*., 2006), and increased susceptibility to disease (Carlén *et al*., 2004; Konig *et al.*, 2008). Sufficient circulating concentrations of P^4 are crucial for pregnancy maintenance (Stevenson *et al.,* 2008; Yan *et al*., 2016), and for full expression of oestrus behaviours (Walker *et al.,* 2008a). Higher yielding cows have been reported to have lower circulating P^4 concentrations, due to increased metabolic clearance rates of steroid hormones (progesterone and oestradiol) (Sangsritavong *et al*., 2002; Vasconcelos *et al*., 2003; Roche, 2006). Therefore, detecting oestrus in high yielding cows is becoming increasingly difficult. Oestrus expression, and detection is also affected by lameness; lame cows display different behaviours from non-lame cows (Navarro *et al*. 2013).

Lameness has a detrimental effect on reproductive function (Garbarino *et al*., 2004), and oestrus behaviour (Walker *et al*., 2008a), including reduced oestrus intensity (Walker *et al.,* 2010). Lameness not only has a detrimental effect on reproductive function, but it also affects behaviours such as those typically

exhibited during oestrus (e.g. Walker *et al.,* 2008a; Walker *et al*., 2010). Intensity and duration of oestrus is lower in lame cows (Walker *et al*., 2010) with reduced P^4 levels.

As dairy herds are increasing, farmers have reduced time available to observe cows. Presently, there are numerous methods to detect oestrus in dairy cattle other than the use of visual observations for mounting activity. Some include; activity monitors, radiotelemetry, pressure activated mount detectors, teaser animals, tail-paint or chalk, intravaginal and vulvar electrical impedance, and body temperatures. Electronic devices have major implications in increasing the accuracy and efficiency of oestrus detection and have been able to establish the optimum time for artificial insemination in relation to the time of first onset of oestrus. Optimal timing can be achieved using continuous monitoring of oestrus behaviour.

Twenty-four-hour monitoring can be beneficial with contradictory evidence as to time of day oestrus is expressed (night (Hall *et al.,* 1959; Orihuela *et al.,* 1983; Mattoni *et al*., 1988), early morning or during the day (Hurnik *et al.,* 1975; Amyot and Hurnik, 1987; Gwazdauskas *et al.,* 1990; Van Vilet and Van Eerdenburg, 1996), no time difference (Esslemont and Bryant, 1976; Esslemont *et al*., 1980; Alexander *et al.,* 1984; De Silva *et al.,* 1998; Xu *et al.,* 1998).

It has been reported that during the day, lame cows displayed a lower proportion of oestrus behaviours in the early morning (Walker *et al.,* 2008a),

and that lame ovulating cows had reduced oestrus intensity, and a lower maximum oestrus score in any 30-min period than their healthy counterparts (Walker *et al.,* 2010). However, identifying particular oestrus behaviours that a lame cow displays more readily/frequently has yet to be reported. In addition to determining if certain oestrus detection methods are more efficient for lame cows. Incorporating a lameness detection aspect into an oestrus detection technology would be beneficial. Therefore, it might be advisable to employ an activity monitor such as the IceQube®, and or NeDap Lactivator RealTime. These devices are activity monitoring systems, which can detect oestrus, while also monitoring cows for lameness. To the knowledge of the author there are no other studies that compare oestrus detection methods for lame cows.

Therefore, this study aims to compare accuracy of oestrus detection methods between lame and non-lame cattle in different housing conditions (access to pasture, and fully housed).

The second aim was to identify cows that were undetected or detected too late by all techniques, using progesterone assays. Further analysis was conducted on this subgroup to determine whether these cows were; infertile, do not express oestrus (silent heats), or whether lameness (as measured by locomotion scoring) is associated with this group.

6.4 Materials and Methods

6.4.1 Animals and data collected

The study was conducted from April 2013 to August 2014 at Rodwell dairy farm (Ipswich, England). The farm had a herd of 130 Holstein Friesian cows with average milk production over 11,000 litres per lactation. The management of these cows is detailed in Chapter 3. Briefly, cows were milked twice daily through a Delaval herringbone 8/8 parlour. Lactating cows were housed during the winter months, and fully pastured during the summer months.

6.4.2 Locomotion Scoring

All cows in the milking herd with the exception of cows in the hospital pen (through illness, or freshly calved) were locomotion scored weekly using the method of Flower and Weary (2006) shown in Table 3-5 in Chapter 3. The mean number of cows' locomotion scored monthly were n=102.5 (±2.4), equating to 5857 locomotion scores given from April 2013-August 2014. The cows were locomotion scored leaving the milking parlour on grooved concrete. The cows were observed walking along the alley way and then turning left into the barn. A detailed description of LCS method is outlined in Chapter 3-section 3.2 All locomotion scoring was carried out by the same observer (AW). Similar to Flower and Weary (2006b), If a cow exceeded the requirements of a particular score, but did not meet all the requirements of the next successive score, a half-integer score was allocated. For example, cows that had improved locomotion scores but still exhibited lame cow characteristics (e.g. arched back) were given a score of 2.5 rather than a 2.

6.4.3 Study animal selection

Cows were chosen based on their locomotion score, and their current reproductive stage. Freshly calved cows with normal reproductive history were enrolled in the study from on average of 25.3 (±0.7) DIM. Cows with clinical conditions such as mastitis, and digestive disorders were excluded from the study. A total of n=73 cows were initially recruited in the study. From the 73 cows, six cows were excluded from the study for the following reasons; one cow perished before oestrus data could be recorded, one cow had a displaced abomasum and required surgery and isolation, two leg pedometers (IceQube®) fell off and were not recovered, and batteries in two in IceQube® devices failed and data could not be recovered. A total of n=67 cows were used in this study. A total of n=33 cows were studied with access to pasture (n=19 lame, n=14 non-lame), and a total of n=34 cows were studied while fully housed (n=12 lame, n=22 non-lame).

Lame cows had a mean locomotion score and parity of 3.1 (±0.2) and 3.9 (±0.1) respectively. Non-lame cows had a mean locomotion score and parity of 1.4 (±0.1) and 3.1 (±0.2) respectively. Lame and non-lame cows were pair matched based as closely as possible based on parity. As lameness increases with increasing parity (Espejo *et al*., 2006; Sarjokari *et al*., 2013), the mean parity of the lame group was higher than the non-lame group. The voluntary waiting period for the herd was 35 days. Healthy cows expressing oestrus at 35 DIM were served, therefore recruiting cows before this time (25.3 DIM (±0.7) ensured oestrus was observed.

6.4.4 Activity monitors

Study cows were fitted with the NeDap activity monitors 10 days' post calving to the front right leg (providing this limb was not affected by lameness) as described by the manufacturer. None of the study cows were affected by lameness on this limb. These devices required 10 days to establish the individual locomotion behaviour of each cow so that fluctuations from typical activity could be identified in the future.

IceQube® Sensors (IceRobotics, Ltd) were fitted 20 days' post calving to the right hind leg (providing this limb was not affected by lameness) as described by the manufacturer. None of the study cows were affected by lameness on this limb. The right side of the cow was easily accessible when the cows were in the crush, therefore the limbs on the right-hand side were used to attach the activity monitors.

6.4.5 Mount Detectors

Three different mount detectors were used on each cow Kamar® (Cox Agri, Stanley, Co Durham), Estrotect™ scratch cards (DairyMac Limited, Wickham, Hampshire, England), and tail chalk (All-weather PaintStik, LA-CO Industries Inc., Illinois, USA) (plate 6-1). Both adhesive mount detectors were fitted 30 days' post calving as described by the manufacturer. Tail chalk was applied when the cow entered the milking herd after parturition. Chalk was checked and applied daily to the tail head of each cow by one of two experienced AI Technicians. The mount detectors did not interfere with one another. Detectors were checked twice daily either by the herdsmen, and/or the AI technician/researcher. The AI technician, and herdsmen routinely checked the

mount detectors for any signs of activation, and this was recorded daily in a designated notebook. Additional notes were made regarding the percentage of chalk removed, whether the Kamar® was activated fully/partially or missing, and the percentage of how scratched the Estrotect™ scratch cards was, or if it was missing. These notes were checked by the researcher. A record was made of those cows where their chalk is removed and/or the detectors turned colour, and their tail heads were re-chalked, and new detectors were applied after insemination.

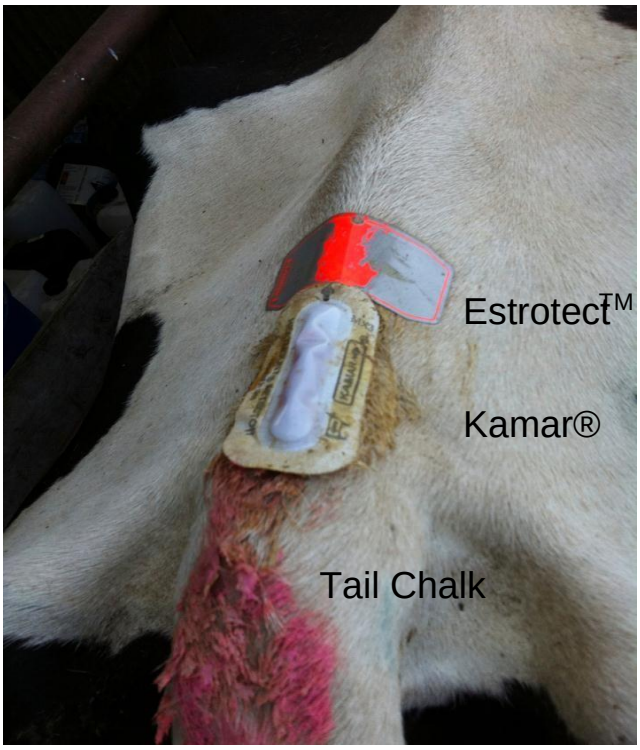

Plate 6- 1 Study cow fitted with three different mount detectors (Estrotect™, Kamar®, and Tail Chalk)

6.4.6 Visual Observations

A closed-circuit television system (CCTV) was installed to assist in visually identifying oestrus behaviours in housed cows without the presence of a human, frequently throughout the day (see Chapter 3, Figure 3-4 for CCTV barn diagram). The main oestrus behaviour that was expected to be recorded

was when a cow stands to be mounted. However secondary behavioural signs were also identified using the Dutch points scale (Table 6-1). Cows were marked with non-hazardous line marking paint (Richey sprayline coloured stock marker) in order to easily identify study cows from a distance, and on CCTV (see Plate 6-2).

Plate 6- 2 Study cow painted with marking paint (Richey sprayline coloured stock marker)

The files were transferred to an external memory source for analysis. Day observations at pasture were carried out by the researcher via binoculars to minimise intrusion. The herdsman also made note if a cow was seen bulling.

Table 6- 1: Dutch point scoring scale for oestrus behaviour

Behaviour	Points
Mucous vaginal discharge	3
Flehmen	3
Restlessness	5
Sniffing the vulva of another cow	10
Mounted but did not stand	10
Resting chin on the back of another cow	15
Mounting the rear of another cow	35
Mounting the head of another cow	45
Stood-to-be-mounted (STBM)	100

(van Vliet and van Eerdenburg, 1996)

6.4.7 Milk Sampling and P^4 analysis

Milk sampling started when a cow entered the study 25.3 (±0.7) DIM, and stopped 10 days after oestrus was observed. This would enable individual analysis of progesterone profiles prior to, during, and after oestrus. Milk samples were collected three times weekly for use in milk progesterone (P^4) analyses (Monday, Wednesday, Friday), at afternoon milking from the right rear quarter, with one exception (cow 185) as the right rear quarter was dry, therefore the sample was taken from the left rear quarter. Samples were collected from one quarter to reduce interference with the herdsmen's milking routine. Teats were dipped prior to sampling using iodine teat disinfectant followed by wiping to ensure hygienic conditions for animal welfare and milking purposes. Gloves were worn for sampling, and initial stripping's (foremilk) were discarded before samples were obtained. Milk samples were collected into 25ml test tubes containing a preservative (Lactab Mark III; Thompson and Cooper Ltd., Runcorn, UK). Cow number and date were written on the side of

the test tubes, and were inverted to mix until the tablet was dissolved. Milk samples were placed in a test tube stand in a cool bag, and were chilled immediately in the farm fridge (4°C), until freezing (within 3.5 hours). Frozen milk samples were stored at Writtle University College laboratory until P^4 analysis could be carried out.

Progesterone was analysed using the Ridgeway ELISA-kit (Ridgeway Science Ltd., Rodmore Mill Farm, Alvington, Gloucestershire, UK), which is an accepted technique for P^4 analysis (Gillis *et al*., 2002; Roelofs *et al*., 2006; Gorzecka *et al*., 2011; Nyman *et al*., 2014; Blavy *et al*., 2016; Adriaens *et al*., 2017; Daems *et al*., 2017). Milk sample handling analysis was carried out following the manufactures protocol, except for incubation period which is described in Chapter 3 (Section 3.6). Each cow had 10 samples analysed, 5 samples prior to oestrus, the day of oestrus and 4 samples post oestrus. A total of n=670 milk samples were analysed. Detailed methodology for progesterone analysis is described in Chapter 3.

6.4.8 Time of day oestrus displayed

Days were divided into three time periods. Early morning 02:00-08:00, mid-day 08:00-16:00, evening 16:00-02:00. These data were obtained from both the NeDap, and the IceQube activity monitors.

6.4.9 Data handling and analysis

6.4.9.1 Mount detectors (Kamar®, Estrotect™, Tail chalk)

Mount detector data were entered into Excel three times per week after visiting the farm. Information collected included if the detectors (Kamar®; Estrotect™)

were activated, partially activated, not activated or were missing. Information regarding tail chalk included if the chalk was fully removed, partially removed, or if no removal occurred. It was then determined if the cow was likely to be in oestrus through a collective assessment of the detection methods employed. If a cow was not physically seen in oestrus, determining if oestrus occurred involved examining the mount detectors/chalk for activation/removal, reading notes made in research books by the herdsmen/AI technician, and checking the NeDap system for any activity alerts made. Partially activated mount detectors were included under 'activated' for statistical analysis. As the cows were confirmed as being in oestrus with the use of milk progesterone, partially activated detectors could be used as an indicator of oestrus. The accuracy of the mount detectors were calculated by dividing the number of accurate detections by the total number of detection events x 100. The sensitivity of the mount detectors was calculated by the true positives/ (true positives detection events + false negatives detection events the method) x100%. A chi-squared test of association was carried out examining the frequencies that the oestrus detectors were correctly activated between lame and non-lame cows, and between when the cows were housed cows or at pasture. Where significant associations were found individual contributions to chi-squared were inspected to identify main effects. All statistical analysis was performed using GenStat (18^{th} edition).

6.4.9.2 Activity monitors

The NeDap activity monitor system was checked for activity alerts. These data were entered into Excel three times per week after visiting the farm. Information collected included; increased activity triggering a suspicious alert, and

increased activity triggering an oestrus alert. IceQube®'s were downloaded wirelessly weekly and files were made for each cow. Days that were analysed for oestrus activity were 10 days prior to oestrus, the day of oestrus, and 10 days post oestrus. Data were found to be normally distributed using the Pearson's Skewness test. The effect of lameness on activity (IceQube® data) and progesterone concentrations were analysed using repeated measures ANOVA. Repeated measures were the days at successive times: 10 days prior to oestrus, the day of oestrus and 10 days post oestrus. Factors assessed were treatment (Lame*Non-lame), time and interaction. This was carried out for each activity measure (step count, motion index, standing times/lying times, number lying bouts and average duration of lying bout). Activity parameters from lame and non-lame cows under different housing conditions (pastured, housed) were analysed with a two-way ANOVA using an unbalanced design. The accuracy of the activity monitors were calculated by dividing the number of accurate detections by the total number of detection events x 100. All statistical analysis was performed using GenStat (18th edition).

6.4.9.3 Oestrus behaviour

To assess if there was a difference in the time of day oestrus was expressed, a one-way analysis of variance was used followed by a Tukey test for post hoc comparison. A chi-squared test of association was performed examining the frequency that cows (lame and non-lame; pastured and housed) displayed oestrus behaviours during specific times of the day (early morning 02:00-08:00, midday 08:00-16:00, evening 16:00-02:00. Where significant associations were found individual contributions to chi-squared were inspected to identify main effects. For duration of oestrus, activity was analysed using one-hour time

periods. Onset of oestrus was defined as 3 consecutive periods of increased activity compared with baseline (4-d rolling average (ninety-six 1-hour periods) before onset of increased activity). Activity increase at oestrus was the average of the 3 periods with the highest activity during the oestrus period, divided by baseline activity, expressed as a percentage. Duration of oestrus, activity increase at oestrus (step counts and motion index) from lame and non-lame cows under different housing conditions (pastured, housed) were analysed with a Two-way ANOVA (using an unbalanced design). Comparison of activity increases (%) between step counts and motion index were analysed to assess if one activity measure would be more suitable to use as an indicator of oestrus. This was done using paired t-test (data were confirmed as being normally distributed using the Pearson's Skewness test.). All statistical analysis was performed using GenStat (18th edition).

6.4.9.4 Milk Progesterone

Oestrus was verified through milk progesterone assay analysis. When pro-oestrus begins milk progesterone will drop from >10ng/ml to <3 ng/ml (Döcke, 1994). During oestrus, and ovulation progesterone levels drop to <0.5 ng/ml (Wiltbank *et al*., 2014). Therefore, an oestrus was assumed to have occurred if the progesterone concentration decreased from >1 ng/ml to <1 ng/ml over one to three sampling periods. The effect of lameness on progesterone concentrations were analysed using repeated measures ANOVA. Repeated measures were the days at successive times: 10 days prior to oestrus, the day of oestrus and 10 days post oestrus. Factors assessed were treatment (Lame*Non-lame), time and interaction.

6.5 Results

6.5.1 Mount Detectors Frequency

6.5.1.1 Pasture vs Housed

The frequency of correctly activated, inactivated, or missing oestrus detectors for housed and pastured cows is presented in Table 6-2. There was a significant association between housing and the accuracy of Estrotect^{TM} scratch cards (χ^2 = 6.58, df=2, N=67, p=0.037). Estrotect^{TM} scratch cards were more accurate at pasture. With the percent correctly activated making a significant contribution to the overall chi square (3.431).

Table 6- 2 Percent of detectors correctly activated for all cows housed and pastured

Variables	Housed			Pasture			*P*-Value (χ^2)
	Yes, (%)	No, (%)	Missing, (%)	Yes, (%)	No, (%)	Missing, (%)	
KaMar	10 (29)	5 (15)	19 (56)	19 (58)	3 (9)	11 (33)	0.067
Estrotect	11 (32)	8 (24)	15 (44)	21 (64)	4 (12)	8 (24)	0.037
Chalk	32 (94)	2 (6)	n/a	30 (91)	3 (9)	n/a	0.617
NeDap	34 (100)	0		32 (97)	1 (3)		0.306
IceQube	34 (100)	0		33(100)	0		

6.5.1.2 All Non-Lame and lame cows housed and pastured combined

The frequency of correctly activated, inactivated, or missing oestrus detectors for all lame and non-lame cows (pasture and housed combined) is presented in Table-6-3. There was no significant association between lameness and accuracy of any of the mount detectors.

Table 6- 3 Percent of detectors correctly activated for all cows' non-lame and lame, pastured and housed

Variable	Non-Lame n=36			Lame n=31			*P*-Value (χ^2)
	Yes, (%)	No, (%)	Missing, (%)	Yes, (%)	No, (%)	Missing, (%)	
KaMar	13 (36.1)	3 (8.3)	20 (55.6)	14 (45)	7 (23)	10 (32)	0.099
Estrotect	21 (58)	2 (6)	13 (36)	16 (52)	6(19)	9 (29.2)	0.218
Chalk	33 (92)	3(8)	n/a	28 (90)	3 (10)	n/a	0.848
NeDap	36 (100)	0	n/a	30 (96.8)	1 (3.2)	n/a	0.278
IceQube	36 (100)	0	n/a	31 (100)	0	n/a	n. s

6.5.1.3 Pasture lame and non-lame cows

The frequency of correctly activated, inactivated, or missing oestrus detectors for all lame and non-lame cows at pasture is presented in Table-6-4. There was no significant association between pastured lame and non-lame cows and the accuracy of any of the mount detectors

Table 6- 4 Percent of detectors correctly activated for all non-lame and lame cows at pasture

variables	Non-Lame			Lame			*P*-Value (χ^2)
	Yes, (%)	No, (%)	Missing, (%)	Yes, (%)	No, (%)	Missing, (%)	
KaMar	7 (50)	1 (7.1)	6 (42.9)	12(63)	2(11)	5(26)	0.605
Estrotect	8 (57)	2 (14)	4 (29)	13 (68)	2 (11)	4 (21)	0.801
Chalk	13 (93)	1 (7)	n/a	17 (89)	2(11)	n/a	0.738
NeDap	14 (100)	0	n/a	18 (94.7)	1 (5.3)	n/a	0.383
IceQube	14 (100)	0	n/a	19 (100)	0	n/a	n. s

6.5.1.4 Housed lame and non-lame cows

The frequency of correctly activated, inactivated, or missing oestrus detectors for all housed cows, both lame and non-lame is presented in Table 6-5. There was no significant association between housed lame and non-lame cows and the accuracy of any of the mount detectors.

Table 6- 5 Percent of detectors correctly activated for all non-lame and lame housed cows

	Non-Lame			Lame			*P*-Value (χ^2)
	Yes, (%)	No, (%)	Missing, (%)	Yes, (%)	No, (%)	Missing, (%)	
KaMar	7 (31)	2 (9.1)	13 (59.1)	3 (25)	3 (25)	6 (50)	0.455
Estrotect	9 (40.9)	3 (13.6)	10 (45.5)	2 (16.7)	5 (41.6)	5 (41.7)	0.133
Chalk	21 (95.5)	1 (4.5)	n/a	11 (92)	1 (8)	n/a	0.654
NeDap	22 (100)	0	n/a	12 (100)	0	n/a	n. s
IceQube	22 (100)	0	n/a	12 (100)	0	n/a	n. s

6.6 Activity

6.6.1 Pastured cows

Repeated measures ANOVA found there was a significant difference in step counts (p=0.013) (Figure 6-1) and motion index (p=0.004) (Figure 6-2), with an effect of time (p<0.001) before, during or after oestrus in lame and non-lame dairy cattle with access to pasture. Time effect demonstrates a sharp increase in activity (steps, and motion index) on the day of oestrus followed by resumption of baseline activity.

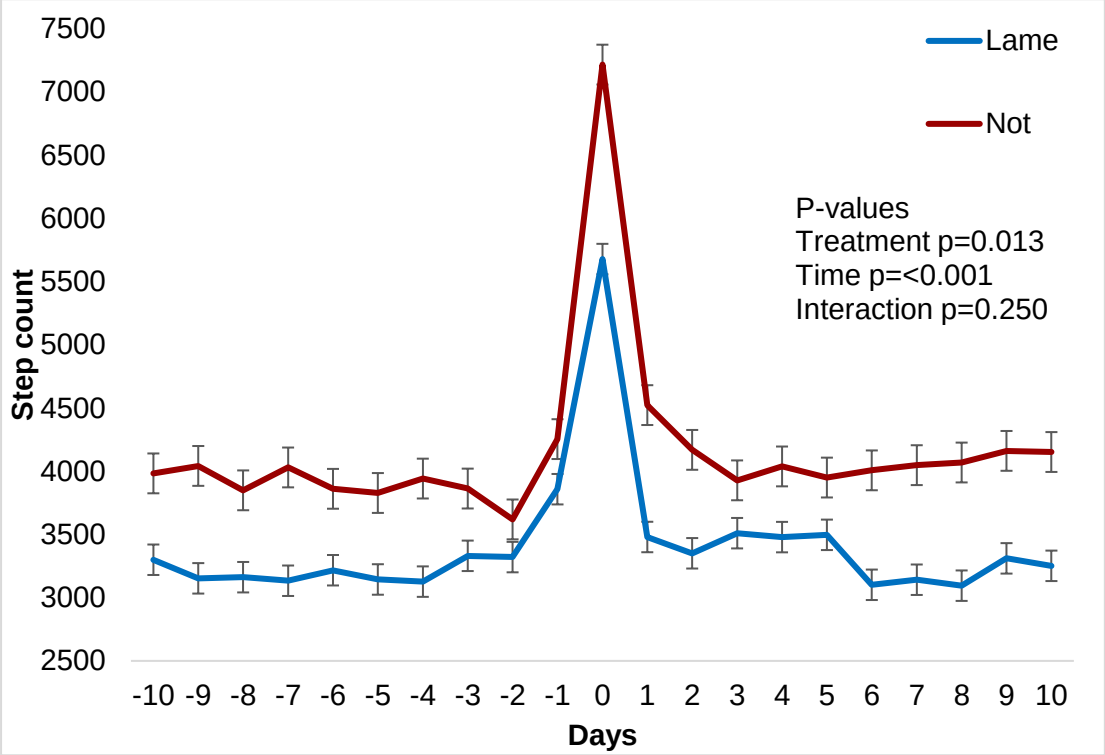

Figure 6- 1 Step count for pastured lame and non-lame dairy cattle before, during, and after oestrus

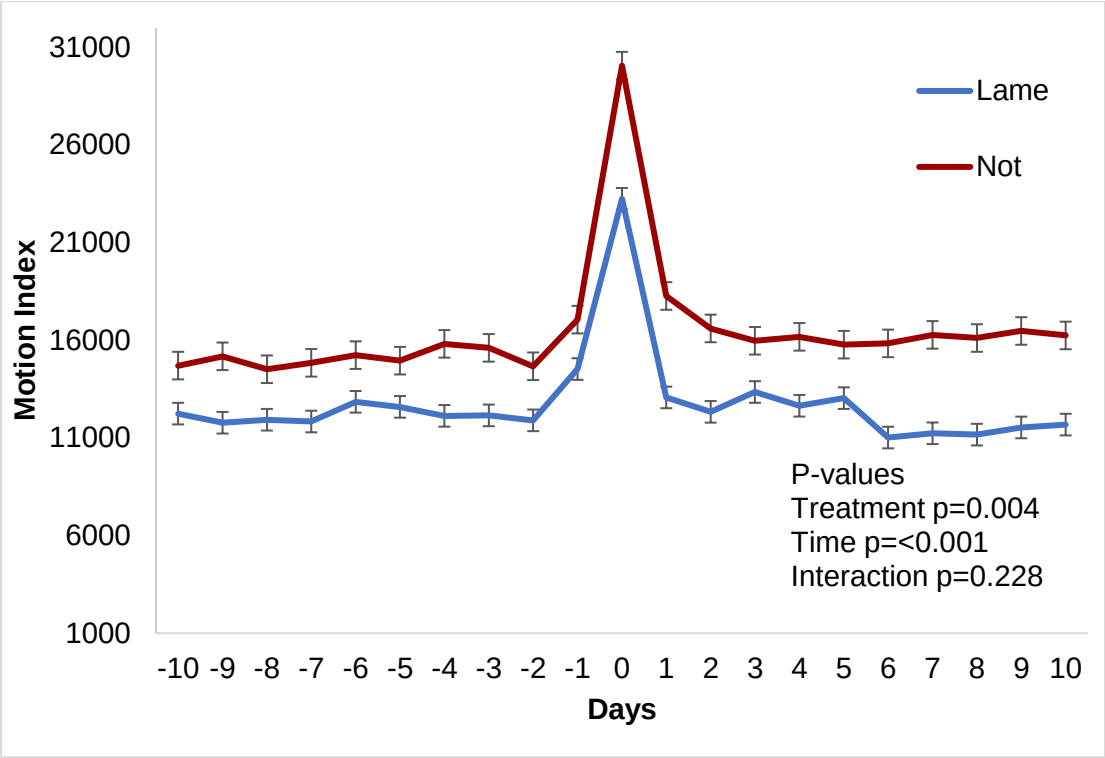

Figure 6- 2 Motion index for pastured lame and non-lame dairy cattle before, during, and after oestrus

There was no significant difference in standing times (Figure 6-3) between lame and non-lame dairy cattle with access to pasture. There was an effect of time ($p<0.001$), with a sharp increase in standing times on the day of oestrus followed by resumption of baseline activity.

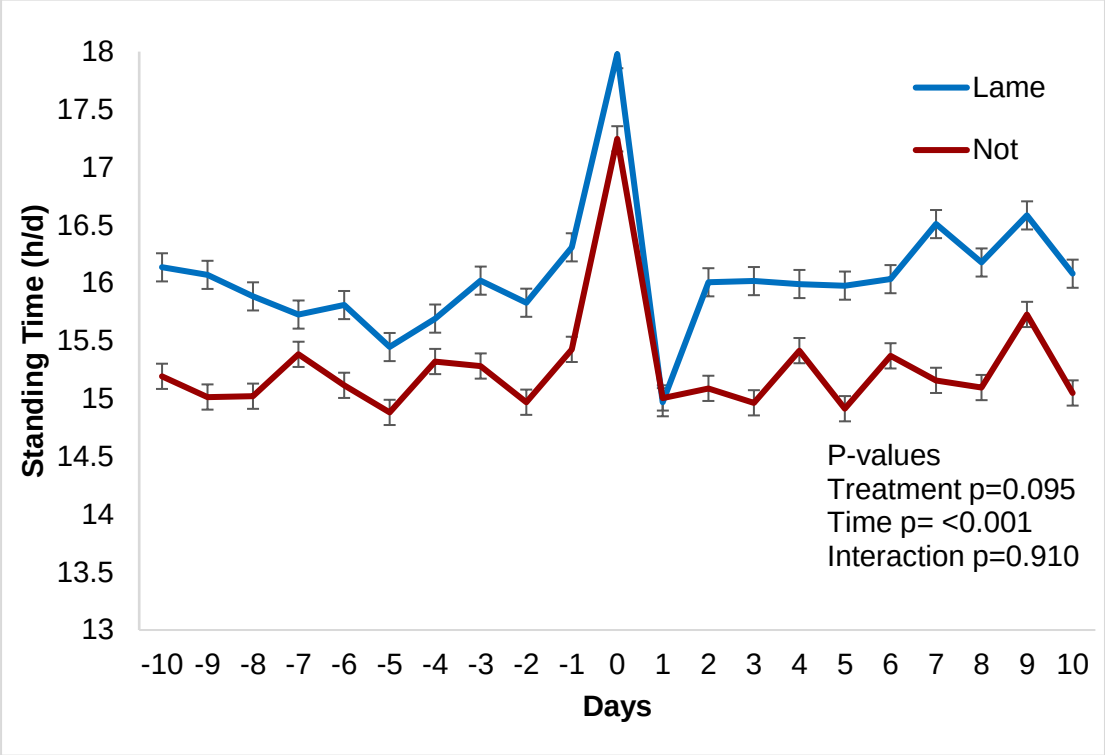

Figure 6- 3 Standing time (h/d) for pastured lame and non-lame dairy cattle before, during and after oestrus

There was no significant difference in lying times (Figure 6-4) between lame and non-lame dairy cattle with access to pasture. There was an effect of time ($p<0.001$), with a sharp decrease in lying times on the day of oestrus followed by resumption lying times.

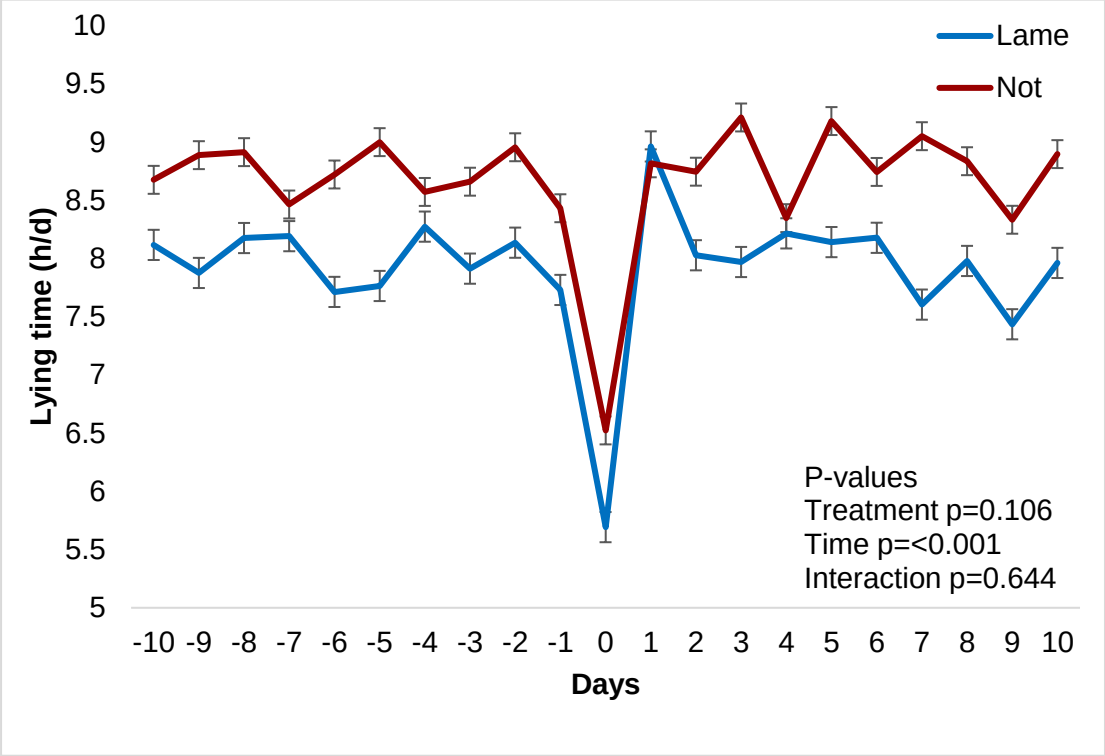

Figure 6- 4 Lying time (h/d) for pastured lame and non-lame dairy cattle before, during and after oestrus

Lame cows had significantly fewer lying bouts when compared to non-lame cows (p=0.05) (Figure 6-5).

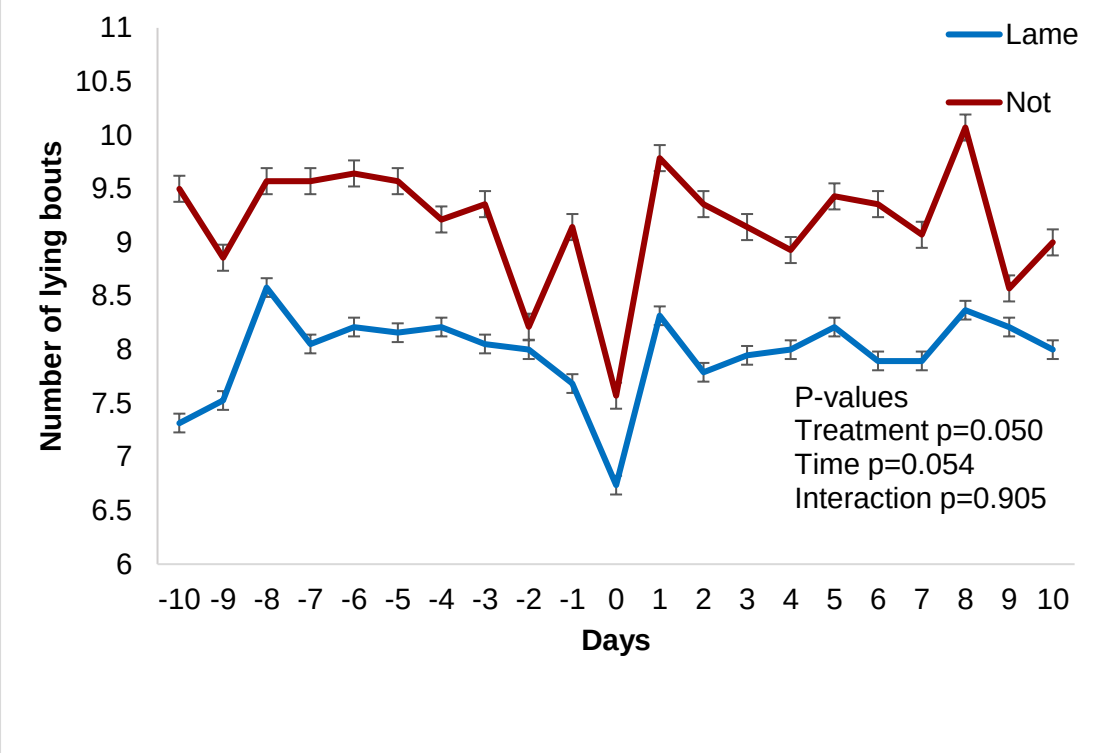

Figure 6- 5 Number of lying bouts for pastured lame and non-lame dairy cattle before, during, and after oestrus

Mean lying bout lengths were not significantly different (Figure 6-6). However, two days prior to oestrus non-lame cows increased lying bout length. Whereas a day after standing oestrus lame cows increased their bout length, and non-lame cows bout length decreased.

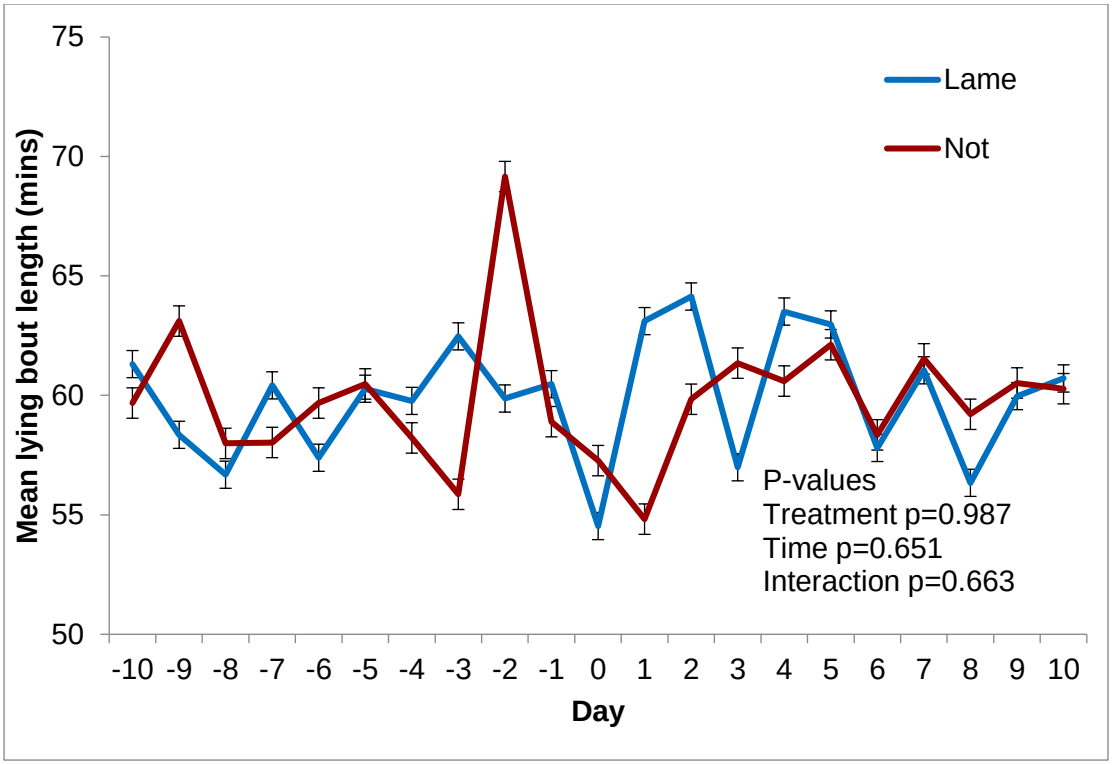

Figure 6- 6 Lying bout length for pastured lame and non-lame dairy cattle before, during, and after oestrus

6.6.2 Housed cows

There was a significant difference in step counts (p=0.027) (Figure 6-7), with an effect of time (p>0.001) before, during and after oestrus in housed lame and non-lame dairy cattle. Time effect demonstrates a sharp increase in activity (steps) on the day of oestrus followed by resumption of baseline activity.

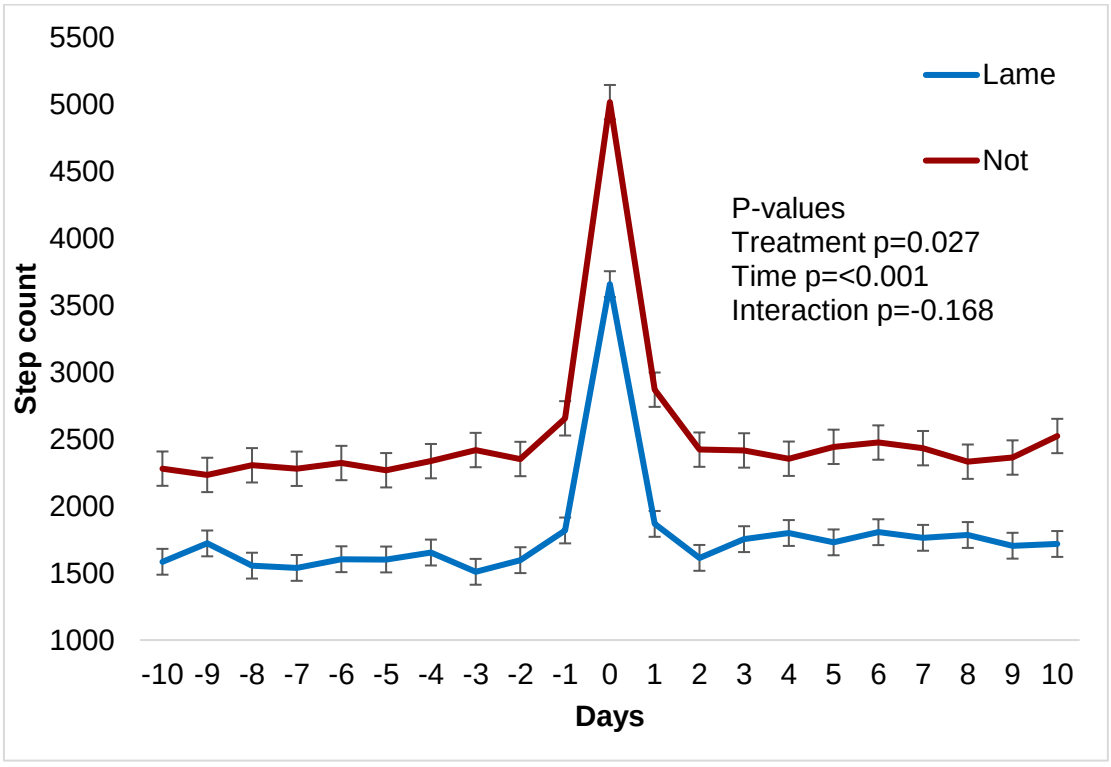

Figure 6- 7 Step count for housed lame and non-lame dairy cattle before, during, and after oestrus

There was a significant difference in motion index (p=0.033) (Figure 6-8), with an effect of time (p>0.001) before, during and after oestrus in housed lame and non-lame dairy cattle. Time effect demonstrates a sharp increase in activity (motion index) on the day of oestrus followed by resumption of baseline activity.

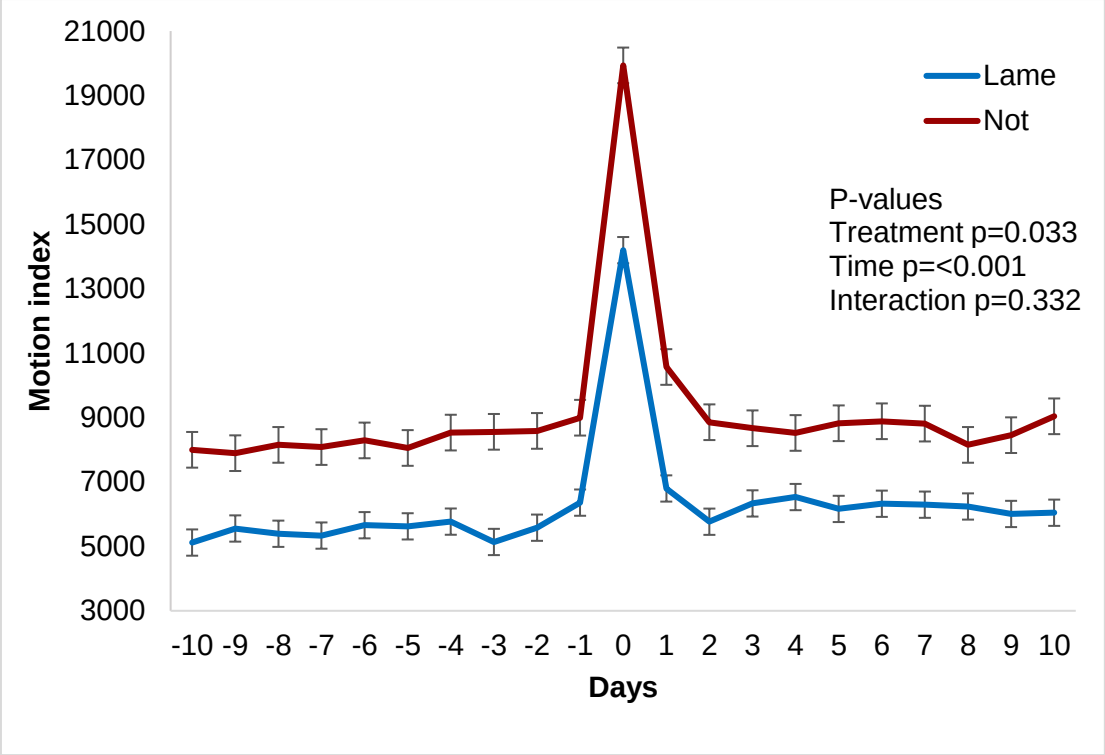

Figure 6- 8 Motion index for housed for lame and non-lame dairy cattle before, during, and after oestrus

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There was no significant difference in standing times (Figure 6-9) between housed lame and non-lame dairy cattle, there was an effect of time (p>0.001). Time effect demonstrates a sharp increase in standing times on the day of oestrus followed by resumption of baseline activity.

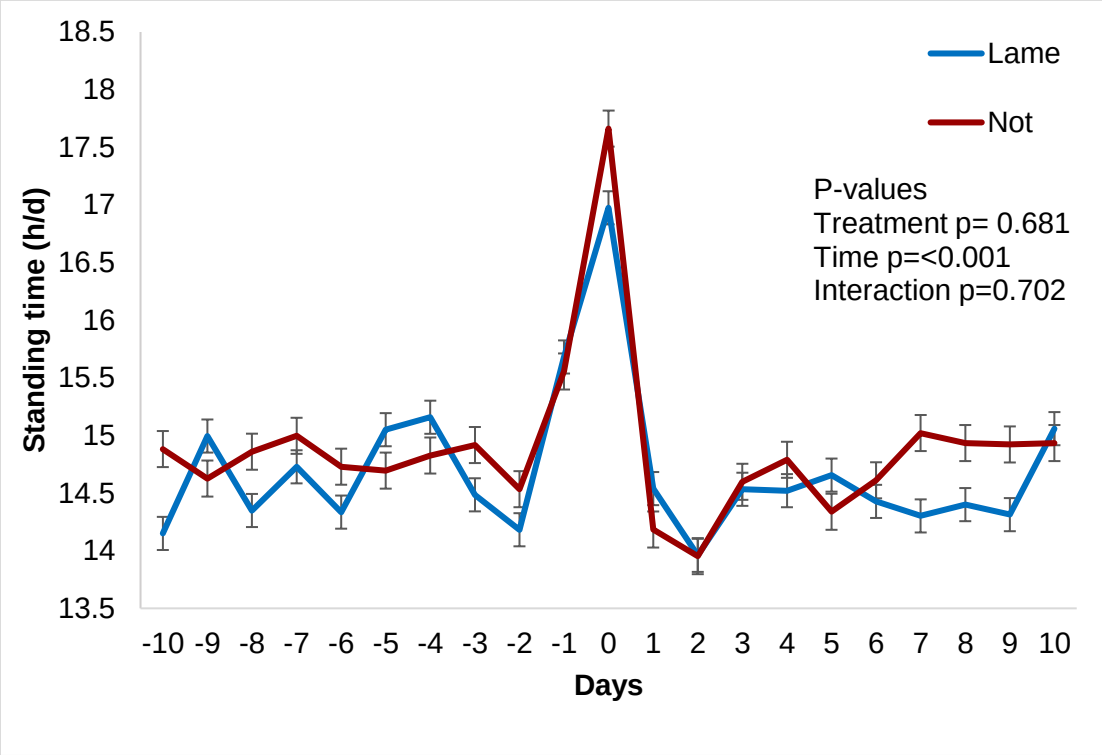

Figure 6- 9 Standing time (h/d) for housed lame and non-lame dairy cattle before, during, and after oestrus

-

There was no significant difference in lying times (Figure 6-10) between housed lame and non-lame dairy cattle, there was an effect of time (p>0.001). Time effect demonstrates a sharp decrease in lying times on the day of oestrus followed by resumption of baseline activity.

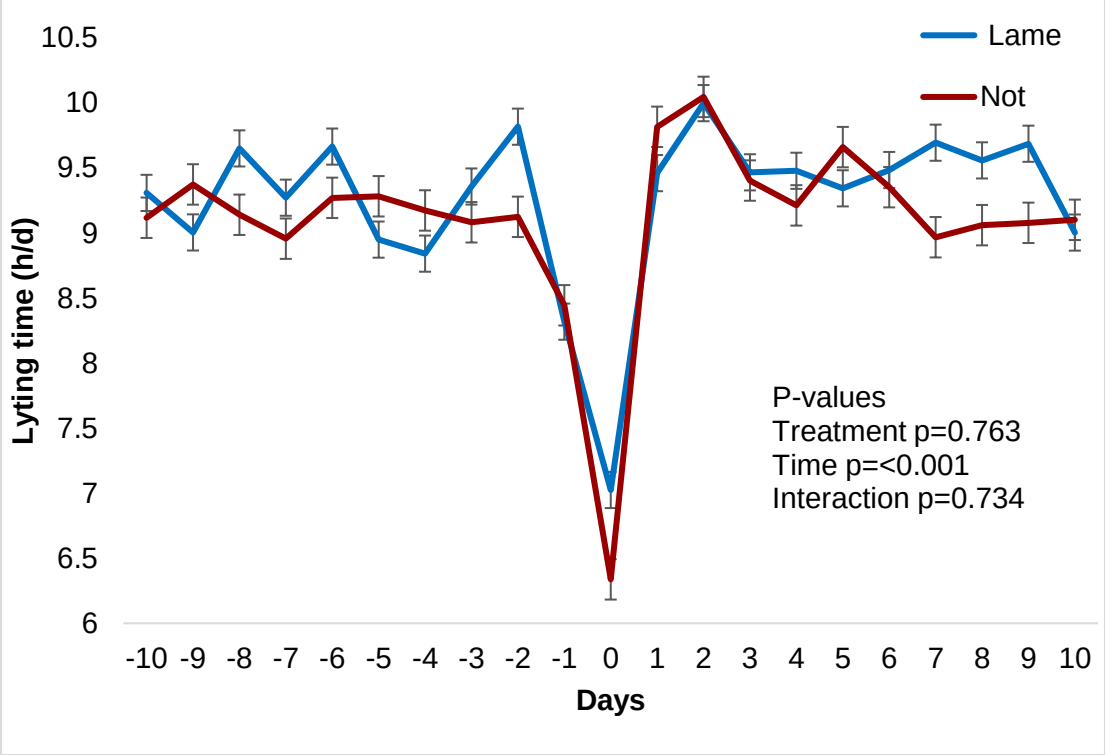

Figure 6- 10 Lying time (h/d) for housed lame and non-lame dairy cattle before, during, and after oestrus

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Housed lame cows had significantly fewer lying bouts (p=0.014) (Figure 6 11) when compared to non-lame cows.

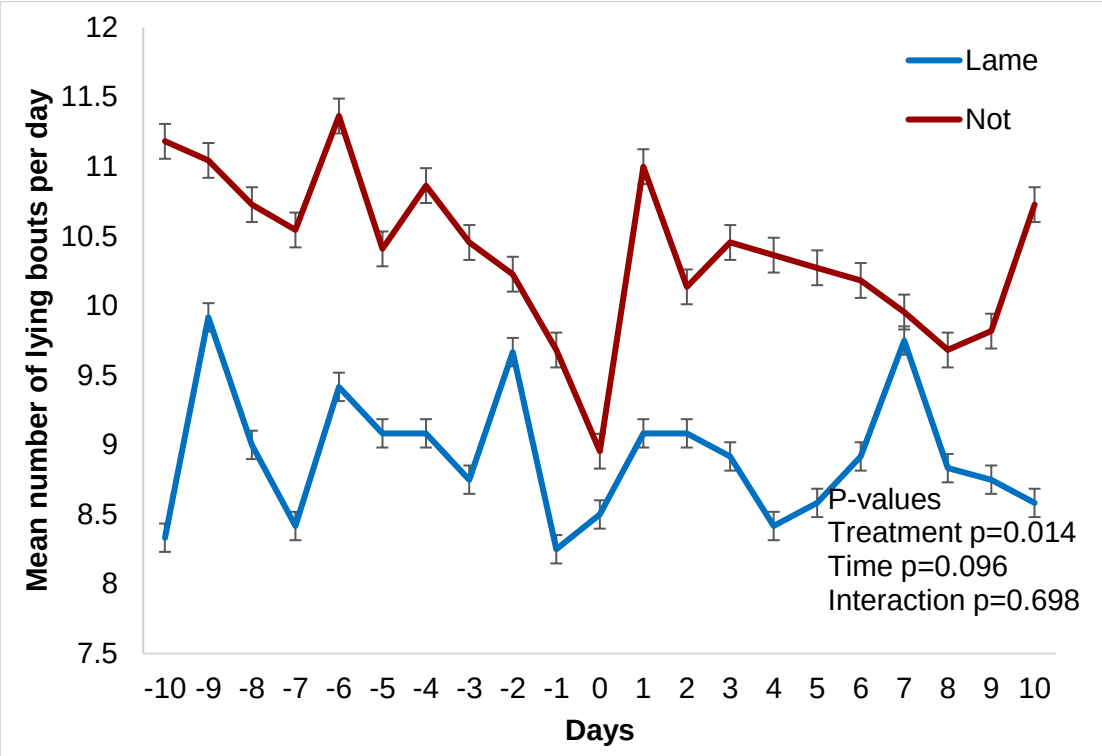

Figure 6- 11 Mean number of lying bouts for housed lame and non-lame dairy cattle before, during, and after oestrus

Mean lying bout lengths were significantly different between housed lame and non-lame cows (p=0.012) (Figure 6-12). Following oestrus, housed lame cows increased their lying bout length (Table 6-6), but not number of lying bouts (Table 6-7) when compared to non-lame cows.

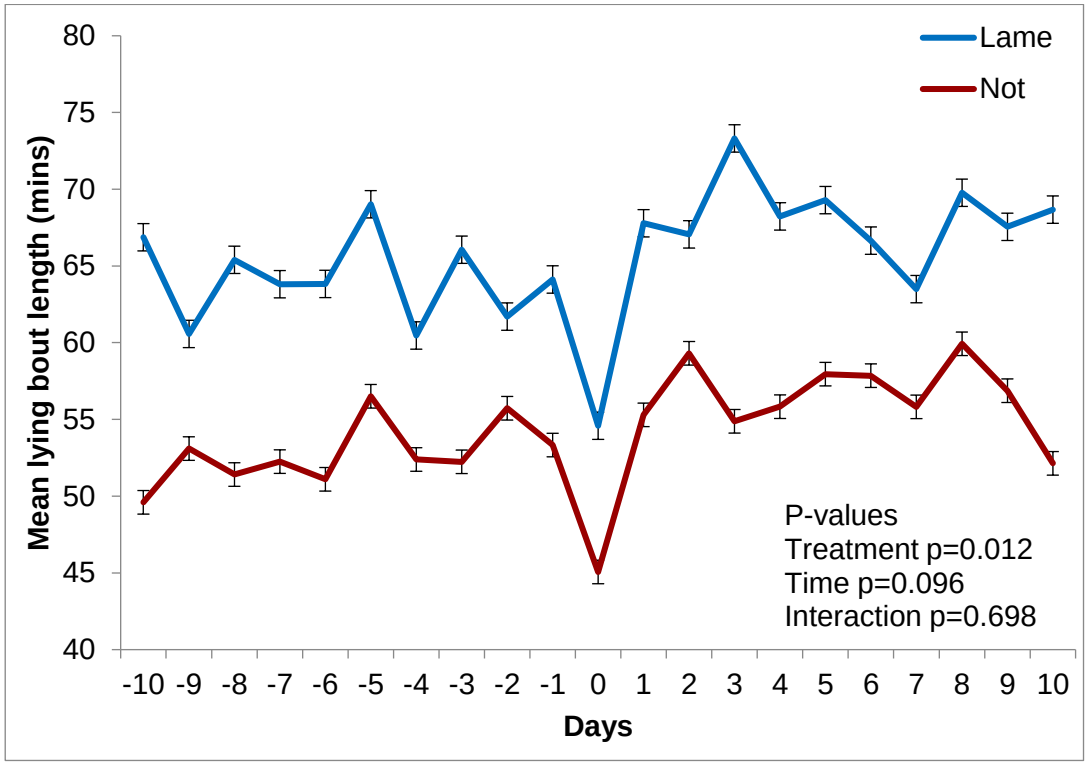

Figure 6- 12 Mean lying bout length for housed lame and non-lame dairy cattle before, during, and after oestrus

Table 6- 6 Mean lying bout length (minutes) for lame and non-lame housed cows two days before, the day of, and two days after oestrus

Mean lying bout length (mins)			
Day	Lame	Non-lame	p value
-2	61.7 (±4.5)	55.7 (±2.5)	0.214
-1	64.1 (±7.0)	53.3 (±2.0)	0.161
0	54.6 (±4.3)	45.1 (±3.0)	0.076
1	67.8 (±5.0)	55.3 (±3.3)	0.037
2	67.1 (±6.9)	59.3 (±1.7)	0.295

Table 6- 7 Number of lying bouts for lame and non-lame housed cows two days before, the day of, and two days after oestrus

	Number of lying bouts		
Day	Lame	Non-lame	p value
-2	9.7 (±0.7)	10.2 (±0.6)	0.573
-1	8.3 (±0.6)	9.7 (±0.6)	0.123
0	8.5 (±1.2)	9 (±0.7)	0.717
1	9.1 (±0.7)	11 (±0.5)	0.028
2	9.1 (±0.8)	10.1 (±0.3)	0.251

6.6.3 Lameness and housing effects on activity

Overall lameness caused significantly lower mean step counts in lame cows ($F_{1,63}$ P=0.047) including the day of oestrus ($F_{1,63}$ P<0.001). Housed cows had significantly lower mean step counts, including the day of oestrus ($F_{1,63}$ P<0.001). There was no significant interaction of lameness and housing ($F_{1,63}$ P>0.05) (Table 6-8).

Overall lameness caused a significantly lower mean motion index in lame cows ($F_{1,63}$ P=0.047), however the day of oestrus was not affected. Housed cows had a significantly lower mean motion index, including the day of oestrus ($F_{1,63}$ P<0.001). There was no significant interaction of lameness and housing (Table 6-8).

Overall lameness did not affect mean standing times, or mean standing times on the day of oestrus. Housed cows had significantly lower mean standing times ($F_{1,63}$ P=0.010), mean standing times the day of oestrus was not affected. There was no significant interaction of lameness and housing (Table 6-8).

Overall lameness did not affect mean lying times, or mean lying times on the day of oestrus. Housed cows had significantly higher mean lying times ($F_{1,63}$ P=0.007), mean lying times on the day of oestrus was not affected. There was no significant interaction of lameness and housing (Table 6-8).

Overall lameness caused fewer mean lying bouts in lame cows ($F_{1,63}$ P<0.001), however the day of oestrus was not affected. Housed cows had significantly more mean lying bouts ($F_{1,63}$ P=0.010), as well as more mean lying bouts on the day of oestrus ($F_{1,63}$ P=0.045). There was no significant interaction of lameness and housing (Table 6-8).

Overall lameness caused longer mean lying bout lengths in lame cows ($F_{1,63}$ P=0.014), however the day of oestrus was not affected. Housing did not affect mean lying bout lengths, or mean lying bout lengths on the day of oestrus. There was a significant interaction of lameness and housing on mean lying bout lengths ($F_{1,63,}$ P=0.017), the day of oestrus was not affected (Table 6-8).

Overall lameness did not affect mean P^4 concentrations, or mean P^4 concentrations on the day of oestrus. Housing did not affect mean P^4 concentrations. Housed cows had significantly lower P^4 concentrations on the

day of oestrus when compared to pastured cows. There was no significant interaction of lameness and housing on mean P^4 concentrations (Table 6-8).

Table 6- 8 Mean activity parameters not in oestrus, and during oestrus (day 0) for lame and non-lame dairy cattle in different housing conditions

	Housed		Pasture				
Mean activity parameters	Lame	Non-lame	Lame	Non-lame	Lameness SED	Housing SED	LxH SED
Mean steps	1781	2530	3412	4168	216.6*	215.9**	305.5
Steps day 0	3659	5020	5678	7216	492.6**	491**	694.7
Mean motion index	6296	9136	12776	16496	875*	872.1**	1234
Motion index day 0	14197	19934	23243	30060	2234	2227**	3151
Mean standing time (h)	14.7	14.8	16.1	15.3	0.316	0.315**	0.446
Standing time day 0	17	17.7	18	17.3	0.51	0.509	0.719
Mean lying time (h)	9.3	9.1	7.9	8.7	0.309	0.308**	0.436
Lying time (h) day 0	7	6.4	5.7	6.5	0.496	0.494	0.7
Mean lying bouts	8.9	10.4	8	9.2	0.412**	0.411**	0.581
Lying bouts day 0	8.5	9	6.7	7.6	0.765	0.763*	1.08
Mean lying bout length	65.6	54.2	59.9	59.9	2.31**	2.3	3.26*
Lying bout length day 0	54.6	45.1	54.3	57.4	3.54	3.53	4.98
Mean P4	1.3	1.5	1.4	1.9	0.217	0.216	0.307
P4 day 0	0.281	0.351	0.471	0.487	0.067	0.067*	0.095

*Indicates significance at P<0.05; ** indicates significance at P<0.01

6.6.4 Duration of oestrus, increase in activity (percentage), and time of day oestrus expressed

Overall lameness did not affect the duration of oestrus, or the percentage of activity increase based on step counts, and motion index (Table 6-9). Housing did not affect the duration of oestrus, however housed cows a greater percentage increase in activity than cows at pasture (steps; $F_{1,63,}$ P=<0.001; Motion index: $F_{1,63,}$ P<0.001). There was no significant interaction of lameness and housing on the duration of oestrus, percentage increase in activity steps, or the percentage of activity increase motion index. Comparisons between the percentage increase of step counts and motion index revealed a significant difference (paired t-test:t_{66}=-4.40, p<0.001).

Table 6- 9 Duration of oestrus and percentage increase in step counts and motion index on the day of oestrus

	Housed		Pasture		Lameness SED	Housing SED	LamenessX Housing SED
	Lame	Not	Lame	Not			
Duration (h) of activity increase	8.3 (±0.5)	10.2 (±0.6)	8.5 (±0.4)	9.3 (±0.6)	0.419	0.086	0.073
Activity (steps) increase at oestrus (%)	652 (±54.1)	647 (±40.0)	436 (±43.0)	486 (±50.1)	0.622	<0.001	0.562
Activity (Motion index) increase at oestrus (%)	831 (±79.0)	727 (±58.3)	504 (±62.7)	525 (±73.1)	0.558	<0.001	0.372

The time of day oestrus was displayed between lame and non-lame cows and in different housing conditions is shown in Table 6-10. There was no association between lameness and the time of day oestrus was displayed. There was no association between the type of housing and the time oestrus was displayed (Table 6-10).

Table 6- 10 Time of day oestrus expressed from lame and non-lame dairy cattle in different housing conditions (pastured/housed)

	Early, n, (%)	Mid, n, (%)	Late, n, (%)	Total, n	*P*-Value (χ^2)
Non-lame	16 (44)	7 (20)	13 (36)	36	0.15
Lame	7 (23)	10 (32)	14 (45)	31	
Pastured	11 (34)	7 (22)	15 (44)	33	0.64
Housed	12 (35.3)	10 (29.4)	12 (35.3)	34	

6.7 Progesterone analysis

6.7.1 Housed cows

There was no significant difference in P^4 concentrations between housed lame and non-lame cows however there was an effect of time. Time effect demonstrates a decline in progesterone concentration leading up to oestrus (day 0), followed by a gradual increase in P^4 concentrations after day 0 ($p<0.001$) (Figure 6-13). Mean values for all of the above parameters are listed in Figure 6-13.

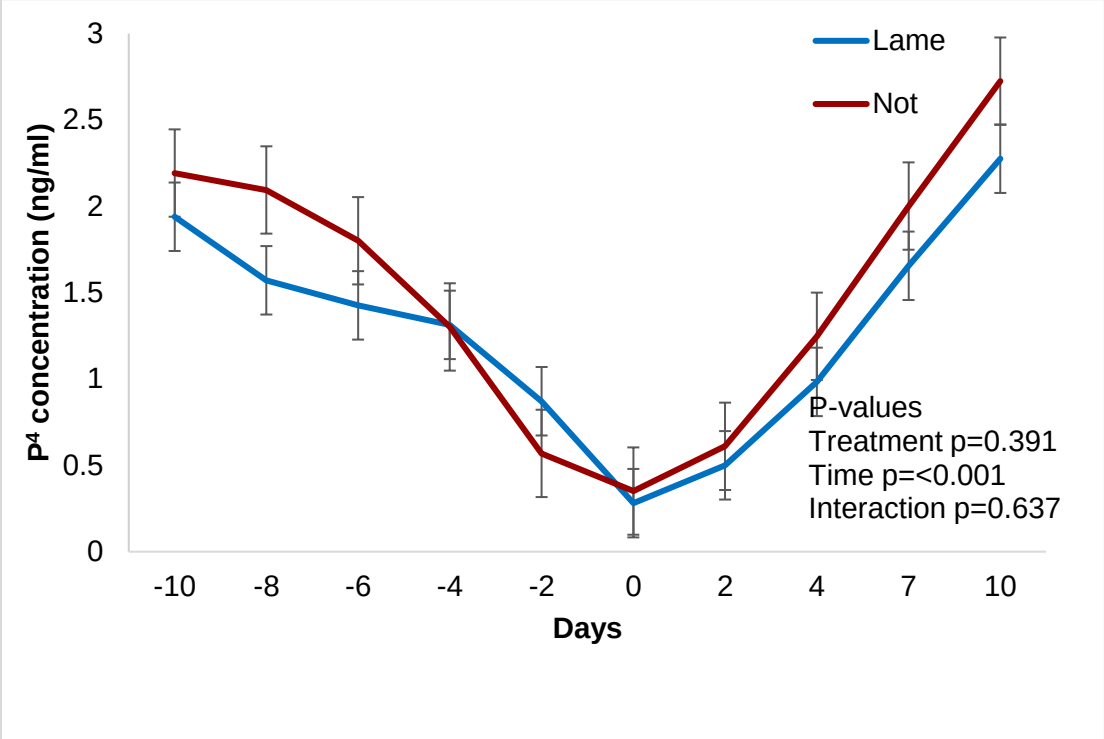

Figure 6- 13 P^4 concentration (ng/ml) for housed lame and non-lame dairy cattle before, during, and after oestrus

6.7.2 Pastured cows

Overall there was no significant difference in P^4 values between lame and non-lame cows, there was an effect of time ($p<0.001$) (Figure 6-14). Time effect demonstrates a decline in progesterone concentration leading up to oestrus (day 0), followed by a gradual increase in P^4 concentrations after day 0. Seven-days and 10-days post oestrus lame cows had significantly lower P^4 values than non-lame cows ($p<0.05$). Mean values for all of the above parameters above are listed in Figure 6-14.

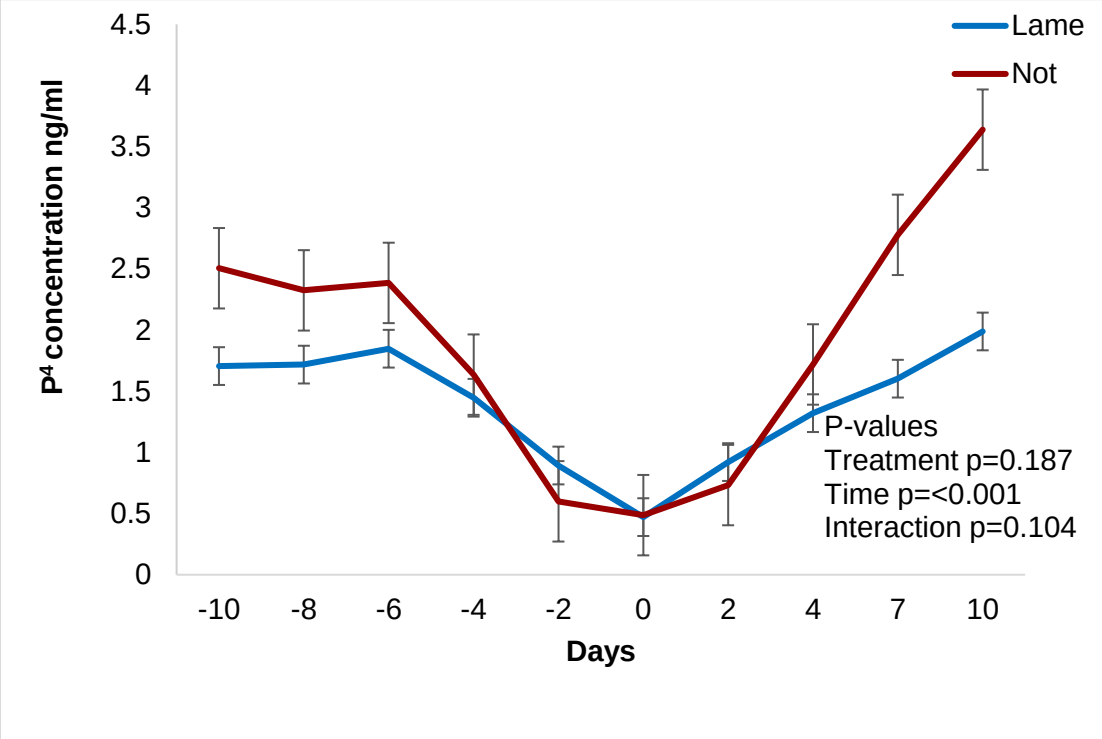

Figure 6- 14 P^4 concentration (ng/ml) for pastured lame and non-lame dairy cattle before, during, and after oestrus

6.7.3 Lame vs non-lame

Overall there was no significant difference in P^4 values between lame and non-lame cows, there was an effect of time ($p<0.001$) (Figure 6-15). Time effect demonstrates a decline in progesterone concentration leading up to oestrus (day 0), followed by a gradual increase in P^4 concentrations after day 0. Seven-days and 10-days post oestrus lame cows had significantly lower P^4 values ($p<0.05$ and $p<0.001$ respectively). Mean values for all of the above parameters above are listed in Figure 6-15.

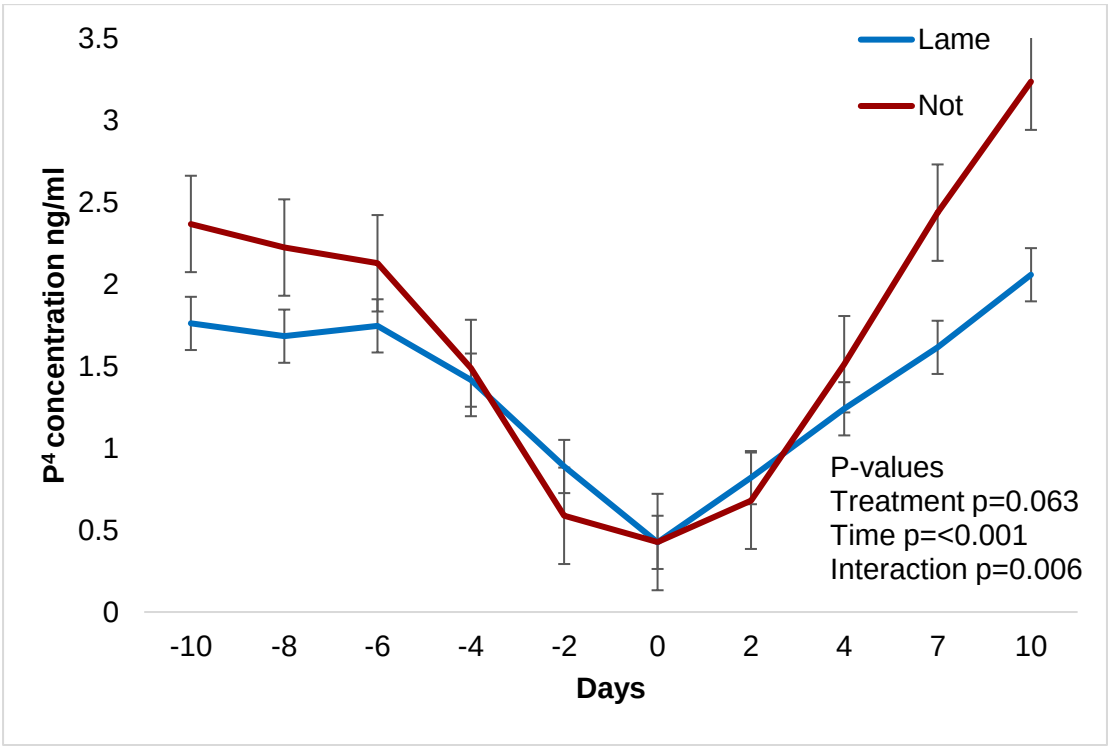

Figure 6- 15 P^4 concentration (ng/ml) for all lame and non-lame dairy cattle before, during, and after oestrus

6.8 Discussion

6.8.1 Mount Detector frequency: All cows (lame and non-lame)

6.8.1.1 Kamar®

On the day of oestrus 36.1% of Kamar® mount detectors were correctly activated in non-lame cows, whereas 45% (incl. 9.7% partial) were correctly activated in the lame cow group. Typically, lame cows reduce standing behaviour (Walker *et al*., 2008a), so these results are unexpected. However, modern cows are reducing oestrus expression (Dobson *et al.,* 2008). Despite the non-lame cows not having mobility issues, other factors such as body condition and milk production could be reducing oestrus expression. Johnson *et al.* (2012) reported that 88.4% (38/43) of the first ovulations postpartum were not accompanied with behavioural signs of oestrus. A significant risk factor for silent ovulations at the second, third, and/or fourth ovulations postpartum has been linked to high milk production (Ranasinghe *et al.,* 2010). Typically, high milk producing postpartum dairy cows are in a negative energy balance throughout early lactation, which can reduce oestradiol production in the preovulatory follicle, thus reducing the sensitivity of the hypothalamus to oestradiol, subsequently resulting in an increased incidence of silent ovulations (Isobe *et al.,* 2004; Ranasinghe *et al.,* 2010). As oestrogen is responsible for initiating the behavioural expression of oestrus, a reduction in the sensitivity/concentration of this hormone may result in reduced oestrus behaviour. A study by Lopez *et al.* (2004a) reported that cows producing more than 39.5 kg of milk per day had reduced serum oestradiol concentration on the day of oestrus, and that the expression of oestrus was greatly reduced when compared to lower yielding cows (>39.5 kg/d). It should be noted that in

high yielding cows, metabolic clearance of steroid hormones is elevated with increased feed intake (Sangsritavong *et al.,* 2002). Therefore, both the concentration of oestradiol, and increased metabolic clearance of oestrogen associated with high producing cows may account for decreased oestrus expression. It could be a possibility that the reduced percent of correctly activated Kamar®'s in non-lame cows is attributed to cow factors such a milk yield. However daily milk production from each cow was not recorded, therefore more research is required to determine if this was the cause.

It should be re-mentioned that partial activation was considered as 'activated', as suggested by the manufacturer (Kamar®, 2017). Although the lame cow group has a slightly higher percentage of correctly activated Kamar®'s, 9.7% were partially activated. This may be indicative that the cow moved away from mounting attempts, or that secondary behaviours (chin resting) caused activation. These devices require a mount to last a minimum of 3 seconds in order to fully burst the chamber, thus releasing all of the red ink. Therefore, the partial release of ink could be caused by failed mounting attempts (<3 seconds), or insufficient pressure received from chin rests from herdmates. Cows with foot problems are more reluctant to express oestrus behaviours such as mounting (Boyle *et al.,* 2007). Walker *et al.* (2008a) reported that lame cows were just as restless as non-lame cows during oestrus, however the reduction in oestrus intensity is not the result from impaired physical movement, but that lame cows dedicate a smaller proportion of their daily activities to expressing oestrus behaviour.

The oestrus detection rate for Kamar® detectors varies from 50 to 85% (Ball and Peters, 2004). A study by Holman (2011) identified Kamar® detectors gave a higher incidence of false positives, thus causing misinterpretation of mounting behaviour. Another study reported a rate of false positives of 26% (AHDB Dairy, 2017b). On the day of oestrus, the percentage of Kamar® detectors that were missing was 55.6% for non-lame cows, and 32.3% for lame cows. This can suggest that mounting behaviour or secondary oestrus behaviours caused the detector removal, as the oestrus event was verified with milk progesterone.

During the initial part of the study numerous Kamar® mount detectors were dislodged shortly after being applied (13 times). A study by Gwazdauskak *et al*. (1990) reported that the loss of rump mounted detectors (including Kamar®) exceeded 40%. The detectors in this study may have be falsely triggered, or removed due to the surrounding environment. Factors such as stall bars, other cows, cow brushes, (Borsberry, 2011), trees, and bushes etc. may cause the detectors to become dislodged. Detectors can be removed by other cows through curiosity. Cows have been observed to lick, and sniff detectors applied to their herd mates, thus increasing the risk of false positives and/or removal of the detectors all-together. Prior to this study the cows never had mount detectors applied before (apart from chalk), therefore the novelty of such devices may have been the cause for the detectors falling off soon after application. During this study the researcher (AW) observed an incident where a cow was licking a Kamar® detector shortly after application. False positives may also occur if a cow is trapped in a stall when being mounted by a cow that is in oestrus. The cow cannot escape the mount attempt, and therefore the

detector may indicate that she is standing to be mounted, when she is not. This behaviour was observed twice during the study via CCTV (Plate 6-3).

Plate 6 3 Cow in oestrus mounting another cow trapped in a stall

Other potential reasons for the device being triggered falsely may be overcrowding in the collecting yard causing chin resting (this was observed on farm). Dislodged detectors may be a result of human error, not correctly applying the right amount of adhesive to the cow or the adhesive glue provided was not stored properly. The detectors were shipped though the post, therefore it is unknown how the kits were stored prior to arrival. However, upon inspection it had been noted that the detectors were completely removed including underlying hair. This indicates that the glue was not faulty. Repeated mounting, increased secondary oestrus behaviours (licking and chin resting) or environmental factors (trees or cubicle bars) could have caused the removal of the detectors. Interestingly after new adhesive was purchased, the number of

dislodged detectors was greatly reduced. However, a use by date for the adhesive was not present on any of the packaging.

Lame cows had an increased percentage of Kamar® detectors that were not activated on the day of heat when compared to non-lame cows (23% vs. 8.3%). This could indicate that the lame cows did not stand to be mounted unlike their non-lame counter parts. This may also be due to a shortened duration of standing oestrus; therefore, lame cows would not receive as many mounts from herd mates. Lame cows may be less “attractive” to herdmates; they may emit a lesser quantity/quality of sexual pheromones (acetic and propionic acids, and 1-iodoundecane (Sankar and Archunan, 2008)) or even stress-related pheromones (Walker *et al*., 2008a). It has been documented that cattle perceive an increased state of stress (from urine from stressed cows) in herdmates by olfactory cues (Boissy *et al.,* 1998). Little is known about which components in urine are responsible for the observed effects (Boissy *et al.,* 1998). Therefore, lame cows may not be as sexually attractive to other cows (Walker *et al.,* 2008a), thereby not attracting attention to be mounted.

6.8.1.2 Estrotect™ scratch cards

On the day of oestrus 58% of Estrotect™ scratch cards were correctly activated in non-lame cows, whereas 52% (incl. 19.4% partial) were correctly activated in the lame cow group. The reported accuracy of Estrotect™ scratch cards varies widely in the literature. For example, the results from this study are considerably lower than those reported by Perry (2005), who reported Estrotect™ scratch cards correctly identified 91% of oestrus events (synchronised beef cows). However, the results from this study are higher than

those reported by van den Berg (2014), who reported that 25% of Estrotect™ scratch cards were correctly activated. Another study by Holman *et al.* (2011) reported that 36% of Estrotect™ scratch cards were correctly activated on the day of oestrus. On the day of oestrus, the percentage of Estrotect™ scratch cards that were missing was 36% for non-lame cows, and 29% for lame cows. Missing stickers could be due to repeated mounting, or increased secondary oestrus behaviours such as licking and chin resting. Both groups of cows had inactivated stickers on the day of heat (19% lame, 6% non-lame).

Successful oestrus detection using these devices requires proper placement (Youngquist and Threlfall, 2007). Although it was observed the mount detectors did not interfere with one another, placement of the mount detectors could be a potential source of error. However, this was minimised in the study in this thesis through the same individual (AW) applying the adhesive mount detectors.

6.8.1.3 Chalk

Tail chalk had the highest percent of correct activation on the day of heat. Chalk was correctly removed in 92% (incl. 8.3% partial) of non-lame cows on the day of heat, whereas lame cows had 90% (incl. 25.8% partial). Oestrus detection rates using tail chalk vary. For example, Fulkerson *et al*. (1983) reported an oestrus detection rate of 66%, and Palmer *et al*. (2010) reported a range of 26 to 65%, which are both lower than the present study. The use of chalk also has limitations due to false positives. Daily social interactions (licking, rubbing behaviour) can lead to the chalk being removed thereby resulting in a false positive (Skenandore and Cardoso, 2017). There was a higher incidence of

partially removed chalk in lame cows (25.8%) than non-lame cows (8.3%). As with the mount detectors, chalk may have not been fully removed due to the cows not having full mounts, but attempted mounts, or licking/chin resting behaviour. It has been reported that heifers with large follicles receive rump licks, chin rests and anogenital sniffs (Skenandore and Cardoso, 2017). It has been reported that the paint/chalk may not always be removed during mounting activity (Firk *et al.,* 2002). Therefore, the cows may have received mounts, however the chalk was only partially removed. Without continual observation, it is not known which oestrus behaviours caused the partial activation in this study.

6.8.2 Activity monitors: All cows (lame and non-lame)

In this study the IceQube® activity monitors correctly identified standing oestrus 100% of the time in lame and non-lame cows. Whereas the NeDap activity monitors correctly identified standing oestrus 100% of the time in non-lame cows, and 96.8% of lame cows. The lame cow from this study that was not identified in oestrus by the NeDap activity monitor underwent further investigations. It was determined through veterinary examination and P^4 analysis that the cow had a silent oestrus. It should be noted that this cow did receive a 'suspicious' alarm by the NeDap activity system on the day of oestrus. This indicates the system did recognise a subtle increase in activity, however the increase was not longer than 2 hours, which is what is required for an 'attention' alarm to be sent. Studies have reported a range in oestrus detection accuracy from activity monitors. Fricke *et al.* (2014) reported that 70% of cows were identified in oestrus using an activity monitor, whereas McGowan *et al*. (2007) reported that 93% of cows were identified with a 20% false positive rate.

6.8.3 Pastured cows: Lame and non-lame

Separating the cow groups based on the different housing conditions, lame pastured cows had 63% (incl. 15.8% partial) of Kamar®'s (11% not activated, 26% missing), 68% (incl. 26.3% partial) of Estrotect™ scratch cards (11% not activated, 21% missing), 89% (incl. 21.1% partial) of chalk (11% not activated), 94.7% NeDap activity monitors and 100% of IceQube®'s correctly indicate standing heat.

Non-lame pastured cows had 50% of Kamar®'s (42.9 missing, and 7.1% not activated), 57% (incl. 5.7% partial) of Estrotect™ scratch cards (14% not activated, 29% missing), 93% (incl. 14.3 partial) of chalk (7% not activated), 100% NeDap activity monitors and 100% IceQube®'s correctly indicate standing heat.

Based on the overall findings Kamar® mount detectors, and Estrotect™ scratch cards were less efficient at identifying standing oestrus in both groups of cows compared to the activity monitors or chalk. As oestrus was confirmed through P^4 analysis, it could be that partial activation or removal of the detectors was due to primary or secondary oestrus behaviours.

The lame cows that did not have any mount detectors activated may not have expressed oestrus overtly, thus they went undetected by herd-mates. It is possible that the standing to be mounted period was either shortened (receiving fewer mounts from herdmates), or that the cows did not stand to be mounted

without moving. The activity monitors did indicate oestrus, however there was no mounting activity documented. This may suggest they were in too much discomfort to stand to be mounted (Boyle *et al.,* 2007), insufficient hormones were present to initiate oestrus expression (Walker *et al.,* 2008a) or that the herdmates were not attracted to her scent.

Lame cows do not receive as many mounts as their non-lame counterparts (Walker *et al.,* 2010). Although repeated mounting can cause the detectors to become dislodged, it can also be suggested that the secondary behaviours such as licking and head resting may cause the detectors to become removed. As lame cows have been reported to avoid mounting activity, this may be why a higher percentage of Kamar®'s were not activated, and a large percentage missing potentially due to secondary behaviours. It is also possible that environmental factors such as trees may have removed the detectors, however as the heats were correctly identified through P^4 analysis it is likely the detectors were disturbed through oestrus behaviours. Without continual observation, it cannot be explicitly determined if the detectors were removed due to repeated mounting, or from increased secondary behaviours received from herd-mates.

IceQube activity monitors correctly identified all cows, NeDap identified all but one. Overall lame cows on a pasture-based system did not have a significant difference in oestrus detection efficiency when using the mount detecting aids when compared to non-lame cows. Tail chalk had high rates of accurately determining oestrus when partial removal was included. Using this product is

inexpensive, but requires time to apply and check the chalk for removal. As determined in Chapter 4, a large proportion of producers consider cost and ease as important factors when choosing an oestrus detection method. Chalk is cost effective; however, 'ease' is subjective. If large herds use tail chalk it might not be time efficient for the producer to use chalk, unless an outside reproduction management company is used. If chalk is used, cows with partial removal should be observed closely as they may be in oestrus despite minimal chalk removal. However as lame cows receive fewer mounts (Walker *et al*., 2010), it might be more suitable to use an activity-based oestrus detection method. Activity monitors had higher efficiency at detecting oestrus in all cows, and therefore would be a more suitable product for correctly identifying oestrus in both lame and non-lame cows.

6.8.4 Housed cows: Lame and non-lame

Housed lame cows had 25% of Kamar®'s activated (25% not activated, and 50% missing), 16.7% (incl. 8.3 partial) of Estrotect™ scratch cards (41.6% not activated, 41.7% missing), 92% (incl. 33.3 partial) of chalk (8% not activated), 100% NeDap activity monitors and 100% of IceQube®'s correctly indicated standing heat.

Non-lame housed cows had 31% of Kamar®'s (59.1% missing, and 9.1% not activated), 40.9% (incl. 9.1 partial) of Estrotect™ scratch cards (13.6% not activated, 45.5% missing), 95.5% of chalk (4.5% not activated), 100% NeDap activity monitors and 100% IceQube®'s correctly indicated standing heat.

Based on the overall findings Kamar®, and Estrotect™ scratch cards were less efficient at identifying oestrus in both groups of housed cows compared to the activity monitors or chalk. A larger percentage of mount detectors were dislodged on the day of heat (lame (50%) non-lame (%59.1) It is possible that the detectors were dislodged by stall bars, or social behaviours. However, accompanied with P^4 analysis to confirm the heat, it is likely that the cause for removal of the detectors was due to oestrus behaviours, either primary or secondary. Lame cows had higher percentages of mount detectors not being activated on the day of oestrus. This could be due to the flooring as oestrus expression (including mounting behaviour) is reduced on concrete when compared to dirt surfaces (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996). Lame housed cows could be reluctant to engage in oestrus behaviours as there is an increased potential cost of injury through falling and slipping (Palmer *et al*., 2010). Overall lame cows in a housed based system had reduced oestrus detection efficiency when using the mount detecting aids when compared to non-lame cows. Activity monitors correctly identified all cows in oestrus.

6.8.5 Pasture v housed

Estrotect™ scratch cards were significantly more accurate at pasture. Although not statistically significant, Kamar® mount detectors had a higher efficiency at pasture (58%) when compared to housed cows (29%). Interestingly housed cows had 94% (incl. 33.3% partial) of chalk correctly removed, pastured cows had 91% (incl. 35.4% partial).

It is expected that mount detection aids would be more efficient at pasture as research has shown cows increase mounting behaviour on dirt surfaces (Vailes and Britt, 1990). Cows require sufficient space to adequately display mounting behaviour, alongside sturdy, soft footing (Squires, 2010). A study by Palmer *et al.* (2010) determined that when comparing oestrus detection methods (including tail chalk), cows at pasture were detected more efficiently than cows housed indoors. Additionally, they also observed that housed cows were observed slipping and falling when mounting behaviour was attempted, however this was never observed in the pasture-based system. This subsequently caused a reduction in the frequency of standing oestrus behaviour (Palmer *et al.,* 2010). The majority of results from this study supports previous research that oestrus expression is reduced on slippery concrete flooring when compared to dirt surfaces (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996; Squires, 2010; Rao *et al.,* 2013). As lame cows reduce standing behaviour, this reinforces the requirement to interpret oestrus behaviour based on a cow's level of soundness.

Interestingly both lame and non-lame cows had a higher percentage of Kamar® and Estrotect™ detectors missing on the day of heat when housed rather than at pasture. Missing detectors can be associated with repeated mounting behaviour; however, this would not support the theory of other reported studies (De Silva *et al.,* 1981; Britt *et al.,* 1986; Vailes and Britt, 1990; Rodtian *et al.,* 1996; Palmer *et al.,* 2010; Squires, 2010; Rao *et al.,* 2013) that cows housed indoors reduce their mounting behaviour due to unsure footing. Having an increased percentage of missing detectors in housed cows may be due to an increase in secondary behaviours if the cows are reluctant to be mounted, or

mount other cows. To accurately determine the cause of the dislodged detectors, continual observation, or the use of devices such as HeatWatch II® that record the number of mounts received would be beneficial. However, devices such as HeatWatch II® have factors that affect longevity including, but not limited to, number of mounts, heat and humidity, age of the glue, confirmation of the tail head, amount and condition of the hair, time of the year, breed of cow, efficiency of the applicator, and maintenance schedule. Additionally, HeatWatch II® manufacturers recommend under normal circumstances, the devices can last about 30 days on a dairy cow and for about 50 mounts on a beef cow before serious maintenance is needed. Additionally, the range that the devices can be read may not be suitable for extensive pasture-based systems as the range is approximately 1/3 of a mile, and interference may also arise from landscape issues such as hills. Even with the implementation of these devices, they may still become dislodged due to a number of factors. Rorie *et al*. (2002) reported approximately 25% of the HeatWatch II® devices required re-gluing. In the current study cows were visually observed performing mounting behaviours while fully housed, however insufficient data were obtained to include this in the overall analysis.

6.9 Activity

6.9.1 NeDap

As cows typically increase their physical activity during the oestrus period (Van Eerdenburg *et al*., 1996; Firk *et al*., 2002), activity monitors can identify when a cow's activity level increases, indicating that the cow will be coming into oestrus. Schofield *et al.* (1991) reported significantly higher activity rates on the

day of oestrus than on other days, with activity increasing by 200-400% (Ranasinghe *et al*., 2010). In the current study NeDap activity monitors accurately identified all oestrus periods for housed cows. During the pasture period, all but one lame cow from this study received a correct oestrus attention with the NeDap activity monitor. The lame cow received a suspicious alert for the first postpartum oestrus, however an attention for oestrus was not indicated. Upon veterinary examination, it was confirmed that the cow had ovulated around the time she received the NeDap suspicion. Progesterone analysis confirmed that the cow had ovulated, however there was no accompanying oestrus behaviour(s). Therefore, the oestrus event was recorded as silent. It is reported that 50-80% of first ovulations postpartum are silent (Kyle *et al*., 1992; Ranasinghe *et al*., 2010). Although silent ovulations may not be accompanied with observable oestrus behaviours (standing to be mounted, secondary signs etc.) (Palmer *et al*., 2010), Ranasinghe *et al*. (2010) reported that most first ovulations postpartum were associated with 80% to 100% increases in walking activity. The NeDap system did indicate an increase in activity, however the threshold was too low to indicate the cow was in oestrus. The addition of a lameness aspect to the detection system would help identify cows with subtle activity increases related to their relative locomotion score. False positive attentions were sent for cows that were not in oestrus, but were engaging in bulling behaviours with herdmates. False positives were also sent when cows were initially turned out to pasture, which is also reported by Roelofs e*t al*. (2017).

6.9.2 IceQube®

6.9.2.1 Step count

It is documented that the number of steps taken each hour by a cow in oestrus is approximately two to four times higher than during dioestrus (Kiddy, 1977). In the current study both lame and non-lame cows fully housed and at pasture increased their step counts on the day of oestrus. On the day of oestrus lame cows had significantly less step counts when compared to non-lame cows, in both housing conditions. Furthermore, there was a significant difference in step counts between the housing and lameness groups before, during and after oestrus. Cows at pasture had significantly more step counts when compared to housed cows. Although there was a difference in the number of steps between the groups, on the day of oestrus lame cows increased their step counts just as non-lame cows. Suggesting that the intensity of oestrus was affected due to the reduced number of steps in lame cows.

6.9.2.2 Motion index

The motion index indicates the overall activity of the cow calculated using the acceleration on each of the 3 axes (IceRobotics Ltd 2010©). Motion index is the sum of the measured net acceleration in the three dimensions minus an offset for gravity, and as such an expression of leg activity. IceRobotics© states that this is a proprietary measure and is recommended over the step count as a measure of activity. The motion index can be explained as how vigorous an animal makes a movement. Therefore, the more vigorously a movement is made, the higher the motion index, suggesting a more intense display of activity. During oestrus both lame and non-lame cows at pasture and fully housed had an increase in motion index on the day of oestrus. There was a

significant difference in the mean motion index, and on the day of oestrus between lame and non-lame groups, and between pasture and housed cows. Repeated measures ANOVA showed high significance between lame and non-lame cows, whereas the two-way ANOVA showed a moderate tread towards significance. As lame cows had similar motion index values as non-lame cows on the day of oestrus (according to the two-way ANOVA), using this parameter as an oestrus detection method may be more suitable for cows with reduced mobility. If the step count does not increase on the day of oestrus due to environmental conditions or lameness, the motion index may indicate a more vigorous/intense movement, thus detecting oestrus in cows that do not significantly increase the number of steps. This measure would be valuable in detecting oestrus as some animals do not increase overall activity. Or it may help to reduce false positives indicated by increased step counts due to factors such as lameness or other activity. For example, lame cows are reported to have a shorter stride than non-lame cows (Telezhenko and Bergsten, 2005), and therefore may have to take more steps to reach the same distance as a non-lame cow. Therefore, if step counts are used, the lame cow may be falsely identified as being in oestrus. Contrastingly lame cows may reduce the time spent walking, and may not exhibit typical restless behaviour observed. Meaning the lame cow may go undetected. Therefore, the application of a measure that records intensity of the overall movement may assist in detecting these cows more accurately.

6.9.2.3 Lying time

Lying down is a crucial, basic component of a dairy cows' natural behavioural repertoire, and if this behaviour cannot be performed it can induce stress, which

can greatly compromise health and welfare (Andreasen and Forkman, 2012). During this time the cow will ruminate, socially interact, or simply rest (Metz, 1985; Cook *et al.,* 2004). It has been reported that dairy cows are motivated to lie down for approximately 13h/day (Jensen *et al.,* 2005). However, lying times vary between studies. For example, Brzozowska *et al.* (2014) reported that dairy cows spend on average 8-15h/day lying down. Another study reports that cows in free stalls spend approximately 12h/d lying down (EFSA, 2009), whereas another study reports a range of 9 to 14h/d (Charlton *et al.,* 2014). During oestrus cows typically increase their physical activity (Van Eerdenburg *et al*., 1996; Firk *et al*., 2002) thereby decreasing lying time. In the current study both lame and non-lame cows in both housing conditions decreased their lying time on the day of oestrus.

It is generally accepted that when compared to non-lame cows, lame cows alter their time budgets (increased lying time (Hassall *et al.,* 1993; Galindo and Broom, 2002; O'Callaghan *et al.,* 2003; Walker *et al.,* 2008a; Blackie *et al.,* 2011; Navarro *et al.,* 2013), fewer social interactions (Galindo and Broom, 2002; Tadich *et al.,* 2013), less time feeding (González *et al.,* 2008)). Therefore, it has been suggested that lame cows have reduced time available to express oestrus with herd mates. One explanation for increased lying times in lame cows is that although lame cows may walk and stand as much as non-lame cows, during oestrus, when non-lame cows increase walking, lame cows suppress this behaviour and increase lying times (Walker *et al.,* 2008a). Additionally, lame cows may travel with cows that are in standing heat, but may require more rest, thus lying down more and dedicating less time to expressing oestrus behaviours. Interestingly the current study reports no significant

difference in lying times between the lame and non-lame cows in either housing conditions. On the day of oestrus pastured lame cows had a lower overall mean lying time when compared to non-lame cows 5.7 (±0.37) h/d v 6.5 (±0.58) h/d. On the day of oestrus lame housed cows had a slightly higher mean lying time than non-lame housed cows on the day of oestrus 7 (±0.36) v 6.3 (±0.33). There are discrepancies whether or not lame cows rest for longer than their non-lame counterparts. For example, some studies report longer resting times (Walker *et al*., 2008a; Walker *et al*., 2008b; Ito *et al.,* 2010) and some report shorter (Chaplin *et al*., 2000; Cook *et al*., 2004b; Cook *et al*., 2008), or no difference at all (Hassall *et al.,* 1993).

Housed lame cows had mean lying times of 9.3 (±0.45) h/day, and non-lame cows had mean lying times of 9.1 (±0.19) h/day. Lying times from housed cows in this study are considered lower than the norm. It has been suggested that cows achieve 12h of lying per day in order to reduce the occurrence of lameness (O'Driscoll *et al.,* 2015). Additionally, it is accepted that low lying times are an indication of uncomfortable lying, or social conditions (Galindo and Broom, 2010). However, Ito *et al*. (2009) reported lying times for individual cows ranging from 4.2 to 19.5 h/d. Lying times can be influenced from management of housing conditions. For example, it has been documented that lying times increased from 8.8 to 13.8 h/d when replacing wet bedding for dry bedding (Fregonesi *et al*., 2007).

Overall pastured lame cows had a mean lying time of 7.9h/day (±0.1) whereas non-lame cows had a mean lying time of 8.7 h/day (±0.1). Other studies report

pasture lying times ranging from 6-11 h/day (Hassall et al., 1993; Sing *et al*., 1993b; Phillips and Rind, 2001; Hernandez-Mendo *et al*, 2007; Tucker *et al*., 2007; Sepúlveda-Varas *et al*., 2014; O'Driscoll *et al*., 2015). Although it may be unusual for cows to have low lying times, this study is comparable to other studies. Factors that affect lying times for cows at pasture are accessibility of forage (O'Driscoll *et al*., 2015), the distance to the milking parlour (Charlton *et al*., 2014), and environmental conditions (Cook *et al*., 2007; Falk *et al*., 2010). The cows were fed from troughs in various locations within a field, and were provided with water ad lib from automatic water troughs. To avoid damage to the ground, the feed troughs were moved often. Therefore, the cows were required to walk from the milking parlour to the feed and water troughs, and also were required to spend time grazing once the feed ran out. Although reported lying times at pasture are lower than the suggested lying times for optimum welfare (12h/d), it may be that the factors related to reduced lying times in housed environments are redundant at pasture (O'Driscoll *et al*., 2015). Reduced lying times in housed environments increase the risk of lameness (Galindo and Broom, 2000; O'Driscoll *et al*., 2015), and reduced lying times are indicative of uncomfortable lying or social conditions (Galindo and Broom, 2000). Pasture conditions such as soft footing, and freedom of movement means these issues may be irrelevant (O'Driscoll *et al*., 2015). Additionally, it may be that housed cows have fewer requirements to seek resources (food, water, shelter), thereby increasing lying times. Stocking density, and milking time can also affect overall lying times for dairy cows. For example, it has been reported that the mean herd lying time reduces if cows are away from the pen/pasture for more than 3.3h/d (Charlton *et al.* 2014). It has also been reported that during wet rainy conditions cows reduce time spent lying on

pasture (Falk *et al.,* 2012). Additionally, if there is insufficient shelter when cows are housed outside, during hot days' cows increase standing times in order to regulate their body temperatures (Cook *et al.,* 2007). Furthermore, it has been reported that high yielding dairy cows spend less time lying down than low yielding cows (Hasegawa *et al*., 1993), and more time standing and ruminating (Norring *et al*., 2012). Lying times increase as the lactation progresses (Ito *et al*., 2014; O'Driscoll *et al*., 2015). All cows in this study were recruited early in lactation. Therefore, reduced lying times from cows in this study could be due to their stage of lactation. However individual milk yields were not recorded in order to confirm this.

The fact that lame cows from this study did not have different lying times from non-lame cows could be related to the severity of lameness. For example, Yunta *et al*. (2012) did not report significant differences in lying times between moderately lame (cows with score 3 and 4) and non-lame cows (score 1). Ito *et al.* (2010) reported that lying bout duration and total lying time were increased in severely lame cows than in moderately lame cows. Additionally, Miguel-Pacheco *et al.* (2016) investigated the effect of lameness treatment for claw horn lesions on lying times, and reported that lame cows in three of the four treatment groups demonstrated no increase in lying time compared to non-lame controls. Juarez *et al*. (2003) reported increased lying times as the severity of lameness increased. Most of the lame cows in their study were classified as mildly lame at the time of enrolment. This suggests that the impact of lameness does not significantly affect lying times in mild to moderately lame cows. This would explain no significant differences in lying times from lame and non-lame cows in the current study, as lame cows had a mean LCS of 3.1

(±0.2), and four cows had LCS of 4. No cows scored 5 (severely lame). The fact that this herd was given access to pasture may have reduced the effect of lameness on behaviours such as lying times. Despite no difference in lying times, lame cows did have reduced step counts, and extended bout lengths suggesting that lameness still affects behaviour of moderately lame cows. Additionally, housed lame cows lied down for 1.4 hr/day longer than pastured lame cows. Lying behaviour is also influenced by what is causing the lameness. For example, foot lesions that cause the greatest increase in lying behaviour are digital dermatitis followed by sole ulcers (Chapinal *et al.,* 2009; Thomsen *et al*., 2012).

There was a significant difference in lying times between pastured and housed cows. Access to pasture enables the cows to move more freely, thereby increasing oestrus expression, and not restricting movement. Pastured lame cows had a lower mean lying time (7.9 (±0.35)) when compared to lame housed cows (9.3 (±0.45)). In general, pastured cows are reported to have lower lying times when compared to housed cattle (O'Driscoll *et al*., 2015). Hernandez-Mendo *et al.* (2007) reported that moving cows from free-stalls to pasture resulted in gait improvements, despite their lying times being lower than housed cows (10.9 h/d pasture vs. 12.3 housed). Pasture also improves gait score over time (Hernandaez-Mendo *et al.,* 2007), therefore it may be that the lame cows increase their activity due to a reduction in the effects of lameness.

6.9.2.4 Lying bouts

Pastured and housed lame cows had significantly fewer mean lying bouts when compared to non-lame cows. Lame and non-lame cows in both housing

conditions reduced the number of lying bouts on the day of oestrus, however the mean number of bouts were not statistically different. Housed non-lame cows had significantly more lying bouts on the day of oestrus than pastured cows (pasture 9.2 (±0.57) housed 10.4 (±0.30).

The number of lying bouts from non-lame pastured cows in this study (9.2 (±0.57)) falls within the range found in other studies. Arachchige *et al.* (2013) reported 9.4 bouts/d, and Hernandez-Mendo *et al*. (2007) reported 15.3 bouts/d. The number of lying bouts from lame pastured cows in this study 8 (±0.31) is comparable to that reported by Navarro *et al*. (2013), who reported 8.1 bouts/d.

The number of lying bouts from non-lame housed cows in this study (10.4 bouts/d) is within range to that found in many other studies. For example, Ito *et al*., (2009) reported 9 bouts/d, Gomez and Cook (2010) reported 12.9 bouts/d, Deming *et al*. (2013) reported 9.3 bouts/d, Charlton *et al.* 2014 reported 10.5 bouts/d, and Westin *et al*. (2016) reported 9.5 bouts/d. Lame housed cows in this study had 8.9 lying bouts per day. Solano *et al.* (2016) reported that lame housed cows had 9.7 bouts per day (±4.7), whereas non-lame cows had 10.2 (±4.5) bouts per day. Gomez and Cook (2010) reported that cows with moderate lameness had 10.9 lying bouts compared to non-lame cows.

Housed lame cows had longer mean bout lengths than non-lame cows. With lame cows' bouts lasting for 65.6 (±4.3) min, whereas non-lame cows' bouts were 54.2 (± 3.0) min. The bout duration findings are different from those reported by Charlton *et al.* (2014), as they reported an average lying duration

of 72 mins. Yunta *et al.* (2012) reported lying bout lengths of 89.3 ±3.89 in lame cows and 80.7 ±3.90 in non-lame cows. The fact that housed lame cows had fewer lying bouts coupled with longer lying bout lengths suggests that lameness could be affecting the cows' ability to either lay down or stand up. The physical act of lying down and getting up may also cause pain, therefore lame cows extend their bout lengths rather than repeatedly lying down and standing up (Chapinal *et al.,* 2009). Sepulveda-Varas *et al.,* (2014) reported that severely lame cows in their study tended to have more bouts when compared to non-lame cows. The fewer number of bouts in lame cows from this study may be due to the fact that they were moderately lame.

Pastured lame cows did not have different mean lying bout lengths from non-lame cows, Mean lying bout lengths were not different between housing conditions. Non-lame cows did have more mean lying bouts when compared to lame cows, whereas pastured lame cows had fewer lying bouts but no difference in bout length. Grazing may be the reason for similar lying bout lengths between lame and non-lame cows. The cows are fed when they are at pasture, however once the feed runs out the cows then graze. Additionally, the cows have to walk to the water troughs, so the time available to rest is reduced. This may be why lame and non-lame cows have similar lying bout lengths, as the time available to lay down is reduced through management procedures. Having access to pasture is known to improve locomotion scores/reduce lameness (Hernandez-Mendo *et al.,* 2007). This herd also has lower lameness prevalence during the pastured months (Chapter 3), therefore it may be that the effects of lameness are reduced, thereby allowing the cow to lay down as much as non-lame cows.

6.10 Duration of oestrus, increase in activity (percentage), and time of day oestrus expressed

6.10.1 Duration of oestrus

The duration of oestrus in this study was 8.9h (±0.4) for all cows. Ranging from 2h to 16h, which is comparable with other studies that reported an average duration of oestrus of 7 ± 5.4 h (range 5.1h to 10.6h) (Dransfield *et al*., 1998), 8.1h (Løvendahl, and Chagunda 2010), and 10.7h (range 2h to 14h) (Homer *et al*., 2013). Pastured cows had an average duration of 8.3h (±0.6), and housed cows was 9.5h (±0.5). Lame and non-lame cows had the same average duration of 8.9h (±0.5). Walker *et al*. (2010) also reported that lameness did not affect the overall duration of total oestrus behaviours (lame 12.3 ± 1.3 h vs non-lame 15.2 ± 1.3 h), but lameness shortened the period when herd-mates attempted to mount the lame cows (1.83 ± 0.69 h vs 5.20 ± 1.53 h). Although the duration of oestrus was not different between lame and non-lame cows in this study, the intensity of oestrus, and the period when herd-mates attempted to mount the lame cows may have been reduced. For example, pastured lame cows from this study had reduced oestrus detection efficiency when using the mount detecting aids when compared to non-lame cows. This may suggest that the intensity of oestrus, or the duration that they stand to be mounted could have been reduced. However, without continual observation, it cannot be explicitly determined that this is the case.

6.10.2 Time of day oestrus expressed

Although some studies suggest oestrus behaviours occur more frequently at certain times of day (Hurnik *et al*., 1975; Van Vliet and Van Eerdenburg, 1996; Diskin and Sreenan, 2000), this current study reports that there was no significant variation, which is in agreement with Esslemont and Bryant, 1976, and Xu *et al*. 1998. This study also reports that there was no association with lameness, or housing on the time of day oestrus was expressed. Walker *et al*. (2008a) reported that lameness affected the daily pattern of oestrous behaviour because non-lame cows expressed oestrus more frequently early morning compared with lame cows. Although there was no statistical significance, the non-lame cows from this study did display oestrus more frequently early in the morning when compared to lame cows. Lame cow proportions: 23% early morning; 32% midday; 45% in the evening; non-lame cow proportions: 44% early morning; 19% midday; 36% in the evening. The fact that there was no difference between lame cows in this study could be due to farm management, or that cows from this study were not severely lame. The study by Walker *et al.* (2008a) does not specify how many cows were classified as mildly, moderately, or severely lame. It could be that the cows from that study had poorer locomotion scores than cows from this study.

6.10.3 Activity increase during oestrus

The percentage of activity increase for all cows based on step counts was 554% (±25.4), and 640% (±36.4) based on motion index. The percentage increase was significantly higher in the motion index when compared to step counts. Lameness had no effect on the percentage increase from either step counts, or motion index. Housing did affect the percentage increase: Step

count: housed cows: 649% (±37.8); pastured cows: 457% (±24.4); motion index: housed cows: 764% (±60.7), and pastured cows was 513% (±25.0). These percentage increases are comparable to other studies. Homer *et al*. (2013) reported increases ranging from 190-349%. Interestingly the percentage increase in activity is higher in the housed cows than pastured cows, even though pastured cows had taken significantly more steps on the day of oestrus than housed cows. This is likely due to the pastured cows taking more steps in general, thus leading to a lower percentage increase as the housed cows did not take as many steps due to management conditions.

6.11 Progesterone Analysis

On the day of oestrus both lame and non-lame cows had a drop in P^4 values (4 days prior to oestrus (>1.5 ng/ml), down to <0.5ng/ml on the day of oestrus) indicating ovulation. Walker *et al*. (2010) examined the effects of lameness on oestrus by measuring the duration, and frequency of oestrus behaviour in relation to milk progesterone levels. The authors reported that lame cows had lower progesterone concentrations during the 6 days prior to oestrus, in addition to a decreased intensity of oestrus, and reduced periods whereby herd-mates mounted the lame cows, and a lower intensity of sexual behaviours (Walker *et al.,* 2008a). It has been documented that cattle perceive stress in herdmates by olfactory cues (Boissy *et al.,* 2008). Therefore, lame cows may emit stress related pheromones in addition to emitting lower quality of sexual pheromones (Walker *et al.,* 2008a). However, it has been reported that even in the presence of an adequate oestradiol surge during stress is enough to decrease, or completely inhibit oestrus associated behaviours such as mounting activity (Hein and Allrich, 1992; Allrich, 1994). Elevated stress related

hormones such as adrenocorticotropic hormone increases blood concentrations of both cortisol and progesterone (Bolanos et al., 1997; Hein and Allrich, 1992), therefore stress may lead to inadequate periestrous progesterone concentrations, consequently altering the appropriate oestradiol/progesterone ratio resulting in reduced oestrus intensity (Allrich, 1994; Duchens *et al.,* 1995). Lame cows from the current study did not have significantly different P^4 values prior to oestrus from non-lame cows. However, 7- and 10-days post oestrus lame cows P^4 concentrations were significantly lower (differences of 1.2±0.2 ng/ml and 1.7±0.2 ng/ml respectively). Cows from the currently study were moderately lame. It may be that the lameness was not severe enough to affect pre-oestrus P^4 values. Confirmation of, and progress of pregnancies were not monitored in this study. As the lame cows had lower P^4 values post oestrus, these cows could have reduced embryo survival rates. Chronic stressors such as lameness, are strongly associated with poor reproductive performance (Whay *et al.,* 2003; Melendez *et al.,* 2003; Hernandez *et al.,* 2005a), including lower including P^4 concentrations which affects early embryo survival rate (Beltman *et al*., 2009). For example, Mann *et al*. (2003) demonstrated that cows with low P^4 at a time when the embryo is at the morula to blastocyst stage have smaller embryos that produce less interferon-τ on days 13-15. It has also been shown that there is an increased probability of embryo survival in heifers and cows that have elevated P^4 on Days 4-6 (Diskin *et al*., 2006). Yan *et al*. 2016 demonstrated an increase in the chance of pregnancy in cows with relatively poor fertility supplemented with progesterone during the period of the postovulatory progesterone rise (Day 3–7) after mating at natural oestrus. Additionally, research has shown that oestrus

detection improved when cows were treated with progesterone (Carrick and Shelton, 1969; Stevenson *et al*., 1977). As lame cows have reduced fertility and P^4 concentrations, perhaps supplementing lame cows could increase oestrus expression and pregnancy rates. However, a study by Beltman *et al*. (2009) also examined the effect of early P^4 supplementation post conception on embryo survival in beef heifers, and they reported that mild or subclinical lameness significantly affected embryo survival rate. Suggesting that P^4 supplementation did not increase the chance of pregnancy in lame cows. However, this study excluded cows with LCS ≥4, and it also did not examine if the additional P^4 increased oestrus expression. It could also be that the embryo survival rates were poor when compared to the non-lame cows, but the study does not comment if administration could benefit lame cows on a wider scale.

Interestingly mean P^4 concentrations on the day of oestrus were significantly lower in housed cows when compared to cows at pasture. Lower circulating P^4 concentrations from housed cows in this study may be attributed to higher milk production, as it is reported that cows at pasture generally have lower milk yields (Fontaneli *et al*., 2005). Cows with higher milk yields have been reported to have lower circulating P^4 concentrations, as the increased milk production is associated with a corresponding increase in dry matter intake, which increases blood flow to the liver and subsequently increases metabolic clearance rates of steroid hormones (progesterone and oestradiol) (Sangsritavong *et al*., 2002; Vasconcelos *et al*., 2003; Roche, 2006), thereby reducing the P^4 concentrations.

In this study progesterone analysis assisted in detecting silent heats. Milk samples from cows that were not in standing heat but showed signs of oestrus such as slightly increased step count, combined with missing partially and/or fully active detectors were analysed. A total of three lame cows (2 pasture, 1 housed) were identified as having oestrus, accompanied with subtle oestrus expression behaviours. Due to limited resources, it was not possible to assess every study cow for an extended period of time. If analysis was possible for all milk samples collected and not just before, on and after the first observed heat, it may have been possible to detect more silent heats. As silent heats are common for the first postpartum ovulation (Ranasinghe *et al*., (2010), more may have been detected if all the milk samples were analysed.

6.12 Conclusions

Lame cows did not have significantly different detection efficiencies for various oestrus detection methods when compared to non-lame cows. Estrotect scratch cards were more efficient at pasture than in housed conditions. Overall the activity monitors were most accurate at correctly identifying oestrus events. Including a silent oestrus in a lame cow. The high incidence of missing detectors makes for a labour-intensive heat detection method. Lame cows took fewer steps on the day of oestrus when compared to non-lame cows. Lame cows did not have a significant difference in motion index on the day of oestrus when compared to non-lame cows. Pastured and housed lame cows had significantly fewer mean lying bouts when compared to non-lame cows. Housed non-lame cows had significantly more lying bouts on the day of oestrus than pastured cows. There was no difference in lying times for lame and non-

lame cows. Based on previous research that farmers do notice altered oestrus behaviour in lame cows (Chapter 4), and that research has shown lame cows to alter their oestrus behaviour (Walker *et al.,* 2008a), either through reduced intensity or frequency of standing behaviours, the application of activity-based systems can assist in detecting the problem or lame animal. Ongoing challenges in oestrus detection has led to advances in oestrus detection aids. Evaluating a novel oestrus detection method for lame and non-lame cows will determine its practicality for all cows.

Chapter 7: Evaluation of a novel oestrus detection method (Infrared thermal imaging) in high yielding lame and non-lame Holstein-Friesian dairy cattle

7. 1 Infrared Thermal Imaging

The use of Infrared thermography (IRT) in the animal industry has become more popular over the years. IRT has been used to monitor pain, and stress in animals (Jerem, *et al.,* 2015), and is a promising non-invasive oestrus detection method (Hurnik *et al.,* 1985; Jones *et al.,* 2005; Talukder *et al.,* 2014; Perez Marques *et al*., 2019). Obtaining IRT readings from areas such as the head, eye, and body are favourable due to the ease of collection. An area of great interest is the eye (lacrimal caruncle), as it has been shown to be closely representative of the core body temperature (Stewart *et al.,* 2005; Stewart *et al.,* 2008a; Gloster *et al.,* 2011; Valera *et al.,* 2012. Therefore, studies observing the effect of stress and pain in animals have used IRT eye temperatures to document the physiological reaction to stressors such as dehorning (cattle) (Stewart *et al.,* 2008a), equine sporting events (Valera *et al.,* 2012), veterinary handling procedures (Travain *et al.,* 2015), or housing conditions (Foster and Ijichi, 2017).

Bovine body temperatures fluctuate during the oestrus cycle (Kyle *et al.,* 1998), approximately 2 days before oestrus the body temperature decreases before increasing during the luteinising hormone (LH) surge (Fisher *et al.,* 2008). IRT measurements for oestrus detection in dairy cows have been made on various areas such as the flank (Hurnik *et al.,* 1985; Perez Marquez *et al*., 2019), vulva and muzzle (Talukder *et al.,* 2014; Perez Marquez *et al*., 2019), tail head, rump,

feet, eye, cheek, neck and withers (Perez Marquez *et al*., 2019). Areas such as the ear, eye, vulva and muzzle have been investigated for the detection of ovulation (Talukder *et al.,* 2015). Additionally, it has been reported that cattle eye temperatures are not affected by ambient temperature, and can therefore be used as in indicator of core body temperature in varying environments (Gloster *et al.,* 2011).

To determine if infra-red thermography (IRT) could be used as an oestrus detection method for lame and non-lame cows, a small pilot study was carried out. The pilot study had two aims; 1) to see if lame cows had different body temperatures from non-lame cows, and 2) to determine if lameness affects the circadian rhythm of body temperatures, by assessing if the time of day the measurements were taken affected the recorded temperatures.

7.2 Pilot study

7.2.1 Materials and methods

7.2.1.1 Animals and data collected

The pilot study used two groups of cows. The first group used 13 (9 multiparous and 4 primiparous) cows. The second group of cows in the pilot study used 19 cows. All cows were high yielding Holstein Friesian dairy cows from Rodwell dairy farm (Ipswich, UK). Average parity of both groups of cows was 2.3 (±0.38).

Detailed management of these cows is in Chapter 3 (3.1.1). All cows were locomotion scored using the method of Flower and Weary (2006) (Chapter 3,

Table 3-5). The cows were locomotion scored leaving the parlour on grooved concrete. Each cow was given a locomotion score from normal to severely lame (locomotion scores 1 to 5, respectively). Cows were then assigned to either a lame or non-lame group.

The first group had a total of n=9 cows were recorded as non-lame, and n=4 were recorded as lame. Non-lame cows had a mean LCS and parity of 1.2 (±0.14) and 2 (±0.23) respectively. Lame cows had a mean LCS and parity of 3 (±0.15) and 3.25 (±1.0) respectively. Cows were randomly selected to have both core (rectal) and thermal eye temperatures measured.

In the second group of cows a total of n=9 cows were recorded as non-lame, and n=10 were recorded as lame. Non-lame cows had a mean LCS and parity of 1.2 (±0.14) and 2 (±0.23) respectively. Lame cows had a mean LCS and parity of 3 (±0) and 3.25 (±1.0) respectively. These cows had their temperatures recorded over time from 5 locations on their body (core, pocket, pinbone, ear, eye). Temperatures were recorded every 84 (±0.01) minutes from 10:00-16:00.

All core temperatures were obtained using a digital rectal thermometer (©Nettex digital thermometer, Rochester, Kent, UK). The thermometer was wiped clean after each use. All thermal images were measured with an infrared thermal camera (FLIR, 60b series: FLIR Systems Co. Ltd., St Leonards, NSW, Australia). These Data were collected within a freestall barn to minimise environmental effects such as wind or solar radiation (Church *et al.,* 2014). All

cows were scanned with the thermal camera from the same side (right), angle (90°) and distance of approximately 0.5-1m. The pocket area under the tail was wiped clean with a damp paper towel to remove faecal material. Thermal images were analysed using the FLIR software.

Before each thermal scanning session, the emissivity value was set to 0.98, and thermograph resolution was calibrated to ambient temperature and humidity as per manufacturer's recommendation using a wireless weather station (Oregon Scientific International Ltd., Los Angeles, CA, USA). The pocket area is located under the tail above the anus (Plate 7-1). The pinbone area measured is shown in Plate 7-2.

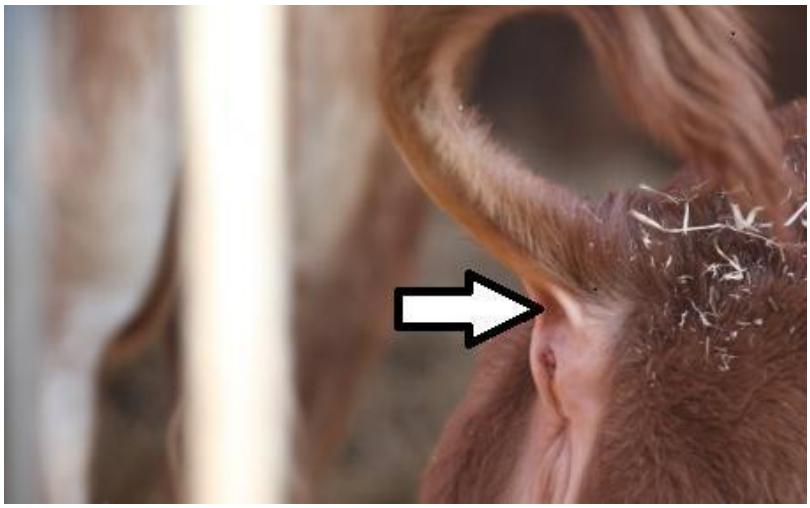

Plate 7- 1 Pocket area of cow (Source: Nima Stock, no date)

Plate 7- 2 Pinbone area of cow

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7.2.3 Data analysis

Thermal images were stored in a memory card and then transferred to a computer for analysis using ThermaCAM Researcher Professional 2.9. The software enabled the user to determine the surface temperature in a user-defined field of interest on the image and calculated the minimum, maximum, and average temperatures and SD (through software recognition of each pixel within the defined area) for each of these "fields." Maximum IRT temperatures were used in line with previous studies (Sykes *et al.,* 2012; Talukder *et al.,* 2014; Talukder *et al.,* 2015).

Core and eye temperatures were compared using a paired t-test, whereas analysis of core temperatures from lame and non-lame cows, and eye temperatures from lame and non-lame cows were analysed with two sample t-test. All analysis was done with GenStat, 18th edition.

Temperatures recorded over time were analysed with repeated measured ANOVA (GenStat, 18th edition).

7.2.4 Results

Mean core and thermal eye temperatures from all subjects was 38.4 °C (±0.08) and 37.4°C (±0.12) respectively. Core and eye temperatures had a difference of 1°C, and were significantly different ($p<0.001$). Core temperatures from lame and non-lame cows were not significantly different, whereas eye temperatures were significantly different between lame and non-lame cows (Table 7-1).

Table 7- 1 Comparison of core and eye temperatures between lame and non-lame dairy cattle

	Lame	Non-lame	SED	P-value
Core temperatures	38.4	38.4	0.180	n. s
Eye temperatures	36.9	37.6	0.209	0.014

Results from the repeated measures ANOVA show that the temperatures were taken were not statistically affected by the time of day, F (1.61, 22.48) =1.620, $p=0.221$.

7.3 Method development

Based on the results from the pilot study the measurements obtained from the eye, and pocket are representative of the cows' core body temperature, whereas temperatures recorded from the ear and pinbone area were

significantly different. The cows body temperature did not significantly fluctuate throughout the day. Daily circadian rhythm differences (day v night) were previously reported, with the lowest temperature early in the morning (05:00 to 07:00), and the highest around midnight (Bitman *et al.*, 1984; Lee *et al.,* 2016). No significant differences in the current pilot study may be due to the fact that the temperatures were recorded outside of these times (10:00-16:00).

Based on the results from the pilot study, the use of IRT as an oestrus detection method between lame and non-lame cows will be further evaluated from more animal subjects in the experiment detailed below (7.4).

7.4 Aims

The aims of this experiment were to further evaluate IRT measurements from different areas of the body (ear, eye, pinbone, pocket) from a larger sample size, to determine if the data could be used to determine oestrus in dairy cattle. The second aim was to assess temperatures between lame and non-lame cows to determine if their baseline and oestrus temperatures were different. Although other studies have assessed the use of IRT readings from the ear and eye for oestrus and ovulation (Talukder *et al.,* 2015; Perez Marques *et al*., 2019), to the knowledge of the author this is the first study to evaluate core body temperatures, and IRT scans of the eye, ear, pocket, and pinbone area from lame and non-lame cows as an oestrus detection method.

7.5 Materials and methods

7.5.1 Animals and data collected

The study was conducted between 13th of March and 25th of August 2014. Three separate farms were included in this experiment. Farm 1 was located in Ipswich Suffolk, United Kingdom; farms 2 and 3 were located in Lacombe, Alberta, Canada. Farms from Canada were included to increase sample size. All cows in the milking herd with the exception of cows that were in hospital pens (through illness or very freshly calved) were locomotion scored using the method of Flower and Weary (2006) (Chapter 3, Table 3-5). The cows were locomotion scored leaving the parlour on grooved concrete. Cows were then assigned to either a lame or non-lame group. Lameness prevalence was calculated for each farm.

Data collected were management/housing conditions; cow information (DIM, parity, BCS, LCS, diet), external body temperatures in °C (FLIR, 60b series: FLIR Systems Co. Ltd., St Leonards, NSW, Australia), and internal core temperatures in °C (©Nettex digital thermometer, Rochester, Kent, UK). Full biosecurity procedures were followed between farms. A total of n=47 heats were recorded; n=23 from farm 1, n=10 from farm 2, and n=14 from farm 3.

7.5.1.1. Farm 1 Rodwell Dairy Farm

Data collection from this farm was between 13th of March and 25th of August 2014. Rodwell Dairy is located in Ipswich, Suffolk, UK. The management of these cows is detailed in Chapter 3, Section 3.1.1. Briefly, the herd consisted of 130 Holstein Friesian cows, with average milk production over 11,000 litres

per lactation. During the winter months, lactating cows were housed in a freestall barn with cubicles bedded with sand. During the summer months' cows were fully pastured. Cows were milked twice daily at 0500 and 1500. All multiparous cows' breeding was managed by Genus Reproductive Management Systems (RMS). An RMS technician visited daily to identify cows in oestrus by using records and tail chalking. These records were checked by the researcher (AW), and cows were also observed in person for oestrus behaviour for a minimum of 30 minutes (2pm). Observations were recorded using the Dutch points scale (details in Chapter 3, Table 3-10, van Vliet and van Eerdenburg, 1996). A total of n=23 cows with a mean parity of 3.5 (± 0.2), mean LCS of 1.7 (±0.2), and mean DIM 149 (±15.6) were included in this experiment. From this group n=5 were lame (mean parity 4 (±0.3), mean LCS 3 (±0), mean DIM 152.6 (±45.3), and n=18 were non-lame mean parity of 3.3 (±0.3), mean LCS of 1.4 (±0.1), mean DIM 147.9 (±16.3). A total of n=230 temperature measurements were collected from these cows.

7.5.1.2 Farm 2 Thornspyc Dairy farm

Data collection from this farm was between the 16th and 21st of July 2014. Thornspyc Dairy farm is located in Lacombe Alberta Canada. The management of these cows is detailed in Chapter 3, Section 3.1.3. Briefly, the herd consisted of 167 Holstein milking cows with average milk production over 10,000 litres per lactation. The Lactating cows were housed in a freestall barn with stalls equipped with rubber matting and topped with chopped straw. Cows were milked twice daily (03:30 and 15:30). The barn had an automatic scraper, and fans to cool the cows. Cows were seen every two weeks by a veterinarian, and they were managed on a PreSynch OvSynch synchronisation protocol for timed

AI (Protocol detailed in section 3.1.3). Cows were observed for oestrus behaviours twice daily in person by the same observer (AW) for a minimum of 30 minutes twice daily (05:00 and 14:00) for the following 6 days. Observations were recorded using the Dutch points scale (details in Chapter 3, Table 3-10, van Vliet and van Eerdenburg, 1996). A total of n=10 cows with a mean parity of 2.6 (±0.5), mean LCS of 1.5 (±0.3), and mean DIM 144.4 (±25.1) were included in this experiment. From this group n=2 were lame (mean parity 3.5 (±1.1), mean LCS 3 (±0.4), mean DIM 106 (±88.8), and n=8 were non-lame mean parity of 2.4 (±0.6), mean LCS of 1.1 (±0.1), mean DIM 154 (±30.3). A total of n=100 temperature measurements were collected from these cows.

7.5.1.3 Farm 3 Lucky Hill Dairy Farm

Data collection from this farm was between the 16th and 21st of July 2014. Lucky Hill dairy farm is located in Lacombe Alberta Canada. The management of these cows is detailed in Chapter 3, Section 3.1.2. Briefly, the herd consisted of 230 Holstein milking cows with average milk production over 10,000 litres per lactation. Fresh cows were housed in a separate pen temporarily post calving, and later added to the main milking herd. Lactating cows were housed in a freestall barn with stalls equipped with gel mats and were topped with sawdust. Cows were milked three times daily (05:00, 13:00 and 20:00). The barn had an automatic scraper, with water misters and fans to keep the cows cool during the summer months. The oestrus detection method used at farm 3 was the Heat Time program, which monitors cow activity. Alerts are sent to the control panel, which was checked twice daily for 6 days to identify cows in heat. Cows were also observed for oestrus in person twice daily by the same observer (AW) for a minimum of 30 minutes (06:30 and 15:30pm).

Observations were recorded using the Dutch points scale (details in Chapter 3, Table 3-10, van Vliet and van Eerdenburg, 1996). A total of n=14 cows with a mean parity of 2.7 (±0.3), mean LCS of 2.4 (±0.4), and mean DIM 175.3 (±37.4) were included in this experiment. From this group n=7 were lame (mean parity 3 (±0.4), mean LCS 3.7 (±0.2), mean DIM 212.3 (±55.9), and n=7 were non-lame mean parity of 2.3 (±0.3), mean LCS of 1.1 (±0.1), mean DIM 145.3 (±54). A total of n=140 temperature measurements were collected from these cows.

A total of n=47 cows from the three farms were included in the experiment. From this group n=14 were lame and n=33 were non-lame. Lame cows had a mean parity, LCS, and DIM of 3.6 (±0.3), 3.4 (±0.1), and 175.8 (±32.8) respectively. Non-lame cows had a mean parity, LCS, and DIM of 2.8 (±0.2), 1.3 (±0.1), and 147.4 (±15) respectively. All cows combined had a mean parity of 3 (±0.2), mean LCS of 1.9 (±0.2), and mean DIM 155.8 (±14.3).

7.6.2 Temperature measurements

7.6.2.1 Core temperature measurement

Core body temperatures were recorded using a rapid rectal digital thermometer (©Nettex digital thermometer, Rochester, Kent, UK). Each farm had a designated rectal thermometer to reduce the risk of spreading disease. The thermometer was labelled with the farm name, and stored in a labelled plastic bag (Polybags Limited., Lyon Way, Greenford, Middlesex). During rectal measurements, the tail was held aside and the area was gently wiped with dry paper towels to remove faecal debris. Core measurements were recorded twice, once when the cow was in oestrus, and once 48 hours' post-oestrus in order to obtain a baseline temperature for each cow. Determination of oestrus

is detailed in the farm information above. Calibration checks were made on the digital thermometers each day using an ice bath, and a certified calibrated reference thermometer (Ever-safe ® Laboratory Thermometers, Certified, Canadawide Scientific Ltd, Ottawa, Ontario). After each core measurement, the thermometer was wiped clean and disinfected with Virkon.

7.6.2.2 External temperature measurement

External body temperatures were recorded with an infrared thermal imaging camera (FLIR, 60b series; FLIR Systems Co. Ltd., St Leonards, NSW, Australia) from 4 locations; eye (Plate 7-3), ear (Plate 7-4), pocket (Plate 7-5), and pinbone (Plate 7-6). Before each thermal scanning session, the emissivity value was set to 0.98. and thermograph resolution was calibrated to ambient temperature and humidity as per manufacturer's recommendation using a wireless weather station (OregonScientific International Ltd., Los Angeles, CA, USA). The temperatures were recorded when the cows were standing in a stall within the house. During thermal scanning of the pocket area, the tail was held aside and the area was gently wiped with dry paper towels to remove faecal debris. The pinbone area was brushed to remove dirt before the IRT measurement was made. During thermal scanning of the eye and ear, all cows were scanned from the same side (left), at an angle if (90°), and from a distance of approximately 0.5-1m. The maximum temperature is identified within the square, and displayed in the top left corner of each image. The brighter colours (red, orange, yellow, white) indicate warmer temperatures (more heat emitted), while the green, blue/black indicate cooler temperatures (less heat emitted). The colour scale at the bottom of the images illustrates the range of colours in

relation to the temperatures detected (coldest on the left and hottest on the right).

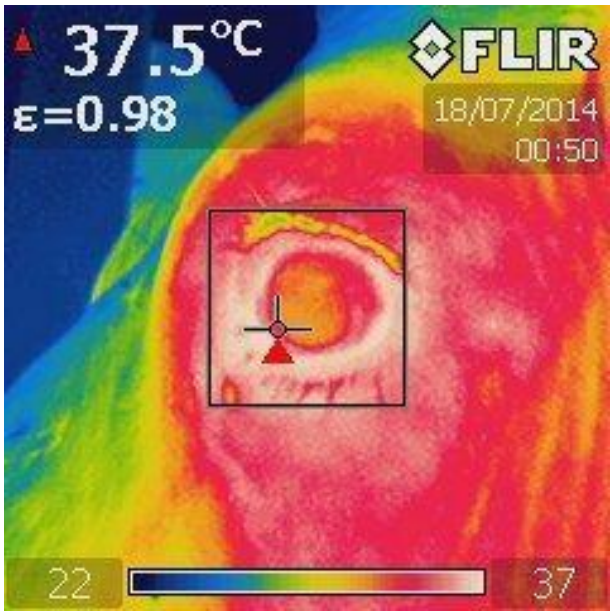

Plate 7- 3 Infrared image from eye

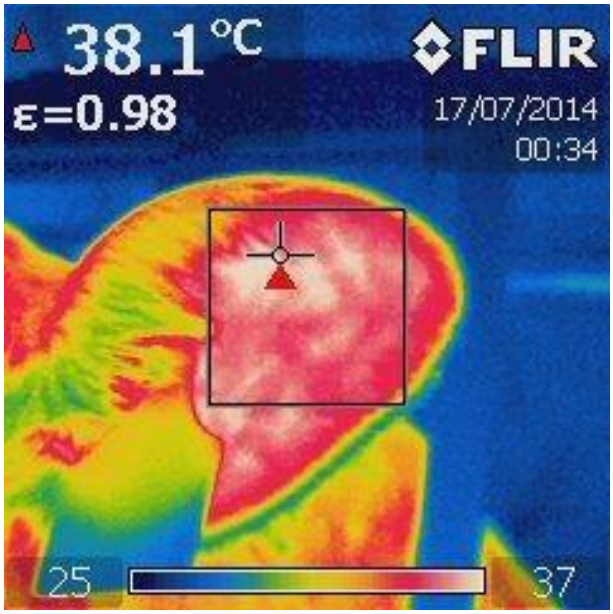

Plate 7- 4 Infrared image from ear

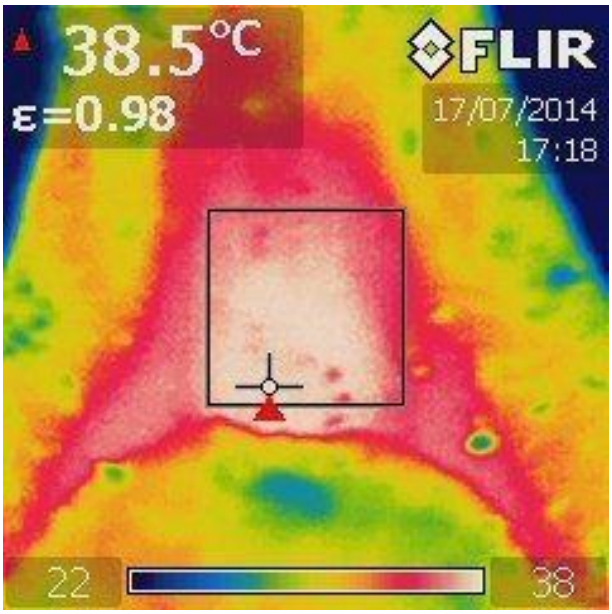

Plate 7- 5 Infrared image from pocket

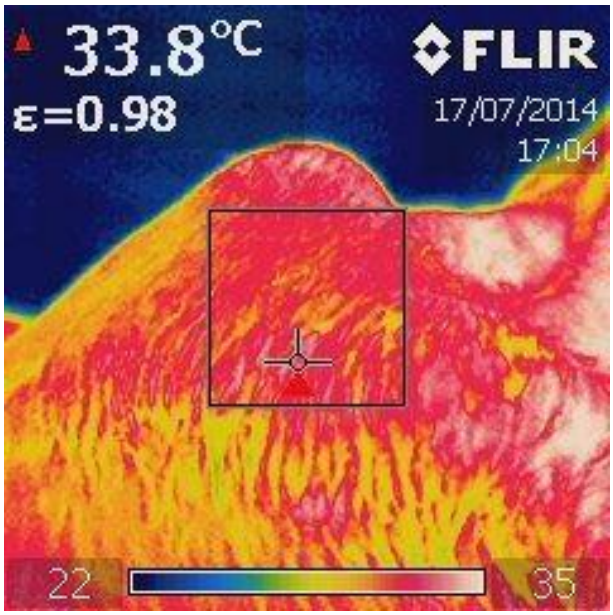

Plate 7- 6 Infrared image from pinbone

7.7 Data handing and analysis

Images were stored on a memory card and then transferred to a computer for analysis using ThermaCAM Researcher Professional 2.9. The software enabled the user to determine the surface temperature in a user-defined field of interest on the image and calculated the minimum, maximum, and average temperatures and SD (through software recognition of each pixel within the defined area) for each of these “fields”. In line with previous studies (Gloster *et al.,* 2011; Sykes *et al.,* 2012; Valera *et al.,* 2012; Talukder *et al.,* 2014; Talukder *et al.,* 2014; Jarem *et al.,* 2015), maximum eye temperatures were used to eliminate lower temperature readings from non-target areas (e.g. skin).

A total of n=47 heats were recorded; n=23 from farm 1, n=10 from farm 2, and n=14 from farm 3. A total of n=470 temperature measurements were recoded from all cows (n=94 core measurements; n=376 IRT). Data were confirmed as being normally distributed using the Pearson’s Skewness test.

To determine the differences in temperatures recorded from various locations on the body a one-way analysis of variance with farm as a block was used followed by a Tukey test for post hoc comparison. The temperature differences (oestrus and not in oestrus) from each location were calculated with Excel.

The correlation and regression of temperatures were calculated with simple linear regression. Coefficient of variation was calculated.

Two-way ANOVA was performed with farm as a block to determine if oestrus and lameness had an effect on the temperatures recorded.

To determine if the farms had significantly different body condition scores a Kruskal-Wallis One-way ANOVA was performed as these data were non-parametric (Tested for normality using Shapiro-Wilk test, GenStat 18th edition).

To determine if body condition score affected oestrus and baseline temperatures a Kruskal-Wallis One-way ANOVA was performed as BCS were non-parametric (Tested for normality using Shapiro-Wilk test, GenStat 18th edition).

To determine if body condition score was affected by lameness a Mann-Whitney U Test was performed as BCS data were non-parametric.

All statistical analysis was performed using GenStat 18th edition.

7.8 Results

Overall the baseline mean eye temperatures recorded from n=47 (n=33 non-lame, n=14 lame) cows was 37.2°C (±0.1) and mean core temperatures were 38.3°C (±0.1). Core and eye temperatures had a difference of 1.1°C and were significantly different (p=0.029).

Core and eye temperatures had a moderate positive correlation relationship (Pearson correlation r_{92}=0.673) (regression y= 16.47-0.5903x, $F_{1,92}$=78.02,

$P<0.001$; $R^2=0.453$). Core and ear temperatures had a weak positive relationship (Pearson correlation $r_{92}=0.387$) (regression y= 32.12-0.1786x, $F_{1,92}=17.37$, $P<0.001$; $R^2=0.15$), Core and pocket temperatures had a moderate positive correlation relationship (Pearson correlation $r_{92}=0.643$) (regression y= 22.05-0.4358x, $F_{1,92}=66.83$, $P<0.001$; $R^2=0.414$), Core and pin temperatures had a weak positive relationship (Pearson correlation $r_{92}=0.305$) core and pin (regression y= 35.33-0.0944x, $F_{1,92}=10.54$, $P<0.05$; $R^2=0.093$).

The differences in temperatures from various locations on the body is in table 7-2. Oestrus temperatures recorded from the core and pocket, and pocket and eye were not significantly different from each other. The ear and pin were significantly lower than all other temperatures recorded. Non oestrus temperatures recorded from the pocket and eye were not significantly different from each other. The ear and pin were significantly lower than all other areas. Temperatures increased during oestrus by 0.44°C -0.66°C depending on body location.

Table 7- 2 Oestrus and non-oestrus temperature (°C) differences from various body locations

Mean Temperature	Core	Ear	Eye	Pin	Pocket	SED	P-value
Oestrus	38.9^{d}	36.6^{b}	37.8^{c}	34.9^{a}	38.3^{cd}	0.21	<0.001
Not in oestrus	38.3^{d}	36.1^{b}	37.2^{c}	34.5^{a}	37.7^{c}	0.20	<0.001
Oestrus and Non-oestrus differences	0.59	0.48	0.58	0.44	0.66		

[a]different letters in the same row indicate Tukey post hoc test significant difference

The coefficient of variation from the different location's temperatures were recorded is in table 7-3. The most reliable point to take the measurements is from the core, pocket and eye.

Table 7- 3 The coefficient of variation (CV%) from different temperature locations

Area	CV %	SE
Core	1.0	0.4
Eye	1.3	0.5
Ear	2.9	1.0
Pocket	0.6	1.7
Pin	4.2	1.5

Temperatures that were significantly affected by both lameness and oestrus were in the core, eye, and pocket. Temperatures that were significantly affected only by oestrus were from the ear. Temperatures that were significantly affected only by lameness were from the pin location. (Table7-4).

Table 7- 4 Baseline and oestrus temperatures from lame and non-lame cows from different areas of the body

Area of measurement	Baseline Temperatures		Oestrus Temperatures		Lameness SED	Oestrus SED	LxO SED
	Lame (n=14)	Non-lame (n=33)	Lame (n=14)	Non-lame (n=33)			
Core	38.1 (±0.1)	38.4 (±0.1)	38.6 (±0.1)	39 (±0.1)	0.09**	0.08***	0.12
Eye	36.9 (±0.1)	37.3 (±0.1)	37.5 (±0.1)	37.9 (0.1)	0.11***	0.08***	0.12
Ear	35.8 (±0.3)	36.2 (±0.2)	36.4 (±0.3)	36.7 (±0.2)	0.24	0.15*	0.33
Pocket	37.2 (±0.2)	37.9 (±0.1)	38 (±0.2)	38.5 (±0.1)	0.15***	0.13***	0.21
Pin	33.9 (±0.4)	34.8 (±0.3)	34.6 (±0.4)	35.2 (±0.3)	0.34*	0.3	0.46

*Indicates significance at P<0.05; ** indicates significance at P<0.01; *** indicates significance at P<0.001

There was no significant difference in BCS from the 3 separate farms (Figure 7-1).

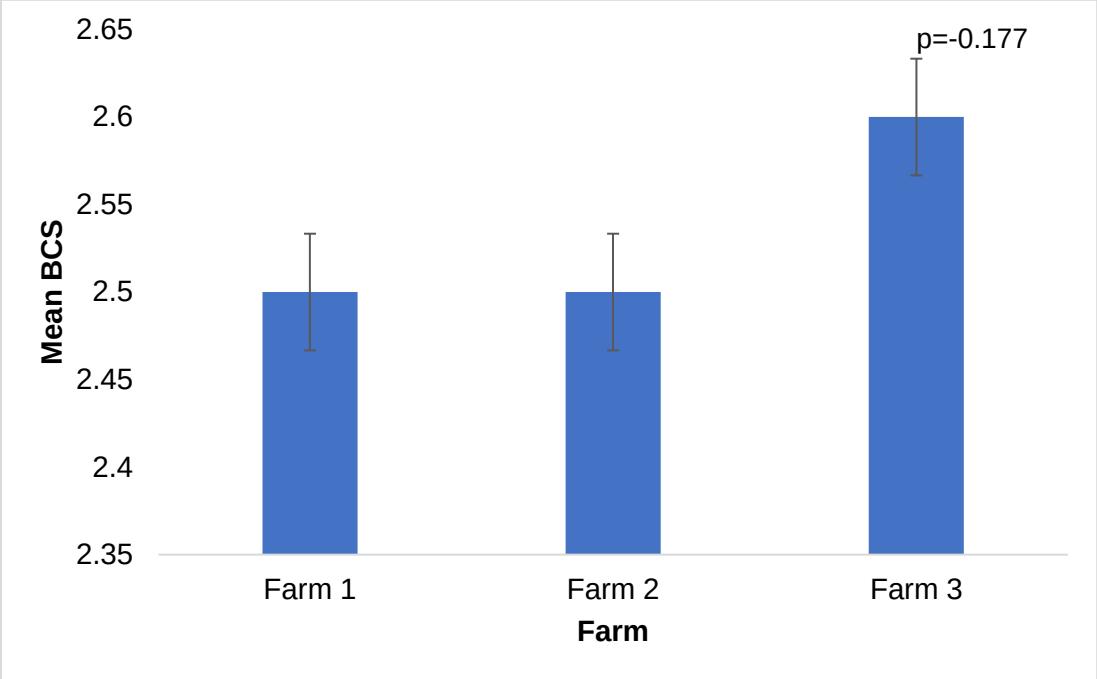

Figure 7- 1 Mean body condition scores of study cows from three separate farms

Body condition score significantly affected baseline temperatures from the eye and pin locations (Table 7-5).

Table 7- 5 Mean baseline temperatures recorded for different body condition scores

Area	BCS				
	2 n=7	2.5 n=31	3 n=9	SED	P-value
Core	38.4	38.3	38.4	0.16	0.348
Ear	36.9	35.9	35.8	0.46	0.108
Eye	37.4[b]	37.3[b]	36.8[a]	0.16	0.015
Pin	36.4[b]	34.1[a]	34.5[ab]	0.71	0.004
Pocket	38	37.6	37.6	0.32	0.218

Body condition score significantly affected oestrus temperatures from the core, eye and pin (Table 7-6). It was determined that lame cows (n=14) had a higher mean BCS (2.7±0.3) than non-lame cows (n=33) (BCS 2.5±0.3) (U=145.0, p=0.023).

Table 7- 6 Mean oestrus temperatures recorded for different body condition scores

Area	BCS				
	2	2.5	3		
	n=7	n=31	n=9	s.e. d	P-value
Core	39.5[b]	38.8[a]	38.8[a]	0.19	0.023
Ear	36.9	36.5	36.6	0.51	0.534
Eye	38.3	37.7	37.6	0.25	0.175
Pin	36.3[b]	34.5[a]	35.6[ab]	0.69	0.006
Pocket	38.8	38.2	38.3	0.27	0.158

7.9 Discussion

This current study determined that eye temperatures were 1.1°C lower than core body temperatures (37.2°C (±0.1) v 38.3°C (±0.1)). Although this is significantly different, a study by Gloster *et al.* (2011) reported eye temperatures were approximately 2°C lower than rectal temperatures, and were not significantly affected by ambient temperature. However, Dunbar *et al.* (2009) reported no significant difference in body temperature from eye temperatures of well-focused IRT images from mule deer. Perhaps the images captured in this study were not as high quality as those captured in the study by Dunbar *et al.* (2009). Additionally, the eye and core had a moderately strong relationship with one another. Despite the 1.1°C difference reported in this study, eye temperatures can be a useful proxy for core body temperature (Gloster *et al.,* 2009).

Comparison of baseline temperatures with temperatures recorded when the cow is in oestrus revealed a significant increase in the core (+0.59°C), eye (+0.58°C), ear (+0.48°C), and pocket (+0.66°C) temperatures. Previous studies also report elevated body temperatures on the day of oestrus or during oestrus (Piccione *et al.,* 2003; Fisher *et al.,* 2008; Suthar *et al.,* 2011). Results from the pilot and main study indicate that the use of IRT scans of the eye, and pocket area have the potential to be used for oestrus detection due to the significant increase in temperature recorded. However, based on the coefficient of variation the pocket, core and eye are also the most reliable point to take the measurements from. Furthermore, during oestrus, the measurements from the core and pocket were similar, as were IRT measurements from the pocket and the eye. Therefore, IRT locations from the eye and pocket are appealing due to their close relationship to core temperatures. However, the eye is more practical for obtaining repeated IRT measurements. This will be discussed in greater detail below.

The baseline pinbone temperature was not significantly different from the temperature detected at oestrus. Temperatures from this location remained low regardless of oestrus (below 36°C). One explanation could be that debris/dirt on the fur interfered with accurate IRT readings (McManus *et al.,* 2016). Thickness of the hair coat, and body condition score can also significantly affect IRT readings. Variations between body condition of cows could lead to significantly different readings. From the current study baseline and oestrus pinbone temperatures from cows with BCS of 2 had higher IRT temperatures recorded than cows with a BCS >2.5. Increased subcutaneous fat covering the

pin bone area is associated with higher BCS, and could lead to lower body temperature readings (Cilulko *et al.,* 2013). Interestingly baseline and oestrus temperatures recorded from cows with a BCS of 3 were not significantly different from BCS 2 or 2.5. The pinbone area is not a reliable measure of body temperature. Therefore, the differences are likely related to coat colour, (Hellebrand *et al.,* 2003; McCafferty, 2007), cleanliness of the coat, and handling errors. Environmental conditions are important factors for thermoregulation and skin temperature. Air temperature, humidity, and solar radiation can influence surface body temperatures (David and Mader, 2003; Norris and Kunz, 2012; Herbut *et al*., 2013; Sorensen and Pedersen 2015). The distance and angle from the pinbone area could potentially be a source of error however this was minimised in the studies in this thesis through the same individual (AW) recording all IRT temperatures. Cow movement could have also affected the distance to the IRT. However, this was minimised in the studies in this thesis by recording the temperatures while the cow was standing in a free stall. Although these sources of error were minimised, complete elimination is unlikely. During oestrus cows with BCS of 2 had higher mean core, and pinbone temperatures compared to cows with a BCS of 2.5 or 3. It could be that cows with lower BCS were able to move around more freely, thereby increasing heat production through exercise. In the current study lame cows had significantly higher BCS compared to non-lame cows, which supports the theory that cows with lower BCS were less lame. However, without observations studying activity (step count), it is not known if the increased temperatures are linked to activity. The results contradict previously reported information regarding BCS and lameness. Cows with BCS >2 have a decreased risk of developing lameness (Randall *et al*., 2015; Solano *et al*.,

2015). Furthermore, it has been reported that lame cows have reduced BCS due to increased lying times, and reduced time feeding (Singh *et al*., 2018). It is possible that lameness in the cows from this study was not severe enough to affect feed intake, thereby not affecting their BCS. Sample size could also have affected these results. There were more cows with a BCS of 2.5 (66% of study cows). Therefore, more research is required to validate these findings.

The oestrus related temperature rise could be an effect from increased thermogenesis associated with increased oestrus activity (Harris and Starnes, 2001; Piccione *et al.,* 2003). Rises in core body temperature during exercise have been previously reported (Harris and Starnes, 2001; Wendt *et al.,* 2007). Interestingly core, eye and pocket temperatures from lame cows during oestrus were significantly lower than non-lame. Cows in oestrus typically increase their physical activity. However, lame cows have been reported to have reduced oestrus behaviour (Walker *et al.,* 2008a), and may not be as active as non-lame cows. Therefore, their body temperature does not increase as much as non-lame cows. However, other studies suggest that hormonal changes during oestrus are responsible for an increase in body temperature, not an increase in physical activity (Fisher *et al.,* 2008, Suthar *et al.,* 2012). Fisher *et al*. (2008) reported that temperature elevation during oestrus was associated with the luteinising hormone (LH) surge. Suthar *et al.* (2011) reported a significant increase in body temperature in cows with restricted movement (tie-stalls) during oestrus, which also suggests that the rise in body temperature is associated with the preovulatory LH surge, and not with increased physical activity. Interestingly, it is documented that lame cows have lower LH pulse frequency leading up to oestrus (Morris *et al.,* 2011). Therefore, lameness

affects the preovulatory LH surge that is associated with an increase in body temperature during oestrus (Fisher *et al.,* 2008). This may explain why lower core, eye and pocket temperatures were recorded from lame cows during oestrus in this study.

Additionally, prior to oestrus lame cows' core, eye, pocket, and pinbone temperatures were significantly different from non-lame cows, however their ear temperatures were not significantly different. Although lame cows had lower mean core temperatures than non-lame cows (38.1°C (±0.1) v 38.4°C (±0.1)), they were within the normal range reported for adult dairy cattle (38°C -39.3°C) (Ortiz *et al.,* 2015; Salles *et al.,* 2016). It may be that the lame cows restrict their overall activity when compared to non-lame cows, thereby causing lower baseline temperatures. Acute lameness and stress can reduce IRT skin temperature in various animal species such as chickens (Edgar *et al.,* 2013), rat tails (Marks *et al.,* 2009), human fingers (Vinkers *et al.,* 2013), rabbit ears (Yu and Blessing, 2009), and cows' eyes (Stewart *et al.,* 2008a). As lame cows are subjected to stress, it could be that the reduced temperatures are an effect of the sympathetically-mediated vasoconstriction. Although sympathetically-mediated vasoconstriction causes a rapid drop in skin temperature during an acute stressor, this typically causes an increase in core temperature (Oka *et al.,* 2001; Marks *et al.,* 2009) through stress mediated thermogenesis (Herborn *et al.,* 2015). Although chronic or long-term stress has been reported to reduce fingertip temperature in humans (Lin *et al.,* 2011), little research is available to determine if a chronic stressor such as lameness could cause lower core temperatures in dairy cattle.

Kovács *et al.* (2016) reported that dairy cows suffering from chronic stress caused by lameness had significantly lower heart rates than non-lame cows. They hypothesised that the reduced heart rates may have been caused by the reduced activity and food intake, as lame cows tend to increase lying times (Galindo and Broom, 2002; Blackie *et al.,* 2011). A potential source of error for core temperatures in the current study could be related to the fact that three different thermometers were used for the three different farms (Bio-security purposes). Although the same brand (©Nettex digital thermometer, Rochester, Kent, UK) was used, and the thermometers were calibrated each day, there could have been differences between individual thermometer readings. The procedure of taking core temperatures could potentially be a source of error as Burfiend *et al*. (2010) reported the penetration depth into the rectum can bias the results (up to 0.4°C). However, this was minimised in the studies in this thesis through the same individual (AW) recording all temperature measurements. Movement of the cow while the temperature was being recorded could potentially affect the penetration depth of the thermometer. Defecation can also slightly affect core temperature readings, Burfiend *et al*. (2010) reported that rectal temperature before and after defecation were minor (<0.1°C).

As the IRT device was handheld, temperature variations can occur if the distance to the target area change, and any camera movement can affect the accuracy of the readings (Hoffmann *et al.,* 2013). This may be why measurements from the ear and pinbone area were not significant when the other temperatures were. The distance and angle from the target area could potentially be a source of error however this was minimised in the studies in

this thesis through the same individual (AW) recording all IRT temperatures. Ear temperatures may increase when the cow is in oestrus, however increased blood flow can also be due to physiological changes to aid in cooling their body temperature during hot weather (Klopcic *et al.,* 2009). Cows with thick coats have lower IRT temperatures recorded, therefore if the hair in the ears is quite long it can interfere with accurate temperature recordings. Therefore, the use of IRT on body areas such as the pinbone, and ear are not consistently accurate.

The pocket area is close to internal core body temperature, however from a practical point of view, obtaining an accurate temperature reading from this area would be challenging. The area would need to be cleaned if any debris/faecal material was on the skin, and the tail must be lifted to record the temperature. Lifting the tail pinches the vertebrae and caudal nerves (Weaver, 2010), which could become uncomfortable after time. Additionally, if the tail is held up for an extended period of time the skin surface cools, especially if there are any environmental factors such as cool winds. Therefore, the use of eye measurements is appealing due to its accessibility and relatively close temperature relationship to the core. The search for reliable oestrus detection methods that do not rely on standing oestrus is important. Not only due to a decrease in oestrus expression, but also in tie-stall housing systems. Felton *et al*. (2012) were unable to detect oestrus using activity monitors in a tie-stall environment despite previous studies finding them to be effective in free stalls (Roelofs *et al*., 2005), and in tie-stall facilities that allow out of stall exercise (Kiddy, 1977; Redden *et al*., 1993; Kennedy and Ingalls, 1995). Although tie-stalls are banned, or are being phased out of some countries (Bergsten *et al*.,

2015), 61% of Canadian dairies (Denis-Robichaud *et al*., 2016), and nearly 40% of dairies in the USA are tie-stall establishments (USDA, 2014). Therefore, the use of alternative oestrus detection methods that do not rely on activity are essential. IRT sensors could be positioned in tie stalls, feeding areas, or the milking parlour. Further investigation is required to determine the ideal placement of such sensors in a dairy facility.

Dairy cows subjected to increased ambient temperatures will have physiological changes that thermoregulate their core temperatures (Min *et al.,* 2015). These responses include vasodilatation (increasing peripheral blood flow), increased sweating rate, and increased respiratory rates to enhance heat loss (Ammer *et al.,* 2016). Heat stress can lead to hyperthermia, subsequently causing a rise in core body temperature (Min *et al.,* 2015). Genetics can also play a role in how sensitive a cow is to ambient temperature changes. Daltro *et al*. (2017) found that purebred Holstein cows are more sensitive to ambient heat than crossbred cattle (Holstein x Girolando). Therefore, utilising IRT as an oestrus detection method in hot climates, or during warm summer months may not be suitable, and would need further investigation.

Although cattle eye IRT temperatures are not affected by ambient temperatures (Gloster *et al.,* 2011), they can be influenced by wind and direct sunlight (Church *et al.,* 2014). If the cattle are exposed to sunlight immediately before an IRT measurement, this may cause a false positive for increased body temperature. The temperatures recorded in this study were collected in the freestall house, away from direct sunlight. Cooling systems such as fans, or

natural wind can cause lower readings, which could also lead to a false negative (Church *et al.,* 2014). One farm in this study used cooling fans in the summer months. This could have affected the IRT readings collected from these cows. Fans could result in cattle with elevated body temperatures going undetected by the IRT system, potentially missing oestrus events. If an automated system was developed to monitor IRT eye temperatures the system would have to record these temperatures in areas free of sunlight and draughts, such as a feed trough in the milking parlour. Environmental factors such as ambient temperature and relative humidity can affect the accuracy of IRT readings from the body (Taulkder *et al.,* 2014). Various studies have investigated the ideal ambient temperature for performing IRT. For example, Love and Linsted (1976) suggested that an ambient temperature of approximately 20°C is most appropriate for the use of IRT. However, another study by Turner *et al*. (1986), reported that 30°C is an acceptable ambient temperature for IRT. Sykes *et al*. (2012) observed noticeable vulval temperature changes in gilts, between oestrus and di-oestrus groups at ambient temperatures below 10°C. Temperatures collected from cows from the current study were carried out in various ambient temperatures. Farm 1 (U.K.) had temperatures collected from March-August, whereas the Canadian farms data collection took place in July. The mean temperature in March was 5°C, whereas the mean temperature in August was 16°C. Both the Canadian and UK mean temperatures in July was 16°C. Despite these differences, before each thermal scanning session, the emissivity value was set to 0.98. and thermograph resolution was calibrated to ambient temperature and humidity. Therefore, these effects would have been minimised. Statistical analysis in this study accounted for farm variation by blocking to reduce residuals. Relative

humidity alters the components of the atmosphere, which needs to be accounted for during IRT (Kastberger and Stachl 2003). At particular wavelengths, certain components of the atmosphere such as vapour and carbon dioxide absorb IR radiation (Kastberger and Stachl 2003; Stelletta *et al*., 2012). If environmental humidity is not accounted for, the temperature recorded may be overestimated (Kastberger and Stachl 2003). Ambient temperature and humidity are potential barriers to the accuracy of the surface temperatures recorded. However, if each device is calibrated for each specific farm, the effect of these factors would be reduced. IRT needs to be evaluated in different ambient conditions.

Additionally, cows that are not in oestrus themselves may interact with cows that are in oestrus, which may potentially cause false positive oestrus alerts as increasing physical activity can elevate the cows' body temperature (Harris and Starnes, 2001; Piccione *et al.,* 2003). Further research would be beneficial to measure temperatures of cows that are not in oestrus, but are engaging in oestrus activities to assess if their body temperatures significantly increase.

As one farm included in this study synchronised their cows, this may have had an effect on the temperatures recorded. Research has shown that fertility is increased in cows that express oestrus at fixed-time AI (Galvão *et al*., 2004; Pereira *et al*., 2014; Pereira *et al*., 2016). Oestrus intensity is greater and duration of oestrus lasts longer with increasing numbers of cows in oestrus simultaneously (Sveberg *et al*., 2013 ; Zebari *et al*., 2019). In non-synchronised cows' there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. There is a possibility that the synchronised cows

had increased activity when compared to the other farms, which could affect temperature readings. However, as previously mentioned studies suggest that the rise in body temperature is associated with the preovulatory LH surge, and not with increased physical activity (Fisher *et al*., 2008; Suthar *et al.*, 2011). This is supported from the findings of Perez Marques *et al*. (2019) as they investigated IRT increases from synchronised cows in a tie stall environment. Further research would be required to identify if synchronisation affects IRT and core readings.

Timing of temperature collection during oestrus varied for all cows between the farms. Due to the design of this study, it was not possible to determine the duration of oestrus for each cow, or how far into oestrus the cow was at the time of temperature collection. Studies have shown that cows have varied oestrus durations. For example some studies report an average duration of oestrus of 7h (range 5.1h to 10.6h) (pressure sensitive radio frequency transmitter) (Dransfield *et al*., 1998), 8.1h (activity monitor) (Løvendahl, and Chagunda 2010), 10.7h (range 2h to 14h) (Ultra-wideband technology, visual and milk progesterone) (Homer *et al*., 2013), and 16h (accelerometer) (Valenza *et al*., 2010). Talukder *et al.* (2014) reported a duration of IRT oestrus of 13.6h. However different oestrus detection methods used in these studies would be expected to create discrepancies (Talukder *et al*., 2014). Furthermore, reported time and extent of body temperatures rises during oestrus vary in the literature. For example, core body temperature rises of 0.3°C to 1.1°C (Kyle *et al*., 1998), 0.9°C to 1.3°C (Piccione *et al*., 2003), and maximum of 0.5°C (Suthar *et al*., 2011) during oestrus have been reported with durations of elevated temperatures ranging from 7 (Redden *et al*., 1993; Rajamahendran e*t al*., 1989)

to 11 hours (Mosher *et al*., 1990). The current study reported an increase of core body temperature of 0.59°C which is in agreement with previous studies. Studies evaluating IRT report a sharp decrease in body temperature at several body points (muzzle, vulva, cheek, neck, eye, withers) 48 hours before ovulation followed by a significant rise (+0.5°C and 1.20°C) 24 hours before ovulation (Talukder *et al*., 2014; Perez Marquez *et al*., 2019). The pre-oestrus decrease in body temperature is most likely related to regression of the corpus luteum (Kyle *et al*., 1998). Although eye and pocket IRT increases (+0.58°C, 0.66°C respectively) in the current study are within the range of those reported by Talukder *et al*. (2014), they are slightly lower. Differences in temperature increases and oestrus duration may be attributed to different housing and management, (pen housing, tie stall, cooling systems, milk yield etc.). Furthermore Talukder *et al*. (2014) reported that cow factors such as body condition score (BCS), parity, locomotion score (LCS) and milk yield affect the IRT oestrus duration and time to ovulation. They reported that cows with a BCS of 2.5 had a longer interval between IRT oestrus alert and hours after PGF2α treatment compared with cows with a BCS of 3.0 or more. They also reported that primiparous cows had longer average (8 hours) duration of oestrus when compared to multiparous cows, and cows with a LCS of 1 had a longer duration of oestrus compared to cows with a LCS of 1.5. Additionally, with each 1 kg increase in milk yield there was a 0.48 hours average decrease in oestrus duration. This decrease associated with increased milk yield could be attributed to a reduction in oestradiol during the follicular phase as a result of hepatic steroid metabolism (Sangsritavong et al., 2002; Wiltbank *et al*., 2006). Although Talukder *et al*. (2014) reported cows with a LCS of 1.5 had a decreased

duration of oestrus, Walker *et al*. (2010) reported that lameness did not affect the overall duration of oestrus, but lameness did shorten the period when herd-mates attempted to mount the lame cows. Interestingly Talukder *et al*. (2014) excluded cows with LCS greater than 2, so all animals in their study were classified as non-lame. However, as Talukder *et al*. (2014) investigated IRT oestrus alerts, it may be that IRT detects subtle temperature increases, thereby indicating a longer duration of oestrus compared to other detection methods. The differences in oestrus duration between LCS could also be attributed to the relatively small sample size in their study (n=20).

Although cows in this study were visually observed for oestrus behaviours prior to temperature collection, determining the length of oestrus for each cow would have been beneficial. The lower IRT temperature increases in this study may be related to the time the temperatures were collected in relation to oestrus duration. As Talukder *et al*. (2014) reported different oestrus durations for non-lame cows with a half score difference, it would be interesting to determine the IRT durations for a range of LCS. Obtaining detailed temperature profiles would be beneficial to determine if lame cows' temperatures increase for as long as non-lame cows, or if lame cows have a different temperature peak from non-lame cows. This study did not collect core or IRT temperatures at various time points. To minimise disturbances to the cows, minimal invasiveness was exercised. As restraining cows can cause stress that alters temperatures (Bewley *et al*., 2008), it was decided not to restrain the cows more than necessary, thereby deciding to obtain two core measurements. Standardising temperature collection requires further investigations such as continuous monitoring and observations (temperature and behavioural), uterine ultrasound

scans (to monitor ovarian activity), and blood analysis (to monitor progesterone and LH). This would enable a detailed description and pattern of the physiological differences between lame and non-lame cow.

Based on the results from this study, using IRT scans from areas other than eyes has limitations for oestrus detection. IRT scans of the eyes has potential for the development of an automated system. The use of IRT is not only promising as an oestrus detection aid, but it also has potential as a lameness detection tool. As lame cows from this study have reduced temperatures, these animals could be detected from the main herd. More research is required with more animals to identify if this pattern is present among all lame cows. These results give insight to the effect of lameness on fertility, as lame cows had lower temperature readings than non-lame cows. Lameness affects locomotion and hormonal parameters, both, which also affect core temperatures. By using IRT as an oestrus detection aid, cows can be detected and inseminated, while also being identified as lame based on the lower temperature readings. If routine locomotion scoring is not carried out, lameness may go undetected. Additionally, it is reported that farmers can underestimate lameness in their herds (Alawneh *et al*. 2012; Fabian *et al.,* 2014), or fail to accurately identify lame cows all together (Whay *et al.,* 2003). Implementing IRT may assist in diagnosing lameness without missing an oestrus event, leading to fewer days open, or reducing premature culling. Lame cows may go undetected using conventional oestrus detection methods if they do not exhibit normal oestrus behaviour (standing to be mounted, increased activity). The use of IRT may be able to identify these animals, potentially reducing the negative economic impact of lameness on fertility. Further research would be beneficial to identify

if the concentration of preovulatory LH is associated with the locomotion score (1-5), and temperature readings. As lame cows have been reported to have lower progesterone concentrations leading up to oestrus (Walker *et al.,* 2010), it would be beneficial to assess if progesterone concentrations are also associated with temperature readings. Monitoring eye and core temperatures continuously would be beneficial to determine if, and at what point the onset of lameness affects the temperature readings of lame cows. Recent research has determined that IRT readings from the forehead are closely associated with core body temperature (Salles *et al.,* 2016), therefore it would be beneficial to investigate this as a means of oestrus detection, and if lameness would affect outcomes.

7.10 Conclusions

During oestrus temperatures recorded from the core, eye, ear, and pocket areas were significantly higher than baseline temperatures in all cows. Lame and non-lame cows had significantly different baseline temperatures from the core, eye, pocket and pinbone areas. Lame and non-lame cows had significantly different temperatures when in oestrus from the core, eye and pocket areas. IRT can be implemented to identify oestrus in lame and non-lame cows, in addition to potentially detecting lameness based on temperature readings. Further research is required on multiple farms with more animals to further validate findings from this study.

Chapter 8: General Discussion and Conclusions

8.1 Research Findings

8.1.1 Lameness and Fertility

It is well documented that lameness affects fertility, including oestrus behaviour and expression. This study set out to investigate the efficiency of different oestrus detection methods between lame and non-lame dairy cattle. One of the aims of this research was to assess dairy producers' perception of reproductive efficiency between lame and non-lame cattle, how they manage oestrus detection in their lame cattle, if they use different methods for lame and non-lame cows, and what influences their choice of oestrus detection method. The results presented in this thesis show that lame cows have reduced reproductive efficiency (Chapter 4 and 5), increased number of inseminations to conception (Chapter 4), lower progesterone concentrations up to 7 and 10 days before ovulation (Chapter 5 and 6), increased number of days from calving to first service, and from calving to conception (Chapter 5). These results are common among lame cows and are consistent with many other studies (Sprecher *et al.,* 1997; Whay *et al.,* 1997; Dobson and Smith, 2000; Hernandez *et al.,* 2001; Melendez *et al*., 2003; Garbarino *et al.,* 2004; Walker, 2008a; Olmos *et al.,* 2009; Walker *et al*., 2010; Morris *et al*., 2011). These results presented in this thesis indicated that the oestrus detection methods chosen are influenced by housing systems. It was determined from the answers that lame cows require more inseminations to conception, however n=67 did not know, or do not keep track of the average number of inseminations for lame cows, and n=25 of respondents reported the same number of inseminations required for lame and non-lame cows.

Additionally, when asked who locomotion scores the cows n=67 said themselves (mean lameness prevalence 5.6 (±0.8), and n=23 responded that no locomotion scoring is carried out (mean lameness prevalence 8.1 (±2.3). Lameness can be underestimated by farmers (Hollenbeck, 1978; Wells *et al*., 1993; Whay *et al.*, 2002; Espejo *et al.,* 2006; Alawneh *et al*., 2012; Fabian *et al.,* 2014), therefore the impact of lameness on fertility may not be fully identified by producers if locomotion scoring is done themselves, or not at all. It was determined from the answers that lame cows alter their oestrus behaviour(s). However, the majority of respondents use the same oestrus detection methods for all animals, despite marked behavioural differences between lame and non-lame cows. Additionally, producers that take additional measures to ensure conception (e.g. isolation/recovery pen) in lame cows may be faced with oestrus detection limitations when using conventional detection methods.

8.1.2 Lameness, Activity and Oestrus Expression

The results presented in this thesis determined that lame cows had reduced activity, and lower progesterone concentrations prior to ovulation when compared to non-lame cows (Chapter 5 and Chapter 6). These results are common among lame cows and are consistent with previous studies (Dobson *et al*., 2008; Walker *et al*., 2008a; Walker *et al*., 2008b; Walker *et al*., 2010). A reduction in oestrus activity could be partly explained by the decrease in progesterone priming which reduces responsiveness to oestradiol, thereby reducing oestrus intensity (Fabre-Nys, and Martin, 1991; Walker *et al*., 2008b). Or that the physical pain causing the lameness reduces walking and mounting activity. Interestingly the studies from this thesis report no difference in lying

times between lame and non-lame cows which contradicts previous studies. It is generally accepted that lame cows have increased lying times compared to non-lame cows (Hassall *et al.,* 1993; Galindo and Broom, 2002; Walker *et al.,* 2008a; Blackie *et al.,* 2011; Navarro *et al.,* 2013). The fact that lame cows from this thesis did not have different lying times from non-lame cows could be related to the severity of lameness. Other studies report no significant differences in lying times between moderately lame (cows with score 3 and 4) and non-lame cows (score 1) (Ito *et al*., 2010; Yunta *et al*. 2012; Miguel Pacheco *et al.* 2016). For example, Ito *et al.* (2010) reported that lying bout duration and total lying time were increased in severely lame cows rather than moderately lame cows. This would explain no significant differences in lying times from lame and non-lame cows in the studies from this thesis, as lame cows had a mean LCS of 3.1 (±0.2), and four cows had LCS of 4. No cows scored 5 (severely lame). The fact that this herd was given access to pasture may have reduced the effect of lameness on behaviours such as lying times, as seen in Juarez *et al*. (2003) and Hernandez-Mendo *et al*. (2007). Even though lameness did not affect lying times in this study, it is evident that oestrus activity was reduced even in mildly/moderately lame cows. Another research aim from this thesis was to assess if improvements in locomotion score affected oestrus expression. Chapter 5 reports that lameness prevalence reduced during the summer months, when seasonal grazing took place. There was also significant improvement in locomotion scores (by 0.21 units/week) after pasture access for all of study cows (both lame and non-lame). It is generally accepted that cows managed under zero-grazed systems have higher lameness rates compared to grazing herds (Haskell *et al*., 2006; Ranjbar *et al*., 2016). Other

studies also report improved LCS after pasture access (Hernandez-Mendo *et al*., 2007; Cook and Norlund, 2009; Olmos *et al*., 2009; Somers *et al*., 2015). To the knowledge of the author, there is no other study examining the relationship between improved LCS and oestrus activity over time. Results showed that as the study cows LCS improved, subsequent oestrus activity (step counts, motion index) also increased. Cows with a LCS of 1 at the time of oestrus had significantly more steps than a LCS of >2.5 during an oestrus event., whereas cows with a LCS of 2 were not significantly different from the other LCS. One reason LCS 2 was not significantly different could be that the cows were transitioning from LCS 3 to 2, and the lameness still could have been affecting activity. As oestrus activity improved alongside LCS, this study reinforces the recommendation for reducing lameness rates, which can be achieved through management protocols, such as pasture access.

8.1.3 Oestrus expression and detection

To the knowledge of the author, there is no other study comparing oestrus detection methods between lame and non-lame cows. The results from this study determined that there was no significant difference in the efficiency of oestrus detection methods in lame and non-lame cows. Estrotect™ scratch cards were more efficient at pasture than in housed conditions, which agrees with previous studies (Vailes and Britt, 1990; Palmer *et al*., 2010). The activity monitors were most accurate at correctly identifying oestrus activity, followed by Chalk. Chalk is inexpensive and easy to apply; however, this method requires time to check the chalk and reapply as needed. Although there was no statistical significance in the efficiency of oestrus detection methods between lame and non-lame cows, the activity-based systems (NeDap,

IceQube) assisted in detecting a silent oestrus in a lame cow, when the mount detectors did not. As lame cows had a mean LCS of 3.1, it could be possible that the severity of lameness did not affect oestrus detection efficiency. However, it is possible that if all milk progesterone samples were analysed, more silent heats could have been detected. As the first oestrus postpartum is associated with reduced oestrus activity (Ranasinghe *et al*., 2010) using a lower activity threshold (increases of 80-100%; Ranasinghe *et al*., 2010) paired with progesterone analysis may assist in detecting more oestrus events. Using more than one oestrus detection method may not be viable on most farms. However, the questionnaire data from Chapter 4 determined that 51% of respondents used two oestrus detection methods, 35% used one, and 14% used more than three. If using more than one oestrus detection method proved to be efficient and cost effective, the uptake may be higher. Chapter 4 revealed that producers consider more than one factor when choosing an oestrus detection method, which agrees with Garforth *et al*. (2006). The results from the questionnaire in this thesis determined that cost and ease were the most important factor when choosing an oestrus detection method, followed closely by cost, ease, and accuracy. With growing dairy herd sizes accompanied with increasing milk production, and poor reproductive performance (including reduced oestrus expression) perhaps implementing automated oestrus detection systems with a lameness aspect would improve productivity, and animal welfare. The study in this thesis used milk progesterone assays as the gold standard to verify ovulation, which was 100% accurate. Similar to a study by McLeod *et al.* (1991) that determined milk progesterone testing accurately predicted 99% of ovulations in their study group. Muasa *et al*. (2017) reported that cow side milk progesterone testing was more accurate at detecting oestrus

than Estrotect™ scratch cards. In-line milk progesterone analysis would eliminate the need to apply oestrus detectors. Furthermore, cows that have disrupted cycles, or are experiencing silent heats would be identified earlier. A study using in-line milk progesterone analysis for a herd of 93 cows successfully reduced the number of services to conception by 0.2, reduced the calving index by 12 days, and eight fewer cows were culled for infertility, saving a total of £6829, or £73/cow (Mann, 2000). Although the integration of a system like this may be costly, annual savings for improved fertility and reduced culling rates may cover the cost. It is possible that lameness could be incorporated into the system, as progesterone is lower in lame cows (Walker *et al*., 2010). Another automated oestrus detection method that would be able to identify lameness is infrared thermography (IRT). Recent developments of infrared thermography (IRT) for oestrus detection (Talukder *et al.,* 2014; Talukder *et al.,* 2015; Perez Marques *et al*., 2019) led to further investigations in this thesis including lame cows. To the knowledge of the author this is the first study to evaluate core body temperatures, and IRT scans (core, eye, ear, pinbone, and pocket) from lame and non-lame cows as an oestrus detection method. It was determined that during oestrus, temperatures recorded from the core, eye, ear, and pocket areas were significantly higher than baseline temperatures in all cows. These results indicate that detecting oestrus using IRT body temperature scans is achievable, which is in agreement with other studies (Talukder *et al.,* 2014; Talukder *et al.,* 2015; Perez Marquez *et al*., 2019). The eye is an easily accessible area, therefore the development of IRT scans from this area is promising. The results from this thesis indicate that using IRT scans of the eye can be a useful oestrus detection method for both lame and non-lame cows, as their IRT eye temperature significantly increases from their baseline

temperature when in oestrus. Furthermore, lame cows had significantly lower baseline (core, eye, pocket and pinbone), and oestrus temperatures (core, eye and pocket) than non-lame cows. Previous research determined that acute pain and stress can affect body temperature (Stewart *et al.,* 2008a), but there is limited information on the effect of chronic stress on dairy cow temperatures. Kovács *et al.* (2015) reported that dairy cows suffering from chronic stress caused by lameness had significantly lower heart rates than non-lame cows. Therefore, the temperature differences between lame and non-lame cows in this thesis may be attributed to the chronic stress experienced with lameness. In addition to oestrus detection, IRT can be implemented to detect lameness based on temperature readings.

8.2 Research Implications and Future studies

Oestrus activity and behaviour was recorded using NeDap and IceQube activity monitors which have been used in scientific studies to date (Dolecheck *et al*., 2015; Roelofs *et al*., 2017). However, the present studies comparing oestrus detection methods in lame and non-lame cows were to the authors' knowledge some of the first to be carried out. The findings from this thesis determined that although lame cows did not have different oestrus detection efficiencies, they did have lower activity measures (Chapter 5 and 6), lower milk progesterone values (Chapter 6), and lower baseline and oestrus temperatures (Chapter 7). The severity of lameness in cows from this study may be why common oestrus detection aids were not different between lame and non-lame cows (Chapter 6). As the cows from this study were classified as mildly lame, further research would be beneficial to examine the efficiency of oestrus detection methods in

cows with more compromised locomotion. Therefore, comparisons could be made from different herds and numerous cows to further identify if different oestrus detection methods are more efficient in detecting the problem or lame cow. Furthermore, continuous observation during oestrus, or devices that record the number of mounts received would be useful to identify if the mount detectors (Chalk, Estrotect^{TM} scratch cards, Kamar®) are being activated from actual mounts received, or secondary oestrus behaviours (Licking, chin resting).

Although lameness had no effect on oestrus detection efficiencies in this study, it was determined that after pasture access locomotion scores improved, which resulted in increased oestrus activity during successive oestrus events (Chapter 5). Therefore, even mild lameness affects oestrus activity despite no difference in other activity measures (lying time). Other studies report improved locomotion scores after pasture access (Hernandez-Mendo *et al*., 2007), however to the knowledge of the author there are no other studies that have examined multiple oestrus events in lame and non-lame dairy cows. The results from this thesis can be useful for future research to perhaps develop a management protocol to improve LCS with the aim to also improve oestrus activity, and oestrus detection rates. Future studies could assess if the increase in oestrus activity/expression associated with improved locomotion score (LCS) after pasture access (discussed in Chapter 5) is also correlated with progesterone concentrations. It would also be useful to compare different oestrus detection methods as lame cows' transition from different LCS, and to see if primary oestrus behaviours increase with improved LCS. This could be done by implementing an oestrus detection system that counts the number of

mounts received. These findings also highlight that the impact of lameness is significant even on a mild level. Disseminating the findings to dairy producers can reiterate the importance of preventing and treating lameness to minimise its impact on reproductive performance.

As other studies report lame cows not increasing their activity, or standing to be mounted, the findings from Chapter 7 (IRT) can be useful for future research. As IRT has been evaluated by other researchers for oestrus detection, the findings that lame cows have lower temperatures can assist future studies using IRT. For example, the results from this thesis can be used to further develop the use of IRT for lameness, and oestrus detection in the lame cow. As lame cows can have lower progesterone (Walker *et al.,* 2008a), and luteinising hormone (LH) (Morris *et al.,* 2011) concentrations leading up to oestrus, future studies could include further testing to determine if these hormonal parameters in lame cows is associated with lower core and IRT eye temperature readings on the day of oestrus. It is also reported that progesterone supplementation in cows (during postovulatory progesterone rise) with poor fertility increases the chance of pregnancy (Yan *et al*., 2016). Therefore, it would be worth investigating if supplementing lame cows with progesterone increases oestrus expression and their chance of pregnancy. Additionally, monitoring eye and core temperatures over a long period of time would be beneficial to ascertain at what point the onset of lameness affects the temperature readings of lame cows. These should be tested on different herds with numerous cows to determine any associations. This thesis determined that cows within a housed system did not have fluctuating body temperatures (Section 7.2), therefore further investigations to ascertain if cows within a

pasture-based system have fluctuating core and eye (IRT) temperatures would be beneficial. Recent research has determined that IRT readings from the forehead are closely associated with core body temperature (Salles *et al.,* 2016), therefore it would be beneficial to investigate this as a means of oestrus detection, and if lameness would affect outcomes.

Section 7.2 of this thesis recorded temperatures over time to see if there were significant differences throughout the day. The findings determined that there were no significant differences from housed cows, therefore future studies can record temperatures during these times without the need to take multiple measurements. The findings from Chapter 7 determined IRT scans from the pinbone area were not representative of core body temperature, the pocket area is not practical, and the ear temperatures can be affected by hair. Eye temperatures are easily accessible, and can be a useful proxy for core body temperature (Gloster *et al.,* 2009). Therefore, a method has been developed for future studies, for example eliminating the need to record temperatures from areas of the body that are not practical, or representative of the core body temperature.

8.2.1 Practical Implications

A practical benefit of using Ridgeway milk progesterone assays to detect oestrus is that it can also identify cows with disrupted cycles, pregnant cows, and cows that have silent heats. The drawback for this type of progesterone analysis is that the kit is designed for laboratory use. However other ‘on farm’ milk progesterone kits are available. These also have limitations as the milk

sample has to be collected for each cow, and the test strip may take up to 5 minutes to reveal a result. Movement towards automated in-line milk progesterone systems would eliminate the time required to test each cow.

NeDap activity monitors are effective at detecting activity increases and decreases, however care must be taken when using this device on seasonally grazed cattle. The increase in activity after initial pasture access can cause false positive alerts. If this system is used on seasonally grazed herds, additional observations should be made to ensure any cows that are in oestrus do not go undetected.

A practical benefit of using Infra-red thermography (IRT) to detect oestrus is that it can also be used to identify disease and lameness. Underestimation of lameness in dairy herds is common, and developing an automated lameness detection system that can also detect oestrus would be useful. The drawback for this technology is that it can be affected by environmental conditions, however the practicality of implementing an automated IRT system will improve with further refining.

8.3 Overall Conclusions

Findings from this study has highlighted that even mild lameness can affect reproductive performance, and oestrus activity in dairy cattle. Pasture access improved locomotion score, and increased oestrus activity in subsequent oestrus events. This work has highlighted solutions for improvement of oestrus expression and detection. This work also evaluated a modern technology to improve oestrus detection, in both lame and non-lame cows. These studies

demonstrated that IRT can accurately identify cows in oestrus, and has the potential to identify lame cows, as they had reduced temperatures when compared to non-lame cows. This study provides an insight of the potential of infrared thermography for increasing oestrus detection, and for automated lameness detection. This could assist in minimising lameness rates worldwide, which could reduce the decline in dairy cow fertility contributing to sustainability of the dairy industry.

Chapter 10: References

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Appendix 1: IceQube Validation Graphs Pasture conditions

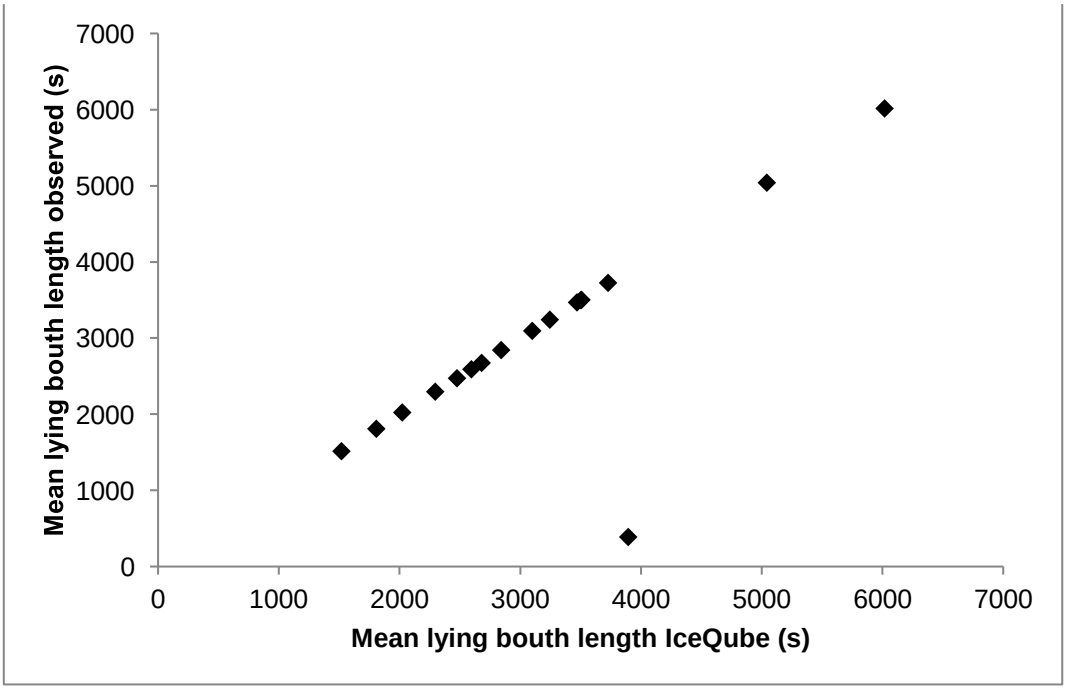

Figure 11- 1: The relationship between lying bout length determined from IceQube and CCTV footage from cow 43

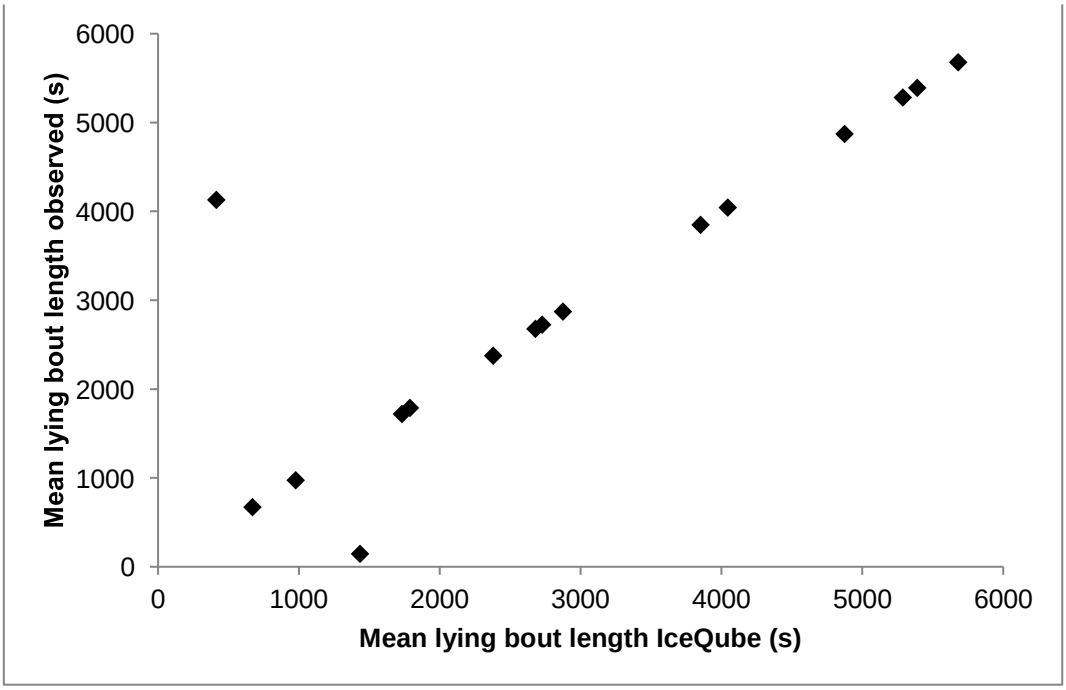

Figure 11- 2: The relationship between lying bout length determined from IceQube and CCTV footage from cow 140

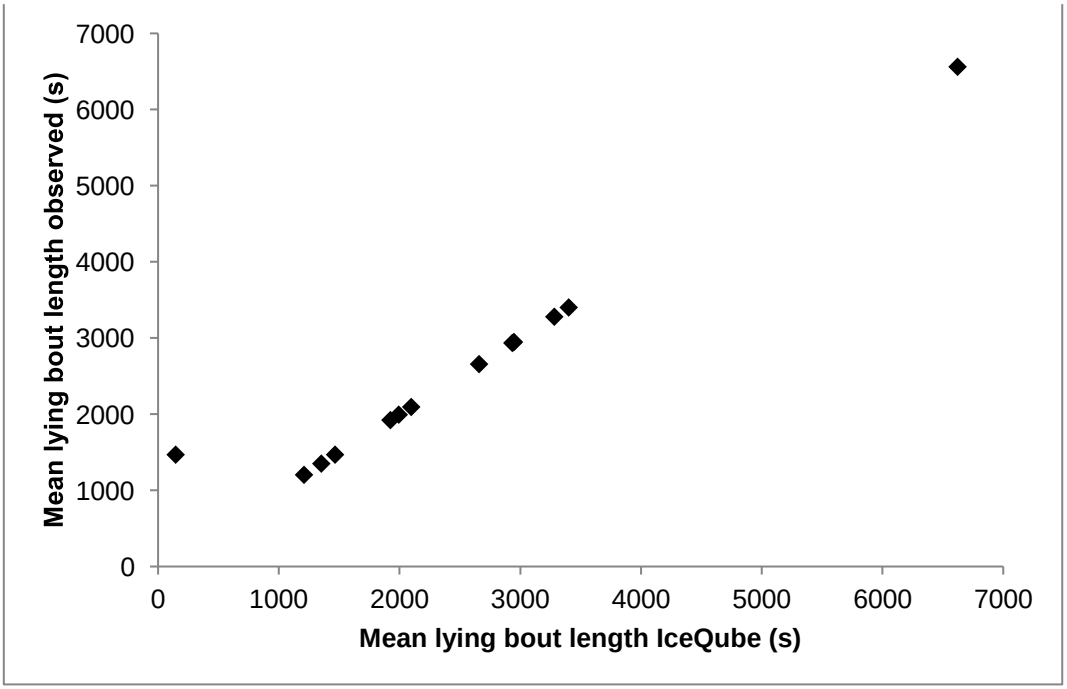

Figure 11- 3: The relationship between lying bout length determined from IceQube and CCTV footage from cow 2

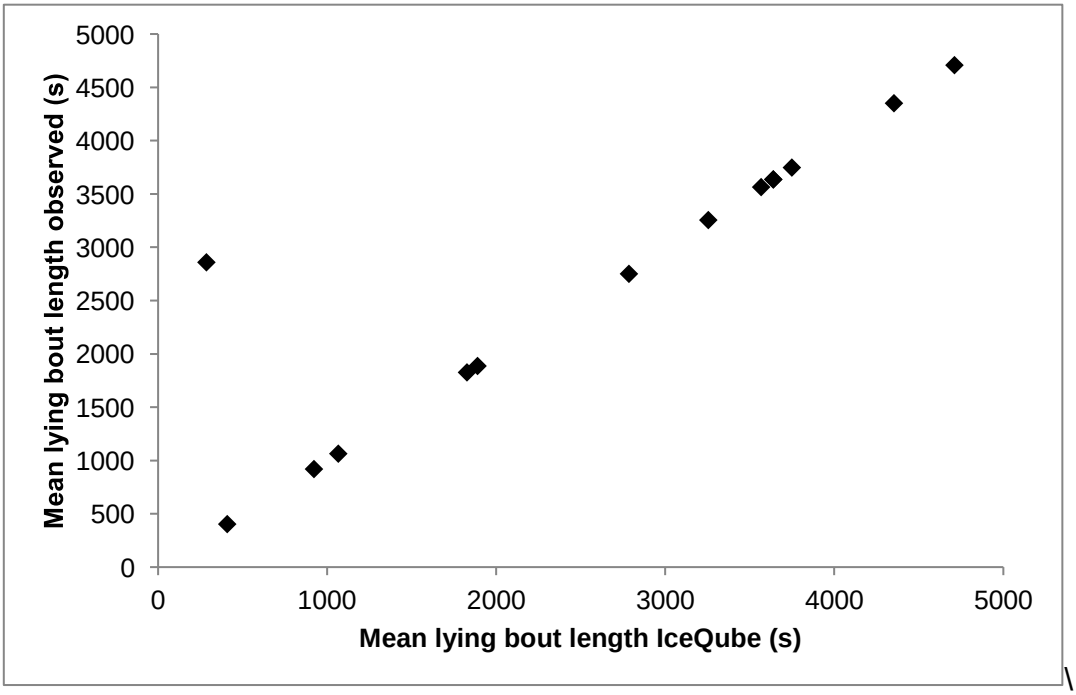

Figure 11- 4: The relationship between lying bout length determined from IceQube and CCTV footage from cow 186

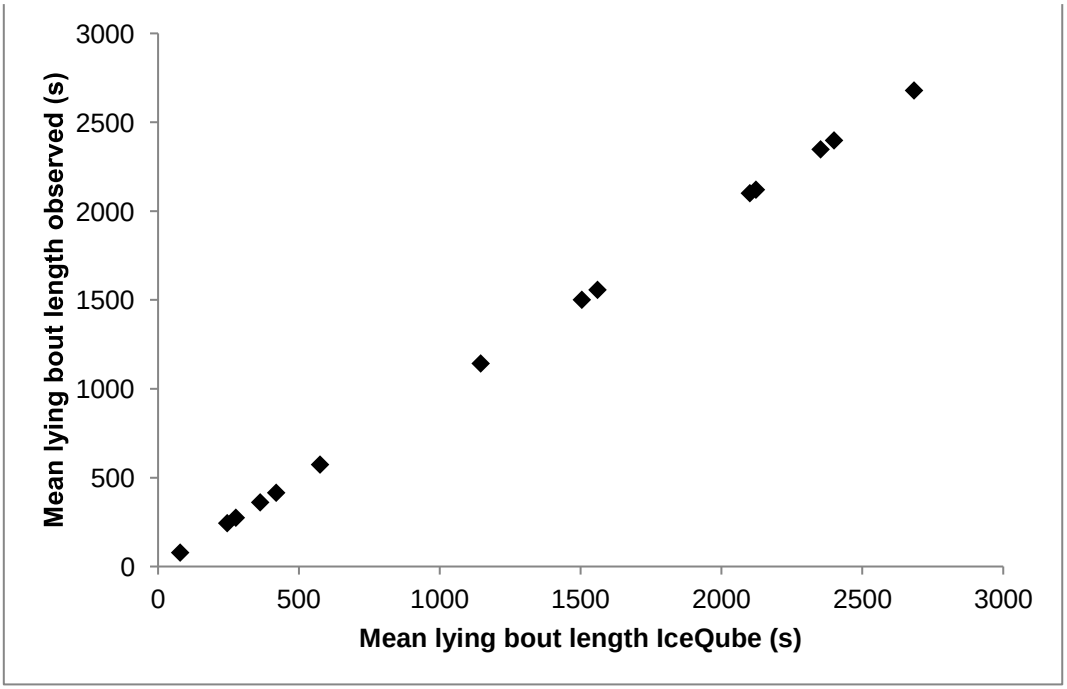

Figure 11- 5: The relationship between lying bout length determined from IceQube and CCTV footage from cow 164

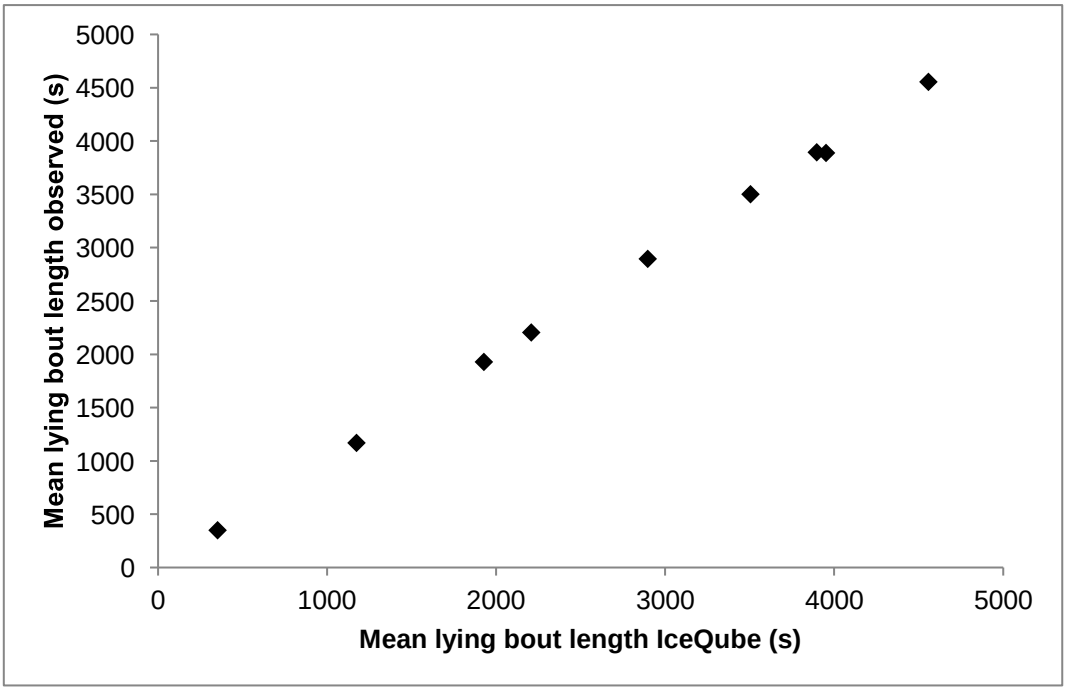

Figure 11- 6: The relationship between lying bout length determined from IceQube and CCTV footage from cow 145

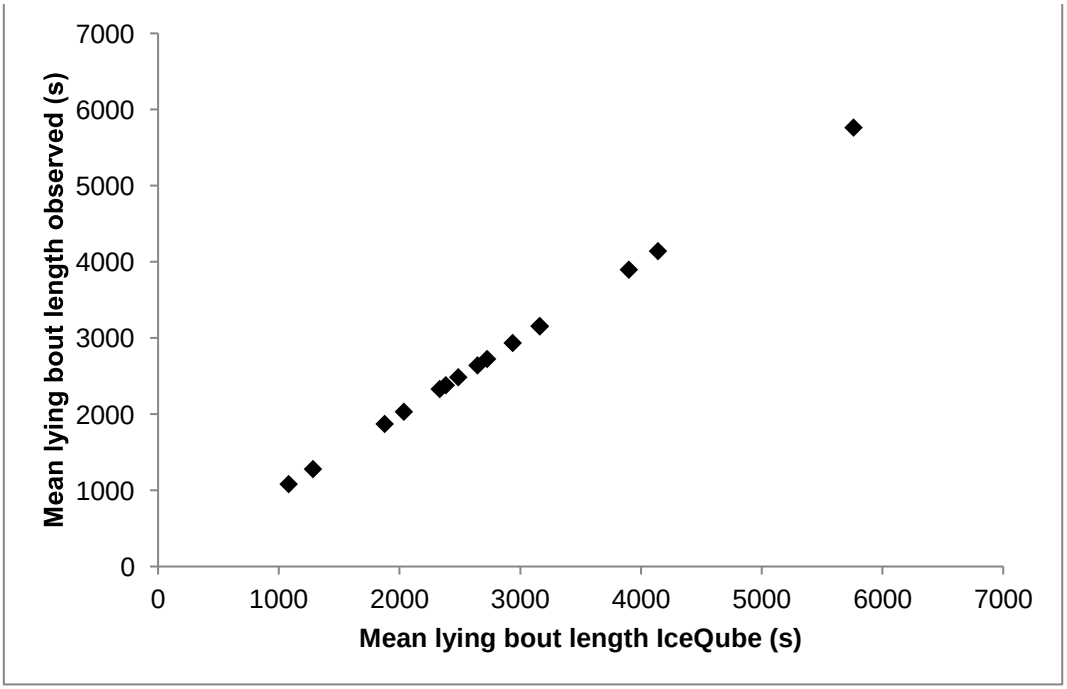

Figure 11- 7: The relationship between lying bout length determined from IceQube and CCTV footage from cow 62

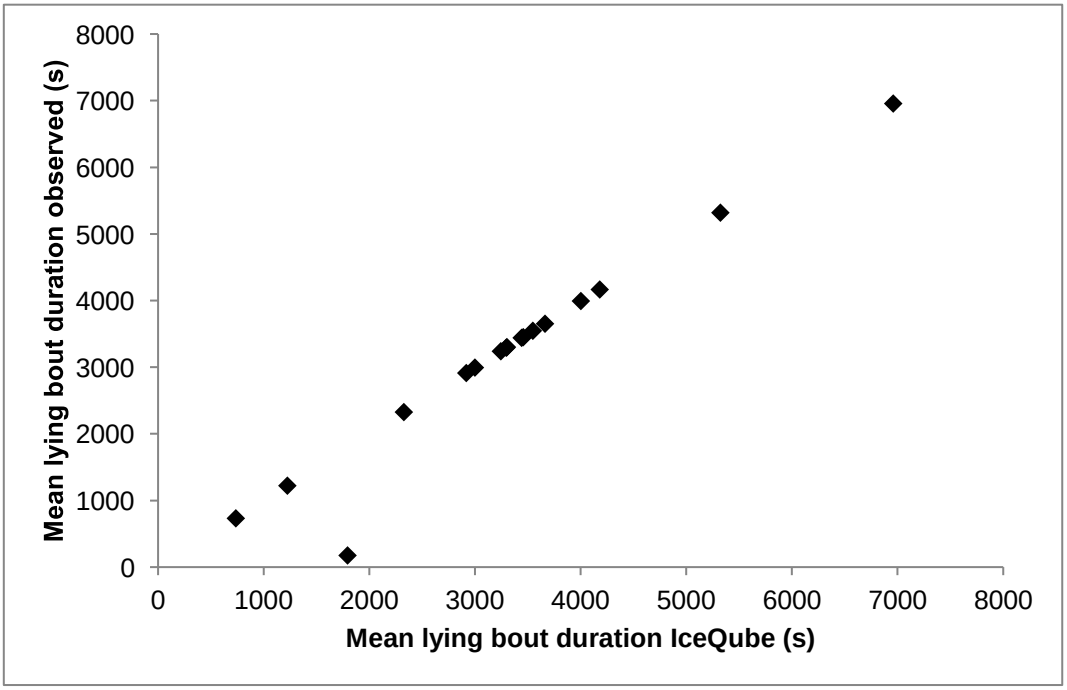

Figure 11- 8: The relationship between lying bout length determined from IceQube and CCTV footage from cow 47

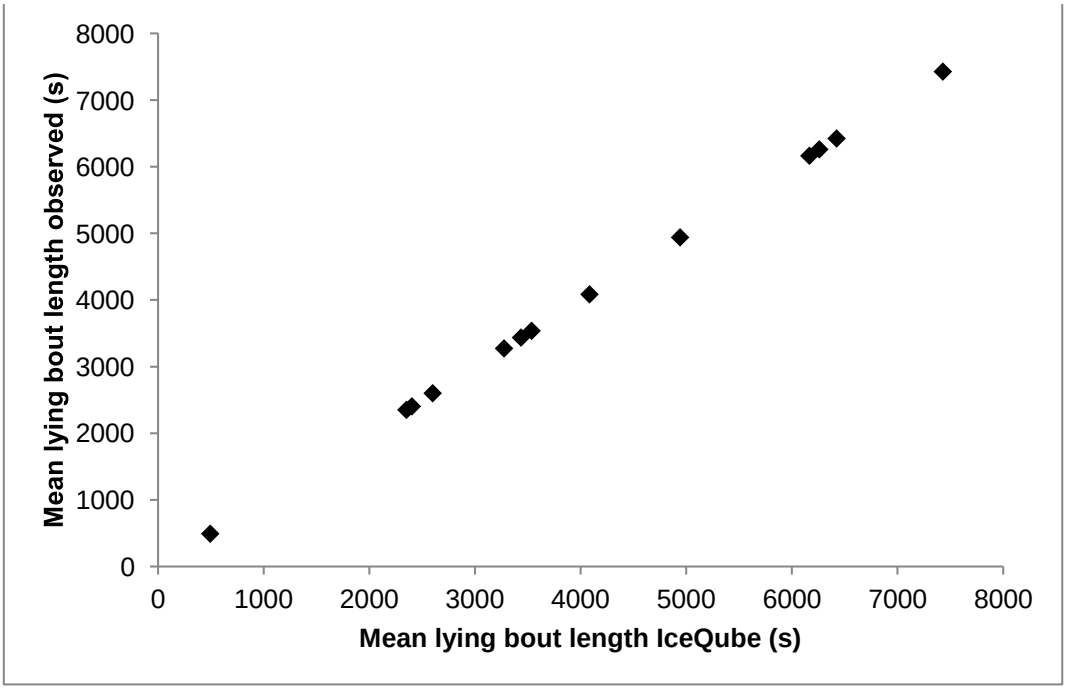

Figure 11- 9: The relationship between lying bout length determined from IceQube and CCTV footage from cow 36

Appendix 2: IceQube Validation Graphs Housed Conditions

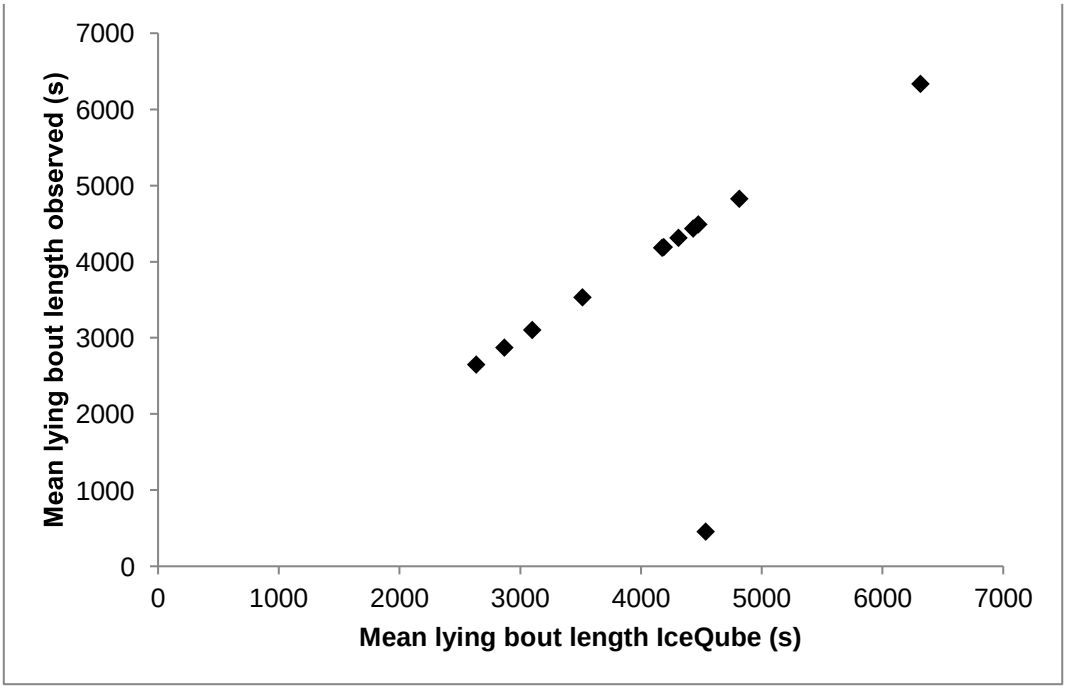

Figure 11- 10: The relationship between lying bout length determined from IceQube and CCTV footage from cow 7

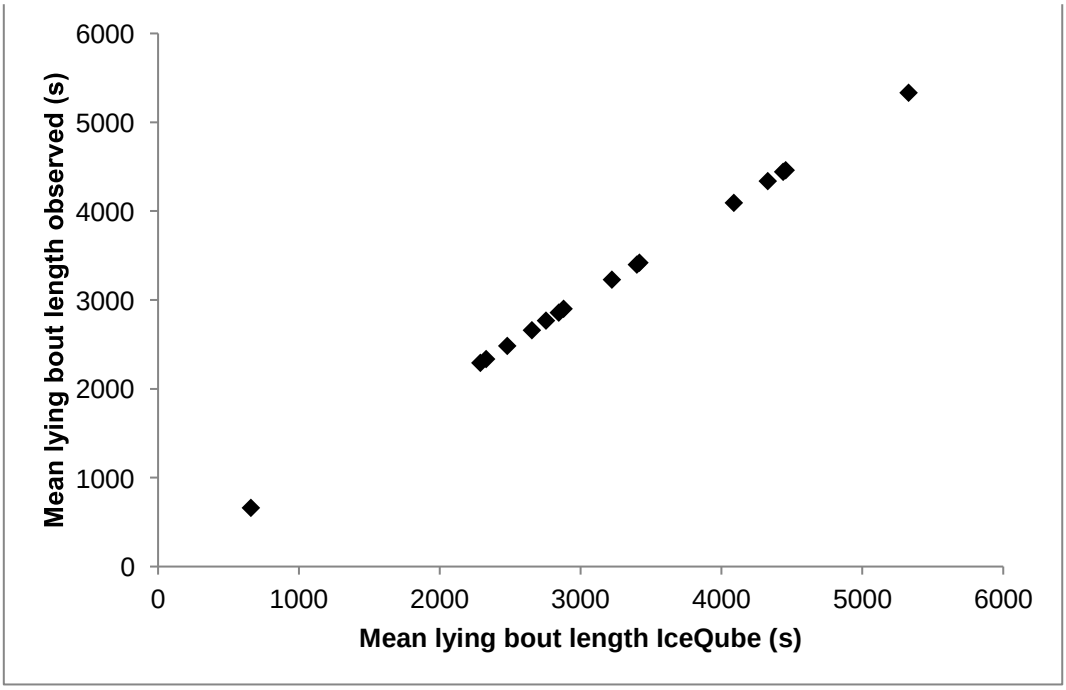

Figure 11- 11: The relationship between lying bout length determined from IceQube and CCTV footage from cow 59

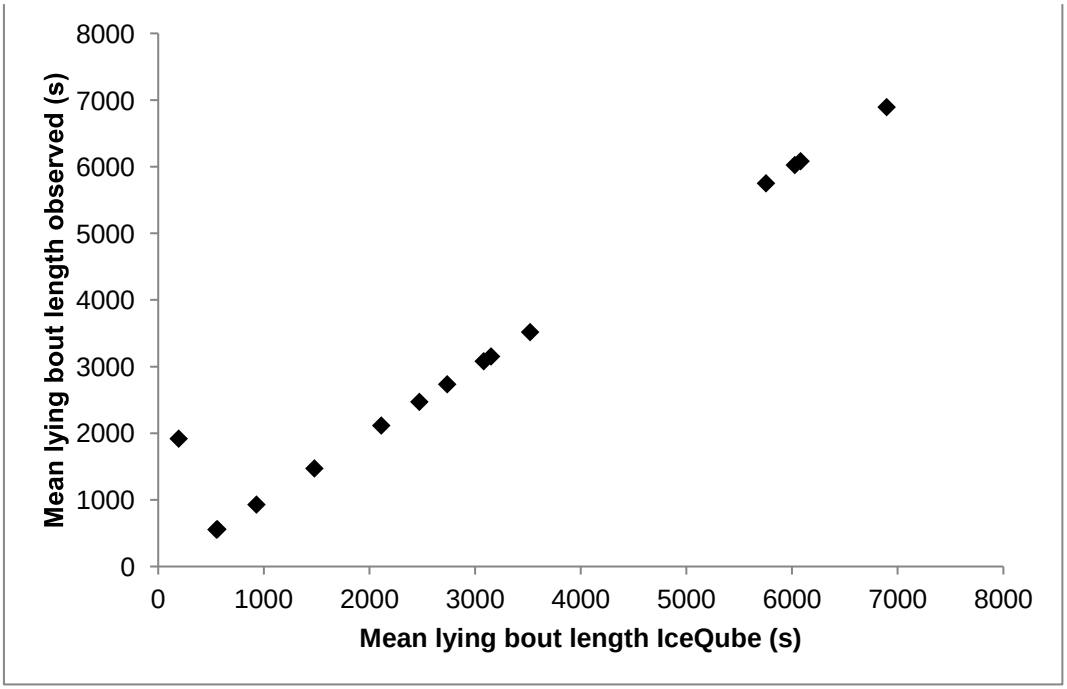

Figure 11- 12: The relationship between lying bout length determined from IceQube and CCTV footage from cow 88

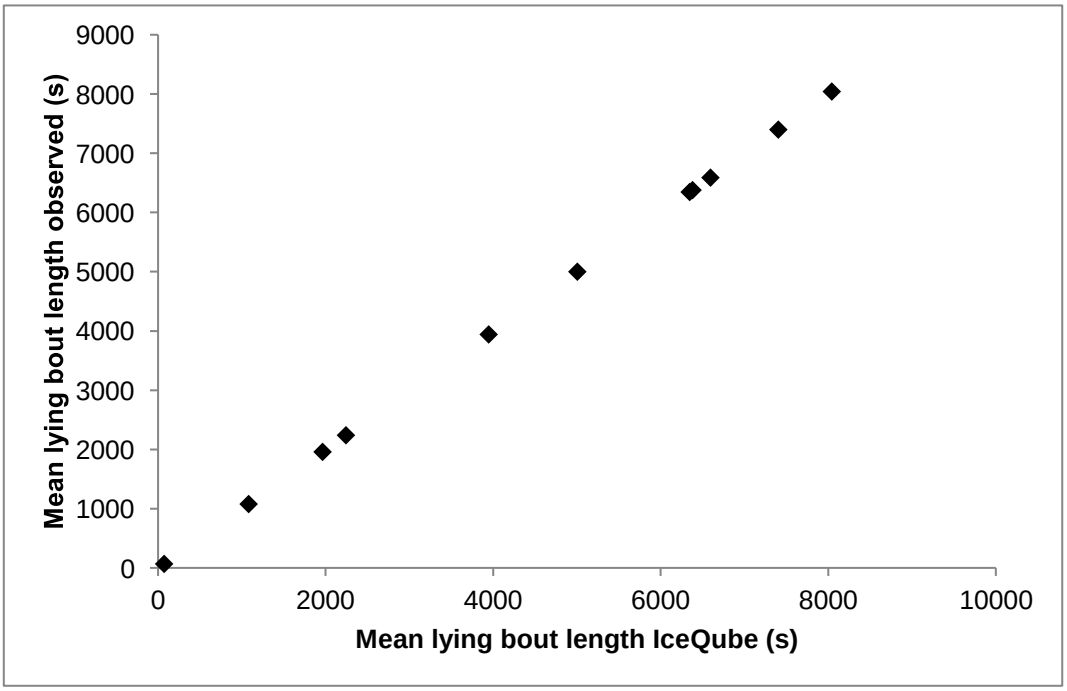

Figure 11- 13: The relationship between lying bout length determined from IceQube and CCTV footage from cow 99

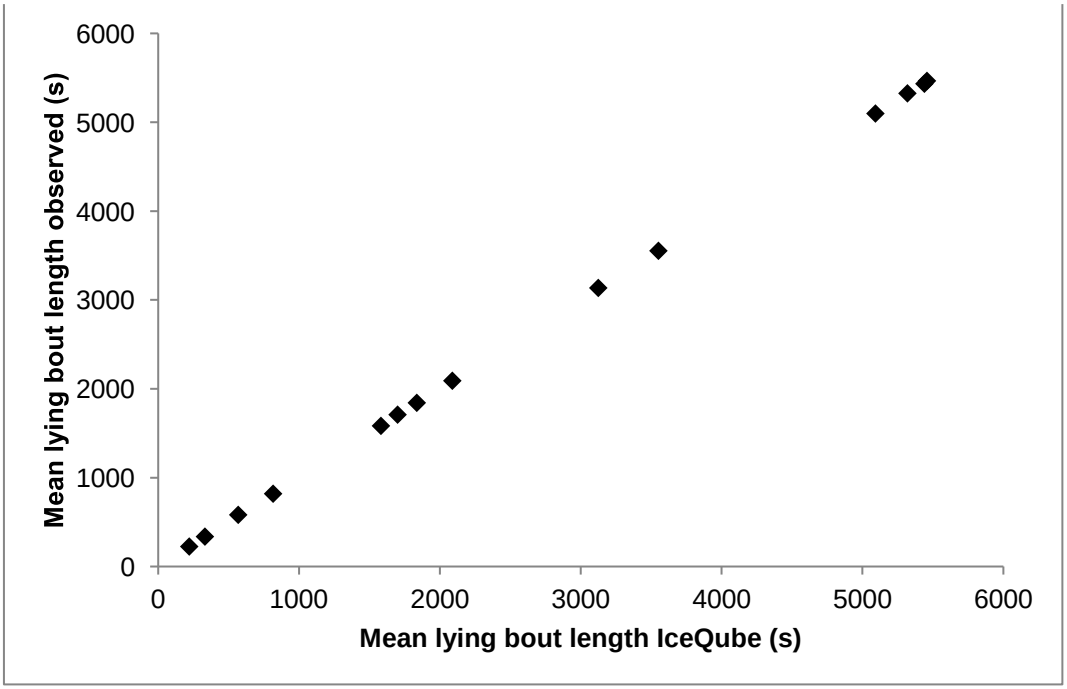

Figure 11- 14: The relationship between lying bout length determined from IceQube and CCTV footage from cow 106

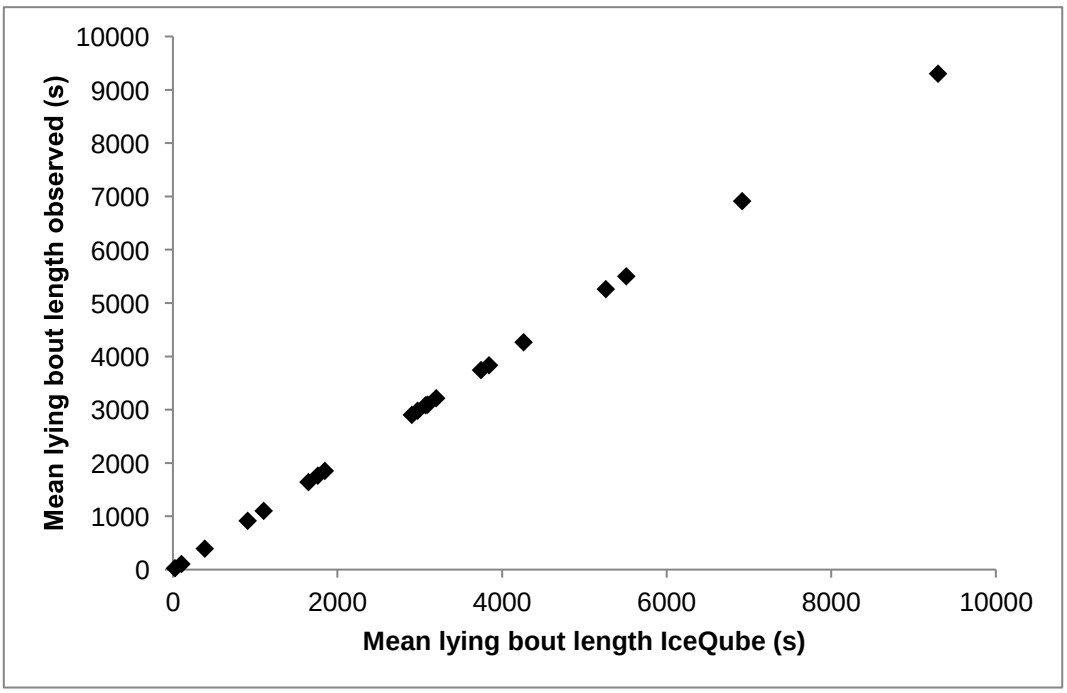

Figure 11- 15:
and CCTV footage from cow 138

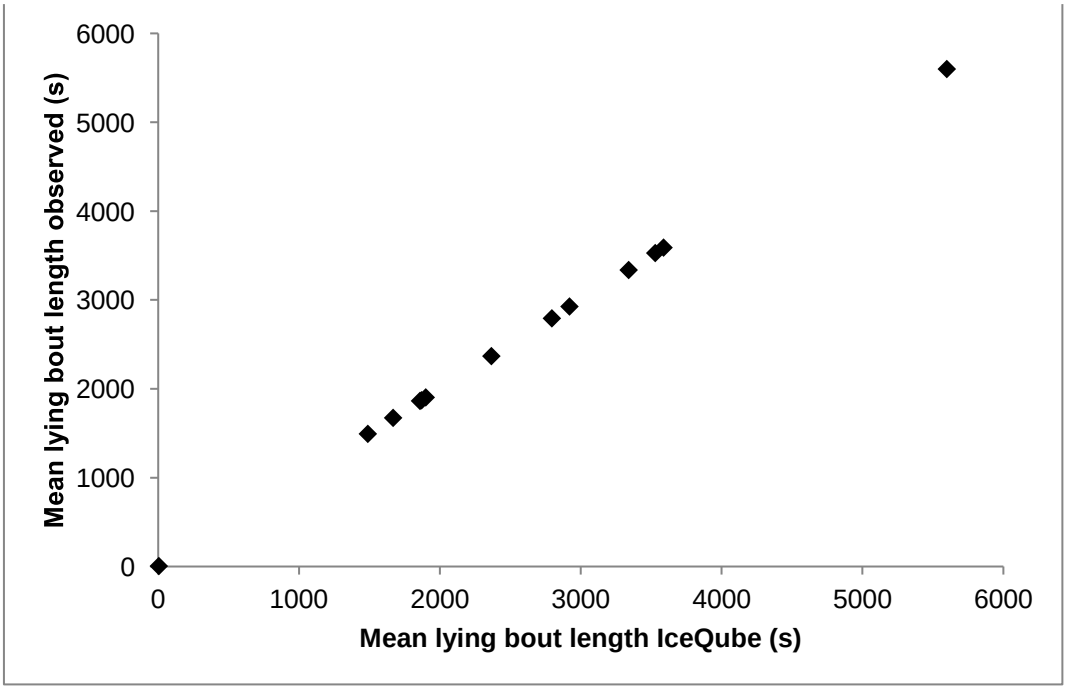

Figure 11- 16: The relationship between lying bout length determined from IceQube and CCTV footage from cow 145

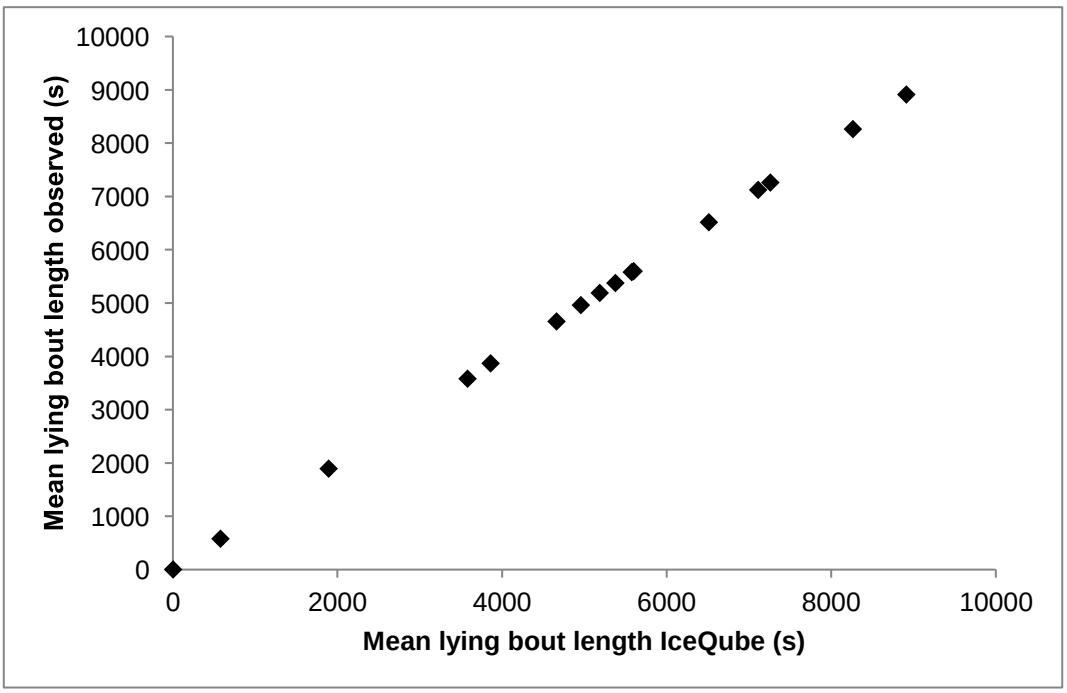

Figure 11- 17: The relationship between lying bout length determined from IceQube and CCTV footage from cow 165

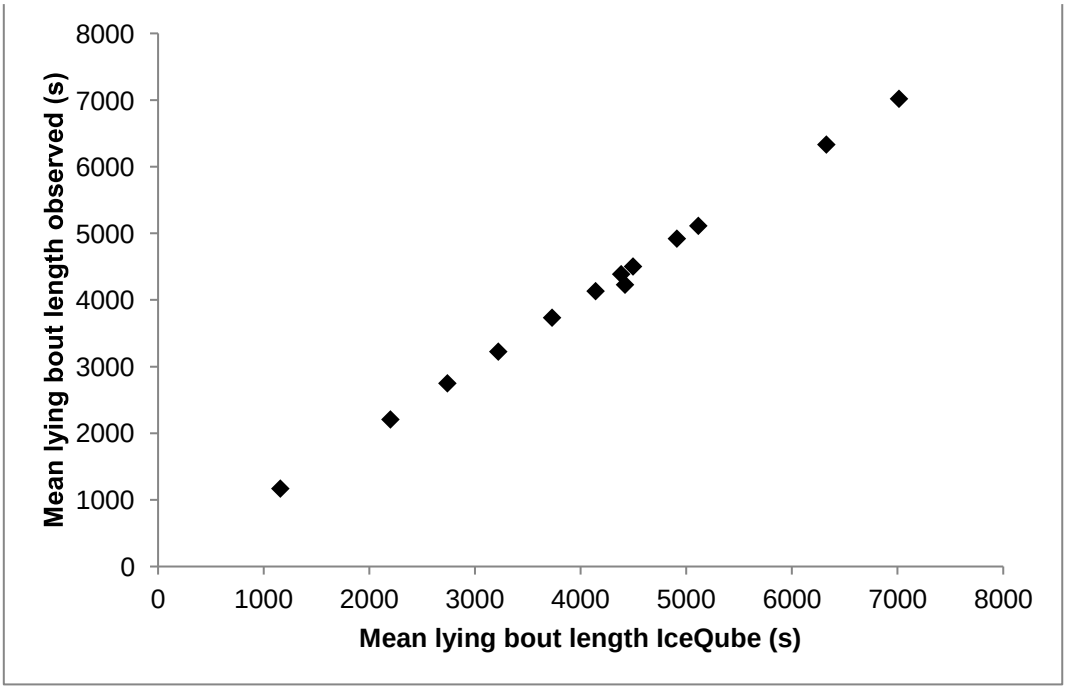

Figure 11- 18: The relationship between lying bout length determined from IceQube and CCTV footage from cow 166

Appendix 3: Questionnaire for dairy producers

1. General information

   Herd size

   Breed

2. Location

   Country

   Province, state, or country

3. Production type

   - Seasonal
   - Year round
   - Other, please specify

4. Housing system

   - Zero grazed
   - Partial grazing
   - Fully pastured
   - Other please specify

5. If the cows have access to pasture, how many hours per day?

6. Stall type for indoor housing systems
    - Tie stall
    - Free housed
7. Flooring type and bedding (Please state floor material and type of bedding if provided)
8. Stocking density for free housing systems (ratio of stalls:cows)
9. Diet provided (forage type, concentrate etc.)
10. Average annual milk production
    - Litres
    - Kilograms
11. Number of milkings per day
    - 1
    - 2
    - 3
    - Other
12. Breeding information- What oestrus detection method(s) do you use? (tick all that apply)
    - Visual observation
    - Tail paint
    - Chalk
    - Mount detector
    - Pedometer devices
    - Radiotelemetry devices
    - Teaser animals
    - Other please, specify

13. Reason for method used (tick all that apply)
    - Cost effective
    - Most accurate
    - Easy to use
    - Other, please specify

14. Oestrus detection rate for NON-LAME cows
15. Breeding method
    - Artifical insemination
    - Natural/bull

16. If you AI, what is the average number of insemintations to conception for NON-LAME cows

17. If you AI, who is responsible for imseminations? (Please list all individuals that perform AI on your farm).

18. Are they trained to perfrom AI> If so, who trained them?

19. Lameness and Fertility
    - Approximate lameness in herd (%)?
    - Main cause (if known)
    - Who mobility scores your cows?
    - Oestrus detection rate for LAME cows?
    - Average number of inseminations to conception for LAME cows?

20. Do you notice altered behaviour and/or reduced oestrus expression in LAME cows?
    - Yes
    - No

21. If so, what do you notice? (e.g. increased lying time, no mounting etc.)

22. Are the same oestrus detection methods used for all cows (lame and non-lame)?
    - Yes
    - No

23. If no, what differing methods do you use?

24. Are any precautionary measures taken for lame cows to ensure conception? E.g. monitored more, kept in pen, double AI?

25. Any other comments?