**University of Alberta**

# Evaluation of a pedometer system for prediction of estrus and parturition in dairy cows housed in a tie-stall barn

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Animal Science

Department of Agricultural, Food and Nutritional Science


Fall 2011
Edmonton, Alberta

## DEDICATION

I dedicate this thesis to my mother, Judith, who has taught me to follow my dreams no matter what obstacles I have to overcome. Thank you for your unconditional encouragement, enthusiasm, love and support.

**ABSTRACT**

Physiological changes associated with estrus and parturition in dairy cows are manifested by changes in activity and behaviour. Accurate and timely detection of cows in estrus and those approaching parturition is very important. The research objective was to determine the efficiency of the Afimilk pedometer system to facilitate the prediction of estrus and parturition through activity changes, in dairy cows continuously-housed in tie-stalls. The Afimilk pedometer system was first validated against simultaneous video recordings. Two studies were then conducted to determine if pedometers could predict estrus and parturition in dairy cows continuously- housed in tie-stalls. Afimilk pedometers were accurate in monitoring steps, lying bout frequency, and duration of lying. However, due to minimal activity change during estrus and high variability in prepartum activity, the Afimilk pedometers did not facilitate the accurate prediction of estrus or parturition, in the studied dairy cows continuously-housed in tie-stalls.

**ACKNOWLEDGEMENTS**

I sincerely thank…

My advisor, Dr. Divakar Ambrose, who took a leap from his research "comfort zone" and provided me this opportunity. His continuing encouragement, guidance, time and support have been invaluable.

My Advisory Committee, Drs. Clover Bench, Masahito Oba, Colleen Cassidy St.Clair, and Derek Haley for their time and advice.

Dr. Marcos Colazo, his enthusiasm and expertise for research, reproductive physiology, and ultrasonography is inspiring; his time, technical assistance, and support are greatly appreciated.

Dr. Patricio Ponce-Barajas whose enthusiasm for science continues to encourage; his time and technical assistance is greatly appreciated.

Dr. Laki Goonewardene for his advice and assistance with statistics.

Jamie Kratchkowski, and all the student volunteers for their time and dedication to these projects.

The SSDS instructors: your patience, understanding, and advice have helped every step of the way.

Most importantly, Andrew Ambrocichuk, my family and friends: your love, encouragement and the fun times we've shared have continuously supported me. You have enriched this experience in countless ways, and I am forever grateful.

The Government of Alberta Queen Elizabeth II Graduate Scholarship and the Government of Alberta Graduate Scholarship, and The Agriculture Funding Consortium for the support during my program.

Lastly, I acknowledge the animals that continue to amaze and inspire.

Financial support for this research was provided by Alberta Milk, Alberta Innovates- BioSolutions, Alberta Livestock and Meat Agency, Agriculture and Food Council of Alberta, and Alberta Agriculture and Rural Development.

The donation of equipment by Afimilk (S.A.E. Afikim, Kibbutz Afikim, Israel) and the assistance provided by Alon Arazi (Afimilk) are gratefully acknowledged.

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| | |
|---|---|
| AI | Artificial insemination |
| ACTH | Adrenal corticotrophin |
| CL | Corpus luteum |
| DIM | Days in milk |
| DRTC | Dairy Research and Technology Centre |
| DVR | Digital video recorder |
| $E_2$ | Estradiol |
| FSH | Follicle stimulating hormone |
| GnRH | Gonadotropin-releasing hormone |
| LH | Luteinizing hormone |
| NEB | Negative energy balance |
| $P_4$ | Progesterone |
| TMR | Total mixed ration |

## Chapter 1. General introduction

For the dairy industry to remain viable, efficient reproductive performance is essential. Estrus (the period when a cow is sexually receptive) and parturition (the act of giving birth) are two main stages in the reproductive life cycle that have dramatic impacts on performance. Currently, estrus detection rates are as low as 35%, (Leblanc, 2005; Ambrose and Colazo, 2007). With the majority of the dairy industry using artificial insemination (AI) to breed cows, it is very important that estrus is accurately and efficiently detected. Failure to detect estrus and perform AI increases the interval from calving to conception. This then prolongs days open (days not pregnant) and reduces profits for producers. Parturition-related disorders, for example perinatal mortality, account for economic losses approximated at $125 million a year in the United States of America (Mee, 2004). Therefore, these are two areas, which require improvement in order to enhance reproductive performance of dairy herds.

During both estrus and parturition, cows experience several physiological changes, which are expressed through changes in activity (Kiddy, 1977; Noakes et al., 2001). Pedometers are small mechanical devices that are attached to the leg or neck of a cow and record activity based on movement. Using pedometers to monitor activity changes, estrus and parturition could be automatically predicted in dairy cows to improve reproductive performance.

In North America, dairy cows are commonly kept in three main types of housing: free-stall, tie-stall, and loose-housing. A free-stall dairy facility is one where cows are not tethered to a stall; they have well-bedded individual stalls for lying but can walk around freely and access feed and water as needed. In a free-stall barn cows are free to choose any stall when they need to rest. A loose-housing barn has open bedded area(s) common to all cows and a separate area provided with shared feed and water troughs. In contrast, tie-stall dairies house cows in designated individual stalls, in which the cow remains tethered for the majority of the day. Tie-stalls have individual feed troughs, are bedded and either have separate or shared automatic water bowls. In tie-stall barns it is common to let cows out for exercise in an open pen or pasture for periods ranging from 2 to 8 h.

Estrus detection is more difficult in tie-stalls compared to free-stalls because cows, in tie-stalls, are restricted to express estrus-related activities such as mounting and standing estrus (standing to be mounted by another cow) except during exercise periods (Pollock and Hurnik. 1979). By automatically predicting estrus using pedometers in dairy cattle housed in tie-stalls, efficiency of estrus detection would increase, thereby improving reproductive performance. Poor reproductive performance is costly and is the number one reason cows are culled in Canada. Throughout the Western provinces 27% of dairy cows are culled for poor reproductive performance (CanWest DHI, 2010). When a cow has poor reproductive performance, such as increased services per conception, increased days open, and increased calving intervals, it results in higher breeding costs, increased involuntary culling rates, increased replacement costs and reduced milk production (Pryce et al., 2000; Sewalem et al., 2008). Therefore, it is important to improve estrus detection efficiency in order to improve reproductive performance in dairy cows.

Improving the ability to predict parturition can also improve reproductive performance. Tie-stall facilities run the risk of in-stall calvings, which are unsafe because of the potential dangers (injuries from manure scraper, risk of calf being trampled by neighbouring cow) and pathogens (located in manure) that are present in the area behind the cow. If pedometers could predict parturition in dairy cattle housed in tie-stalls, preparturient cows would be moved into maternity pens prior to parturition, thus reducing in-stall calvings. Moving cows into maternity pens prior to the onset of parturition is recommended (Mee, 2004) as it provides the cow with a clean untethered area for parturition to take place.

The majority of published research has focused on using pedometers to monitor activity changes in dairy cattle housed in free-stall facilities (Liu and Spahr, 1993; Roelofs et al., 2005; Ranasinghe et al., 2010). Activity monitoring in tie-stalls is important as 75% of Canadian dairy herds and 49% of American dairy operations are housed in tie-stalls (United States Department of Agriculture, 2007; Canadian Dairy Information Centre, 2011). To our knowledge, it has not been determined if pedometers could predict estrus or the onset of parturition in dairy cows continuously- housed in tie-stalls. Therefore two projects (Chapters 3 and 4) were completed for this thesis. The first project (Chapter 3) objectives were to determine if the Afimilk pedometer system could accurately monitor cow activity and facilitate estrus detection in dairy cows continuously- housed in tie-stalls. The second project (Chapter 4) objectives were to determine if the Afimilk pedometer system could facilitate the prediction of parturition, and secondly, determine if parity affects activity during late gestation and early lactation.

This thesis begins with a literature review (Chapter 2), discussing the physiological mechanisms that control estrus- and parturition-related activities. The literature review also describes the use of pedometers and how they could be used to improve reproductive performance. Chapters 3 and 4 describe the two studies completed for this thesis. Lastly, Chapter 5 discusses the limitations with the pedometer system, a summary of the findings from the two studies, and suggestions for future research to improve knowledge surrounding estrus and parturition-related activity.

## Chapter 2. Literature review

### Part 1. Estrus[1]

Cattle are polyestrous species, characterized by having estrous cycles throughout the year until pregnancy occurs, which can happen regardless of season. The first estrous cycle in a well-nourished dairy heifer occurs at approximately 9-11 mo of age (Chelikani et al., 2003). Estrous cycles vary in length and on average last 21±3 d in cows and 20±3 d in heifers (Ginther et al., 1989).

Estrus is the stage in an estrous cycle when a cow is receptive to mating. Estrus is a preovulatory state; it is the only stage, in the estrous cycle, that is visible to the human eye. The most primary and reliable sign of estrus is standing estrus, which is defined by a cow standing still while being mounted by a bull or a female herd mate (Baker and Seidel, 1985). Estrus is also characterized by increased activity, restlessness, chin resting, vulva sniffing, mounting, and increased vaginal mucus discharge (Kiddy, 1977; Esslemont et al., 1980). The duration of estrus in lactating dairy cows is on average 7 to 8 h (Dransfield et al., 1998; Lovendahl; Chagunda, 2010) and about 9 h in heifers (Lovendahl and Chagunda, 2010).

During estrous cycles, a Graafian follicle grows by the stimulation of pituitary gonadotrophic hormones (Roelofs et al., 2010). When the Graafian follicle grows it increases the production and concentration of estradiol ($E_2$)

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[1] For more information regarding estrous cycles and the endocrine control of estrous cycles refer to Appendix 1.

(Roelofs et al., 2010). Estradiol is processed in the hypothalamus and stimulates estrous activity and behaviour (Vailes et al., 1992).

**Estrous behaviour**

The behavioural changes cows experience during estrus are regulated by the endocrine system and processed through the brain. Increased concentration of $E_2$ is processed in the brain. Neurons (in the hypothalamus) produce behaviour specific neurotransmitters, which are transferred to the midbrain. The speed of the impulse increases and directs the signals to the medulla (Senger, 2003). In the medulla the signal is integrated and the nerves synapse with motor neurons, which run on nerve tracts to the spinal cord, producing estrus behaviors such as, standing estrus and mounting (Senger, 2003).

After the onset of standing estrus, ovulation will occur within 24-30 h. Therefore, the ideal time to breed a cow is between 11 and 16 h after the onset of estrus (Roelofs et al., 2005). There are occurrences, for example first postpartum estrous cycle, when cows do not show standing estrus therefore, it is important to detect estrus using secondary behavioural changes (Esslemont et al., 1980; Isobe et al., 2004).

Increased activity surrounding estrus is an important behavioural change used in estrus detection. With the help of pedometers, increases in activity can be monitored. During estrus, cows housed in tie-stalls and free-stalls increased their activity, on average, by 282% and 396%, which is a 2.75 and 4.0 fold increase, respectively (Kiddy, 1977). Behavioural changes associated with estrus allow producers to detect the optimal time to inseminate.

**Factors affecting estrous behaviour**

There are several factors, which affect cow behaviour and expression of estrus; these can originate from group of cows, a management problem, an environmental issue, or a physiological condition. An important factor, which controls the intensity of estrus behaviour, is the size of the sexually active group. When 1, 2, and 3, animals were in estrus Hurnik et al. (1975) found that the mean number of initiated mounts increased (11, 36, and 53 mounts, respectively). Management and housing factors such as loud noises, low ceilings, and type of flooring (Hurnik et al., 1975; Britt, et al., 1986) can all affect the frequency, duration, and expression of mounting and standing activity.

A physiological factor that impacts estrus detection is silent ovulation or silent estrus. Silent estrus is when cows do not express sexual behaviours or do not increase their walking activity in association with estrus (Ranasinghe et al., 2010). Ranasinghe et al. (2010) used pedometers to record changes in activity surrounding estrus in a free-stall facility; 55.2, 23.8, 21.3, and 10.5% of cows had silent estrus in their first, second, third, and fourth ovulation postpartum, respectively. For the first estrous cycle after calving cows commonly do not express estrous behaviours. This is because the hypothalamus is not sensitive to $E_2$ due to the high $E_2$ concentration in late gestation and the decreased production of $E_2$ from the preovulatory follicle (Allrich, 1994).

There are certain factors that increase the risk of silent estrus. One factor that contributes to silent estrus is the state of negative energy balance, which occurs during early lactation. During energy deficits the production of $E_2$ may be reduced in the Graafian follicle along with lowered hypothalamic sensitivity to $E_2$ (Isobe et al., 2004). Lower $E_2$ production or lower sensitivity to $E_2$ would result in decreased expression of estrus-related behaviours or silent estrus.

A second contributing factor to silent estrus is when cows produce moderate to high milk yields. An increased risk of silent estrus in the second, third, and fourth ovulation was observed in cows producing ≥27.8 kg/d (Ranasinghe et al., 2010). Lactating dairy cows have a 54% increase in blood flow rate through the liver compared to non-lactating dairy cows (Lomax and Baird, 1983; Sangsritavong et al., 2002). Increased blood circulation (associated with high feed intake) increases metabolic clearance rate and reduces the circulating concentration of $P_4$ and $E_2$ in lactating cows; thus increasing the risk of silent estrus (Sangsritavong et al., 2002). On the day of estrus high producing cows (≥39 kg/d) had lower circulating $E_2$ concentrations shorter standing events, shorter standing time and a shorter duration of estrus compared to lower producing cows (Lopez et al., 2004).

Management, environment, and physiology can also impact the expression of estrous behaviour. Although expression of estrus can be low the detection of estrus is an important requirement for a successful reproductive program.

**Estrus detection**

The greatest limiting factor in a successful reproduction program is estrus detection failure (Stevenson, 2001), this is because estrus detection rates vary and are affected by numerous factors. On average the estrus detection rates are as low as 35% (LeBlanc, 2005; Ambrose and Colazo, 2007). Inaccurate detection of estrus and untimely insemination reduce conception rates, and increase calving intervals, thus increasing costs (Firk et al., 2002). By improving efficiency of estrus detection from 50 to 60%, decreasing days to first service from 80 to 60 d, and increasing conception rates from 35% to 50%, in a herd milking 300 cows, a combined net increase in profit of $18,485 was generated (Hady et al., 1994). This demonstrates that improving estrus detection efficiency could benefit reproductive performance in cows and improve profits for producers.

There are several methods to detect estrus; a few examples are visual observation, video recording, tail chalk or paint, activity monitoring systems such as pedometers, and both mechanical and electronic pressure sensitive devices that are activated based on actual mounting events. Senger (1994) suggested that the highest detection rates come from continuous observation either through electronic, chemical, or visual methods. Visual observations are time-consuming and require trained personnel, however can result in accurate detection if done frequently and with diligent attention. Using visual observations (2x/d) with tail paint in a loose pasture-based herd, Xu et al. (1998) found efficiency and accuracy rates of 98.4% and 97.6%. Although visual observations are effective, with dairy farms consistently growing in size, producers have less time to monitor individual cows; therefore, an automatic monitoring system (e.g. pedometer or mount detector) will be valuable.

With poor estrus detection rates, there has been an increase in the use of timed-AI programs. Timed-AI programs synchronize ovulation, with hormones, and cows are inseminated at a predetermined time without the need for estrus detection. Timed-AI programs such as, Ovsynch may be beneficial to dairy herds that have poor estrus detection rates (Ambrose, 1999).

## Part 2. Parturition[2]

During parturition cows experience physiological changes, which are expressed by changes in activity. It has been well documented that cows express increased restlessness in the days prior to parturition (Owens et al., 1985; Bao and Giller, 1991; Huzzey et al., 2007; Miedema et al., 2011a; Miedema, et al., 2011b). Increased restlessness can be observed through increases in standing time, number of walking bouts, walking duration, number of lying bouts or decreased lying duration (Bao and Giller, 1991; Huzzey et al., 2007; Miedema et al., 2011a; Miedema et al., 2011b). If the onset of parturition were predicted accurately, then preparturient cows, particularly those housed in tie-stalls, would get moved from their tie-stalls into maternity pens prior to parturition – thereby avoiding in-stall calving. Moving cows into a maternity pen prior to parturition improves overall dam welfare by providing a clean, safe, and untethered environment for calving.

### Activity changes surrounding parturition

Cows experience changes in activity and behaviour in the weeks surrounding parturition. From 3 wk before parturition, duration of feeding, ruminating and total feed intake declines in pregnant cows (Bao and Giller, 1991) Within 1 wk of parturition, a sudden decline in feeding and ruminating occurs. Once parturition is complete, cows begin to increase their feeding and ruminating

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[2] For more information of the endocrine control of gestation or the stages of parturition refer to Appendix 2 and 3, respectively.

time (Bao and Giller, 1991). Feeding and ruminating changes surrounding parturition are commonly observed as well as changes in physical activity.

Standing duration and frequency are increased in the weeks approaching parturition. The average standing time in the preparturient period (10 d before parturition to 1 d before parturition) was 12.3 h (Huzzey et al., 2005). Standing time increased to 14.4 h during the parturition period (1 d before to 1 d after parturition; Huzzey et al., 2005). The number of standing bouts (interval between two lying events), and the frequency of walking bouts (period of walking separated by periods of standing or lying) increased in the parturition period compared to the pre and post parturition periods (Huzzey et al., 2005; Miedema et al., 2011b). Increased standing time, standing bouts and walking bouts (increased restlessness) are likely due to discomforts prior to parturition. During the week after parturition standing time decreases (Bao and Giller, 1991) and may be associated with increased calmness as discomforts are reduced after parturition.

Once cows are moved into a maternity pen, exploration of the new environment is usually the first behaviour change observed (Wehrend et al., 2006). Other behavioural changes noticed during the 24 h prior to expulsion of the calf are increased tail-raising, ground-licking, kicking at the belly, standing with arched back and raised tail, smelling the ground, and nest-building (Noakes et al., 2001; Wehrend et al., 2006; Miedema et al., 2011b).

Activity levels are highly variable within cows and between parities. Activity surrounding parturition can differ between primiparous and multiparous cows. Primiparous and multiparous cows were classified during the first stage of parturition as calm, restless, or very restless. Primiparous cows were either classified as restless or very restless whereas cows were classified as either calm, restless, or very restless; suggesting that primiparous cows are more restless during parturition than cows (Wehrend et al., 2006).  Primiparous cows expressed increased tail-raising up to 4 h before parturition compared to multiparous cows who expressed tail-raising 2 h before parturition (Miedema et al., 2011a).

Activity differences between parities can inform producers what to expect during parturition in heifers and multiparous cows. These differences may be valuable in accessing if intervention during parturition is required. This is important because assisting a cow before it is required could lead to added difficulty and unnecessary increased inflammation during parturition. Producers should have a good understanding of activity changes during parturition and be able to recognize the signs of cows with eutocia (normal calving) compared to cows with dystocia (difficult calving or calvings that require assistance).

**Prediction of parturition**

It is important to predict parturition in order to ensure cows calve in a clean maternity pen; however, with North American dairy herds growing in size this may increase difficulty in predicting parturition as producers have less time to monitor cows individually (United States Department of Agriculture. 2007; CanWest DHI, 2010). With less time to monitor cows, an increase of in-stall calvings may occur.

In-stall calvings reduce the health, safety, and comfort of dams as well as restrict normal parturition-related behaviour. In-stall calvings also increase the risk of injury to dams. When the dam lays in a recumbent position, common during parturition (Edwards and Broom, 1982), and is coupled with contractions, she may be forced to pull against the tether, which can lead to choking (personal observations). Preparturient cows normally explore their calving area prior to parturition as well as express nest-building behaviours (Wehrend et al., 2006); however, when cows are forced to calve in-stall these behaviours get inhibited. Cows should be loose, not tethered, during parturition and placed in a maternity pen bedded with clean deep straw, woodshavings or other nonslip material, as well as feed and water (Mee, 2004).

In-stall calvings also reduce the health and safety of the calf. Dams that calve while tethered cannot lick their calves clean. Licking calves after parturition is important for stimulating the calf's activity, breathing, circulation, urination and defecation (Metz and Metz, 1986). Licking the calf clean also dries the calf's coat therefore, reducing evaporative heat loss (von Keyserlingk and Weary, 2007). Calves that are born in-stall may have a higher risk of disease compared to those born in a clean maternity pen. This is because the calf may be delivered into an area (e.g. on gutter) where there is manure and urine from the dam and neighbouring cows. This area is also dangerous for the calf due to unsafe equipment for example, a manure scraper. Calves born in-stall also have an increase risk of injury from being trampled by neighbouring cows or calves stumbling into moving equipment. By predicting parturition, in-stall calvings could be prevented thereby improving the health and safety of the dams and their calves.

**Part 3. Pedometers**

**How pedometers work**

Pedometers are small electronic devices that record physical activity by counting steps. They are located inside waterproof hard shell cases, which are usually attached to the hind leg of a cow by a nylon strap. In order for activity data to get transmitted to the software, an antenna scans over the pedometer to download the stored data. This scanning activity identifies the individual pedometer and transmits the pedometer ID, and activity log through a controller into a computer (Calderón, 2002). This information gets stored and computed in the herd management software. The software computes averages for each cow's stepping and lying activity; if the cow's activity deviates from its average activity the system alerts the operator.

The AfiMilk Pedometer Plus™ system (S.A.E. Afikim, Kibbutz Afikim, Israel) (Afimilk pedometer) is an innovative product; it not only records stepping activity (alike all other pedometers) but also records lying activity through a newly developed sensor (Behaviour Tag®; Arazi et al. 2010). Afimilk pedometers monitor activity by counting steps (the number of significant leg movements), lying bouts (number of lying events), and lying time (duration of a lying event). Afimilk pedometers record activity using a mechanic analog device. When significant leg movements occur, a moving mechanical part hits a stationary mechanical part and a step is counted (Alon Arazi, Afimilk Applied Research Group Manager, personal communication). The force of the movement in order to count a "step" has been validated for cattle.

The Afimilk pedometer detects lying activity based on the angle of the cow's leg. If the angle is above a predetermined threshold, and 3 min have elapsed, the pedometer begins to count this as lying time (in min). The 3 min wait period is to filter out mock lying events (aggressive leg movements) and to guarantee the cow is in a recumbent position. Once the cow stands, the angle of the pedometer changes and the lying time counter ends (Alon Arazi, Afimilk Applied Research Group Manager, personal communication). The period between two standing events (i.e., standing, lying, standing) is counted as a lying bout.

Pedometer data can be downloaded anytime and as many times per day as needed as long as the pedometer gets scanned by the antenna reading zone (up to approximately 1 m). In a commercial herd that uses a milking parlor or robotic milking machine, the pedometer antenna would be located on a gate at the entrance of the milking area to facilitate automatic collection of pedometer records. In a tie-stall facility where cows remain in their stall 24 h/d, the antenna can be located on a portable stick with a fixed connection to the controller, and data has to be procured by manual scanning.

**Technology advancements**

Farris (1954) was the first to critically describe the relationship between physical activity increases during estrus in dairy cows using mechanically activated pedometers. From then it took approximately 20 years before the next study was released examining pedometer-monitored activity increases during estrus in dairy cattle housed in a tie- and free-stall facility (Kiddy, 1977). Since then pedometer use and technology has grown and advanced significantly over the years. Kiddy (1977) used pedometers that were designed for humans, enclosed in handmade waterproof cases. Throughout Kiddy's experiment, activity changes were recorded manually twice daily at milking. Activity averages were calculated by subtracting the amount of steps that occurred in between milking periods.

Nowadays, pedometers have evolved to encompass the following: 1) they are designed and validated for cattle, 2) they have large storage capabilities, 3) they have internal power sources to continuously count activity, 4) they have self-contained computerized counters that are accessed remotely using an antenna, 5) they have specialized software that automatically computes deviations from the norm, and 6) they can automatically alert producers when deviations from norm occur by a notification sent to the herd management software (Senger, 1994). Technological advances have improved the usefulness of pedometers because they can accurately identify the cow as well as onset of estrus (Nebel et al., 2000). These advances in technology have permitted pedometers to work efficiently in animal production facilities and improve the accuracy of individualized cow monitoring.

**Pedometers in dairy cattle management**

Pedometers are used to monitor changes in activity and to predict different stages of production or disease in a cow's life. Estrus is one stage pedometers commonly predict in free-stall facilities. With the use of pedometers, estrus detection efficiency can range between 51 and 87% (Rorie et al., 2002; Roelofs et al., 2005). A sample of studies that focused on pedometer estrus detection efficiencies is located in Table 1. Cows may get occupied by barn activities such as eating, milking which may results in a reduction in the time spent mounting; therefore, pedometers can be used to monitor activity changes based on activity and not mounting (Nebel et al., 2000).

Pedometer estrus (estrus measured with pedometers) is defined by activity thresholds, which affect the accuracy of detection rates (Roelofs et al., 2005). With a high threshold or increases (3- or 4-fold verses 2-fold) estrus detection accuracy may be high however, detection efficiency rates may be low. With low thresholds detection rate efficiency will be high however, a rise in false positives (lower accuracy) may occur (Rorie et al., 2002; Roelofs et al., 2005; Lovendahl and Chagunda, 2010).

No single detection method is 100% accurate or efficient; therefore, by combining and refining methods, accuracy and efficiency of detecting estrus will improve (Peralta et al., 2005). Pedometers will help to improve estrus detection because they monitor activity changes automatically, thereby reducing the responsibility of the producer to monitor or detect estrus. By improving estrus detection, with pedometers, a subsequent improvement in reproductive performance will occur because cows are more likely to be inseminated at the correct time.

Pedometers have been used since the 1970's to detect estrus activity changes in cows (Kiddy, 1977). Along with estrus, pedometers have also been successful in measuring distances traveled in grazing cattle (Walker et al., 1985), predicting lameness (Mazrier et al., 2006), and metabolic disorders such as ketosis, left displaced abomasum and digestive disorder 7 to 8 d earlier than visual diagnosis (Edwards and Tozer, 2004). By monitoring changes in cow activity pedometers detect estrus, record grazing distances and predict disease in dairy cattle. Based on this same principal, monitoring activity changes, pedometers could be used to predict parturition as cows express increased restlessness surrounding parturition. Little research has investigated pedometers in the prediction of parturition; however, the prediction of parturition would be valuable (Lidfors et al., 1994).

Pedometers could automatically monitor activity changes and alert producers when activity deviates from the norm. This alert could be used to predict imminent parturition; the producer could then move the cow from the tie-stall into a maternity pen prior to parturition. Timely movement of preparturient cows into maternity pens is important; if cows are moved too early the calving pen will become dirty and increase risk of metritis and mastitis; however, if the cow is moved too late it may prolong parturition causing dystocia because of the sudden increase in stress due to the change of environment.

No known research has determined if pedometers can predict estrus and the onset of parturition in continuously-housed cattle in tie-stall barns. Therefore, the two main objectives, for this thesis, are to determine if Afimilk pedometers can: 1) accurately predict estrus in dairy cows housed continuously in a tie-stall barn and 2) predict the onset of parturition in dairy cows housed in tie-stalls.

**Table 1. A sample of studies on estrus detection using pedometers in different housing systems from 1977-2007.**

| Reference | Housing | Parity | Exercise allowed in tie-stall | Activity increase (threshold) | Estrus detection efficiency (%) | False positive (# or %) | Estrus observations |
|---|---|---|---|---|---|---|---|
| (Kiddy, 1977) | Tie stalls<br>Free-stall | - | Yes | Mean+ 3 SD | 79<br>84 | - | Twice daily at milking |
| (Redden, et al., 1993) | Tie-stall | Primiparous and multiparous | Yes | >50% | 80 | 4 | Twice daily at milking |
| (Kennedy and Ingalls, 1995) | Tie-stall | Primiparous and multiparous | Yes | 2 -3 X | 67 | 22 | 2 h intervals |
| (Roelofs et al., 2005) | Free-stall | Primiparous and multiparous | - | SD 2.5 | 87 | 16% | 2 h intervals |
| (Sakaguchi et al., 2007) | Pasture<br>Paddock<br>Tie-stall | Heifers | No | 1.4 X<br>1.3 X<br>1.4 X | 100<br>92<br>92 | - | Every hour |

## Chapter 3. Evaluation of the Afimilk Pedometer Plus system for detecting estrus in dairy cows housed in tie-stalls

### Introduction

Accurate and efficient detection of estrus is a key component to successful reproductive management. Nevertheless, failure to detect estrus in dairy cattle is one of the largest limiting factors in reproductive performance (Nebel and Jobst, 1998). Annual loss to the US dairy industry over failure to detect or incorrectly detecting estrus is estimated at over $300 million (Senger, 1994). When estrus goes undetected or is falsely detected, it causes increase days open, income lost, and herd losses (Firk et al., 2002).

Estrus detection rates vary in accuracy (the percentage of estruses observed that are true estruses) and efficiency (the percentage of possible estruses that were observed over a given time period) (Heersche and Nebel, 1994). Estrus detection rates in Canadian herds are currently as low as 35% (LeBlanc, 2005; Ambrose and Colazo, 2007); therefore, up to 65% of estrus events go undetected. Several factors impact the expression of estrus and subsequently behavioural estrus detection. These include: the type of housing (tie-stall vs. free-stall), flooring surface, detection method (live: visual; assisted: colour markers, heat-mount detectors, pedometers or other electronic aids) and the management (number, duration, and time of observations) (Kiddy, 1977; Britt, et al., 1986; Xu et al., 1998; Roelofs et al., 2005).

Poor estrus detection may be associated with increased herd sizes as producers have less time for individual monitoring of cows. Estrus detection, traditionally used in tie-stalls, is tedious and incurs high labor costs (At-Taras and Spahr, 2001). Pedometers can automatically monitor cow activity, and assist producers in identifying cows in estrus (At-Taras and Spahr, 2001; Roelofs et al., 2005).

Pedometers are automatic activity monitors attached to the leg of a cow. They record changes in walking activity based on steps. During estrus, free-stall and tie-stall-housed cows are reported to have increases in activity of 4 and 2.75 times, respectively, that of cows not in estrus (Kiddy, 1977). In free-stall facilities pedometers have estrus detection rates between 79 and 87% (Roelofs et al., 2005; Roelofs et al., 2010). Little research has been done to determine the efficiency of pedometers in detecting estrus in lactating cows continuously- housed in tie-stalls. This is important, as 75% of Canadian dairy herds are tie-stalls (Canadian Dairy Information Centre, 2011) and some tie-stall facilities do not provide consistent out-of-stall periods due to harsh weather, staff shortages, or space restrictions.

Tie-stalls restrict the expression of estrus-related behaviours such as: increased walking, mounting, or standing heat, unless observed out-of-stall. Although pedometers regularly facilitate estrus detection by recording changes in stepping or walking activity, there are other measures of activity (lying and standing behaviour) (Pollock and Hurnick, 1979; Walton and King, 1986) that change during estrus and could be automatically measured. The AfiMilk Pedometer Plus™ system (Afimilk pedometer), used in this study, is unique in that in addition to the number of steps, they have an additional sensor (Behaviour tag®), which records lying bouts (number of lying events) and lying time (duration of a lying event), which is beneficial in a tie-stall barn to monitor changes in activity besides increased walking.

Therefore, this study aimed to determine if Afimilk pedometers could: 1) accurately record cow activity, and 2) facilitate estrus detection in tie-stalls. We hypothesized that Afimilk pedometers would: 1) accurately record cow activity by comparing pedometers records to video records and 2) retrospectively, facilitate estrus detection by recording changes in estrus-related activity (increased steps and increased frequency of lying bouts, and decreased lying times).

**Materials and Methods**

This study was conducted with lactating Holstein cows at the Dairy Research and Technology Centre (DRTC), a 146-cow tie-stall facility at the University of Alberta, Edmonton, Alberta. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009). The University of Alberta's Animal Care and Use Committee approved the experimental protocols used is this study.

**Activity monitoring**

Pedometer system

The AfiMilk Pedometer Plus™ system (S.A.E. Afikim, Kibbutz Afikim, Israel) is an electronic monitoring device designed to facilitate estrus detection in free-stall dairy cows by measuring changes in activity; number of steps, number of lying bouts and duration of lying time. Each pedometer had an individual identification number, which was programmed into the Afimilk herd management software for each cow ID. Each pedometer was manually scanned using a portable hand-held antenna. The antenna had a wired connection to the data logger, which in turn was connected to a computer wherein the herd management software was installed. At each scan, the pedometer ID was captured along with the activity records; this information was downloaded onto the Afifarm herd management software. For this study, the software was not used to process activity changes because of the limitation that activity thresholds for estrus in tie-stall dairy cows are unknown. If threshold values for predicting estrus in tie-stall cows were known, data would be processed automatically and the software would indicate if a cow had achieved a set activity threshold, and alert which cows are suspected to be in estrus based on changes in activity. To monitor activity changes this study used unprocessed activity measurements (number of steps, frequency of lying bouts in 24 h, and duration of lying in 24 h) obtained from downloading the raw pedometer measurements after each scan and calculating differences in activity between periods using Microsoft excel.

Video surveillance

Eight tie-stalls, equipped with 24 h video surveillance, were used in this study. There were 2 monochrome cameras (Sony Super HAD ccd, SSC-M183 Sony Canada, Toronto, On, Canada) installed such that each camera captured 4 stalls. Cameras captured a rear-end image in order to record rear leg stepping and lying activity. Cameras were connected into a 4-channel digital quad (Robot MONOCHROME QUAD, MV47, Sensormatic Electronics Corporation, Tyco International Ltd.Princeton, NJ, USA). The quad allowed up to 4 video images to be viewed on 1 screen. Video was recorded through a pinnacle video transfer device (Pinnacle Systems, Mountain View, CA, USA) and stored on digital external hard drives (IPRO Drive, Futureshop, Edmonton, AB, Canada). The Pinnacle device continuously recorded 4 h of video and saved the video into compressed video formats (mp4). The compressed video format allowed for continuous recording.

**Animals and experimental design**

The experimental design and treatment schedule are shown in Figure 1. There were 37 Holstein cows used in this study, 19 postpartum estrus-induced cows and 18 pregnant cows used as controls. Parity of the estrus-induced cows ranged from 1 to 6; 10 cows were in their first lactation, 6 were in their second lactation, 1 was in her third lactation, 1 was in her fourth lactation and 1 was in her sixth lactation. The estrus-induced cows had an average of 77 days in milk (DIM), parity of the pregnant cows ranged from 1-4 with an average of 244 DIM. Cows were housed in tie-stalls continuously for 9 d with unrestricted access to water and fed a total mixed ration (TMR) once daily formulated for lactating dairy cows according to NRC guidelines (National Research Council, 2001). Main ingredients in the TMR were silage (barley and alfalfa), grain (barley or corn), and hay (alfalfa or grass), and mineral supplements. Feed intake was measured daily. Cows were milked in-stall twice daily between 0400 and 0600 and 1530 and 1730. Milk yield was recorded twice daily for 10 d.

This study was conducted between October 2009 and January 2010 in 5 replicates with 7-8 cows per replicate. On day -3 (3 d before PGF) cows were moved from their original stall, and relocated into 1 of the 8 video-monitored stalls. An Afimilk pedometer was then attached to their rear right leg. Cows were given a 3 d adaptation period (days -3, -2, and -1) to ensure they were habituated to the sampling protocol, pedometer, new stall, and neighbour. During this adaptation period, pedometers were scanned and temperatures taken. Pedometers were scanned 6 times per day (0630, 0830, 1230, 1430, 1930, 2130 h) from day -3 to day 6. Rectal temperatures were taken twice daily (0830 and 2130 h) using a digital thermometer (PharmaSystems Inc, Markham, ON, Canada).

**Estrus synchronization, blood collection, and ultrasonography**

To synchronize estrus, each cow received 2 injections, 12 h apart on day 0, of 500μg cloprostenol prostaglandin $F_{2\alpha}$ (PGF) (Estrumate; Schering-Plough Animal Health, Pointe-Claire, QC, Canada). Control cows were given 2 sham injections 12 h apart. Plasma progesterone ($P_4$) concentrations were measured from blood samples collected twice daily (0830 and 2130 h) for 3 consecutive d. Progesterone was measured in order to determine if the treated cows responded to the PGF, cows that respond would have had a large decline in $P_4$ after the PGF was administered. The first blood sample (10 mL) was collected immediately before the PGF was given; samples were collected by coccygeal venipuncture into evacuated tubes containing sodium heparin (Vacutainer, Beckton Dickinson and Co., Franklin Lakes, NJ, USA) and were immediately placed on ice and centrifuged within 1 h at 4°C for 20 min at 1500 X g. Plasma was separated and stored at -20°C. Progesterone concentrations were determined using solid phase radioimmunoassays (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA, USA). Transrectal ultrasonography (Aloka-500V scanner equipped with a 7.5 MHz linear transducer, Aloka Co., Tokyo, Japan) was performed on day -1 until ovulation was confirmed. Control cows had their manure manually removed to simulate a transrectal ultrasonography scan.

**Validation of pedometers**

To determine if pedometers could accurately monitor cow activity in a tie-stall, 24 h video records were analyzed for 17 estrus-induced cows for 6 d (2 d before PGF, the day of PGF, and 3 d after PGF). Observers watched and recorded frequency of lying bouts (definition: the number of events a cow changes her position from standing to laying in 24 h) and lying times (the duration from when a cow's hindquarters touch the ground to the time when the cow lifts her hindquarters from the ground) for all 17 cows. Video records were then compared to pedometer records for subsequent times. Due to poor video quality stepping activity was not validated using the above method.

In order to validate the pedometers for stepping activity observers had to determine what the pedometer counted as a "step". An observer counted the right rear leg movements (where the pedometer was attached) then compared live visual records to pedometer records. The rear leg movements were divided into three categories: 1) stride – defined when a cow lifted her right rear foot off the ground and placed it back on the ground in any forward, sideways or backward position. In order for the observer to count the movement as a stride the cow's body had to move in either a forward, sideways (left or right), or backward direction, 2) lift up – defined by a cow lifting her foot off the ground and placing it back into a similar position, cows body did not have to move in a direction, and 3) weight shift – the cow shifts her weight side-to-side without lifting the foot off the ground. By individually counting the 3 separate movements (stride, lift up, and weight shift) and comparing live-records to pedometer records it was determined that the pedometer counted a "step" using the definition of a "stride" and "lift-up" combined; as a result, a "step" was defined as, when the right rear foot lifted completely off the ground and was placed back to the ground in any location with or without body movement.

In order to validate the pedometers for steps, a preliminary validation study was completed. Two cows were used, and their pedometers were scanned every 3 min for a total of 10 3-min intervals. During these intervals, cows were also video recorded. Using the above definition of a "step" video was analyzed and steps were counted then compared to pedometer records. After obtaining this preliminary data and determining a high correlation between pedometer and video records, a second stepping study using more cows and more pedometer scans was completed.

The second study started at 3 separate time periods throughout the day, 0800 h (during feeding – lots of activity in barn), 1200 h (before PM milking – little activity in barn) and 1700 h (after PM milking - no activity in barn). Four cows had pedometers attached to their right rear leg. Pedometers were scanned every 3 min for a total of 10 3 min intervals. These 10 3-min intervals were recorded then analyzed by an observer counting each step. Between the preliminary study (60 min) and the second study (360 min) a total of 420 min of video data were validated.

**Statistical analysis**

All statistical analyses were performed using SAS software (Release 9.2, SAS Institute, Inc., Cary, NC). To determine if Afimilk pedometers could accurately monitor in-stall cow activity Pearson correlations were used to determine the relatedness of Afimilk pedometer records to video records. To determine the reproducibility between Afimilk pedometers and video records concordance correlation coefficients were used. Concordance correlation coefficients are used to determine the agreement between paired readings (Lin, 1989). It is a measure of precision multiplied by a measure of accuracy and can be used to validate the reproducibility of an instrument (Lin, 1992).

Daily activity data were tested for the assumption of normality; however, activity data were not normally distributed. Steps were successfully transformed using a log10 transformation; therefore, PROC MIXED was used with a repeated statement to determine activity changes over time. Lying bouts and lying time could not be successfully transformed; therefore, PROC GENMOD was used because normality assumption is not necessary (SAS Institute Inc). Least square means were calculated for day, treatment, and treatment by day interactions. A similar model was used to determine changes in feed intake, rectal temperatures, and milk production.

Data was normalized to day of PGF treatment (day 0) for estrus-induced cows and to the day of the sham injection (day 0) for controls. Luteolysis was confirmed when P4 concentration declined to <1 ng/ml, 24 – 96 h after PGF administration (Hittinger et al., 2004). Any cow that did not respond to the PGF was removed from all statistical analysis.

Peak estrous behaviour is expected to occur 18.5 to 48.5 h (average 30.0 h) prior to ovulation (Roelofs et al., 2005); therefore, estrus-induced activity data was also normalized to the day of ovulation (day 0). PROC GENMOD and PROC MIXED procedures using a repeated measures statement were used to determine activity changes preceding ovulation (days -9 to 0) in estrus-induced cows. Cows that did not ovulate were removed from all statistical analysis. Feed intake, rectal temperatures and milk production were analyzed using the same model for estrus-induced cows normalized to day of ovulation.

*P* values of $\leq 0.05$ were considered significant, *P* values between 0.051 and 0.099 were considered trends, and P values $\geq 0.100$ were considered not significant.

**Results**

Plasma $P_4$ concentrations confirmed that luteolysis occurred in 17 of the 19 estrus-induced cows (89.5%) the day after PGF (day 1; Figure 2) Ultrasonography confirmed that 16 of the 19 estrus-induced cows (84.2%) ovulated within 9 d of PGF. Six (37.5%) cows ovulated on day 4, 4 (25.0%) on day 5, 2 (12.5%) on day 6, 2 (12.5%) on day 7, and 2 (12.5%) ovulated on day 9.

**Validation of Afimilk pedometers**

Pedometer records were also highly correlated (P=0.01) to video records for steps (r=0.88) frequency of lying bouts (r=0.94) and lying time (r=0.91). The concordance correlation coefficients (reproducibility) for steps, frequency of lying bouts, and lying time were ($r_c$=0.85), ($r_c$=0.94), and ($r_c$=0.89), respectively (Figure 3, Figure 4, and Figure 5).

**Estrus detection**

**Data surrounding day of PGF or sham treatment**

Overall (over 10 d sampling period) daily (24 h) mean steps and frequency of lying bouts did not differ (P=0.37, P=0.93; respectively) between estrus-induced and control cows; however, estrus-induced cows had lower overall mean lying time for the 10 d sampling period (646.7 vs. 790.8 min; P=0.01) compared to control cows. During the predicted estrus period (48-96 h after PGF) there were no changes in activity (by day) in the estrus-induced cows before or after the PGF injection.

Pedometer records were also analyzed separately for daytime (0630 – 2130 h) and nighttime (2130 – 0630 h). Over the 10 d period, daytime mean steps, frequency of lying bouts and lying time did not differ (P=0.56, P=0.81, P=0.38; respectively) between estrus-induced and control cows, although estrus-induced cows had lower lying time compared to control cows on days -3,-2,0,1,2, and 6 (**Table 2**), Over the 10 d period nighttime steps and frequency of lying bouts did not differ (P=0.19, P=0.52; respectively) between estrus-induced and control cows, yet, over the 10 d period lying time was lower (285.92 vs. 357.17 min; P=0.01) for estrus-induced cows compared to controls.

Feed intake and rectal temperatures did not differ between estrus-induced and controls cows. Estrus-induced cows had higher milk production (38.8 vs. 30.6 kg/d; P=0.02) compared to control cows.

**Data normalized to day of ovulation**

For estrus-induced cows (n=16) Afimilk pedometer records were normalized to the day of ovulation. In the 72 to 96 h preceding ovulation (days -3 to -1) daily (0630–0630 h) steps, frequency of lying bouts, and lying time (

Figure 6, Figure 7, and Figure 8) respectively did not change. Steps, frequency of lying bouts, and lying time did not change throughout the daytime (0630–2130 h) or nighttime (2130-0630 h) activity events. Feed intake, rectal temperature and milk production did not change in the estrus-induced cows surrounding ovulation.

## Discussion

Our first hypothesis was supported; results showed that Afimilk pedometers accurately record steps, frequency of lying bouts, and lying time in tie-stall-housed cows. There was a strong correlation between pedometer and video records for steps, frequency of lying bouts and lying time (Figure 3, Figure 4, and Figure 5) Higginson et al. (2009) completed a pedometer validation study comparing Afimilk pedometers to IceTags pedometers (IceRobotics, UK) and found the two pedometers were highly correlated for steps, frequency of lying bouts and lying times in free-stall housed cattle (Higginson et al., 2009). Afimilk pedometers were closely related to video records, in the present study, and as a result, they accurately record cow activity in a tie-stall facility.

The results of this study did not support our second hypothesis; Afimilk pedometers could not facilitate estrus detection because estrus-induced tie-stall cows expressed no change in steps, frequency of lying bouts or lying time during the predicted estrus period. Unal (1986) found similar result for lying activity by visually observing stanchion-housed cattle throughout a 9 h undisturbed period (1900 to 0400 h). It was determined that lying bouts and standing time increased in only 2 of the 16 estrus periods; concluding that lying bouts and standing time are weak estrus detection aids in stanchion-housed cattle (Unal et al., 1986). Although this suggests that we should not have expected changes in lying activity during estrus, there are important differences between the study by Unal (1986) and the current study. First, cows were visually observed for a 9 h undisturbed period: second, cows were milked in a parlor, and third, cows were given a daily out-of-stall exercise period (Unal et al., 1986). In comparison, in the present study cow activity was automatically monitored for 24 h/d by Afimilk pedometers, and cows continuously remained in a tie-stall (milked in stall) for 9 d. Activity data during an undisturbed 9 h period is not an accurate assessment of 24 h activity. Routine barn activities (milking, feeding etc.) can affect cow activity, and without recording 24 h activity it is difficult to determine activity changes throughout estrus. Cows may have also expressed changes in activity during the unobserved 15 h period or during the out-of-stall milking/exercise period. Because cows had an out-of-stall period and were not observed for 24 h, research was required to determine if activity changes during estrus in continuously- housed cows.

We hypothesized that during estrus an increase in steps, frequency of lying bouts, and a decrease in lying time would occur because of the previously reported information on estrus behaviour. Kiddy (1977) determined that, during estrus, lactating dairy cows housed in tie-stalls exhibit 2.75 times higher activity than cows not in estrus. Redden et al. (1992) and Kennedy and Ingalls (1995) determined, using pedometers, that estrus could be detected in tie-stall cows. The most recent study monitored activity using a newly developed pedometer system (Gyuho; Comtec, Miyazaki, Japan) in continuously-housed (tie-stall) virgin dairy heifers and determined that the estrus detection efficiency and accuracy rates were between 78-87% and 78-83%, respectively (Sakaguchi et al., 2007). Walton (1986) determined, through visual observations and recorded video, that during estrus, lactating dairy cows housed in tie-stalls have increased changes in position from standing to lying (Walton and King, 1986). Pollock and Hurnick (1979) also determined that lying time and eating time decreased in tie-stall cows on the day of estrus (Pollock and Hurnick, 1979). As a result, it was hypothesized that Afimilk pedometers would be able to facilitate estrus detection, based on changes in activity during estrus; however, our results did not support our hypothesis. There were no differences in activity between the estrus-induced and control cows during the predicted estrus period.

The results for the second objective were not expected based on the findings of Kiddy (1977), Walton and King (1986), Redden et al. (1992), and

Kennedy and Ingalls (1995), and Sakaguchi et al. (2010) describing estrus activity; this may be due to differences in the methods between the studies. Firstly, cows in the current study remained continuously in-stall, unlike cows in the previous studies, Kiddy (1977), Redden et al. (1992), and Kennedy and Ingalls (1995). Cows in previous studies were given an out-of-stall exercise period or walked (distances between 30 and 125 m) to an open holding pen to be milked in a parlor. Thus, estrus-related activity could have occurred during these out-of-stall periods, when cows were free to move around interacting with one another. In the previous studies, pedometer activity was recorded during milking. Therefore, these studies cannot be confident that the changes in estrus-related activity described occurred while cows were in-stall or during the out-of-stall periods. There was no opportunity for the cows in the current study to move freely, interact with other cows and express changes in activity during estrus. When cows are able to interact and more than one cow is in estrus at the same time it increases the expression of estrus behaviour and mounting activity (Hurnik et al., 1975). We believe that if the cows in the current study were given an out-of-stall period, changes in activity during estrus would have been observed and detected by the Afimilk pedometers. A recent study monitored activity in continuously-housed (tie-stall) virgin heifers and determined that pedometers could accurately detect estrus at differing rates dependent on the reference period and the threshold set (Sakaguchi et al., 2007). The main difference between the current results and that of Sakaguchi's may be explained because dairy heifers normally express intensified estrus-related activity compared to cows (Sakaguchi et al., 2007).

Therefore the results in Sakaguchi et al. (2007) are not comparable to the results found in the current study which monitored lactating dairy cows (≥1 lactation) not heifers. Continuous restraint of lactating dairy cows may have been responsible for the lack of change in activity; although Hackett et al (1984) found that cows continuously-housed indoors in tie-stalls period had fewer days to first recorded estrus, fewer days to first service and overall more cows observed in estrus compared to cows housed in a loose-housing barn; estrus detection was preformed while the cows were held in the holding pen prior to milking.

There are also physiological explanations that affect activity changes; one in particular is silent estrus. Silent estrus is when cows do not express sexual behaviours or do not increase their walking activity in association with estrus (Ranasinghe et al., 2010). Ranasinghe et al. (2010) used pedometers to record changes in activity surrounding estrus in a free-stall facility and reported that 55.2, 23.8, 21.3, and 10.5% of cows, respectively, had silent estrus in their first, second, third, and fourth ovulation, postpartum. Cows commonly do not express overt estrous behaviours preceding the first ovulation after calving. This is because the hypothalamus is not sensitive to $E_2$ due to the high $E_2$ concentration in late gestation and the decreased production of $E_2$ from the preovulatory follicle (Allrich, 1994). All cows in the current study had resumed cyclicity and were in their second or third estrous cycle postpartum; therefore, an increase in activity was expected. Once $P_4$ acts on the hypothalamus making it sensitive to $E_2$, estrus-related activities are expected in subsequent estrous cycles; as a result, normal estrous-related activity was expected in the cows used in the present study.

There are certain factors that increase the risk of silent estrus. Firstly, during early lactation when cows are in a state of negative energy balance. During energy deficits the production of $E_2$ may be reduced in the Graafian follicle along with lowered hypothalamic sensitivity to $E_2$ (Isobe et al., 2004). Lower $E_2$ production or lower sensitivity to $E_2$ would result in decreased expression of estrus-related behaviours or silent estrus.

Secondly, when cows produce moderate to high milk yields they are a higher risk of silent estrus. An increased risk of silent estrus in the second, third, and fourth ovulation was observed in cows producing ≥27.8 kg/d (Ranasinghe et al., 2010). In the current study estrus-induced cows were producing, on average, 38.8 kg/d; therefore, they would have had an even higher risk of experiencing silent estrus. The estrus-induced cows had higher milk productions than the control cows. As the two groups were at different stages of lactation differences in milk production were expected. Estrus-induced cows were at peak to mid lactation while control cows were in late lactation. On the day of estrus, high-producing cows (≥39.0 kg/d) had lower circulating $E_2$ concentrations (the hormone responsible for estrus-related activity), shorter standing events, shorter standing time and an overall shorter duration of estrus compared to lower producing cows (Lopez et al., 2004). Lactating dairy cows have a 52% increase in blood flow rate through the liver compared to non-lactating dairy cows (Lomax and Baird, 1983). Increased blood circulation (associated with high feed intake) can increase metabolic clearance rate of steroid hormones, thereby decreasing the circulating concentration of estrogen in lactating cows and increasing the risk of silent estrus (Sangsritavong et al., 2002). These silent estrus risk factors may have affected the cows in the current study thereby contributing to the lack of activity change observed during the estrus period. Estradiol was not measured in this study; consequently, it is only speculated that lower circulating $E_2$ concentrations may have been a contributing factor for lack of activity observed during estrus in the estrus-induced cows.

The mean duration of lying time (total for 10 d sampling period) was shorter in the estrus-induced cows compared to the control cows. This means that control cows lay for longer periods than estrus-induced cows, although, there were no day-to-day differences in 24 h records. The overall decrease in lying time for estrus-induced cows in the present study was not attributable to estrus-related activity, as there were no decreases in lying time during the expected estrus period, i.e. during the 72 -96 h preceding ovulation. Our result, that estrus-induced cows (who also produced more milk) had shorter overall lying time and shorter lying times during the daytime period on certain days throughout the study is similar to that of Fregonesi and Leaver (2001) who found that higher yielding cows have shorter lying times. The decrease in lying time in cows producing more milk may be to due to increased time standing to feed in order to meet their higher nutritional requirements during peak lactation (Bewley et al., 2010).

Rectal temperatures did not differ between estrus-induced and control cows. This is supported by Yadav et al. (1986) unpublished data; cited in (Walton and King, 1986) who determined that rectal temperatures did not increase in continuously restrained cattle during estrus; yet, Walton and King (1986) found contrasting results. They determined that rectal temperatures increased on the day of estrus in cows housed in stanchions, but they were not continuously-housed in stalls because they were walked to a parlor for milking. Walton and King (1986) suggested that the increase in rectal temperature is attributed to increased activity expressed preceding the temperature measurement (Walton and King, 1986). Therefore, because estrus-induced cows in this study were continuously-housed in-stalls without changes in activity, during estrus, it is reasonable to expect no change in rectal temperatures.

Feed intake did not change in association with estrus, which is supportive of the results of De Silva et al. (1981) and Kerbrat and Disenhaus (2004). De Silva et al. (1981) found no correlation between feed intake and estrous activity, there were also no changes found in feed intake 3 d before or 3 d after estrus (De Silva, Anderson et al., 1981). Kerbrat and Disenhaus (2004) measured time spent eating; no differences were observed in time spent eating on the day of estrus (Kerbrat and Disenhaus, 2004). Therefore, because the estrus-induced cows did not experience estrus activity changes it may be fair to assume that feed intake would remain constant. These results may also show that there are no relationships between feed intake and estrous activity in dairy cows continuously-housed in tie-stalls.

Tie-stalls restrict the freedom of movement and alter normal activity patterns in dairy cattle (Krohn, 1994). Our results suggest that tie-stalls restrict normal estrus-related activity changes observed in dairy cattle. Out-of-stall cows express estrus by interacting with other cows and displaying sexual behaviours such as: chin resting, mounting, and standing estrus (Esslemont et al., 1980); however, if cows are continuously tethered they are unable to interact sexually and estrus-related activities become restricted. We had expected that the cows continuously-housed in tie-stalls cows would show increased restlessness through increased standing and stepping activity, and an increased frequency of lying bouts; but contrary to our expectation, these changes did not happen.

**Conclusions**

Afimilk pedometers can accurately monitor steps, lying bouts, and lying time in cattle housed continuously in tie-stalls. However, because cows in the present study did not have increased activity during estrus, Afimilk pedometers were unable to facilitate estrus detection in the studied dairy cows that were continuously-housed in tie-stalls.

**Table 2. Average lying time (min) during the day (0630 – 2130 h) between control and estrus-induced cows. Day 0 = sham (control) or PGF (estrus-induced) treatment; estrus-induced cows were expected to be in estrus between 48 and 96 h after PGF treatment, and have an associated reduction in lying time.**

| Day | Control | Estrus-induced | *P* |
|---|---|---|---|
| -3 | 372.4 | 310.5 | 0.05 |
| -2 | 453.4 | 389.3 | 0.04 |
| -1 | 456.2 | 421.9 | 0.27 |
| 0 | 434.4 | 356.3 | 0.01 |
| 1 | 456.7 | 383.4 | 0.02 |
| 2 | 472.0 | 372.2 | 0.01 |
| 3 | 442.5 | 386.3 | 0.07 |
| 4 | 443.6 | 395.1 | 0.12 |
| 5 | 424.5 | 372.8 | 0.09 |
| 6 | 447.5 | 369.5 | 0.02 |

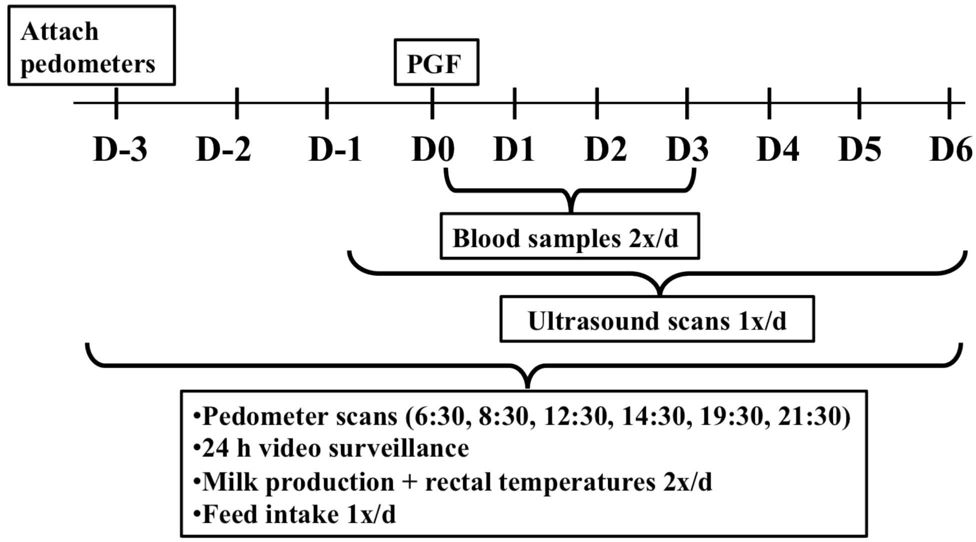



**Figure 1.** Experimental design and treatment schedule. PGF = Prostaglandin F2α.

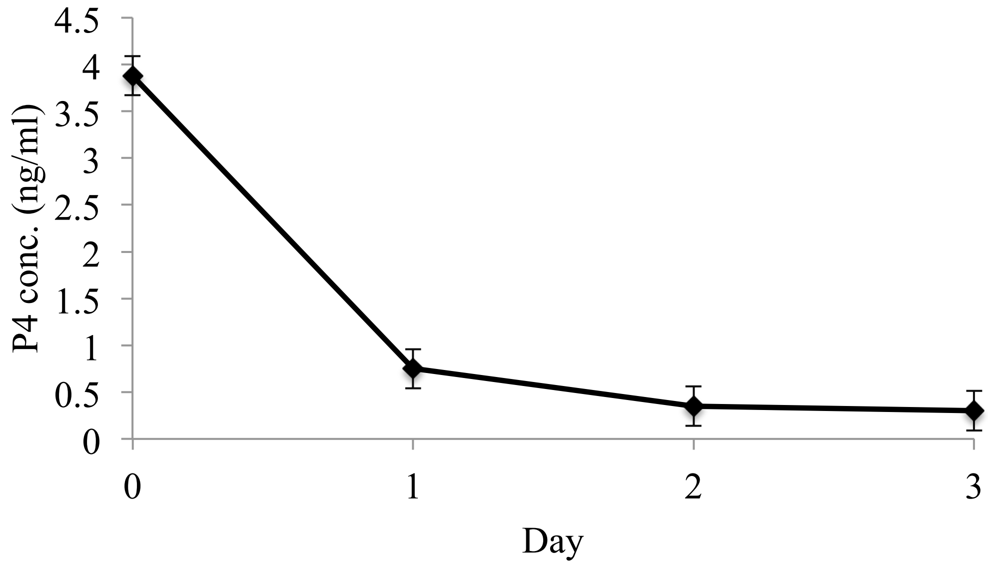



**Figure 2.** Average progesterone ($P_4$) concentrations per day from cows that responded (n=17) to the PGF treatment and regressed their CL (P=0.01).

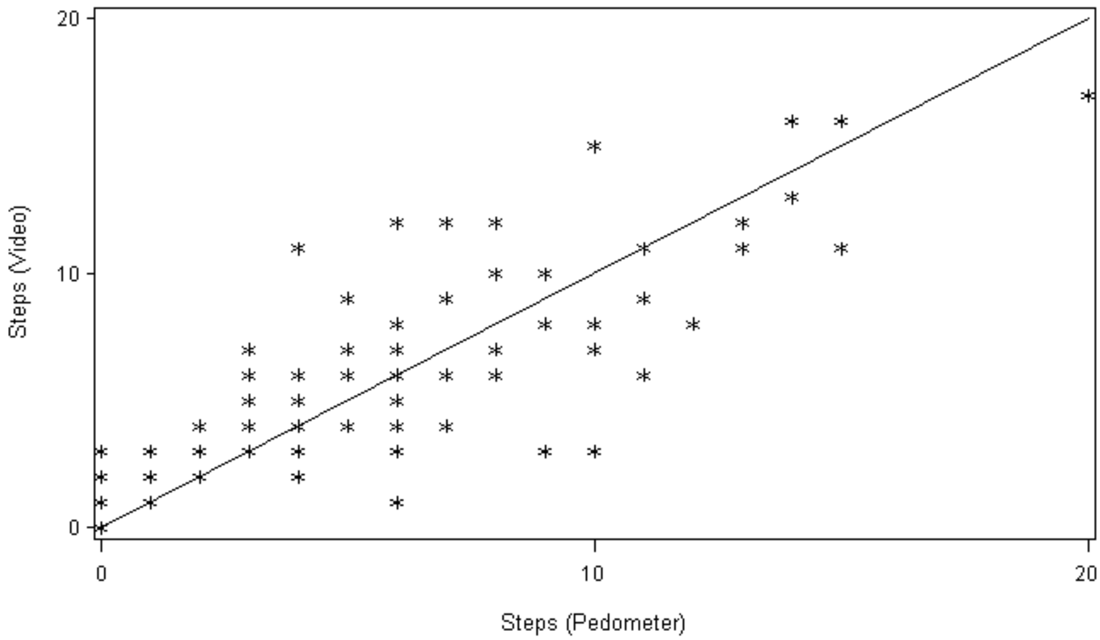



**Figure 3.** Concordance correlation for steps between Afimilk pedometer and video ($r_c$=0.85).

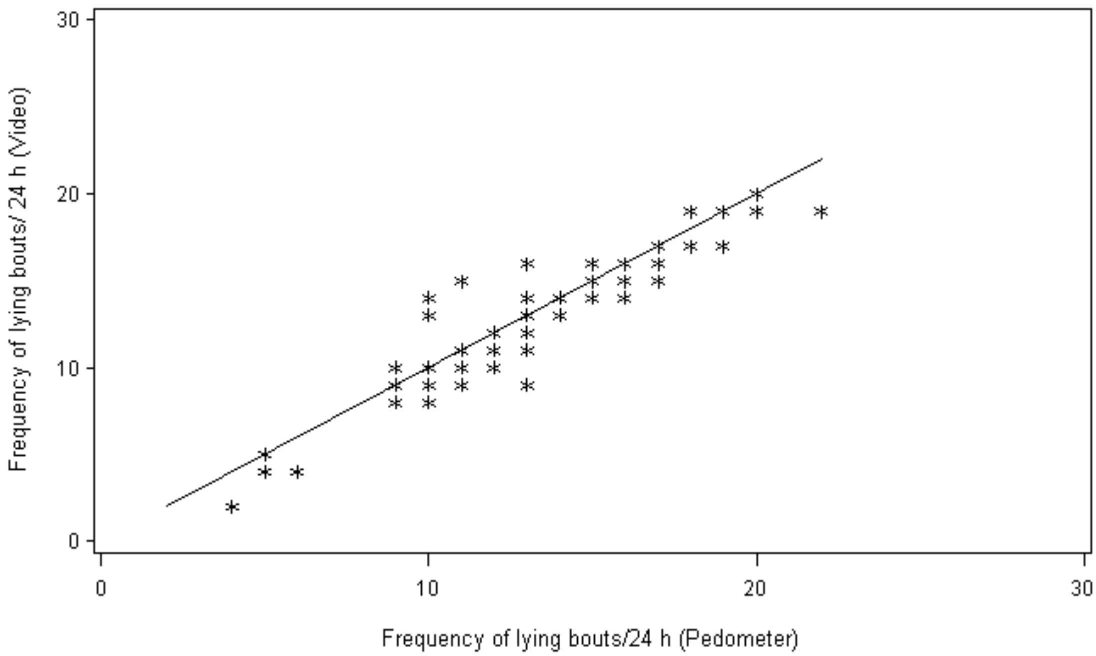



**Figure 4.** Concordance correlation for lying bouts / 24 h between Afimilk pedometers and video ($r_c$=0.94).

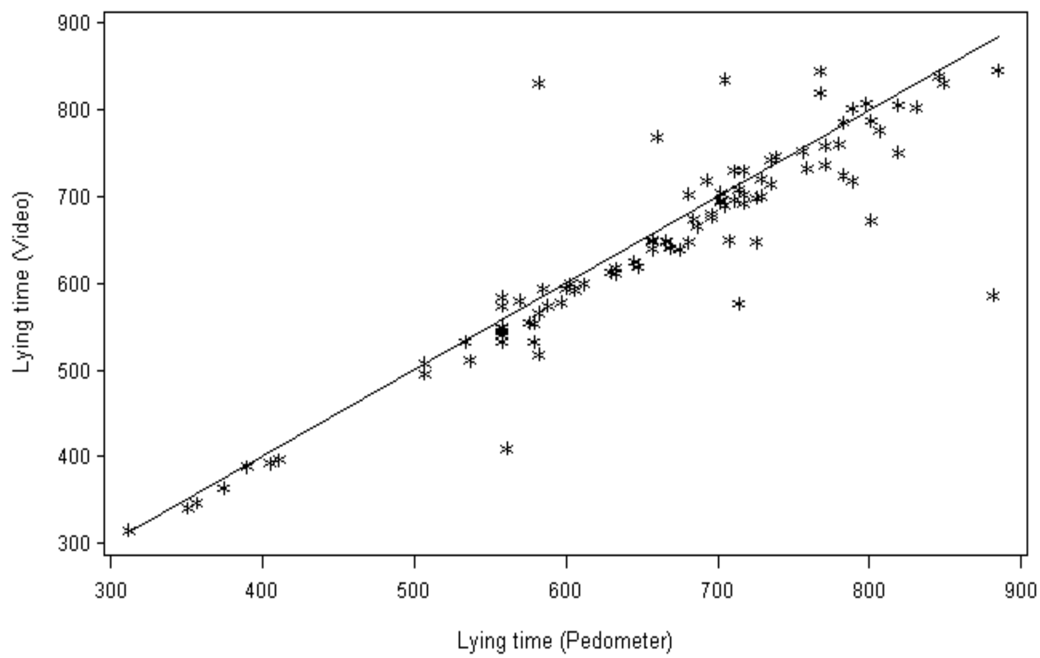



**Figure 5.** Concordance correlation for lying time between Afimilk pedometers and video ($r_c$=0.89).

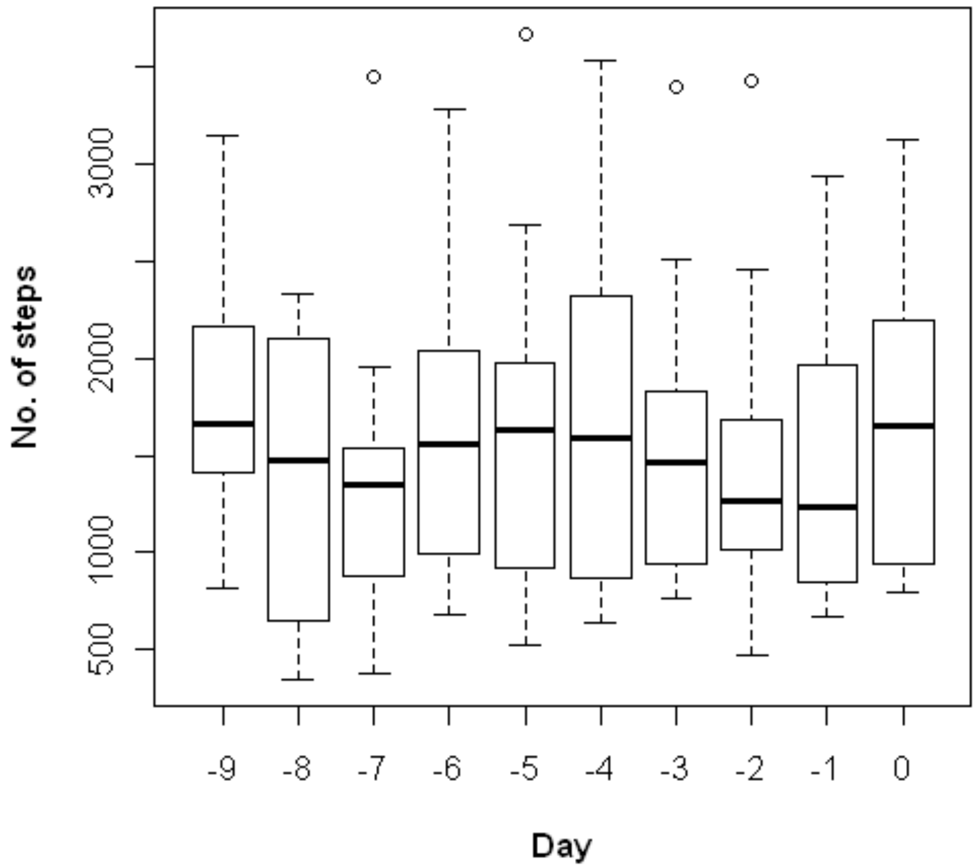



**Figure 6.** Daily steps normalized to ovulation (day 0) in 16 estrus-induced cows. (P=0.13) The box itself represents 50% of the data, while the upper hinge of the box represents the 75$^{th}$ percentile and the lower hinge represents the 25$^{th}$ percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

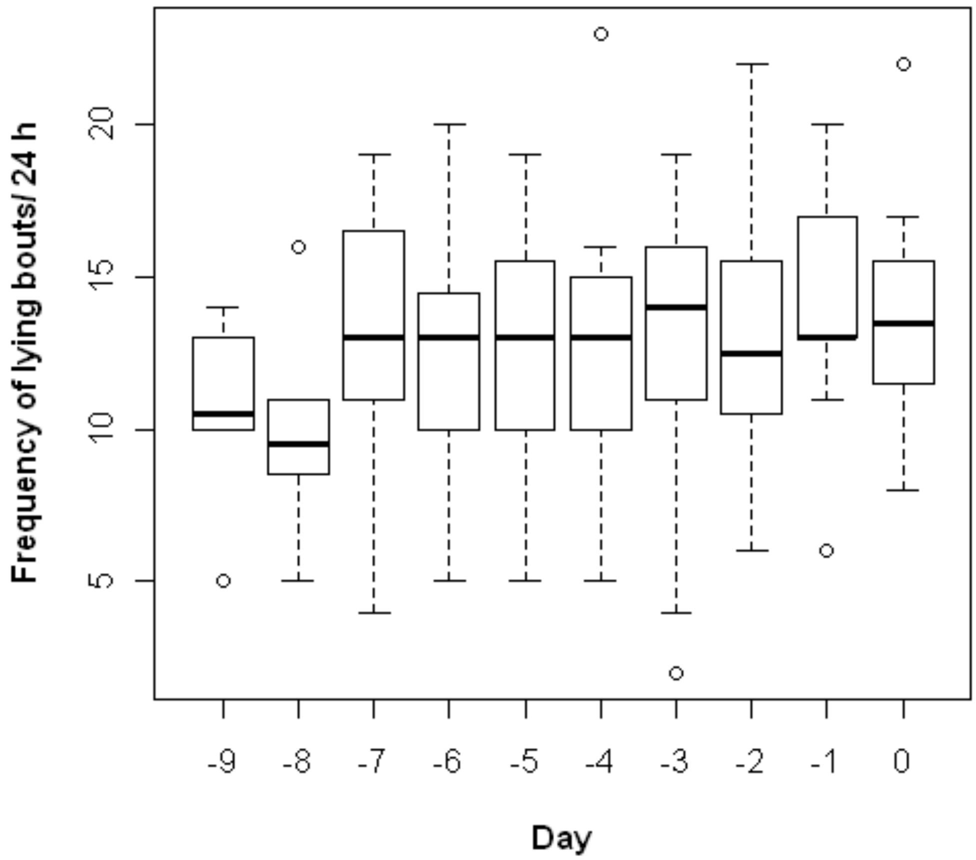



**Figure 7.** Daily frequency of lying bouts normalized to ovulation (day 0) in 16 estrus-induced cows. (P=0.74) The box itself represents 50% of the data, while the upper hinge of the box represents the 75th percentile and the lower hinge represents the 25th percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

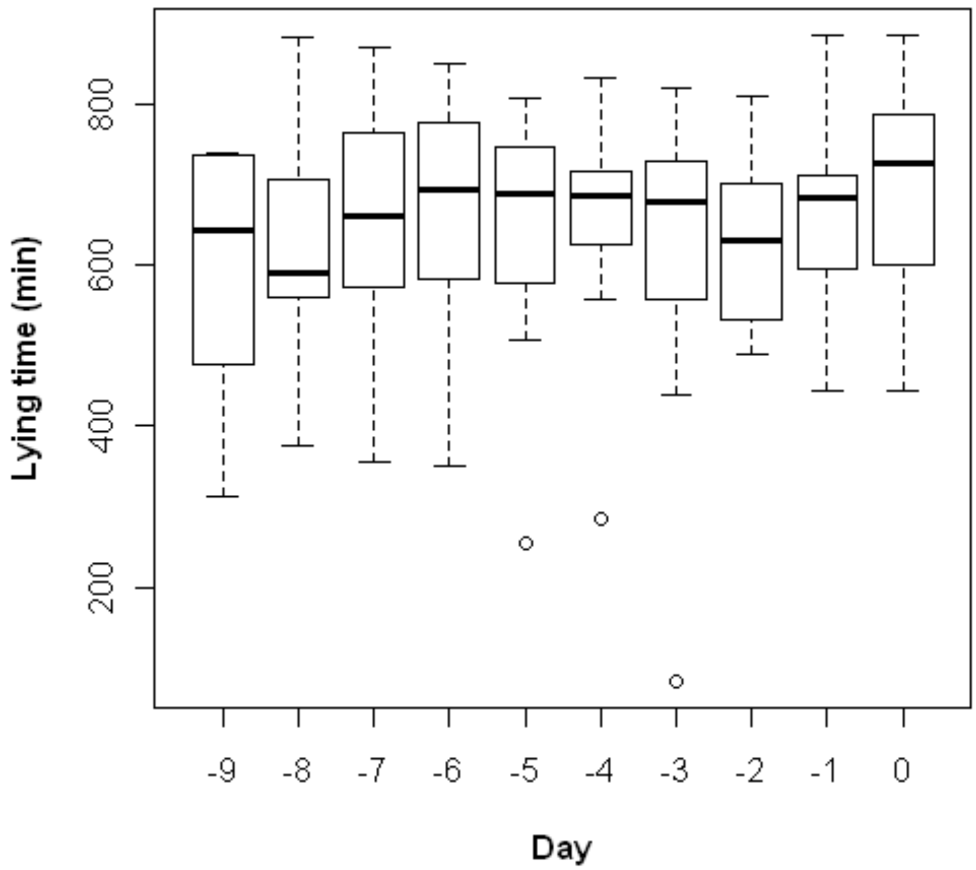



**Figure 8.** Daily lying times normalized to ovulation (day 0) in 16 estrus-induced cows. (P=0.34) The box itself represents 50% of the data, while the upper hinge of the box represents the 75th percentile and the lower hinge represents the 25th percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

## Chapter 4. Evaluation of the Afimilk Pedometer Plus system for predicting parturition in dairy cows housed in tie-stalls

### Introduction

Cows experience physiological changes during parturition; these physiological changes are manifested and expressed through activity changes. Activity changes during late gestation could be used to predict parturition. If the onset of parturition was accurately predicted cows could be moved into a maternity pen prior to parturition, which would improve dam and calf comfort because a maternity pen provides a clean, safe space dedicated for parturition. A maternity pen provides space required for the dam to express natural parturition-related activity without being tethered. By monitoring prepartum activity changes it may be possible to accurately detect the onset of parturition.

Previous research focusing on prepartum activity changes has been conducted on free-stall or group-housed cattle (Huzzey et al., 2005, Maltz and Antler, 2007, Miedema et al., 2011a, Miedema et al., 2011b. Holstein cows, housed in a free-stall barn, had longer standing time and increased number of standing bouts (interval between 2 lying events) during the immediate prepartum period (day -1 to day 1; day 0 = calving) compared to the pre (day -9 to -2) and post (day 2 to 10) partum periods (Huzzey et al., 2005). In a second study cows were housed in a straw-bedded barn. Within 24 h of parturition, the number of lying bouts (periods of lying, separated by periods of standing or walking), and walking bouts (periods of walking, separated by periods of standing or lying) increased while the duration of lying time decreased compared to a prepartum control period (days -4 to -1) (Miedema et al., 2011b). In a third study, 12-preparturient cows were housed in free-stalls. The daily number of steps increased, lying time decreased, and the ratio of steps to lying time increased within 24 h of parturition (Maltz and Antler, 2007). Although these studies demonstrate that activity change associated with imminent parturition is apparent in free-stall-housed cattle, to our knowledge, little research has been done describing prepartum activity changes in cows housed in tie-stalls during late gestation. This information is important as 75% of Canadian dairy herds are tie-stalls (Canadian Dairy Information Centre, 2011).

The AfiMilk Pedometer Plus™ system (Afimilk pedometers) can not only record steps, but they have an additional sensor (Behaviour Tag®), which records number of lying bouts and lying time (duration of a lying event). Cows are more restricted in their movements in a tie-stall vs. a free-stall facility; therefore, these additional features relating to lying bouts and lying time in the Afimilk pedometers may be useful in facilitating the prediction of parturition in cows housed in tie-stalls. Being able to predict parturition and move cows into maternity pens on time will improve dam and calf welfare it also reduces environmental stress.

In order to predict the onset of parturition in both young and mature cows it is important to understand differences in prepartum activity between primiparous and multiparous cows. In one study, primiparous cows and multiparous cows were classified during the first stage of parturition as calm, restless, or very restless. Primiparous only were classified restless or very restless

(Wehrend et al., 2006), indicating that primiparous cows will have increased activity prior to and during parturition. The previous research relating to parity differences on prepartum activity is limited and there is little discussion of activity differences between primiparous and multiparous cows housed in tie-stall-housed cattle.

Monitoring activity changes and predicting the onset of parturition in tie-stall-housed cattle would ensure that cows get moved to maternity pens prior to parturition. Cows should be moved into a maternity pen a minimum of 24 h before expected parturition (Mee, 2004). Moving cows to a clean maternity pen increases comfort and safety, which may increase welfare by reducing environmental stress caused from tethering during calving or disturbances from routine barn work. It would be valuable to automatically predict the onset of parturition especially in dairy facilities that have difficulty observing individual cow activity, thereby reducing in-stall calvings. To our knowledge, no research has been conducted using pedometers to automatically record prepartum activity in order to retrospectively determine if the onset of parturition can be predicted. It is also unclear if activity changes differ between primiparous and multiparous cows during late gestation. Therefore, the primary objectives in this study were to determine, retrospectively: 1) if Afimilk pedometers could assist in the prediction of parturition in cows housed in tie-stalls, and 2) if parturition-associated activity differed between primiparous and multiparous cows. We hypothesized that 1) Afimilk pedometers could facilitate the prediction of imminent parturition by recording increased restlessness (increased steps, increased frequency of lying bouts, and decreased lying time) in the days prior to parturition, and 2) that primiparous cows would exhibit increased restlessness compared to multiparous cows.

**Materials and methods**

This study was conducted with 24 Holstein cows (12 primiparous and 12 multiparous) at the DRTC, University of Alberta, Edmonton Alberta. The DRTC is a 146-cow tie-stall herd. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009), and the University of Alberta's Animal Care and Use Committee approved experimental protocols.

**Activity monitoring**

Pedometer system

The AfiMilk Pedometer Plus™ system (S.A.E. Afikim, Kibbutz Afikim, Israel) is an electronic monitoring device designed to detect estrus in dairy cows through measuring activity (steps, lying bouts, and lying time). Each pedometer has an individual identification number, which is set to the individual cow ID. A pedometer sensor (located for this study on a portable rod with a fixed wired connection to a computer) was used to manually scan each pedometer and capture the recorded activity data; this information was automatically downloaded and stored in the Afifarm herd management software in a dedicated computer. To record activity this study used unprocessed raw activity measurements (steps, lying bouts, and lying time) obtained from scanning each pedometer and calculating the differences between scans.

Video surveillance

Eight tie-stalls, located in the main barn equipped with 24 h video surveillance were used to house 8 prepartum cows at a time. There were 2 monochrome cameras (Sony Super HAD ccd, SSC-M183), each camera captured 4 stalls, and was mounted on a beam running perpendicular to the cows (8 m away from the cows). Cameras captured the cows from a rear view (tail to head) in order to record stepping, lying activity, and signs of parturition. Cameras were connected into a 4-channel digital black and white quad (Robot MONOCHROME QUAD, MV47, Sensormatic Electronics Corporation, ADT security services, Canada). The video quad received video signals from the 2 cameras and allowed multiple video signals to be viewed on a single screen. Video was recorded onto digital external hard drives (iPRO Drive, Futureshop, Edmonton, Alberta Canada) through a pinnacle video transfer (Pinnacle Systems, Mountain View, California USA) which records 4 hours of video and saves it to a compressed file format (mp4). The compressed video format allowed for continuous recording.

A maternity pen (2.7 m by 4.9 m), used for parturition, was located in a building adjacent to the main tie-stall barn. The maternity pen was equipped with 4 colour video cameras (Panasonic colour WV-CP470, CCTV camera) mounted (2.4 m high) at the top of the pen gates at the 4 corners of the pen. There was 24 h video surveillance once a cow was placed inside the maternity pen. Cameras were wired (RG-59 Siamese cable, Premium Power/Video cable) to a stand-alone digital video recorder (DVR) (AverMedia, 4 channel real time MPEG4 DVR, EB1303NET SATA, Milpitas, California USA).

**Animals and experimental design**

Three weeks prior to the estimated parturition date, cows were brought into the barn and housed in 1 of the 8 tie-stalls with 24 h video recording. Cows had unrestricted access to water and were fed a total mixed ration once per day (0800 h) formulated for close-up dry cows according to NRC guidelines (National Research Council. 2001). Main ingredients in the TMR ration were silage (barley), hay (alfalfa), and grain (barley or corn).

Cows were monitored by pedometer scans from approximately 3 wk before parturition to 7 d after parturition. There were differences between predicted and actual parturition dates; therefore, the analysis began 9 d before calving to 7 d after calving. Pedometers were attached to the right rear leg of each cow and scanned twice daily (0800 h and 1700 h). Rectal temperatures were taken once daily (1700 h) using a digital thermometer (PharmaSystems Inc, Markham, ON, Canada). Feed intake was measured daily (0700 h). Cows were taken outside for a 2 h exercise period between 0900 and 1100 h sporadically throughout this study.

A prepartum-scoring index was created to monitor visual changes during the 3 wk postpartum period (Figure 9). Scores were taken once daily (1700 h) and ranged from 0 (udder not full, vulva normal size) to score 4 (stage 2 of parturition, immediately prior to expulsion of fetus, feet or water bag showing). The scoring index (was designed, with the help of the herd manager, to create a consistent scoring system that staff could use to determine when cows should be moved into the maternity pen. Score 3 (reddish mucus hanging from vulva) was the stage barn staff were instructed to move cows into the maternity pen. The score 3 descriptor (reddish mucus) was recommended by the herd manager, as this was the common practice in this herd.

Once in the maternity pen, researchers and staff monitored the cow. The maternity pen had a concrete floor bedded with approximately 15 cm of wood shavings and free access to water. Once a cow was placed in the pen the left over feed from their tie-stall was removed and placed in a trough in the maternity pen. Using their own judgment, staff would intervene to assist in the parturition if sufficient progress was not being made or if the cow appeared to be in distress. There was no means of determining pull-strength between technicians offering calving assistance; therefore, assistance during parturition was not analyzed in this study. No caesarean sections were performed on any of the cows. Once cows calved they were given time to lick their calves clean and then immediately returned to their tie-stall, usually within 2 h after calving.

**Statistical analysis**

All statistical analyses were done using the SAS software (Release 9.2, SAS Institute, Inc., Cary, NC). To determine prepartum activity, daily pedometer records from day -10 to day -1 were analyzed. Activity records were normalized to calving (day 0) then split into prepartum (day -10 to day -1) and postpartum (day +1 to day +7) periods. Day 0 activity records were not analyzed because the objective was to determine if the onset of parturition could be predicted in time to move cows into maternity pens. It is recommended to move cows a minimum of 24 h before the predicted parturition time (Mee, 2004); therefore, day -1 was used as the last activity record prepartum.

To determine changes in activity, steps were log-transformed to fit the test of normality, whereas lying time was normally distributed. A PROC MIXED procedure in SAS was used and contained a repeated measures statement. PROC MIXED was used because it can handle missing data, whereas PROC GLM cannot (Wang and Goonewardene, 2004). Frequency of lying bouts could not be transformed to meet the requirements for the assumption of normality; therefore, PROC GENMOD was used to determine differences in lying bout activity. The results presented for frequency of lying bouts were analyzed using PROC GENMOD. Paired t-tests were used to determine differences in duration of parturition, duration from moving to calving, duration of calving between primiparous and multiparous cows, and differences between calf weights. PROC GLM was used to determine differences between prepartum score and calving duration. *P* values of $\leq 0.05$ were considered significant, *P* values between 0.051 and 0.099 were considered trends and P values $\geq 0.100$ were considered not significant.

Duration of parturition data were obtained from video recordings of the tie-stall or maternity pen from the initiation of straining to expulsion of calf. Pedometer records from 10 d before parturition to 7 d after parturition were analyzed for activity changes. A 15 h (900 min), undisturbed, continuously in-stall period from 17:00 – 08:00 h was used to measure activity changes. The 900 min period is described as the "daily" activity throughout the study. This period was used because cows consistently remained in-stall during this period.

Parity, and location of parturition were used in the SAS model as fixed variables to determine if these affected the independent variables of activity, rectal temperatures or feed intake surrounding parturition. Once it was determined that these variables were not significant they were removed from the model.

Day of parturition was defined as day 0 (from 0800 to 0800 h). Day 0 was defined this way because the time when cows calved varied throughout that 24 h period. One multiparous cow calved outside during the exercise period (unmonitored) between 0800 and 1200 h and was therefore removed from any data analysis-involving day 0 and or duration of calving.

## Results

### Parturition data

Out of the 24 calvings, 12 cows (50%) required assistance during parturition, 7 of which were primiparous and 5 multiparous. Sixteen cows calved in the maternity pen while 7 calved in the tie-stall and 1 calved outside in the exercise pen. The breakdown of parturition information into parity and location is presented in Table 3. There were 4 out of 24 (16.6%) malpresentation calvings (3 were assisted: 2 dead at arrival; 1 unassisted) and a total 3 out of 24 (12.5%) calves were dead at arrival. There were 13 heifer calves and 11 bull calves with average weights of 42.1 and 44.2 kg (P=0.67) respectively.

The duration of parturition was measured from the first time a cow was visibly straining (laying on side with the legs stretched out, straining every 4 to 10 min, increased breathing, noticeable contractions occurring throughout abdomen) until expulsion of the fetus (calf completely outside cows body). The median duration of parturition for primiparous and multiparous cows was similar (P=0.24) at 65.1 min (range 22.1 – 561.7 min) and 58.9 min (range 4.85 – 88.5 min), respectively.

The time when cows were moved into the maternity pen to the time of expulsion of calf varied considerably between animals (range, 32.3 – 803.2 min). Primiparous (n=9) and multiparous cows (n=7) had similar (P=0.72) durations from moving into maternity pen to expulsion of (median 125.8 min (range 32.3 – 803.2 min) and (median 222.4 min (range 78.9 – 765.9 min), respectively. The prepartum index score that cows were given prior to moving from their tie-stall to the maternity pen had no relationship with the duration of calving. The prepartum index score, the number of cows in each group, and the average and range of duration of parturition from moving to expulsion are presented in Table 4.

**Prepartum activity**

Due to malfunctioning of the pedometer scanner during some scans, pedometer activity analysis between day -2 and -1 and between day -3 and -1 were based on only 16 and 17 cows, respectively. Retrospective review of pedometer logs (average for all 24 cows) indicated that stepping began to increase from day -10 by approximately 10% each day until day -2, after which, a mean increase of 34% (range, 32% decrease to 119% increase) occurred from day -2 to day -1 (1259.5 vs. 1684.5; P=0.01). There was a large variation in stepping activity change from day -2 to day -1 from 16 cows that had pedometer records

(Table 5). During the prepartum period frequency of lying bouts stayed fairly constant (Day P=0.50) with a 19% increase (range, 50% decrease to 250% increase) from day -3 to day -1 (7.1 vs. 8.5 bouts/15 h; P=0.05). Lying time decreased by 11% (range, 54% decrease to 350% increase) from day -2 to day -1 (392 vs. 349 min; P=0.11). The degree of restlessness in the days prior to parturition varied considerably within cows and between days (Figure 10, Figure 11, Figure 12 and Figure 13 a,b,c,d,e,f).

There were no day-by-parity effects cows in steps, frequency of lying bouts, or lying time between primiparous and multiparous. Activity was summarized for the entire prepartum period (days -10 to -1); primiparous cows had increased stepping (1383 vs. 1071; P=0.07) and shorter lying time (387 vs. 467; P=0.02) than multiparous cows.

Rectal temperatures and feed intake

There was a slight decrease in rectal temperature from day -2 to day -1 (38.9 vs. 38.7°C; P=0.03). Multiparous cows tended to consume more feed during the prepartum period compared to primiparous cows (22.9 vs. 17.4 kg; P=0.08). Day, parity and location during parturition had no effect on prepartum rectal temperature or feed intake.

**Postpartum activity**

During the postpartum (day +1 to day +7) period daily steps decreased from day 1 to day 2 by 13.4%, (1241.9 vs. 1075.8; P=0.89). Steps stayed fairly constant (P=0.12) with a consistent decrease from day 3 until day 7, average 6.9% per day. Frequency of lying bouts stayed fairly constant between 7.6 and 8.2 bouts per day throughout the postpartum period (P=0.99). Parity had no effect on stepping or lying bout activity. Lying time remained fairly constant throughout the postpartum period (P=0.43). Parity had an effect on lying time; primiparous cows had laid less (346.0 vs. 468.6 min; P=0.01) than multiparous cows throughout the postpartum period.

From day +1 to day +7 rectal temperatures fluctuated between 37.4 and 40.7°C from day +1 to day +7 however, no day-to-day effects were observed. From day +1 to day +7 primiparous cows had higher rectal temperatures compared to multiparous cows (39.1± 0.08 vs. 38.7± 0.08°C; P=0.01), however there were no day-to-day effects between primiparous cows and multiparous cows. During the postpartum period, multiparous cows consumed more feed than primiparous cows (26.6 vs. 18.5 kg; P=0.05). On days +5, +6, and +7 multiparous cows consumed more feed than primiparous cows (20.1, 16.6, 17.0 vs. 25.5, 28.8, 31.0 kg/d; P=0.02). As expected, multiparous cows produced more milk from day +1 to +7 than primiparous cows (25.9 vs. 19.0 kg/d; P=0.01).

**Discussion**

Tie-stall dairy herds that have difficulty observing individual cow activity and consequently experience a high rate of in-stall calvings would benefit from an automated activity system that predicts imminent parturition. If pedometers automatically detect imminent parturition, cows could be moved into maternity pens prior to the onset of parturition. A maternity pen provides a safe, clean untethered area for calving, thus improving cow and calf welfare. The high percent (33%) of calvings that occurred out of the maternity pen during this study demonstrates how the use of an automated system to accurately predict parturition could be useful in certain dairy facilities. Interestingly, of the 9 cows that expressed a ≥20% increase in stepping, between days -2 and -1, 4 calved in the tie-stall and 1 calved outside in the exercise shed. This result demonstrates that cows that calved in-stall or outside did express increases in restlessness 1 to 2 d prior to parturition; however, lack of obvious physical changes, and therefore, due to human error, these cows were not identified and left to calve in-stall or outside.

The primary objectives of this study were to investigate if Afimilk pedometers could retrospectively determine if these changes in activity could be used to predict the onset of parturition in order to move cows into maternity pens prior to parturition. Stepping activity increased between days -2 and -1 on average by 34%. This increase in activity suggested that cows are restless in the days approaching parturition, as found in earlier studies (Bao and Giller, 1991, Huzzey et al., 2007, Miedema et al., 2011a). Increased restlessness prior to parturition could possibly be due to discomforts associated with imminent parturition.

In this study the degree of restlessness varied (Figure 10, Figure 11, Figure 12, and Figure 13 a,b,c,d,e,f) within and between cows in the days prior to parturition. Pedometers would not be able to predict parturition accurately in all cows as the degree of increased restlessness varied day-to-day throughout the prepartum period. Increased stepping activity was not consistent in all cows. The increase in stepping observed between day -2 and -1 suggests cows display increased restlessness prior to parturition. Therefore, if pedometers were set at a stepping increase threshold of 20%, 9 of the 16 (56%) cows would have been correctly identified and moved into a maternity pen at least 24 h prior of parturition (based on stepping activity only from days -2 to -1) (Table 5). However, because this was a retrospective study, day 0 was known; therefore, data could be normalized to day 0 and changes in activity prior to parturition could be analyzed. Day of calving is unknown in a real-time trial; therefore, anytime during late gestation if a cow expressed increased restlessness, which occurred in cows in this study (Figure 13) the pedometer would alert the producer by flagging the cow as being close to parturition. It must be noted that increased restlessness could have occurred on any of the days during the prepartum period, resulting in the cow being inaccurately predicted as being close to parturition and moved to the maternity pen several days before parturition actually occurred. Moving a cow too early into the maternity pen could increase labor associated with cleaning the pen and feeding an extra cow in addition to the regular chores of cleaning and feeding.

The results conclude that increased restlessness is observed in some dairy cows housed continuously in tie-stalls in the days prior to parturition; however the degree of restlessness within cow varies during late gestation (days -10 to -1) and therefore, pedometers would not be accurate in predicting the onset of parturition.

In the current study, the occurrence of assisted parturition was quite high (50%). The average estimate of assisted calving is approximately 28% (Johanson et al., 2011). The high rate of assistance during parturition could be confounded by the subjectivity of barn staff as to when assistance during calving was necessary.

The incidence of fetal malpresentation in the current study was higher (4/24; 17%) than average (<5% in dairy herds) (Mee, 1991a as referenced in Mee, 2008); of the 4 cows that had posterior malpresentation 3 required assistance. Of the 3 that required assistance, 2 of the calves were dead at arrival. The high incidence of assistance during parturition and posterior malpresentation could be due to the cows having little exercise prior to parturition as they remained tethered for the majority of the study. Assistance during parturition and calving-related diseases decrease with exercise (Wautlet et al., 1990; Gustafson, 1993). Therefore to reduce risk of assisted calving preparturient cows should be given access to daily exercise in an open lot. For this study cows were given sporadic (2 h) exercise periods; however, as stated earlier, that activity data was not analyzed because it was not controlled for in every cow. Activity data in this study was from a period when cows were continuously-housed in tie-stalls.

Our second objective was to determine if parturition-associated activity differed between primiparous and multiparous cows. Between days -2 and -1 the decreases in activity were from the primiparous cows while all multiparous cows had increased stepping activity. No day-to-day differences between primiparous and multiparous cows were evident. However, when activity was summarized for the prepartum period primiparous cows had increased steps and a lower duration of lying time compared to multiparous cows, which supports the second hypothesis that primiparous cows express increased restlessness compared to multiparous cows. The unfamiliarity with preparturient changes and of the tie-stalls may have caused increased restlessness in younger cows. Younger cows which have relatively smaller visceral capacity compared to a fully-grown multiparous cow may have added discomforts from a near-term pregnancy (large gravid uterus, fetal movements, etc.) resulting in increased restlessness.

Multiparous cows consumed more on days 5, 6, and 7 postpartum, compared to primiparous cows, which was expected (Hayirli et al., 2002). Multiparous cows consume more feed postpartum because their digestive tract has a larger capacity to meet the higher energy demands associated with higher milk production (Smith and Baldwin, 1974).

A limitation within this project was that the time used for daily activity records was a 900 min period (15 h) compared to a 24 h period. Using only 900 min of activity records from 1700 to 0800 h may have contributed to an imprecise estimate of daily activity, as the activity from 0800 -1700 h was not used. The activity period of 1700 to 0800 h was chosen because it was the only period when all preparturient cows remained continuously in-stall; therefore, activity during this period was accurately measurable. Between 0800 and 1200 h cows could have been taken outside to an exercise lot; therefore, activity data would not have been controlled on the days cows went to the exercise activity. The days that cows had access to exercise would have had increased activity, which would have biased results.

A second limitation was that activity relating to day 0 (i.e., day of parturition) could not be analyzed because moving time (time of day cows were moved into maternity pen), calving time (time of day cows calved, pedometer records were missed) and location of calving all varied and could not be controlled. Although there was a prepartum scoring index it was not always possible to identify score 3 (red mucus) clearly; therefore not all cows were moved at score 3. However there were no association between the prepartum score the cow received when moved and the duration of parturition.

**Conclusions**

Automatic prediction of parturition would be valuable for dairy herds that experience high levels of in-stall calving. Predicting prepartum activity is difficult because activity varies considerably within and between cows. Over the prepartum (day -10 to day -1) and postpartum (day +1 to day +7) period primiparous cows were more restless than multiparous cows. Activity throughout late gestation varied significantly in the 24 cows continuously-housed in tie-stalls; therefore, Afimilk pedometers were not useful in facilitating the accurate prediction of parturition in the cows observed in the present study.

**Table 3. Distribution of calving by location and parity**

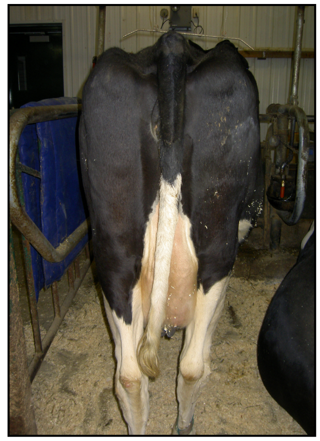
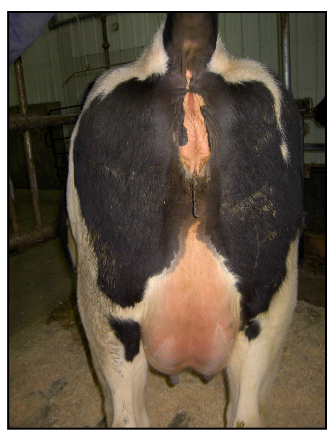
| Parity | Location of calving | No. of cows (%) |
|---|---|---|
| All (n=24) | Maternity pen | 16/24 (67.7) |
| | Tie-stall | 7/24 (29.1) |
| | Outside | 1/24 (4.2) |
| Primiparous (n=12) | Maternity pen | 9/12 (75.0) |
| | Tie-stall | 3/12 (25.0) |
| Multiparous (n=12) | Maternity pen | 7/12 (58.3) |
| | Tie-stall | 4/12 (33.3) |
| | Outside | 1/12 (8.3) |

**Table 4. Prepartum index score when moved into maternity pen, range and average duration of parturition from moving, by parity**

| Parity | Score before moving to maternity pen | n | Range of duration (From moving to expulsion of calf) (min) | Average duration (From moving to expulsion of calf) (min) |
|---|---|---|---|---|
| Primiparous | 2 | 3 | 70.8 – 664.0 | 292.6 |
| | 3 | 3 | 105.9 – 803.2 | 339.2 |
| | 4 | 3 | 32.3 – 452.2 | 220.8 |
| Multiparous | 2 | 3 | 186.6 – 765.9 | 572.7 |
| | 3 | 0 | - | - |
| | 4 | 4 | 78.9 – 265.6 | 183.7 |

Prepartum index score did not affect average duration of calving from moving between primiparous and multiparous cows (P=0.34).

| **Table 5. Percent change in steps from day -2 to day -1 in 16 of the 24 cows (day 0 = parturition).** | |
|---|---|
| Percent change | No. of cows (%) |
| ≤ 0% | 3 (18.7) |
| > 0% - ≤ 10% | 4 (25.0) |
| >10% - ≤ 30% | 3 (18.7) |
| >30% - ≤ 60% | 2 (12.5) |
| > 60% - ≤ 90% | 3 (18.8) |
| > 90% | 1 (6.3) |

| Score | Description | Picture |
| --- | --- | --- |
| **0** | Approximately 2-3 wk from parturition, udder not filled, vulva normal size |  |
| **1** | Udder is beginning to fill, vulva has started to swell |  |
| **2** | Udder looks full and vulva has started to swell.<br>There may be cloudy, thick mucus. |  |

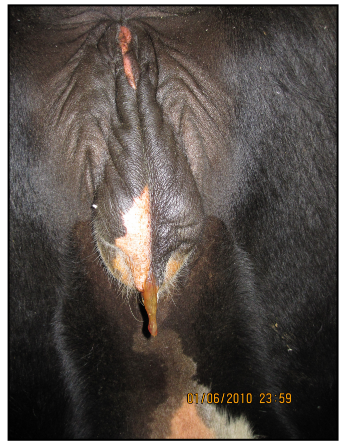
| | | |
|---|---|---|
| **3** | Reddish mucus coming from vagina, swollen vulva, tail is arched (won't lay flat), cow is restlessness (standing up and down, kicking at stomach, looking back at rear<br><br>***** MOVE COW INTO MATERNITY PEN ***** |   |
| **4** | Seeing excess mucus, amniotic sac, or feet from calf |  |

Figure 9. Prepartum scoring index.

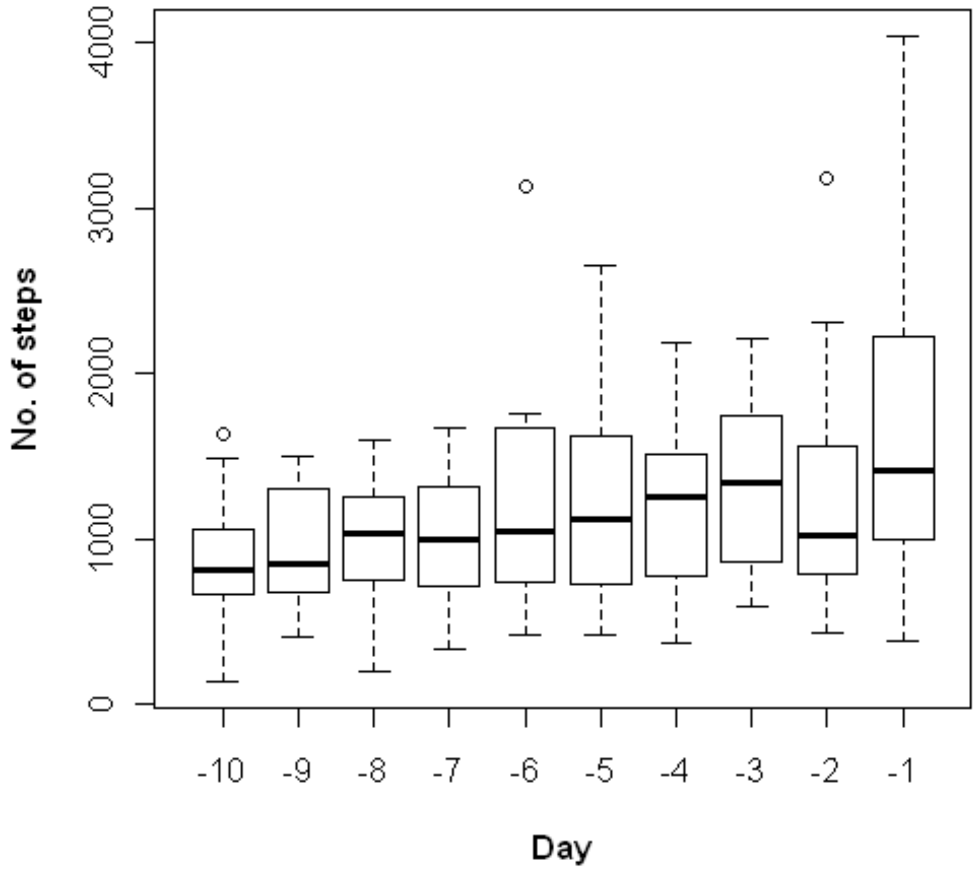



**Figure 10.** Prepartum stepping activity for 900 min/d. The box itself represents 50% of the data, while the upper hinge of the box represents the 75th percentile and the lower hinge represents the 25th percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

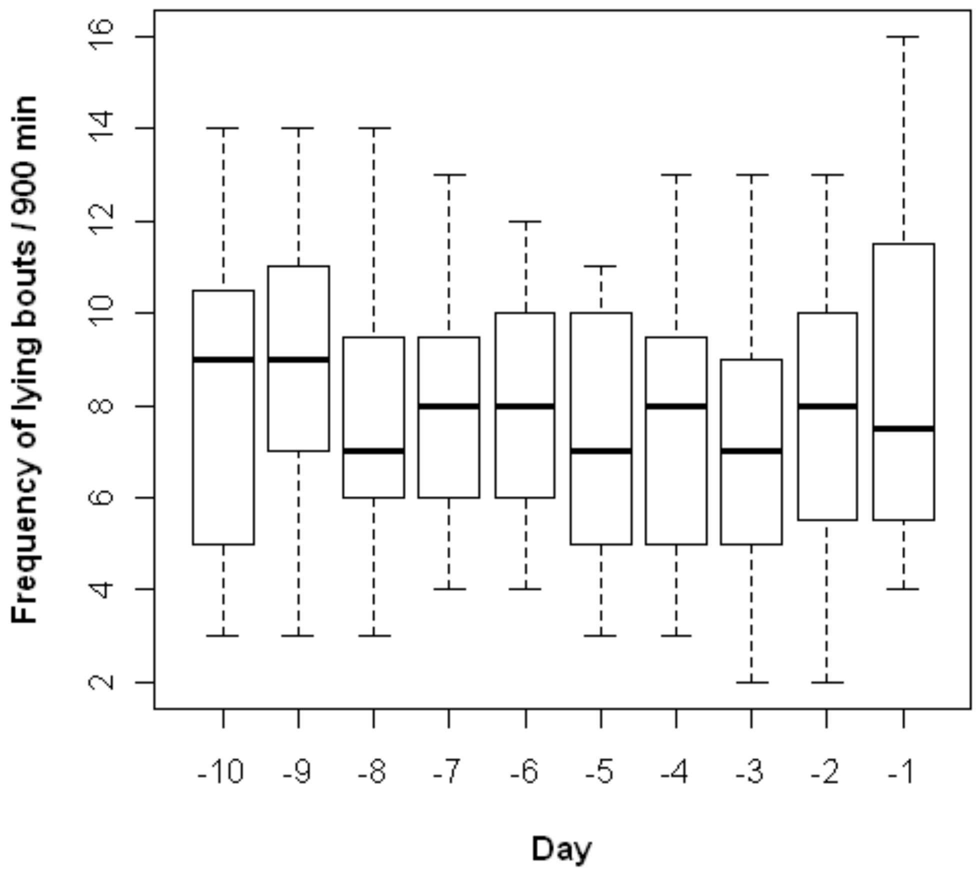



**Figure 11.** Prepartum lying bout activity for 900 min/d. The box itself represents 50% of the data, while the upper hinge of the box represents the 75th percentile and the lower hinge represents the 25th percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

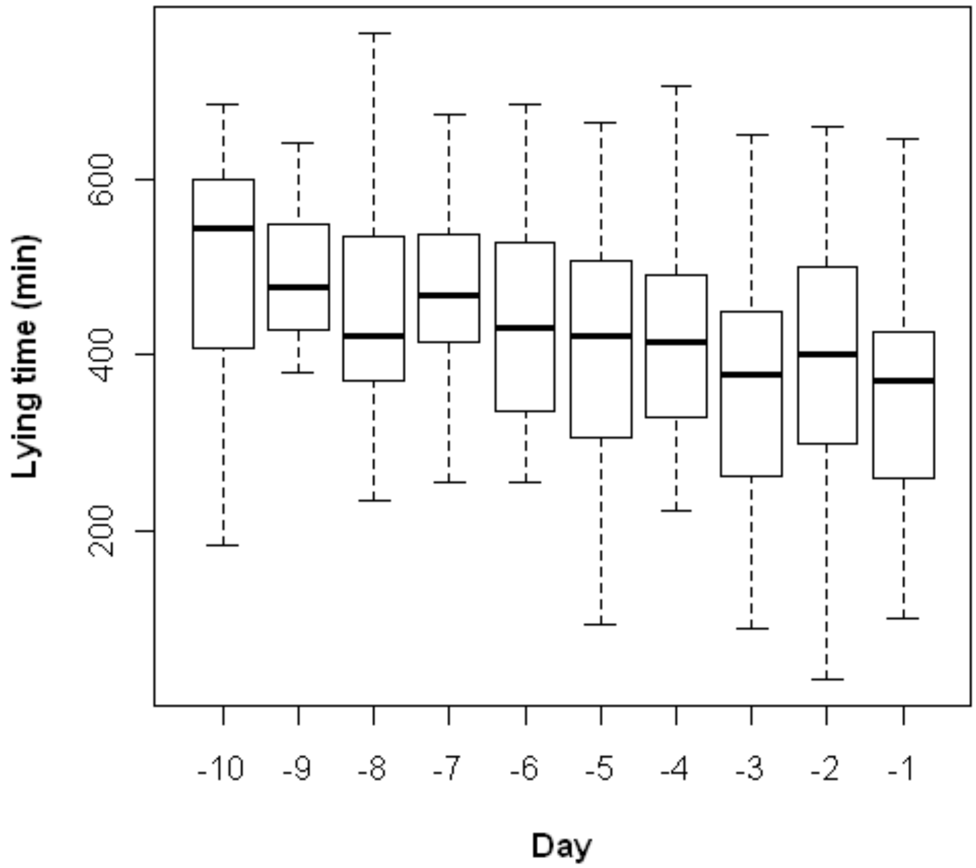



**Figure 12.** Prepartum lying activity for 900 min/d. The box itself represents 50% of the data, while the upper hinge of the box represents the 75th percentile and the lower hinge represents the 25th percentile. The central dark line through the box represents the median. The whiskers (vertical lines outside box) represent the minimum and maximum values. The dots that fall outside the whiskers are outliers.

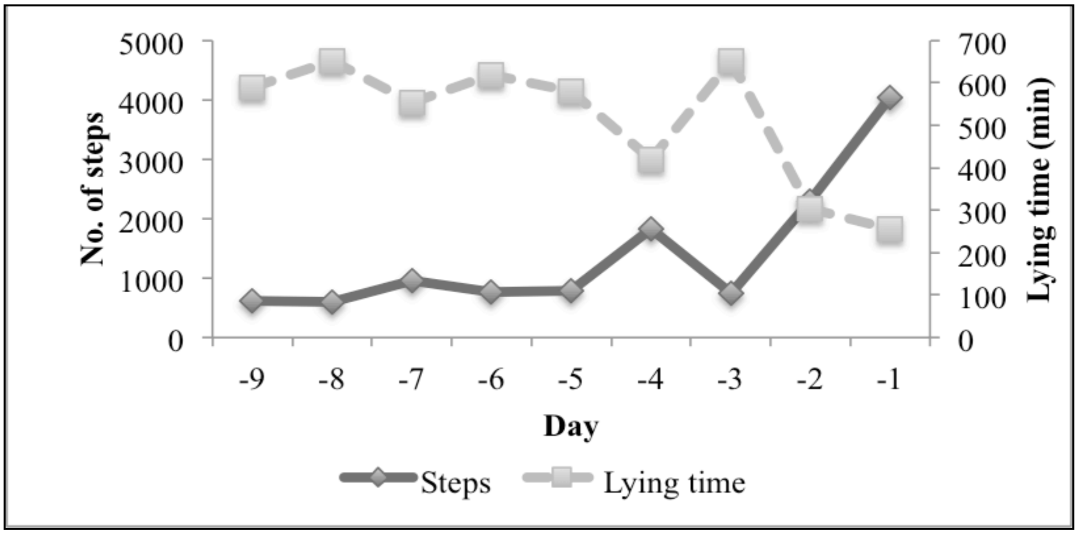



**Figure 13a**. Prepartum stepping and lying activity for 900 min/d from a multiparous cow. Little change in stepping activity occurred before day -5. Between day -5 and day -4 stepping activity increased with a subsequent decrease in lying time. Stepping activity decreased while lying time increased between day -4 to day -3. Stepping activity increased while lying time decreased from day -3 to day -1.

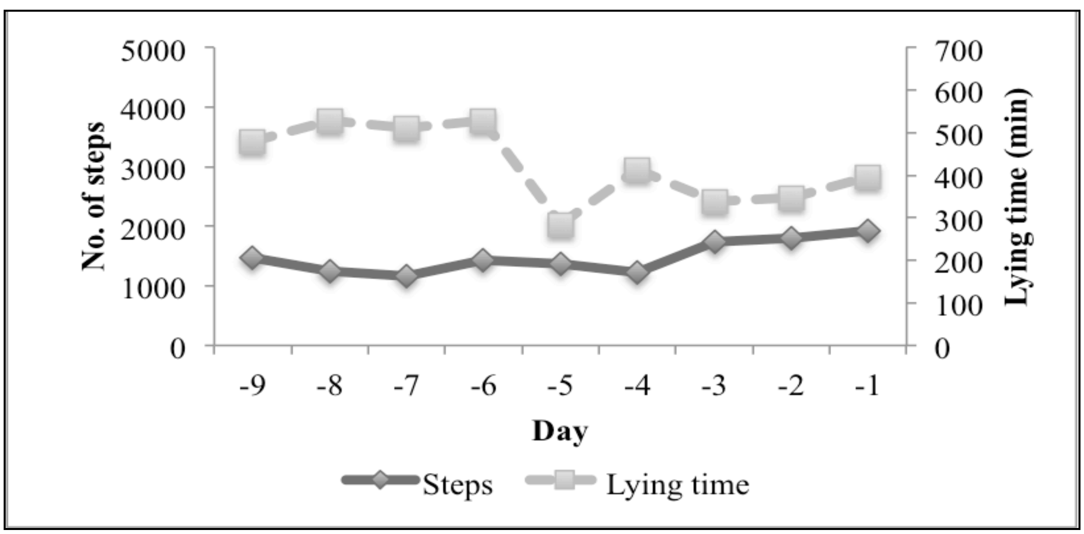



**Figure 13b**. Prepartum stepping and lying activity for 900 min/d from a multiparous cow. There was little change in stepping activity through the postpartum period; however, there was a large decrease in lying time from day -6 to day-5. Lying time then increased from day -5 to day -4 then stayed relatively constant.

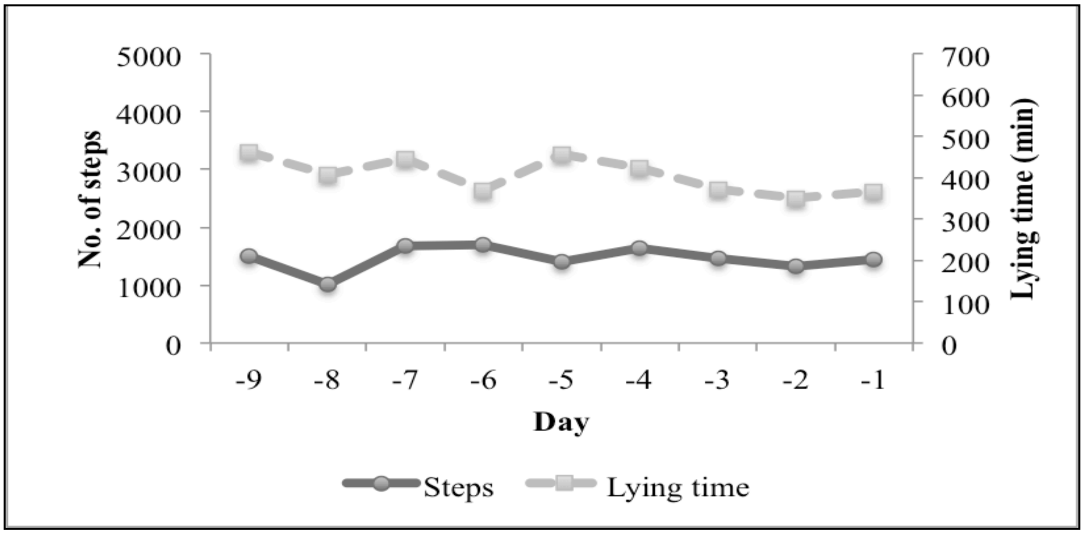



**Figure 13c**. Prepartum stepping and lying activity for 900 min/d from a multiparous cow. Changes in stepping and lying activity were subtle during the prepartum period. A decrease in lying time was observed from day -7 to day -6.

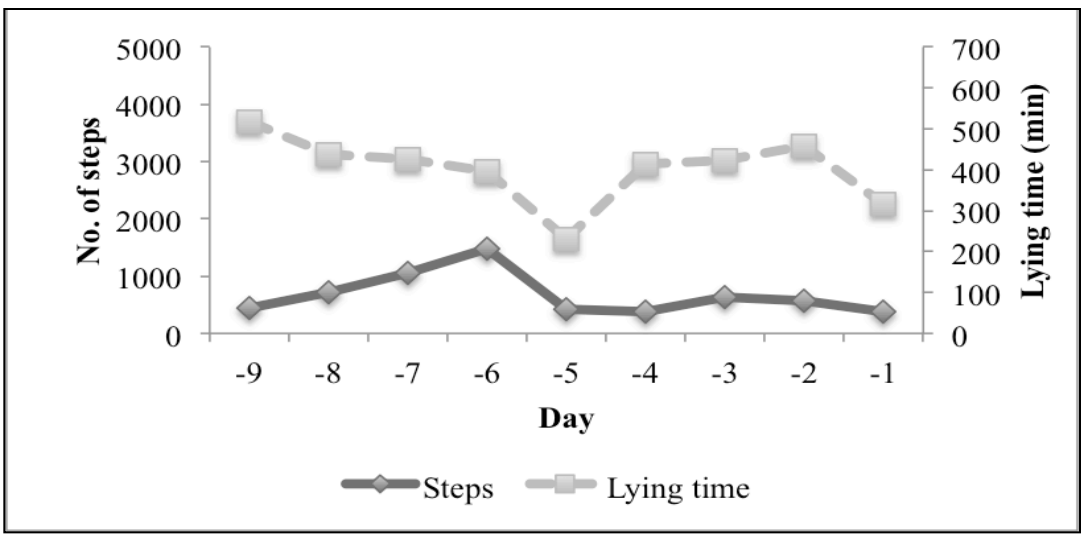



**Figure 13d**. Prepartum stepping and lying activity for 900 min/d from a primiparous cow. Stepping activity increased from day -9 to day -6 then decreased on day -5 and stayed relatively constant until day -1. Lying time gradually decreased from day -9 to day -6. From day -6 to day -5 a large decrease in lying time occurred. From day -5 to day -4 lying time increased and then stayed relatively constant until day -2 where another decline occurred to day -1.

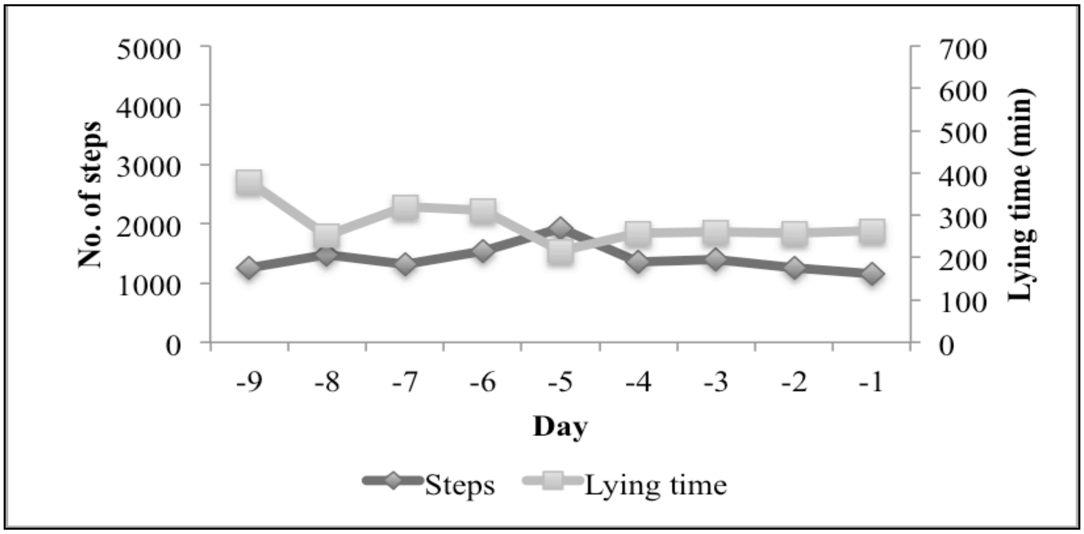



**Figure 13e**. Prepartum stepping and lying activity for 900 min/d from a primiparous cow. Changes in stepping and lying activity were subtle during the prepartum period.

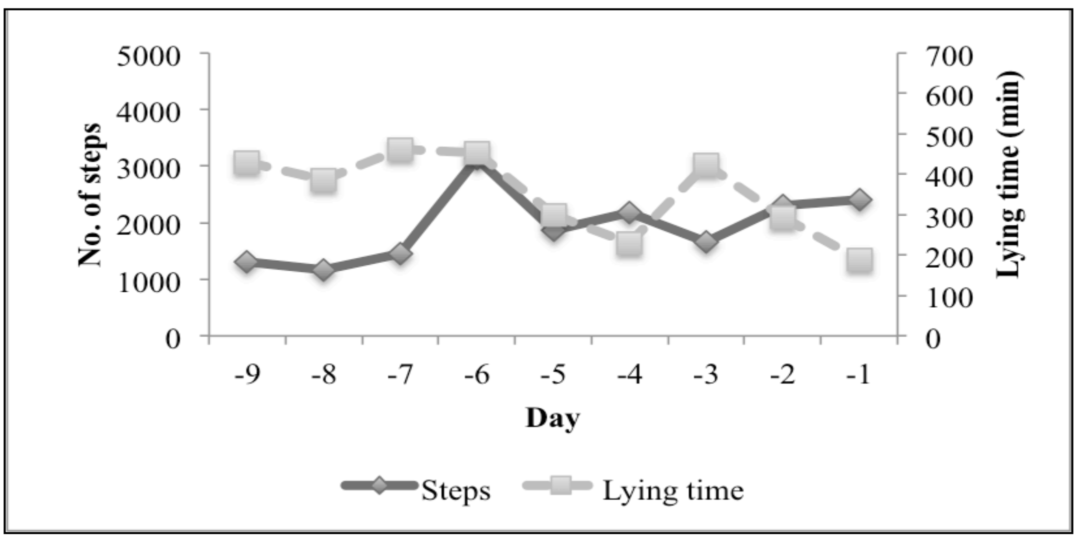



**Figure 13f.** Prepartum stepping and lying activity for 900 min/d from a primiparous cow. Stepping activity increased from day -7 to day -6 then decreased to day -5. After day -5 stepping activity stayed fairly constant. A decrease in lying time occurred from day -6 to day -5. Lying time increased between day -4 and day -3 and then decreased to day -1.

# Chapter 5. General Discussion

## Summary of findings

Two studies were conducted to evaluate the Afimilk Pedometer Plus system for the prediction of estrus and parturition in dairy cattle housed continuously in tie-stalls. The first study had 2 objectives. The first objective was to evaluate if the Afimilk pedometers could accurately monitor cow activity (steps, frequency of lying bouts, and lying time) by comparing daily Afimilk pedometer records to daily video records. Afimilk pedometer records were highly correlated to video records for steps, frequency of lying bouts, and lying time. As a result, it was determined that Afimilk pedometers are accurate in monitoring activity of dairy cows housed continuously in tie-stalls.

The second objective of the first study was to evaluate if the Afimilk pedometers could facilitate estrus detection in dairy cows continuously-housed in tie-stalls. Nineteen cows were treated with PGF to have their estrous cycles synchronized, while 19 pregnant (control) cows received a sham treatment. Estrus was confirmed in 17 treated cows through a decline in $P_4$ concentration; ultrasound scans confirmed ovulation in 17 of the 19 treated cows. Afimilk pedometers recorded daily activity records from 3 d before the PGF treatment (day 0) to 6 d after, or when ovulation was confirmed in the treated cows. Results indicated that activity did not differ between treated and controls through the expected estrus period. Activity also did not change in the days prior to ovulation in the treated cows. It was concluded that Afimilk pedometers would not be useful in facilitating estrus detection because, in the present study, cow activity did not change in cows continuously- housed in tie-stalls even though most cows came into estrus and ovulated.

The second study evaluated if the Afimilk pedometers could facilitate the prediction of parturition. Twelve primiparous and 12 multiparous dairy cows were moved into the tie-stall facility and monitored with Afimilk pedometers from 10 d before parturition to 7 d after parturition. There was an overall average increase in stepping activity in the days prior to parturition (day 0 = parturition); yet, activity varied significantly with some cows decreasing stepping activity. Primiparous cows expressed increased restlessness (increased steps, decreased lying time) compared to multiparous cows. Increased restlessness in the primiparous cows may have occurred because of their unfamiliarity to parturition related changes and of the tie-stalls. This experiment determined that activity varies significantly in preparturient dairy cows housed continuously in tie-stalls; consequently, pedometers were unable to accurately facilitate the prediction of parturition in the present study.

**Limitations with Afimilk pedometers**

Limitations within the Afimilk Pedometer Plus system were discovered throughout the two studies reported in Chapters 3 and 4. The first limitation, found during the validation study reported in Chapter 3, was that the pedometers do not have an automatic built in time stamp. Without a time stamp it was difficult to validate the pedometers against the video data. The limitation occurred when the two sums (one from the pedometer session and one from the video session) differed. When the sums differed, there was no way to determine what activities had been miscalculated because there was no time stamp on the pedometer record informing when the activity occurred. This limitation impacted the correlation analysis used in the first paper to validate the pedometer data against video data. When the sums of the pedometer or video sessions differed it resulted in lower correlations between the video and pedometer. This was more evident with stepping activity.

The second limitation was also found in the validation study reported in Chapter 3. Information regarding what the Afimilk pedometer counted as a step was not available. Therefore, it had to be determined what movement was being counted as a step in order to validate against the video records. Our definition of "step" as defined in the first study (Chapter 2), i.e., when the right rear foot lifted completely off the ground and was placed back to the ground in any location, resulted in a high correlation to the pedometer "step". Yet there were discrepancies between pedometer steps and video steps that lowered the correlation between the two. It is currently undetermined as to what movement activates the pedometer to count a "step" as defined by Afimilk. The monochromatic video recordings did not have a bright clear picture, which made it difficult at certain instances to determine if the cow completely lifted the foot off the ground or if the heel was only lifted. This difficulty would have also reduced the correlation between pedometer and video data. It is important to note that the Afimilk pedometers are designed for free-stall cows. Therefore, if steps had been validated in cows walking (actual steps) the correlation may have been closer to 1. These limitations discussed are important for research and validating purposes.

The third limitation found during these two projects was that the Afimilk scanner stopped working now and then, resulting in missed data during the second project. This caused for several data points to be missed because the data were not captured despite the pedometer being scanned. It is important to note that there was no obvious indication that the pedometer had been successfully scanned. The only way to tell was to stand directly in front of the reader and watch for a small green light to flash. This light could not be observed from where the cow stalls were located. Thus, to be able to confirm that the scanning was successful, two people were necessary at any given time. Therefore, it would be useful for Afimilk to have an audio or visual indication built into the scanner so that a successful scan is confirmed immediately either through an audible or visible signal. If an audio or visual signal failed to register an automatic wireless notification could be sent to the data logger, computer or smart phone to inform the operator which cows' activity data were missed by the pedometer scanner. If Afimilk pedometers were used in a commercial herd the scanner would have been fixed to a crowding gate where cows would walk through during each milking time, thereby facilitating automatic scanning and data capture. If a scan fails to register, the activity data will not be captured and therefore the cow's activity will be incorrectly measured by the pedometer software because of missing data. If activity data were incorrectly measured the software could measure a change resulting in a false positive (indicating a change in activity when there was no change).

**Future research**

The two studies, discussed in this thesis, have provided valuable information regarding activity patterns in dairy cattle continuously-housed in tie-stalls. Future research is warranted to determine why dairy cows continuously-housed in tie-stalls failed to express the expected activity changes during estrus.

There have been studies that describe potential explanations for the surprisingly low activity change observed during estrus; one common explanation is silent estrus. Silent estrus is when cows do not express sexual behaviours or do not increase their walking activity in association with estrus (Ranasinghe et al., 2010). This condition was prevalent in the present study because 17 of the estrus-induced cows underwent CL regression and ovulated; yet, there was no detectable change in activity during estrus. Silent estrus is more common in early postpartum (cows <60 days from calving) compared to cows between 61 to 308 d after calving (Labhsetwar et al., 1963). Early postpartum dairy cows experience negative energy balances (NEB) due to high energy demands caused from lactation. During NEB, $E_2$, which regulates the expression of estrous behaviour, may be insufficiently produced to create estrus activity. Increased blood circulation during milk production and high feed intake increases the metabolic clearance rate of steroid hormones, which reduces the circulating $E_2$ concentration, thereby reducing estrus activity. Future studies could measure $E_2$ concentrations in estrus-induced dairy cows continuously-housed in tie-stalls to determine if low plasma $E_2$ concentrations are responsible for the inactivity expressed during estrus.

It has been reported that prediction of parturition is difficult as there is not one consistent alteration in physical appearance or activity observed in all preparturient cows (Berglund et al., 1987; Metz and Metz, 1987). It has been stated in numerous publications that restlessness is commonly observed prior to parturition (Bao and Giller, 1991; Huzzey et al., 2007; Miedema et al., 2011a). In the second study increased restlessness was observed in some, but not all preparturient cows. Repeating this research with a larger number of cows in several facilities is warranted to determine if variations in preparturient activity would be reduced.

A trend was observed in some of the preparturient cows of steeply decreasing lying time approximately 6 d before parturition. A similar numerical decrease was observed in Maltz and Antler (2007); the average lying time in 15 preparturient cows decreased from day -6 to day -5 (Maltz and Antler, 2007). Using the mean, standard deviation, and change in lying time reported by Matlz and Antler (2007), a power test was performed to determine an effective sample size for future research. Based on this approach, we found that a sample size of 125 cows would be required to confirm if the steep-decline in lying time ~6 d before calving is repeatable in a larger population of preparturient dairy cows. If this trend were clearly established, it could be used as an indicator of imminent parturition.

In the second study daily activity was calculated from 1700 to 0800 h. If activity had been monitored round the clock there may have been more increased restlessness during the daytime period or when cows had the opportunity to go outside for exercise. Future studies need to determine if preparturient cows housed in tie-stalls would experience consistent increases in restlessness if activity was calculated over a 24 h period or if the cows were given a consistent daily exercise period.

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**APPENDICES**

Disclaimer: Appendices 1, 2, and 3 present a synopsis of the bovine estrous cycle, endocrine control of parturition, and the stages of parturition. This supplementary information was compiled primarily from textbooks to enhance the student's understanding and comprehension of these physiological processes, and it should not be considered part of the literature review or used for referencing purposes.

**Appendix 1. Estrous cycle**

**Estrous cycle**

The estrous cycle is divided into 2 phases, (follicular and luteal) and four main stages (proestrus, estrus, metestrus, and diestrus). The follicular phase encompasses 20% of the estrous cycle and is made up of proestrus and estrus. It is characterized by the period from regression of the corpus luteum (CL) to ovulation (typically, from Day 17 to 21, and Day 1 of the estrous cycle). The dominant ovarian structure in the follicular phase is the actively-growing preovulatory follicle, which produces high levels of $E_2$. The luteal phase is approximately 80% of the estrous cycle and includes metestrus and diestrus. This phase covers the period from ovulation to CL regression and its primary ovarian structure is the CL, producing high levels of $P_4$ (Senger, 2003).

Proestrus is the stage directly preceding estrus; it is characterized by increasing amounts of $E_2$ produced from the growing preovulatory follicle. During proestrus, a transition from a $P_4$-dominant stage to an $E_2$-dominant stage occurs with the help of the pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). During proestrus, the female reproductive tract prepares for estrus and mating. After proestrus, estrus occurs.

Estrus is the stage in the cycle that causes a female to be sexually receptive. Estrus is characterized by the animal displaying sexual behaviours. It takes approximately 24 to 32 h from the onset of estrus to ovulation. Once the cow is in standing estrus she displays lordosis (standing behaviour with arched back), signifying she is ready to accept a mate or be inseminated. The predominant hormone throughout estrus is $E_2$ and is responsible for behavioural and physiological changes observed in the cow. At the end of estrus is ovulation, which is the first stage of metestrus.

The period from ovulation to the formation of the early CL is defined as metestrus. During metestrus the CL is formed from the ovulated follicle. The formation of the CL changes the hormonal balance, from $E_2$-dominant to $P_4$-dominant. Metestrus is followed by diestrus.

Diestrus is the longest stage in the estrous cycle, lasting 10-14 d; it lasts from the initiation of production of $P_4$ from the CL to the regression of the CL (luteolysis). The $P_4$ is responsible for preparing the uterus for embryo development and attachment of the conceptus.

**Endocrine control of estrous cycles**

Estrous cycles are controlled by follicular dynamics (ways that antral follicles continuously grow and regress in wave like patterns) (Senger, 2003). These waves lead to the development of a preovulatory follicle. During an estrous cycle, cattle generally have 2 or 3 follicular waves, with the last wave producing the preovulatory follicle (Lucy et al., 1992).

The follicular phase is characterized by the production of a preovulatory follicle; follicles go through developmental stages: recruitment, selection, dominance, and atresia. During recruitment, a small group of antral follicles grow and produce $E_2$ from their granulosa cells. The $E_2$ is secreted into the blood and processed by the hypothalamus-pituitary axis. The hypothalamus produces and releases gonadotropin-releasing hormone (GnRH), which is processed by the anterior pituitary gland. In response to GnRH, the pituitary gland secretes the gonadotropins, FSH (controls follicular growth and development) and LH (causes ovulation and CL formation).

Selection is when a follicle from the recruited group of follicles continues to grow, and avoids atresia (Lucy et al., 1992). Concentrations of FSH are lowest during selection, while LH increases.

During dominance, the selected follicle continues to grow until ovulation. This growing follicle produces increased concentrations of $E_{2,}$ and inhibin (a hormone inhibiting the release of FSH; thus, inhibin suppresses the growth of other antral follicles causing atresia (degeneration of follicles). In order to maintain a dominant follicle increased LH secretion is required (Savio et al., 1993). Increased amounts of $E_2$ create a positive feedback to the hypothalamic surge center, thereby stimulating GnRH neurons, which react by secreting an LH surge causing ovulation.

The luteal phase is the period from ovulation to luteolysis. This consists of CL formation, CL production of $P_4$, and luteolysis (regression of CL). Following ovulation, a CL is formed (luteinization) from the granulosa and theca interna cells of the preovulatory follicle. The CL continues to form and secrete increasing quantities of $P_4$, which is measurable in blood after 3-4 d. The $P_4$ secretion creates a negative feedback on the hypothalamus, which reduces basal GnRH pulses. If insemination is successful and fertilization occurs the CL stays for 6-7 mo throughout pregnancy continuing the production of $P_4$. In the absence of pregnancy, luteolysis occurs between days 15 and 17 of the cycle. Oxytocin and

$P_4$ from the CL and PGF from the uterine endometrium are the hormones that regulate luteolysis.

In the early luteal phase, $P_4$ blocks oxytocin receptors. After 10-12 d the $P_4$ block on oxytocin and GnRH is removed thereby allowing PGF secretion and preovulatory follicular growth. Increasing production of $E_2$ increases endometrial oxytocin receptors, thus increasing PGF secretion. Corpus luteum regression is caused by PGF; PGF is transferred from the uterine vein to the utero-ovarian vein. To minimize metabolism of PGF a counter-current exchange transfers PGF from the utero-ovarian vein into the ovarian artery, which then carries the PGF to the ovary. Once the PGF reaches the CL it can begin regression. This therefore results in a significant drop in $P_4$. Once $P_4$ concentrations are low a new cycle begins (every 17-24 d) in the absence of pregnancy. It is important to understand how the endocrine system controls and regulates estrous cycles in order to know when and what to observe during estrus.

**Physiological changes during estrus**

During follicular growth $E_2$ concentrations peak, while $P_4$ concentrations are suppressed from CL regression (Chenault et al., 1975). This peak in $E_2$ is responsible for the behavioural and physiological changes observed during estrus. Due to a rise in plasma $E_2$, there is an increase in blood flow through the uterus and reproductive tract (Abrams et al., 1973), resulting in increased genital swelling, leukocytosis, increased mucosal secretion, initiation of uterine gland growth, elevated myometrial tone, and changes in tissue electrical conductivity. These physiological changes prepare the reproductive tract for fertilization through increased mucus secretions, protection against foreign material, and transportation of sperm. These changes also help with detection of estrus by increased vaginal mucus, swelling of the vulva, and through measuring electrical conductivity (Senger, 2003).

Physiological changes can help producers detect estrus. Blood plasma and milk samples can identify changes in $P_4$ concentrations, which strongly correlate to estrus (Firk et al., 2002). Changes in milk yield, milk temperature, feed intake, and body temperature are practical on farm techniques that may be used to detect estrus although with limited accuracy. The normal body temperature of a cow is 38.6°C, during estrus body temperatures increase between 0.1 to 0.5°C (Boyd (1984) and Geers et al. (1997) as cited in (Firk et al., 2002). Walton and King (1986) observed increases in rectal temperatures (1.5% increase in 9 cows) the night before estrus, decreased milk yield and decreased feed intake on the day of estrus.

**Appendix 2. Endocrine control of gestation to parturition**

After a cow is successfully inseminated and fertilization occurs, maternal recognition of the pregnancy is required so that luteolysis is prevented to support embryonic development and implantation. The critical period for maternal recognition occurs between days 15 and 16 after ovulation. In order to inhibit luteolysis the blastocyst produces non-specific glycoproteins (particularly, interferon-tau). Interferon-tau acts on the endometrial cells of the uterus and block the production of oxytocin receptors (Senger, 2003). Once oxytocin receptors are blocked endometrial cells can no longer synthesize PGF ($P_4$ block) therefore, inhibiting luteolysis. Once maternal recognition has occurred the conceptus begins to attach to the uterus 18-20 d after ovulation; full attachment does not occur until 40 d after ovulation.

The placenta is the attachment site between the dam and the fetus; it is an endocrine organ responsible for stimulating ovarian function, maintaining pregnancy, influencing fetal growth, stimulating mammary function and it assists in parturition (Senger, 2003). The placenta is made up of a maternal component of uterine endometrium and a fetal component made from the chorion. Cows have a cotyledonary placenta, which is comprised of 70-120 button-like structures called cotyledons; these are the placental transfer sites between the dam and fetus. The placenta produces placental lactogen responsible for promoting fetal growth and stimulating the dam’s mammary gland for lactation.

Gestation is a dynamic process that involves endocrine control from the dam and the fetus. In cows gestation lasts approximately 280 d with a standard deviation of 7.5 d (Meyer et al., 2001). Progesterone is produced from the corpus luteum (CL) and is the primary hormone throughout gestation. Progesterone provides the stimulus for elevated secretion of endometrial glands thereby assisting in development and growth of the embryo (Senger, 2003). The placenta produces placental lactogen responsible for promoting fetal growth and stimulating the dam's mammary gland for lactation. The $P_4$ block inhibits myometrial contractions thus maintaining a quiescent myometrium (Senger, 2003). After 6-8 months the placenta takes over $P_4$ production from the CL, yet the CL continues to produce $P_4$ throughout gestation. The regression of the CL is important for the initiation of parturition.

Parturition is a dynamic process involving several endocrine changes in the dam and fetus. Parturition can occur only once the mechanisms that have maintained pregnancy are reversed or removed. Activation of the fetal hypothalamus-pituitary-adrenal axis initiates parturition; however how this is activated remains uncertain (Noakes et al., 2001). It is believed that towards the end of gestation the fetal size reaches the uterus' space limitation and subsequently the fetus becomes stressed (Senger, 2003); consequently, the fetus releases adrenal corticotrophin (ACTH) from its anterior pituitary. ACTH stimulates the production of corticoids from the fetal adrenal cortex (Senger, 2003). Fetal corticoid concentration rises and causes two main endocrine changes in the dam.

The first change is the removal of the $P_4$ block, which causes a decrease of maternal plasma $P_4$ to approximately <1.2 or 1.3 ng/ml within 24 h of parturition

(Matsas et al., 1992). Fetal corticoids increase the synthesis of enzymes that convert $P_4$ to estradiol; therefore, a $P_4$ decline occurs. Once $P_4$ concentration declines myometrial contractions are enabled.

The second change, increased levels of fetal corticoids cause is the increased synthesis of placental PGF. Placental PGF completely removes the rest of the $P_4$ block, luteinizes the CL and increases myometrial contractions. Increased fetal corticoids also cause increased production of reproductive tract secretions in order to prepare for parturition (Senger, 2003).

## Appendix 3. Stages of parturition

There are three stages of parturition; the first stage is initiated by the fetus and defined as the initiation of myometrial contractions. This occurs from the removal of the $P_4$ block discussed previously in the section above. As $P_4$ concentrations decline estradiol and PGF concentrations increase and myometrial contractions become frequent. This is associated with elevated pulse and respiratory rates (Noakes et al., 2001). The myometrial contractions change from isolated, uncoordinated waves during late pregnancy and increase to more regular coordinated peristaltic type contractures near delivery (Noakes et al., 2001). Pressure being exerted on the cervix from the fetus increases myometrial contractions. This pressure causes oxytocin to be released; thus, increasing the force of contractions.

The structure of the cervix loosens and dilates in order to ready itself for parturition. The fetus rotates itself so its front feet and head are positioned to the posterior of the dam (Senger, 2003). The first stage of parturition is complete once the fetus is in the cervical canal; stage 1 of parturition can take from 2 to 6 h in the cow.

The second stage of parturition is defined by the expulsion of the fetus. The second stage begins when abdominal contractions are visible and coupled with more frequent myometrial contractions between 24 and 48 per h (Gillette and Holm, 1963). Frequency of contractions increase from 2 every 10 min to 4 every 10 min, in the last 2 h they increase up to 8 every 10 min at birth (Gillette and Holm, 1963).

Gillette and Holm (1963) identified 3 distinct features in stage 2 of parturition. First, contractions from the abdomen only occur once the feet of the calf are in the cervix or vagina. Second, breaking of the allantoic sac causes a large increase in abdominal contractions. Third, a large increase in abdomen contractions or straining occurs once passage of the head, shoulders, and hips occur through the pelvis (Gillette and Holm, 1963; Noakes et al., 2001).

Straining continues as the amnion moves into the vagina appearing in the vulva. This is referred to as the "water-bag"; the water-bag may rupture by the feet of the calf (Noakes et al., 2001). If rupture occurs, amniotic and allantoic fluid serves as lubricant in the birth canal. Once the feet are through the vulva, the head of the fetus becomes present coupled with peak intensity contractions of uterine and myometrial muscles. Contractions continue causing the fetal thorax to pass through the vulva followed by the hips and hindlimbs (Noakes et al., 2001). Stage 2 of parturition is counted from approach of water-bag can take anywhere between 30 min to 4 hours, average is approximately 70 min (Noakes et al., 2001).

Stage 3 of parturition is defined as the expulsion of fetal membranes. Once the calf is expelled uterine contractions cease while myometrial contractions continue. These myometrial contractions are required for expulsion of fetal membranes. Vasoconstriction of arteries and villi and myometrial contractions help separate the chronic villi from the crypts on the maternal side of the placenta (Senger, 2003). Once the afterbirth is detached, straining is stimulated and expulsion of the fetal membranes occurs. This last stage of parturition lasts between 6 and 12 h. The dam will immediately begin to lick her calf clean and it is not uncommon for the dam to eat the afterbirth. If the calf is not removed from the dam the calf will begin suckling within the first hours after birth. This suckling causes a release of oxytocin, which stimulates milk let down. Most commercial dairy herds remove the calf from the dam within a couple of hours of calving.