Estrus Detection Intensity and Accuracy, and Optimal Timing of Insemination with Automated Activity Monitors for Dairy Cows

By

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ABSTRACT

ESTRUS DETECTION INTENSITY AND ACCURACY, AND OPTIMAL TIMING OF INSEMINATION WITH AUTOMATED ACTIVITY MONITORS FOR DAIRY COWS

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This thesis was conducted to evaluate automated activity monitors (AAM) for estrus detection and to identify the time interval after activity alert that optimizes pregnancy risk. A study was conducted on four dairy farms using one of two AAM systems for inseminations before 80 days in milk (DIM). Activity monitors identified 83% of cows in estrous by 80 DIM. Anovular or multiparous cows, cows with purulent vaginal discharge, or lame cows with body condition score ≤ 2.5 had higher odds of not being detected in estrus by 80 DIM. In a sample of inseminations based on AAM, only 3% were not in estrus based on serum progesterone concentration. The probability of pregnancy was higher for primiparous cows inseminated 0 to 8 hours after AAM alert but was not different for multiparous cows inseminated 0 to 8 h, 8 to 16 h or 16 to 24 h after AAM alert.

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STATEMENT OF WORK DONE

The funding for this project was obtained by Dr. Stephen LeBlanc. The methodology of the data collection was discussed and developed between Craig LeRoy and Dr. Stephen LeBlanc. Dr. John Walton helped in the development of the analysis. The data collection was conducted by Craig LeRoy with assistance provided by various summer students and part-time hired assistants. The lab work was completed by Craig LeRoy. Data cleaning and analysis was performed by Craig LeRoy with the help from William Sears. More complex data manipulation was performed by Karen Hand to recreate the activity alert and the time interval to insemination. The writing of this thesis was performed by Craig LeRoy with guidance of Dr. Stephen LeBlanc and additional input and revisions were received from Dr. John Walton.

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LIST OF ABBREVIATIONS

AAM = Automated Activity Monitor
BCS = Body Condition Score
CL = Corpus Luteum
DC305 = DairyComp 305
DHIA = Dairy Herd Improvement Association
DIM = Days in Milk
DNB = Do Not Breed
FSH = Follicle-Stimulating Hormone
GnRH = Gonadotropin-Releasing Hormone
LH = Luteinizing Hormone
$PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$
PVD = Purulent Vaginal Discharge
P4 = Progesterone
P/AI = Pregnancy Per Artificial Insemination
TAI = Timed Artificial Insemination
THI = Temperature Humidity Index
VWP = Voluntary Waiting period

CHAPTER ONE

INTRODUCTION, LITERATURE REVIEW, AND RESEARCH OBJECTIVES

INTRODUCTION

Reproductive performance of dairy herds is crucial for their financial and operational efficiency. Accurately identifying cows to be inseminated in a timely manner is necessary to have them become pregnant, calve, and return to peak milk production, and to produce a consistent supply of replacements (Roelofs *et al.*, 2010). Many methods for detecting estrus have been tested and one of the methods that has been adopted by producers and is proving to be useful is automated activity monitoring (Fricke *et al.*, 2014).

Research evaluating changes in activity at the time of estrus has been taking place for over 70 years (Trimberger, 1943). Over this time, it has been discovered that there are many factors that affect the intensity of activity at estrus. There are both environmental and physiological factors that affect the level of activity a cow will express around estrus (Eradus *et al.*, 1992). This makes it difficult to develop activity monitors able to detect almost all cows that are in estrus, while also producing few false positives.

Cows that are detected in estrus then require insemination at an optimal time that allows the oocyte to encounter a sufficient quantity of fertile spermatozoa in the oviduct immediately after ovulation (Saacke, 2008). Though the optimal time of insemination has been determined in relation to the time of ovulation, few studies have determined the optimal time relative to when a cow exceeds a threshold of activity that is measured and processed by a commercial activity monitoring system.

Estrus detection using activity monitoring systems is a promising tool for the dairy industry. However, further research into their accuracy, interacting factors and best timing of insemination should be done in a commercial setting, and these are the main topics of this thesis.

LITERATURE REVIEW

Normal Estrous Behaviour and Cow Physiology

Estrous behaviours are the visible signals related to ovulation because ovulation cannot be seen visually. Therefore, estrous behaviour plays a critical role in ensuring that cows are inseminated at an appropriate time. Estrus has been observed for over 10,000 years, evidenced by cave paintings of an aurochs bull sniffing the vulva of a cow in Teyjat (Dordogne, France) (Mourant and Zeuner, 1963).

Throughout estrus, there are many hormones that play various roles in coordinating behaviour with the time of ovulation. Estradiol is synthesized by a growing dominant follicle during proestrus and is responsible for the behaviours associated with estrus (Hunter, 2003). This display of estrous behaviour is vital so that cows in estrus are identified by either a bull for natural mating or a herd manager for artificial insemination. Estradiol also plays a role in the positive feedback release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which causes the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Estradiol provides positive feedback to the hypothalamus and anterior pituitary to produce GnRH and eventually a surge of LH to cause ovulation (Kelly *et al.*, 1997). The FSH and LH also cause recruitment of new follicular waves leading to the development of a new dominant follicle after ovulation. Once the follicle has ovulated, a corpus luteum (CL) begins to form from the follicular remnants which produces progesterone (P4). P4 has a negative feedback on the hypothalamus which then prevents estradiol positive feedback and estrus behaviour, and sustains a basal release of GnRH. P4 also prepares the uterus for the support and eventual attachment of the embryo and inhibits uterine contractions. Alternatively, in the non-pregnant cow, once the circulating concentration of P4 reaches a threshold relative to estradiol, the uterus releases prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which regresses the corpus luteum and therefore temporarily eliminates the production of P4. P4 then no longer inhibits the hypothalamus from producing GnRH and the processes begins again. Conversely, if an embryo signals its presence by day15 of the cycle the release of $PGF_{2\alpha}$ is inhibited, the CL remains and continues to produce P4 to support pregnancy. Therefore, at the time of estrus there will always be a low circulating concentration of P4 as the CL was just regressed by the $PGF_{2\alpha}$ (Rasby *et al.*, 2016). The decrease of P4 to basal level (< 1 ng/ml serum P4) during estrus usually lasts for 2 to 3 days during the follicular phase of the cycle. This basal P4 concentration has been used to confirm the potential for a cow to be in estrus in many research trials. A single serum sample with low P4 could be from a cow in estrus or an anovular cow. By taking samples a minimum of 2 times per week a baseline can be developed and when estrus occurs, basal levels followed by a normal luteal phase should be seen. With the possibility of the low P4 only having a duration of 2 days many studies take samples 3 times a week or daily. There are on-farm milking systems available that will measure the level of P4 in the milk for both estrus and pregnancy detection. However, the system has both a high start-up and per-use cost (Herd Navigator, DeLaval, Tumba Sweden).

Though there are many hormonal changes that occur at time of estrus they are often impractical, too expensive, or time-consuming to measure for routine management. Estradiol-17β from the dominant follicle causes the many behavioural signs of estrus, provided P4 is basal (Hunter, 2003). The principal sign of estrus is a cow standing to be mounted (Eradus *et al.*, 1992; Van Eerdenburg *et al.*, 1996; Roelofs *et al.*, 2010) but when estrus was detected using daily milk P4 concentration as the confirmation, only 37% of cows in estrus were recorded as being mounted when observed for 30 minutes every 2 hours (Van Eerdenburg *et al.*, 1996). The increase in activity at estrus has been recognized for many years (Hurnik *et al.*, 1975; Kiddy, 1977). Kiddy (1977) observed that on the day of estrus, cows were four times as active as their normal level of activity. Visual observation can be very accurate detecting over 90% of estrus events (Hall *et al.*, 1959) but can also be inaccurate, missing over 50% of estrus events (Roelofs *et al.*, 2006). The duration and frequency of observations plays a large role in the accuracy of this method of estrus detection (Roelofs *et al.*, 2010). Van Eerdenburg *et al.* (1996) also found that if only 3 of the 30 minutes of observation (1000h, 1200h and 2000h) occurred only 12% of cows would have been observed mounting. This quantity of observations would be closer to what would actually occur on commercial farms. Other studies that only included cows that were detected using observed signs of estrus found that when observed 8 times per day for 30 minutes or 5 times for 10 minutes only 58 and 63% were seen to stand to be mounted, respectively (Cutullic *et al.*, 2009; Roelofs *et al.*, 2005a). Other visible signs of estrus include mounting other animals, increased irritability, increased activity, sniffing, vocalization, vulval swelling/reddening, and vaginal discharge (Eradus *et al.*,1992; Roelofs *et al.*, 2010; Stevenson, 2013). Internal changes that occur around estrus include increased body temperature, pulse, blood flow, pheromone release, and decreased milk yield and feed intake (Stevenson, 2013).

Milk yield has been shown to decrease around time of estrus (Copeland, 1929; King 1963, 1977; Schofield et al, 1991; Gaillard *et al.*, 2016). A drop of milk yield of 2 to 8% on day of estrus compared to a non-estrus day has been documented (Copeland, 1929; King, 1977; Horrell et al, 1985; Schofield *et al.*, 1991). Schofield *et al.* (1991) suggested that the reduction in milk yield was due to a failure in milk ejection rather than secretion. This is supported by Horrell *et al.*, (1985) who found that there was a decrease of milk yield at the first milking at the time of estrus and the following milking had an increase of milk yield by an equivalent amount. However, Eradus *et al.* (1992) concluded that the reduction in milk yield was caused by the increase of restlessness and decreased feed intake that is associated with estrus. Schofield *et al.* (1991) and Schlünsen *et al.* (1987) found that only 33% and 42% of estrus events could be detected by monitoring milk yields, respectively. This is partially due to the fact that it is a relatively small change in daily milk production to be detected, which falls within the expected milking-to-milking variation in milk yield and that the decrease is not seen in all cows (Lewis and Newman, 1984).

Intensity and Accuracy of Detection of Estrus using Activity Monitors

The effectiveness and accuracy of estrus detection are critical for dairy operations (Senger, 1994). A system that has a low sensitivity will not detect all estrus events (Koelsch *et al.*, 1994). This will result in increased intervals to insemination and economic losses due to increased time to pregnancy (Esselmont, 2003). A system with low specificity or a high error rate will have a high number of false detection alerts that are not associated with estrus (Koelsch *et al.*, 1994). This could lead to unnecessary inseminations and insemination of pregnant cows, which could cause iatrogenic abortion (Sturman *et al.*, 2000). The accuracy of activity monitors has been verified in numerous studies (Schofield *et al.* 1991; Roelofs *et al.* 2005a; Aungier *et al.* 2015). To confirm the accuracy of activity monitors, studies use a variety of methods such as milk P4, serum P4 or ultrasound to observe the disappearance of the follicle. To monitor when and if a cow has an estrous cycle, milk or serum progesterone samples have to be collected a minimum of 2 times a week. Collecting milk samples is non-invasive, however does require samples taken at milking time which can be inconvenient. Taking serum progesterone is more invasive but can be done at a time other than milking time which would interfere less with the daily routine of the farm. Using an ultrasound to determine if ovulation occurs is a method that doesn’t require laboratory analysis, however, it is labour intensive to repeatedly monitor cows until ovulation occurs.

Though there are many varieties of hardware and companies marketing activity monitors they all have the common goal of detecting the greatest proportion of estrus events with the fewest false positives. Table 1.1 shows the results of numerous studies over the past 34 years that evaluated the sensitivity, error rate, and specificity of activity monitors (expanded from Firk *et al.* (2002)). The studies summarized have a large variety of samples sizes and referent tests used.

Across the studies in Table 1.1 there is a large variety of thresholds to signal estrus because various companies have developed different algorithms. These studies should have included a measure of variance, such as putting confidence intervals, however many do not report and therefore was not included in the table. This is necessary as different styles of activity monitors, such as leg compared to neck mounted, collect different measures of increased activity at estrus (Favero *et al.*, 1984). The basis of the calculation for determining when a cow is in estrus involves creating a baseline by taking the average activity of each cow in the days preceding the day of estrus. For a cow to be flagged in estrus, activity must either rise above a set percentage of its baseline average or a certain number of standard deviations above the average. Some systems have a varying threshold depending on the baseline value of a specific cow. This is used to help correctly identify those cows with a high baseline that would otherwise have an unrealistic threshold of activity in order to be detected.

Studies using activity monitors have found a variety of relative changes of activity at estrus, ranging from 2 to 4 times more than non-estrus days (Kiddy; 1977; Lewis and Newman, 1984; Schofield *et al.*, 1991; Løvendahl and Chagunda. 2010; Madureira *et al.*, 2015). The mean duration of the activity event captured by activity monitors (i.e. the time above the estrus threshold) has been reported to be between 8 and17 hours (Schofield *et al.*, 1991; Løvendahl and Chagunda, 2010; Valenza *et al.*, 2012; Bombardelli *et al.*, 2015; Madureira *et al.*, 2015).With varying degrees and durations of increased activity at estrus it is challenging to develop a threshold that can account for cows that do not have a large increase at estrus without having false positives from the cows that have a large daily variation in activity. Schofield *et al.* (1991) developed an algorithm that detected all estrus events and produced no false positives in a research setting, however, it was a small sample size in a controlled environment. There are many farm factors that limit the application of these results, such as cows with metabolic problems and changes in daily routine such as foot bathing, and changings in temperature (Koelsch *et al.*, 1994).

Factors Affecting Activity Monitor Performance

There are many internal and ambient variables that affect the level of activity that an animal will exhibit (Eradus *et al.*, 1992). Some factors that have been shown to have an effect on the level of activity at estrus include the number of cows in estrus at the same time, the physical environment such as flooring, parity, milk production, lameness, BCS and ambient temperature (Van Vliet and Van Eerdenburg, 1996; Roelofs *et al.* 2005a; Lopez –Gatius *et al.*, 2005; Madureira *et al.*, 2015)

As the number of animals that are in estrus at one time increases, so does the intensity of estrous behaviour for each individual animal (Hurnik *et al.*, 1975; Esselmount *et al.*, 1980; Helmer and Britt, 1985). Van Vliet *et al.* (1996) found that as the number of cows in estrus increased, the intensity and duration of activity captured by a pedometer also increased. Roelofs *et al.* (2005a) concluded that when there was more than one animal in estrus, pedometers could detect up to 95% of estrus events compared to the average detection rate of 83% in that study.

Heat stress has many negative effects on dairy cows (Cowley *et al.*, 2015). Research has found that cows ovulate without behavioural signs of estrus more often when in hotter environments compared to cooler conditions (Rodtian *et al.*, 1996). This could be explained by the fact that there is less estradiol produced by follicles during heat stress. (Wolfenson *et al.*, 1997). Lopez -Gatius *et al.* (2005) found that there was lower walking activity measured by pedometers and more ovulations without estrus during the warmer months when the average temperature as > 25C.

The effect of parity on the intensity of estrus expression has been evaluated in numerous studies, with mixed results. A less intense increase in activity level at estrus with increasing parity was found in several studies, (Lopez-Gatius *et al.*, 2005; Roelofs *et al.*, 2005a; Stevenson *et al.*, 2014; Madureira *et al.*, 2015). Primiparous cows had 9 to 30% higher peak activity and longer duration above threshold than multiparous cows. Contrary to those results, Van Vliet and Van Eerdenburg (1996) and Walker *et al.* (1996) found that primiparous cows showed increased activity for a shorter period of time compared to multiparous cows. Others found no significant difference between parity groups (Arney *et al.*, 1994; Løvendahl and Chagunda, 2010). The large variety of results supports the need for more research on this topic.

High levels of milk production have been associated with decreased levels of estrus activity (Aungier *et al.*, 2012). The theory behind this is that cows with high levels of production will have an increased feed intake and catabolic rate, which means an increased flow of blood to the liver, which may increase the metabolism of estradiol-17β (Sangsritavong *et al.*, 2002). With increased metabolism of estradiol-17β there will be lower concentrations in the blood which would depress estrous behaviour (Hunter, 2003). Lopez *et al.* (2004) found that cows with average milk production (from 10 days before estrus) < 39.5 kg/d had longer estrus duration, more standing events, and longer standing times when compared to those with average production > 39.5 kg/d. The high production group also had lower estradiol when compared to the low production group. Lopez-Gatius *et al.* (2005) found a decrease of peak activity of around 1.6% for each kg increase of average milk production measured in the 3 days prior to AI. Valenza *et al.* (2012) also found that as milk production increased there was a decrease of activity. These findings could help explain why Aungier *et al.* (2012) found that for each 10 kg/d increase of milk production (calculated every two weeks of lactation) around the day of estrus, a cow was 67% less likely to be flagged by the activity monitor at a time of milk P4 $\leq$ 0.6 ng/ml. These results agree with Holman *et al.* (2011) who saw an overall decrease in sensitivity of activity monitors with an increase of milk production. However, there have also been numerous studies that have explored the effect of the level of milk production on intensity or accuracy of activity monitoring and have found little to no effect (Arney *et al.*, 1994; Lyimo *et al.*, 2000; Van Eerdenburg *et al.*, 2002; Madureira *et al.*, 2015). However, to detect the change in activity a substantial sample size is required as it can be a small change. The effect of milk production on estrus expression and detection by activity monitoring should be further researched.

Lameness is one of the most significant challenges facing the dairy industry (Huxley, 2012). It has been shown that cows with a lameness score of ≥ 3 have increase lying times and therefore a decreased amount of activity overall (Galindo and Broom, 2002). A lame cow may display increased activity at estrus but not a sufficient change to be detected. Aungier at al. (2012) found that lameness was not significant in their model looking at factors that affect the accuracy of activity monitors. However, more research is required on this topic as observing a decrease in the accuracy of detection when monitoring lame cows would make biological sense.

Body condition score (BCS) ≤ 2.5 at calving has been associated with reduced production, reproduction, and health (Roche *et al.*, 2009). A lower BCS has been shown to be associated with a smaller relative increase in activity during estrus and a shorter duration of estrus (Løvendahl and Chagunda, 2010; Madureira *et al.*, 2015). This would explain why Aungier at al. (2012) found that for each additional 0.25 points of BCS, which was measured every 2 weeks, a cow was 1.4 times more likely to be correctly identified by the activity monitor system, using a referent of low milk P4 measured twice per week.

Timing of Insemination with Activity Monitors

To maximize the probability of fertilization and pregnancy, semen must be delivered at the appropriate time relative to ovulation. Walker *et al.* (1996) demonstrated that the time from onset of increased activity to ovulation was 27.6 ± 5.4 hours. Succeeding studies have found that on average, ovulation occurs between 26 and 33 hours after activity surpasses the threshold from various activity monitoring systems (Roelofs *et al.*, 2005b; Hockey *et al.*, 2010b; Yoshioka *et al.*, 2010; Stevenson *et al.*, 2014; Bombardelli *et al.*, 2015). Though not significantly different, Stevenson *et al.* (2014) observed that primiparous animals tended to ovulate earlier than multiparous cows: 25.4 ± 0.5 h and 27.0 ± 0.8 h, respectively. Spermatozoa need 6 to 10 hours for capacitation and transport in the female tract (Wilmut and Hunter 1984; Hawk 1987; Saacke *et al.*, 2000). By inseminating cows between 36 hours before and 12 hours after ovulation and assessing time of ovulation using ultrasonography, Roelofs *et al.* (2006) found that inseminations that occurred 12 to 24 hours before ovulation resulted in the highest number of good quality embryos. Hockey *et al.* (2010b) also used ultrasonography at 0, 12, 24 and 36 hours post AI and found that inseminations that occurred 0 to 16 hours before ovulation had the highest conception rate.

The timing of insemination relative to the first observed sign of estrus has been a topic of research for over 70 years. One of the first guidelines was the “a.m.- p.m. rule” that suggested that if a cow was observed in standing estrus in the morning AI should occur that afternoon, and if observed in estrus in the afternoon, AI should occur the following morning (Trimberger, 1943). This rule is still in use. Since then, there have been studies that found that performing AI once a day could achieve similar conception risk to AI twice a day (Foote, 1978; Nebel *et al.*, 1994). Foote (1978) had records from 44,707 cows and virgin heifers including the time (a.m. or p.m.) of first observation of estrus and the time that the insemination was performed by a professional inseminator. A large proportion (73%) of the cows and heifers were first seen in estrus in the morning and there was no difference in non-return to estrus between those inseminated before 1200h, between 1200h and 1800h or after 1800h. For those cows that were first observed in estrus in the afternoon there was no difference in non-return to estrus whether inseminated before 1200h or between 1200h and 1400h the following day. However, the small sample of inseminations that occurred after 1400h the day after being observed in estrus in the afternoon had a decreased non-return rate. Though cows may not have been observed at the actual beginning of estrus and the actual time of observation was not recorded, Foote (1978) concluded that based on these findings one mid-morning insemination should give near maximum probability of pregnancy. Nebel *et al.* (1994) similarly found that once-daily insemination between 800h and 1100h had the highest non-return rate and was not different to the traditional "a.m-p.m. rule". In this study, 7240 first service inseminations from 166 herds were performed by professional technicians using either the "a.m-p.m. rule" or inseminating once daily. To minimize the effect of herd and season, technicians were randomly assigned to one of the two insemination programs for 3 months and then switched for the following 3 months. The signs of estrus observed and time of both observation and insemination were recorded. Seeing no loss in conception rates, using a once a day insemination program has the benefit of only requiring one farm visit from a hired technician and is an efficient use of labour for farms that inseminate the cows themselves.

There are few studies on the timing of insemination relative to activity thresholds to maximize the probability of pregnancy. Maatje *et al.* (1997) found that the highest probability of pregnancy was achieved 6 to17 hours after the onset of increased pedometer activity, with the estimated optimum at 11.8 h. This study used Boumatic Heat-seeker- TX (Dairy Equipment Co., Madison, WI) pedometers and all of the 171 inseminations included in the trial were associated with signs of estrus, which were observed during the three 30 minute observations per day by the herd manager. A cow was flagged by the system if the cow had an average activity for six 2-hour periods that was double the baseline mean activity from the 2 previous days for the same six periods. The exact time that the alarm began was recorded with the time of insemination. Stevenson *et al.* (2014) concluded that primiparous cows should be inseminated 13 to 16 hours after passing the activity threshold and multiparous cows should be inseminated between 0 and

12 hours. In this study the AAM system Select Detect (Select Sires Inc.) was used to detect estrus on 15 commercial farms totaling 4019 inseminations, excluding cases of lameness or activity periods longer than 33 h. From the system's data, the time of onset of increased activity was calculated and the time until insemination was calculated. The inseminations were based on the activity monitors alone and were not confirmed by other methods. More research is required on the optimal timing of AI to achieve the highest probability of pregnancy with AAM.

Timing of insemination may have an effect on the ratio of female to male offspring (Rorie, 1999). In a trial with 500 cows inseminated between 0 and 12 hours after observed estrus and 500 cows inseminated between 12 and 24 hours after first observed estrus, there was no difference between the groups in the gender ratio of the offspring (Ballinger, 1970). Pursley *et al.* (1998) synchronized cows and found that cows that were inseminated at 0 or 32 h after the second GnRH injection of Ovsynch (ovulation would occur 24 to 32 hours after second GnRH injection) had a higher ratio of females compared to those inseminated at 8, 16 or 24 hours. Overall, the pregnancy per AI was 39% and not significantly different for any of the groups. However, at 32h there was pregnancy loss of 32%, which was significantly higher than the 9% loss for those bred at 0 hours. There are mixed results on whether timing of insemination has an effect on the sex ratio of the offspring.

Activity monitoring versus other approaches to management of reproduction

There have been numerous studies comparing activity monitors to alternative methods of estrus detection. Michaelis *et al.* (2014) compared the use of activity monitors to visual estrus detection or the detection by at least one of the methods. There were 1004 potential periods of estrus, determined retrospectively by ultrasound, serum P4 ≤ 1 ng/ml at insemination or pregnancy to the insemination, from 348 cows monitored by Heatime activity monitors (SCR Engineering Ltd., Netanya). For the visual detection, cows were observed for 30 minutes twice a day. There was no difference in the estrus detection rate between the AAM system and visual detection in the first 21 days of the breeding period but the detection by at least one system was greater than either alone. Overall, the AAM system had a slightly higher efficiency (the number of correctly detected estrus events divided by the total number of possible estrus periods) than visual detection: 35.6% and 34.3%, respectively. This value is much lower than advertised by the manufacturer. The accuracy ((number of correctly detected estruses with P4 < 1 ng/ml / number of true and false estrus signals)*100) of the AAM was 83.8% compared to 75.1% for visual detection. The study showed that compared to visual detection, activity monitors performed well and cows detected by AAM were 1.4 times more likely to be diagnosed as pregnant, but improvements in estrus detection efficiency need to be made.

Timed AI protocols have been adopted in the dairy industry (Fricke *et al.*, 2014). However, with concerns over the use of hormones, alternative estrus detection methods such as AAM need to be further researched. A study comparing the reproductive performance of cows managed using an AAM system compared to using various timed AI breeding programs was performed on 3 commercial farms with the Heatime AAM (SCR Engineers Ltd., Netanya, Israel) (Neves *et al.*, 2012). This study found that at the cow level, there was no difference in time to pregnancy and time to first or second service for the two management programs. Another study that compared using an AAM system (AfiAct Pedometer Plus, Afimilk, Kibbutz Afikim, Israel) with the addition of hormone intervention to timed AI using a Presynch, Ovsynch and Resynch program found no difference in reproductive performance (Dolecheck *et al.*, 2016). This study also took place on three commercial farms and all cows that were in the AAM treatment group received no hormones unless they had no AAM alert by 39 ± 7 days after the VWP (25% of cows) after which they received one injection of either $PGF_{2\alpha}$ or GnRH but no TAI (timed artificial insemination). These studies demonstrate that AAM can be a competitive alternative to using timed AI protocols.

Conclusion

Although there is a growing body of published research on the use of activity monitors there are still questions to be answered. Evaluating the performance of AAM in a commercial setting is very important to inform producers' selection and use of management tools. There are many factors that affect the level of activity that a cow will exhibit at time of estrus. By identifying and quantifying these factors, an algorithm could be developed to correct for them and lead to more effective and accurate estrus detection. Once a cow is identified in estrus, finding the optimal time from passing the activity threshold until AI is important to optimize the performance of these tools.

RESEARCH OBJECTIVES

The objectives of this thesis are to:

1. Validate the accuracy of multiple automated activity monitors to detect cows that have low P4 when signalled as in estrus

2. Quantify the ability of AAM to detect cows in estrus for first AI, and to identify risk factors for not being detected in estrus by activity monitors by 80 DIM

3. Identify the optimal timing of insemination relative to the signalled onset of increased activity around estrus

Table 1.1 Sensitivity, Error Rate and Specificity of activity monitoring systems sorted by the standard to which the system was compared

Author	Number of estrus events	Threshold of Baseline[1] (%)	Sensitivity[2] (%)	Error Rate[3] (%)	Specificity[4] (%)	Study Referent[5]	Activity Monitor
Schofield *et al.* (1991)	12	≥ 2 SD above baseline	100	33		Milk P4 –Daily < 2.5ng/ml	Walker Pedometer, Silva UK Ltd.
Redden *et al.* (1993)	25	≥ 150% of baseline	80	17		Milk P4 –Daily < 1 ng/ml followed by < 1 ng/ml	Pedometer DIGI-WALKER MINI,(EM-201, Yamax Corp., Yokohama, Japan)
Koelsch *et al.* (1994)	29	n/a	72		98	Milk P4-Daily < 1ng/ml	Neck and leg Monitor Modified (Piezoelectric by Gettens)
De Mol *et al.*(1997)	537	95[6]	91		96	Milk P4-Daily < 7ng/ml	Pedometer (Undisclosed)
	537	99[6]	87		97	Milk P4-Daily < 7ng/ml	Pedometer (Undisclosed)
	537	99.9[6]	81		98	Milk P4-Daily < 7 ng/ml	Pedometer (Undisclosed)

Author	Number of estrus events	Threshold of Baseline[1] (%)	Sensitivity[2] (%)	Error Rate[3] (%)	Specificity[4] (%)	Study Referent[5]	Activity Monitor
Maatje *et al.* (1997)	121	≥ 200% of baseline	78	32		Milk P4 2x a week (cut point not reported)	Pedometers Boumatic Heat-seeker (TX Madison, WI)
Cavalieri *et al.* (2003)	43	≥ 2 Activity Ratio[7]	81.4	10		Milk P4 2x/ synchronized estrus < 2 ng/ml	Pedometers Boumatic Heat-seeker (TX Madison, WI)
Hockey *et al.* (2010a) (Dirt Loafing Paddock)	141	≥ 2 SD above baseline	90.8	60.4	90	Milk P4 2x/week < 2 ng/ml	Neck Activity Rescounter II, (Westfalia-Surge, Germany)
(Grazing Paddock)	135	≥ 2 SD above baseline	94.1	59.4	90.4	Milk P4 2x/week < 2 ng/ml	Neck Activity Rescounter II, (Westfalia-Surge, Germany)
Lovendahl and Chagunda (2010)	3674	≥ 0.70 threshold value[8]	74.6		99	Milk P4 2x/week < 4ng/ml (smoothed)	Neck Activity Alpro, (Version 6.60; DeLaval, 2007)
Aungier *et al.* (2012)	221	≥ 5 SD above baseline	72	32		Milk P4 2x weekly < 0.6 ng/ml	Heatime (SCREngineers Ltd., Netanya, Israel)

Author	Number of estrus events	Threshold of Baseline[1] (%)	Sensitivity[2] (%)	Error Rate[3] (%)	Specificity[4] (%)	Study Referent[5]	Activity Monitor
Kamphuis *et al.* (2012)	835	≥ 5.2 threshold Value[8]	77	18	99	Milk P4 2x weekly (cut point not reported)	Heatime Activity and Rumination (SCR Engineering Ltd., Netanya, Israel)
	835	≥ 5.2 threshold Value[8]	62	23	99	Milk P4 2x weekly (cut point not reported)	Heatime Activity only 83(SCR Engineering Ltd., Netanya, Israel)
Chanvallon *et al.* (2014)	179	≥ 170% of baseline	71	29		Milk P4 2x/week < 2.5 ng/ml	AfiTag PM (AfiMilk Packo, France)
	179	n/a	62	16		Milk P4 2x/week < 2.5ng/ml	Heatime (SCR Engineers Ltd., Netanya, Israel)
	179	n/a	61	33		Milk P4 2x/week < 2.5ng/ml	HeatPhone (Medria, France)
Williams et al (1981)	50	≥ 1 SD above baseline	74	42		Blood P4 3x/ week < 0.5 ng/ml	Basic Pedometer
	50	≥ 2 SD above baseline	68	17		Blood P4 3x/ week < 0.5 ng/ml	Basic Pedometer

Author	**Number of estrus events**	**Threshold of Baseline[1] (%)**	**Sensitivity[2] (%)**	**Error Rate[3] (%)**	**Specificity[4] (%)**	**Study Referent[5]**	**Activity Monitor**
Aungier *et al.* (2015)	21	≥ 5 SD for 4 2h blocks	90	17		Included if visually identified, Ultrasound (presence then disappearance of the follicle)	Heatime (SCR Engineers Ltd., Netanya, Israel)
Roelofs *et al.* (2005a)	63	≥ 2 SD above baseline	87	37	97	Ultrasound (disappearance of the follicle) behavioral estrus	Pedometers Nedap Groenlo, The Netherlands
	63	≥ 2.5 SD above baseline	87	16	99	Ultrasound (disappearance of the follicle) and behavioral estrus	Pedometers Nedap Groenlo, The Netherlands
Madureira *et al.*, (2015)	1041	≥ 35 index		10.4		Ultrasound presence of a follicle	Heatime (SCR Engineers Ltd., Netanya, Israel)
	993	≥ 180% of baseline		14.4		Ultrasound presence of a follicle	Pedometers Boumatic Heat-seeker (TX Madison, WI)

Author	Number of estrus events	Threshold of Baseline[1] (%)	Sensitivity[2] (%)	Error Rate[3] (%)	Specificity[4] (%)	Study Referent[5]	Activity Monitor
At-Taras and Spahr (2001)	46	≥ 2 Activity Ratio[7]	87			Heat mount, activity, visual observation, when possible palpation	Pedometers Boumatic Heat-seeker (TX Madison, WI)
Liu and Spahr (1993)	66	≥ 175% of baseline	74	33		Predicted periods[9]	Pedometer Heat Seeker (Dairy Equipmet Company, Madison, WI)

[1] Threshold of Baseline – The threshold that activity must cross for a cow to be flagged as in estrus
[2] Sensitivity – True Positive (TP)/(TP+ False Negative (FN))*100
[3] Error Rate – False Positive (FP)/(TP+FP)*100
[4] Specificity – True Negative (TN)/(TN+FP)*100
[5]Study Referent – Method of determining true estrus
[6]Confidence Interval
[7]Activity Ratio = 2 * (D-0)/(D-1) + (D-2), where

D-0 = average activity for the last 12 h,

D-1 = average activity for the corresponding 12 h from 1 d previous, and

D-2 = average activity for the corresponding 12 h from 2 d previous

[8] Threshold value – Standard proprietary threshold
[9]Predicted Periods – Period in the cycle of 18 to 24 d prior to the date that the cow became pregnant (Sometimes over estimates as doesn’t account for silent estruses)

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Chapter 2: Evaluation of factors affecting the detection of estrus using automated activity-monitoring systems for lactating dairy cows

INTRODUCTION

Inseminating cows in a timely manner is important for efficient dairy production management (Walker *et al.*, 1996). Activity monitors are useful tools for detection of estrus (Fricke *et al.*, 2014) and producers who have installed automated activity monitoring (AAM) systems report satisfaction with their performance (Michaelis *et al.*, 2013). However, there are many environmental and metabolic variables that can have negative effects on the ability of activity monitors to identify cows in estrus.

Temperatures above 25ºC have been shown to reduce the amount of walking activity exhibited by cows in estrus (Lopez-Gatius *et al.*, 2005). Higher milk production has been associated with decreased frequency and duration of standing behaviour during estrus (Lopez *et al.*, 2004). A study found that for every kilogram of increased milk production around the time of estrus, there was a 1.6% decrease in activity at estrus (Lopez-Gatius *et al.*, 2005). This is supported by Aungier *et al.* (2012) who found that for every 10 kg increase in milk production around the time of estrus there was a 6 to 7% decrease in the likelihood of a cow being correctly identified as in estrus by an activity monitoring system. Lameness not only increases lying time and reduces activity but can also subdue behavioural signs of estrus (Galindo and Broom, 2002; Sood and Nanda, 2006). Highly associated with lameness, low body condition score has also been found to reduce the peak of activity at estrus (Løvendahl and Chagunda, 2010; Madureira *et al.*, 2015). Reduced peak activity and reduced duration of activity have also been seen in cows of higher parity (Roelofs *et al.*, 2005; Madureira *et al.*, 2015).

The goals of a dairy farm's reproductive program is to achieve pregnancies in a timely manner postpartum, to reduce the time cows spend with low production levels later in lactation and to produce a consistent supply of replacements (Roelofs *et al.*, 2010). With the goal of having a calving interval of 12 to 13 months and a gestation length of approximately 280 days (Knott, 1932; Jenkins *et al.*, 2015) the target is for cows need to conceive between 85 and 115 DIM. With average conception rates around 32% (Schefers *et al.*, 2009), cows often require more than one insemination to become pregnant and therefore need to receive their first insemination prior to 80 DIM. In a reproductive program that uses activity monitors for heat detection, a cow must have estrus with an associated increase of activity for insemination to occur. To try to capture estrus as accurately as possible, various accelerometers and data processing algorithms have been developed to detect relevant changes in activity. A recent review (Saint-Dizier and Chastant-Mailland, 2012), found that the efficiency of estrus detection (number of cows in estrus detected ⁄ number of cows in estrus * 100) of AAM systems is generally higher than 80%. However, the positive predictive value for these systems can vary greatly. The ability of activity monitors to detect cows in estrus by 80 DIM has not been quantified.

The gold standard for estrus is a cow that is displaying standing to be mounted, has a dominant follicle that subsequently ovulates, has no *corpus luteum* (CL) and therefore has low levels of P4 in serum and milk. If a cow has high P4 while displaying signs of estrus, a CL is present and it can be considered as a falsely-identified estrus (Aungier *et al.*, 2012). However, a cow with low levels of P4 cannot be assumed to be in estrus because it is possible that the cow is anovular. Holman *et al.* (2011) found that 93.5% of the estrus events detected with neck-mounted activity monitors were associated with milk P4 below 0.2 ng/ml, meaning that there were few false positives.

Madureira *et al.* (2015) found a positive relationship between secondary signs of estrus (repeated mounting activity, clear vaginal mucus, and uterine tone) and increased peak activity at estrus. However, there was no association of these signs with pregnancy per artificial insemination (P/AI). In that study signs of estrus were only classified into a weak, normal or strong scoring scheme based on 1 to 3 signs of estrus observed, but these were not evaluated individually.

The first objective of this study was to determine the proportion of cows detected in estrus by AAM alone by 80 DIM, and to evaluate the effects of BCS, milk yield, anovular status, lameness or having purulent vaginal discharge (PVD) on this outcome. The second objective was to evaluate the accuracy of activity monitors (correct identification of cows with low P4 at the time of insemination) and to compare that to timed AI programs. The final objective was to assess the association between producer-recorded signs of estrus for cows detected in estrus by AAM and the probability of pregnancy to that AI.

MATERIALS AND METHODS

Data were collected from four commercial farms in Ontario, Canada, within 100 km of the University of Guelph, between May 2014 and August 2015. To be included, farms were required to have an activity monitoring system (Heatime (SCR Engineers, Netanya, Israel) or AfiAct (Afimilk, Kibbutz Afikim, Israel)) and be using DairyComp305 (DC305; Valley Ag Software, Tulare, CA, USA) for herd management. The size of these herds ranged from approximately 100 to 400 lactating Holstein cows and the cows were all housed in sand-bedded freestall barns. Two of the enrolled herds (those using AfiAct) milked 3 times a day and the other two milked 2 times a day. The farms had a VWP of 55 DIM and used activity monitoring to identify estrus for essentially all inseminations between 55 and 80 DIM, after which the remaining inseminations were based on a combination of activity and various protocols for timed artificial insemination (TAI).

Farms were visited once weekly for sample collection and to retrieve data from DC305. Blood samples were collected from all cows via the coccygeal vessels into an evacuated tube without anti-coagulant (Vacutainer BD, New Jersey, USA) at weeks 5 (32 to 38 DIM), 7 (46 to 52 DIM) and 9 (60 to 66 DIM) postpartum. These samples were allowed to clot and then transported back to the lab and centrifuged at 3000 rpm for 15 minutes. The serum was removed from the sample and frozen at -20 ºC for later analysis of P4 concentration. Cows were examined for PVD using the Metricheck device (Simcrotech, Hamilton, New Zealand) at week 5 postpartum. Any discharge was evaluated using a 0 to 3 point scale similar to Leutert at al. (2012). A cow with a score of 2 or greater was considered to be positive for PVD. At week 7 postpartum, BCS was assigned and lameness scoring was performed. The BCS was based on a 5 point scale (Edmonson *et al.*, 1989) and a cow was considered thin if BCS was ≤ 2.5 at week 7 of lactation. The lameness scoring was also based on a 5 point scale (Sprecher *et al.*, 1997) and a cow scoring ≥ 3 was considered to be lame. The majority of scoring was performed by the primary researcher and assistants were trained and verified to have similar scoring when necessary. Milk, % fat and % protein were also recorded for the first two DHIA (Dairy Herd Improvement Association) tests of the lactation (test 1 mean = 23 DIM ± 12.5, test 2 mean = 59 DIM ± 13.8) from CanWest DHI (Guelph, ON, CA).

Blood samples were also collected from cows that were inseminated or were flagged by the activity monitor as being in estrus on the day of the weekly farm visit.

The serum samples taken from all cows at weeks 5, 7 and 9 postpartum and from the subsample of cows the day of AI were thawed and P4 was measured using the Ovucheck Plasma (Biovet, Saint-Hyacinthe, QC, CA) ELISA test. This 96 well test has a range of quantification from 0.55 ng/ml to 10.45 ng/ml and has been validated for accuracy (Broes *et al.*, 2014). Cows were considered to be anovular if all samples from weeks 5, 7 and 9 postpartum had P4 > 1.0 ng/ml (Stevenson *et al.*, 2006). For samples on the day of AI, cows with serum P4 > 1.0 ng/ml were determined not to be in estrus at insemination.

Managers were asked to record details of insemination on standardized forms (Appendix) supplied to them which included: time of insemination, whether AI was based on AAM signal or TAI, the technician who performed the AI, the ease of insemination, and whether any of mounting or standing to be mounted, increased activity, decrease in milk production or presence of mucus discharge were observed.

Statistical Analysis

Data for AI dates, breeding codes (i.e., based on AAM signal or timed AI protocol), pregnancy diagnosis and culling were extracted from DC305 and exported to Microsoft Excel (Microsoft Corp., Redmond WA) for all enrolled animals. The statistical analyses were performed in SAS (Version 9.4, SAS Institute, Inc., Cary, NC, US). Univariable associations of all variables with the relevant outcomes and interactions were screened using the FREQ procedure and variables with a P-value < 0.2 were offered to the three multivariable mixed logistic regression models (GLIMMIX procedure in SAS).

Model for Detection of Estrus by 80 DIM

The variables parity (first, second, or third or greater), milk production, percent fat and percent protein at first and second test, being anovular, positive for PVD, low BCS and the interactions between them were tested for their association with being detected in estrus and inseminated by 80 DIM. When building the model, the variables that passed the screening and were offered to the model were 1) being anovular, 2) positive for PVD, 3) parity, and the interactions of 4) low BCS with lameness, 5) positive PVD with low BCS, 6) being anovular with being lame, 7) being anovular with low BCS, 8) parity with being anovular, 9) parity with low BCS, 10) parity with being lame and 11) parity with PVD. A random effect for “farm” was included to account for the unmeasured sources of variability between herds and correlation of cows within a herd.

Model for Accuracy of Detection

The outcome variable was a serum P4 < 1 ng/ml on the day of insemination. The predictor variable was the basis of insemination (an activity monitor alert or based on a timed AI protocol) or being from a cow that was flagged by the activity monitor but not inseminated. Both farm and cow were included as random effects.

Model for Observed Sign of Estrus

The variables 1) technician who performed the AI, 2) the ease of insemination, 3) observed mounting, 4) observed mounted, 5) observed increase in activity, 6) observed decrease in milk production and 7) observed presence of mucus discharge were screened for association with pregnancy to the AI. Decrease in milk production, presence of mucus discharge and the interaction between them were offered the model. Both farm and cow were included as random effects.

Sample Size

Sample size was calculated based on estimating the intensity of activity monitors detecting cows in estrus and being inseminated by 80 DIM within ± 3 points with 95% confidence and 80% power. It was calculated that it would require 1000 first inseminations using WINPEPI (Abramson, J.H., 2011).

RESULTS

Detection of Estrus by 80 DIM

There were 1014 cows with blood samples from weeks 5, 7, and 9. Of these cows, 17% were not identified by the activity monitor and not inseminated by 80 DIM. The prevalence of cows being anovular, positive for PVD, having low BCS, and lame was 9%, 14%, 9% and 15%, respectively. In preliminary analyses, first lactation cows had an increased incidence of PVD and cows in lactation three and above had a higher prevalence of lameness, BCS $\leq$ 2.5 and being anovular. However, there were no interactions of these variables in the in the final model of detection of estrus by AAM by 80 DIM.

Cows that were anovular, positive for PVD, multiparous or cows that both had low BCS and were lame had decreased odds of being detected in estrus by the AAM system and inseminated by 80 DIM (Table 2.1). There were no associations of milk (kg), percent fat or percent protein at first or second test with odds of being detected in estrus by AAM by 80 DIM.

Though the interaction between being anovular and being lame was not significant in the final model, of the 149 lame cows 18% were anovular compared to the 7% of non-lame cows. The interaction between being anovular and having low BCS was also not significant in the final model but 26% of cows with a BCS of ≤ 2.5 were anovular and 7% of cows with a BCS > 2.5 were anovular.

The third blood sample to determine if a cow was anovular was taken at 63 ± 3 DIM and therefore there was a window for a cow to start to cycle and be inseminated by 80 DIM but still be considered as anovular. Of the 87 cows classified as anovular, 28 (32%) were inseminated between 67 to 79 DIM and were therefore identified as anovular but apparently began to cycle after the last blood sample. There were 30 anovular cows (34% of the anovular cows or 4% of cows inseminated by 80 DIM) that were inseminated prior to 66 DIM, and therefore would have been inseminated based on a false positive alert.

Accuracy of Detection

Over the course of the study, 919 blood samples were taken from cows that were being inseminated or had been flagged as in estrus by the activity monitor on the day of the weekly farm visit. Of these, 445 samples were from inseminations based on activity, 323 were timed inseminations, and 148 were samples taken from cows that were flagged that day by the AAM system but not inseminated. Of the 148 cows not inseminated, 36 were not inseminated because they had not passed the voluntary waiting period (VWP) of 55 DIM, and 23 samples were from cows designated not to be inseminated (Do not breed (DNB)). The reason for not inseminating the remaining 89 cows was unknown.

There was no significant difference in the odds of a sample having high P4 between those from cows inseminated based on activity and those from cows inseminated based on timed AI (3 ± 1.2 % and 4 ± 1.8 % of samples had P4 > 1 ng/ml, respectively (P = 0.35)). Cows that were signalled as in estrus but not inseminated for an unknown reason had significantly higher proportion of samples with high P4 (43 ± 11.2%) than samples from inseminations based on AAM or TAI (P < 0.0001).

Considering all animals flagged by the activity system (n = 538), both inseminated and not inseminated, 9 ± 2.0% had serum P4 > 1 ng/ml. This was greater than the proportion among cows inseminated based on TAI (4 ± 1.8% P = 0.02).

Observed Signs of Estrus

For 2849 inseminations, observed signs of estrus were recorded by the producers on standardized forms. Of these inseminations, 2151 (76%) were based on an activity monitor alert and 698 (24%) were based on TAI. There were 2060 activity-based inseminations and 667 TAI that were fully recorded by the producer both on the standardized forms and in the herd management software.

For these 2060 inseminations that were based solely on an AAM alert (for all inseminations, first or repeated), 640 (31%) resulted in a diagnosed pregnancy. The signs of estrus that were observed by the producers are presented in Table 2.2. Observation of increased activity, mounting behaviour, standing to be mounted, and ease of insemination were not associated with the probability of pregnancy per AI and were not offered to the multivariable model. Though opposite to what was expected, when tested independently an observed decrease in milk yield or seeing mucous discharge were associated with a decrease in conception risk

(32% vs. 23% (P = 0.002) and 33% vs. 27% (P = 0.01), respectively). However, in the model with random effects for farm and cow, these variables were no longer significant. The associations were primarily driven by one farm that reported a significantly higher number of these observations than the other farms.

DISCUSSION

Cows that were anovular, multiparous, positive for PVD or had both low BCS and lameness were less likely to be detected in estrus by AAM and inseminated by 80 DIM. Of cows that were inseminated based on an activity monitor alert only 3% had a serum P4 concentration incompatible with estrus, which was similar to the cows bred by a TAI protocol. Signs of estrus that happened to be observed by the producer were not associated with the risk of pregnancy.

On commercial farms, Fricke *et al.*, 2014 found that only 56% of cows were detected in estrus by activity monitoring by 65 DIM. In our study, activity monitors detected cows in estrus and had 83% of cows inseminated by 80 DIM without intervention. This indicates that AAM can effectively achieve timely first insemination for a substantial majority of cows, but also highlights that intervention such as a TAI program would be needed for the remainder to achieve this goal.

The prevalence of anovular cows in this study is within the herd-level range of 5 to 45% reported by Walsh *et al.* (2007). However, the mean prevalence is typically 20 to 30% (Gümen *et al.*, 2003; Lopez *et al.*, 2005; Walsh *et al.*, 2007) which is much higher than what was observed here (9%). This could be explained by the fact that this study was conducted in well-managed herds that had low prevalence of lameness and few cows with low BCS, each of which are risk factors for anovular condition. As expected, cows that were found to be anovular were two times less likely to be inseminated by 80 DIM.

The prevalence of PVD in this study was consistent with the 10 to 17% that has been reported by others (Barlund *et al.*, 2008; Dubuc *et al.*, 2010; Denis-Robichaud and Dubuc, 2015). If a cow was diagnosed with PVD there were 40% higher odds of not being detected in estrus and inseminated by 80 DIM. A relationship between having PVD and being non-cyclic has been shown by Maquivar *et al*. (2015), who found that 44% of cows without PVD were anovular by 40 ± 3DIM, but 57% of untreated cows with PVD were anovular. However, in the present study there was no interaction between PVD and being anovular with respect to the probability of estrus detection by AAM for AI by 80 DIM.

Cows in first lactation tended to have increased odds of being inseminated by 80 DIM compared to cows in second or greater lactation. Madureira *et al.* (2015) found that multiparous cows had decreased peak of activity and duration of estrus. Multiparous cows may have decreased activity as there is increased incidence of lameness and low BCS in multiparous cows which are factors that have been shown to have a negative impact on activity levels. This effect could explain why a lower proportion of multiparous cows were detected in estrus by AAM and inseminated by 80 DIM in the present study.

The association between low body condition and lameness has been demonstrated in numerous studies. For example, Solano *et al.* (2015) found that the odds of a cow with a BCS of ≤ 2.5 being lame were 1.6 times greater than a cow with a BCS of > 2.5. In the present study, there were few cows that had both low BCS and were lame at week 7 of lactation, but these cows were more than twice as likely not to be inseminated by 80 DIM compared to well-conditioned, non-lame cows. Though this interaction had a P-value = 0.11, as seen in the Table 2.1, when the two variables are compounded a substantial decrease in the predicted probability of being detected in estrus was observed, therefore this term was kept in the model.

When looking at only the blood samples that came from animals that were inseminated, the proportion of animals signalled by the AAM system that had high P4 at time of insemination (therefore considered not to be in estrus) was low and not significantly different than the proportion with high P4 that were inseminated on a TAI protocol. However, the 25% of cows signalled by AAM as in estrus that were not inseminated demonstrates that the producers were implementing selective insemination when looking at the list generated by the AAM system. It is possible that the producers were observing for signs of estrus and if they were absent would not inseminate the cow. Unfortunately, our data collection did not specifically capture reasons for non-insemination of cows signalled by the AAM system.

The lowest value that the ELISA could detect was 0.55 ng/ml. Of the samples taken from day of insemination that were classified as having low progesterone (below 1ng/ml) 98.7% had progesterone classified at the lowest level of 0.55ng/ml. Therefore, the effect of having varying levels of progesterone between 0 and 1 ng/ml was not evaluated.

Though it was out of the scope of the current study, implementing observation of estrous behaviours in addition to AAM is likely to increase the sensitivity, but perhaps not the accuracy of estrus detection. In the present study, cows that were not inseminated when they had an activity alert had a significantly higher risk of having high P4. While the reasons for non-insemination of eligible cows were not recorded, it appears that in many cases producers correctly applied selection or confirmation criteria to AAM alarms. These apparent false positive estrus signals might have been associated with moving cows to a new pen or non-routine activity in the barn (e.g., hoof trimming). If these cows had been inseminated it would have increased the number of false positives and decreased pregnancy per AI (P/AI). However, overriding the AAM indication of estrus can also have negative effects because 57% of these cows did have low P4 and might have been successfully inseminated. With the addition of the non-inseminated animals, the proportion accurately detected by the activity monitors (based on low serum P4) decreases to 91%, similar to Madureira *et al.* (2015).

The lack of association of chance observation of increased activity, mounting, mucous discharge, or decrease in milk production or of being considered an easy insemination with P/AI is consistent with the findings of Madureira *et al.* (2015), who found that secondary signs of estrus had no influence on pregnancies per AI based on AAM.

The goal of reproductive programs is to have cows inseminated in a timely manner with high P/AI. Previous studies have looked at the accuracy of AAM systems but there was a lack of data on the effectiveness of reliance on estrus detection by AAM for more than one full estrus cycle for first insemination. This provides a benchmark for expectations of AAM relative to the need for interventions such as TAI to achieve targets for timely first AI. Conducting studies such as this on commercial farms provides greater statistical power and generalizability of the results than smaller studies in more controlled research herds. Limitations of using commercial farms include not having all information about management decisions to inseminate a cow or not when flagged by the AAM system. Another limitation to this study relative to the possible value of supplementing estrus detection by AAM with observation of estrous behaviours is that only the signs of estrus that happened to be observed by the producer were recorded. Future studies should investigate the utility and incremental gains in performance from the addition of other estrus detection methods to AAM.

CONCLUSION

Activity monitors can identify over 80% of animals for insemination by 80 DIM. However, anovular or multiparous cows, cows with purulent vaginal discharge or cows with both BCS ≤ 2.5 along and a lameness score ≥ 3 are less likely to be identified in estrus. Identification and treatment of PVD, anovular condition and lameness may improve estrus detection by AAM; these effects should be quantified in future studies. Activity monitors are effective at identifying animals that have low P4 at insemination. Accuracy can apparently be improved with managers' discretion about insemination of cows signalled as in estrus by AAM, but specific signs associated with higher P/AI were not identified

Table 2.1 Descriptive information from enrolled herds

Descriptive parameter	**Herd A**	**Herd B**	**Herd C**	**Herd D**
Average herd size	352	160	94	250
Herd average 305 ME[1]	14474	12541	12354	12366
Stage of pregnancy diagnosis (DIM)	30-40	42-60	35-47	30-40
21 day pregnancy rate (%)	23	15	25	31
Insemination rate (%)	67	67	62	74
Conception risk (%)	33	21	39	44
Number of sires used during trial	80	90	37	22
Milking frequency	3	2	2	3
Activity Monitor System	Afiact	Heatime	Heatime	Afiact

[1] 305–d mature–equivalent milk

Table 2.2 Final mixed logistic regression model, accounting for the random effect of farm, of variables associated with being inseminated by 80 DIM based on detection of estrus by automated activity monitors in 1014 cows in 4 herds.

Variable	Class	n	OR[1]	PPD[2] (%)	β	SE	95% CI	P-value
Anovular[3]	Yes	87	0.379	62	0.97	0.27	0.226-0.638	<0.001
	No	927	Referent	81	-	-	-	-
PVD[4]	Yes	140	0.661	68	0.41	0.24	0.415-1.053	0.08
	No	871	Referent	76	-	-	-	-
Low BCS[5]	Yes	88	0.757	70	0.75	0.45	0.422-1.357	0.35
	No	923	Referent	75	-	-	-	-
Lame[6]	Yes	149	0.403	63	1.38	0.53	0.225-0.722	0.002
	No	862	Referent	81	-	-	-	-
Parity	1	327	Referent	78	-0.43	0.22	0.42-1.01	0.06
	2	221	0.616	68	0.059	0.23	0.679-1.657	0.80
	3+	456	0.653	70	-	-	-	-
Low BCS and Lame	Not Low BCS and Not Lame	804		79	0.95	0.58		0.11
	Low BCS and Not Lame	58		71				
	Not Low BCS and Lame	119		82				
	Low BCS and Lame	30		53				

[1]Odds Ratio
[2] Predicted Probability of Detection in Estrus by 80 DIM
[3]Anovular at week 9 – P4 of < 1ng/ml at weeks 5, 7 and 9
[4]Purulent Vaginal Discharge score of ≥ 2 i.e., muco-purulent
[5]Body Condition Score ≤ 2.5
[6]Lameness – locomotion score of ≥ 3 (Sprecher *et al.*, 1997)

Table 2.3 Prevalence of signs of estrus observed by producers at time of insemination based on a signal from the activity monitor system (n= 2060 artificial inseminations from 898 cows on 4 farms)

Estrus Signal	Prevalence (%)
Observation of increased activity (Visual)	76
Observation of mounting	44
Observation of standing to be mounted	43
Observation of mucus discharge (during AI)	25
Decreased milk production	14
Easy insemination (ease of passage of the AI gun through the cervix)	98

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Chapter 3: Relationship between timing of insemination and pregnancy in lactating dairy cows detected in estrus using activity monitoring systems

INTRODUCTION

Having semen delivered at the optimal time to have viable spermatozoa at the site of fertilization in the oviduct awaiting a viable oocyte is important for pregnancy (Roelofs *et al.*, 2006). The obstacle is identifying when ovulation will occur with sufficient precision. The spermatozoa take approximately 8 h to reach the isthmus of the oviduct and by 12 to 24 h there are few spermatozoa remaining in the reproductive tract (Hawk, 1987). Many methods have been used to identify when an individual cow will ovulate. However, the time of ovulation relative to signs of estrus is variable among cows (Roelofs *et al.*, 2006). This makes optimizing the timing of insemination a challenge. Studies evaluating the optimal time to inseminate cows have been occurring for over 70 years (Trimberger, 1943). The “am-pm rule” whereby cows first detected in estrus in the morning would be inseminated that afternoon and those detected in the afternoon would be inseminated the following morning was widely adopted in the dairy industry. On the other hand, inseminating once a day was also adopted by many herds as it is favourable for those farms where inseminations are performed by a hired technician. Conception risks for once a day AI were reported to be comparable to insemination twice a day (Foote, 1978; Nebel *et al.*, 1994).

To achieve maximum conception, viable sperm must be present to fertilize the newly ovulated and transported oocyte. Once previously frozen and extended semen is deposited in the uterus during AI it takes the spermatozoa 10 to 12 hours for capacitation and transport in the female tract (Hunter and Wilmut, 1983). Inseminating cows 12 to 24 hours before ovulation was found to produce the highest number of good quality embryos (Roelofs *et al.*, 2006). However, predicting when ovulation will occur can be a challenge. The time from the onset of behavioural estrous activity to ovulation has been found to average between 26 and 33 hours (Roelofs *et al.*, 2005; Hockey *et al.*, 2010; Yoshioka *et al.*, 2010; Stevenson *et al.*, 2014; Bombardelli *et al.*, 2015). Stevenson *et al.* (2014) found that primiparous cows tended to ovulate slightly earlier after passing the threshold of activity with an automated activity monitor system than multiparous cows (25.4 ± 0.5 h and 27.0 ± 0.8 h, respectively) but more investigation is needed on that question.

The interval from the first time being mounted (measured by Heatwatch Heat-Watch II; CowChips, Manalapan, NJ, USA, a telemetric mounting sensor to detect standing estrus) to insemination to maximize the probability of pregnancy has been found to be 12 hours (Dalton *et al.*, 2001). Standing to be mounted is a behaviour specifically expressed at estrus and therefore these systems have few false positives. In a review of the performance of these Heatwatch systems, Saint-Dizier and Chastant-Maillard (2012) they found that the accuracy (number of correct estrus detections/number of correct + false-positive estrus detection x 100) of the system was between 87 and 100%. However, studies have found that only 29% to 58% of lactating dairy cows will stand to be mounted during estrus and many estrus events would not be identified even with near-perfect detection of this behaviour. (William, 1981; Roelofs *et al.*, 2008). Therefore, the optimal timing from standing estrus to insemination may not be relevant for other signs of estrus.

There are fewer studies identifying the optimal interval from achieving an activity monitor threshold to insemination. Maatje *et al.* (1997) found that the highest conception risk was achieved 6 to 17 hours after an increase of activity using pedometers. Stevenson *et al.*

(2014) found that there was a difference in optimal interval from onset of estrus (signalled by passing the threshold of activity in a commercial AAM system) to insemination for primiparous cows compared to multiparous cows such that they should be inseminated 13 to 16 hours or 0 to 12 hours after passing the activity threshold, respectively.

With a large variety of activity monitors on the market and many variables that can affect activity measurements, more research on optimal time of insemination following an activity monitor alert is required.

Commercial AAM systems use different algorithms to calculate when a cow is in estrus. However, all employ the basic concept that each cow creates her own rolling mean baseline activity over 7 to 10 days and needs to achieve a threshold of activity relative to the baseline to be flagged in estrus. Also, the method and frequency of data transfer differs among systems e.g., at entry to the milking parlor or at frequent intervals throughout the day. The timing of the data transfer and how often the data are acted upon by managers could be a confounding factors in the interval from estrus signal to insemination.

The objective of this study was to determine the time interval from activity monitor estrus alert to insemination associated with the highest risk of pregnancy. Our hypothesis was that cows would have the highest probability of pregnancy risk if bred within 16 h after the activity alert, based on the findings of Maatje *et al.*, 1997 and Stevenson *et al.*, 2014.

MATERIALS AND METHODS

Four farms within a one hour drive of the University of Guelph (Ontario, Canada) were enrolled in this observational study, which was conducted from May 2014 to August 2015. The herds were a purposive sample of herds with AAM systems that were enrolled on DHIA and were willing to collect data and participate in the study. Two of the farms enrolled in the trial had the AfiAct (Afimilk, Kibbutz Afikim, Israel) leg-mounted activity monitor system and were milked three times a day. The other two farms had neck-mounted Heatime (SCR Engineers, Netanya, Israel) activity monitors, one with Heatime HR and one with Heatime Dataflow; both of these herds milked two times per day. All of the herds enrolled used DairyComp305 (DC305; Valley Ag Software, Tulare, CA, USA) as the herd management software. The farms involved in this study all had sand-bedded freestall barns and the herd size ranged from 100 to 400 lactating cows.

During the study, enrolled farms continued with the reproduction management program that they had in place. This meant that inseminations would be a result of both activity monitor alerts and timed artificial insemination (TAI) protocols. For this analysis, only inseminations based on an activity monitor alert were analysed. For each insemination, producers were asked to record the date, cow identification, the time of insemination and whether the insemination was based on an activity signal or a TAI protocol. Data were collected from DC305 and from the software of the activity monitors during weekly farm visits. Data on inseminations and pregnancy outcomes were extracted from DC305 and exported to Microsoft Excel (Microsoft Corp., Redmond WA).

The raw activity recorded by the AfiAct activity tags was transmitted to the farm computer when the cow entered the parlour at each of the three daily milkings. At this time, the cows that had a deviation in activity since the previous milking would be flagged on the AfiFarm software and put on a list of cows to inseminate. The activity value that was produced by the system at each milking was an average of the hourly activity for the time between the current milking and the one preceding it (i.e., average steps per hour for each 8-h “session”). Before the first milking of each day, a data back-up file was produced and automatically saved on the farm computer with the 3 activity values from the previous day. At the end of the study, the daily files were merged to form a timeline of activity for each cow. These activity data where then used to identify the 8-hour period in which a cow crossed the threshold of activity and was flagged as in estrus by the system. The raw activity did not include a “stamp” of the period in which estrus was first signalled. To re-create when a cow was flagged in estrus the average and SD of the activity of the 10 days before each AI was created. To be flagged as in estrus the activity value must achieve a threshold. According to the system software the threshold value varied with the baseline average number of steps a cow took per hour as seen in Table 3.1. Once a cow crossed the threshold, the onset was considered to be at the time within an 8 h data collection period that the estrus signal appeared on the computer and could be viewed by the manager, which for both of the enrolled herds with AfiAct was considered to be 0600, 1400 or 2200h.

The SCR system produced values for activity in two hour intervals. The PC-based Dataflow system made daily reports of the previous day’s raw activity, producing 12 2-hour intervals of each day, for each cow. These daily files were then merged to produce an activity timeline for each cow. The standalone Heatime HR system had a back-up option to extract all the raw activity for up to one year. This file was converted to an Excel file by the Heatime Batch tool which produced the raw activity timeline for each cow, for each day, in 2 hour blocks.

To calculate when a cow crossed an activity threshold for estrus, activity change, which is a proprietary calculation from SCR, is required. This value was unavailable for the Heatime Dataflow system so therefore it was re-created using a calculation provided by SCR that created a value which is a close approximation of the proprietary algorithm. For uniformity, this calculation was used for both of the SCR herds. It was calculated by first taking the average of the previous 10 hours including the current two hour value to create the *10 Hour Average*. Then, taking the mean of the *10 Hour Average* of the same time period of the past 7 days, the *Week Average* was created. The standard deviation was then taken of the 7 values in the *Week Average* to create the *Week Standard Deviation*. A logical test was then performed: if the W*eek Standard Deviation* divided by the *week average* was < 0.05 then *Minstd* was equal to the *Week Average* multiplied by 0.05, and if not the *Minstd* was equal to the *Week Standard Deviation*. Another logic test was performed: if the *Week Standard Deviation* divided by the *Week Average* was greater than 0.2 then the *Newstd* was equal to *Week Average* multiplied by 0.2 and if not *Newstd* was equal to *Minstd*. The *Activity Change* value was then calculated by subtracting the *Week Average* from the *10 hour Average*, dividing that by *Newstd* and then multiplying by 5. Once the *Activity Change* was calculated, it along with the raw activity, was processed using an Excel macro that was developed and validated by University of British Columbia to identify the 2h periods when the onset and peak estrous activity occurred (and the number of 2h periods above the estrus threshold).

The re-created times of onset of estrus from both systems were then merged with the insemination dates and times that were recorded by the producers. The interval between the time of estrus signal from the AAM and insemination was then calculated. To be comparable between systems, the intervals from estrus alarm to AI were classified as 0 to 8 hours, 8 to 16 hours or 16 to 24 hours.

Statistical Analysis

The statistical analyses were performed in SAS (Version 9.4, SAS Institute, Inc., Cary, NC, US). The FREQ procedure was used to screen univariable associations of all variables with the probability of pregnancy per AI (P/AI) and those with a P-value < 0.2 were offered to a multivariable mixed logistic regression model (GLIMMIX procedure in SAS). The variables farm, activity monitor system (Afi or SCR), parity, time to insemination (0 to 8 h, 8 to 16h and 16 to 24 h) and the interactions among them were tested with pregnancy as the outcome for each AI. The variables time to insemination, parity and the interaction of time to insemination with parity passed the screening and were offered to the model. Farm was included as a random effect. The unit of analysis was the insemination and because some but not all cows contributed more than one AI, cow (nested within farm) was also included as a random effect.

The sample size was calculated on the basis of detecting a difference in conception risk from 32% to 38% with 95% confidence and 80% power which would require 990 inseminations for each pairwise comparison of the three breeding intervals (0 to 8 h, 8 to 16 h and 16 to 24 h) when calculated using WINPEPI (Abramson, J.H., 2011).

RESULTS

Throughout the study 2739 inseminations where recorded by the producers. Of those, 673 were based on a timed artificial insemination (TAI) protocol and therefore were not included in the calculations, leaving 2066 AI that were based on activity. Due to inseminations that occurred at the beginning of the study and those that occurred during a period when one of the farms had their computer fail, 473 inseminations did not have a complete baseline of activity and were excluded from the study. This left 1593 inseminations, of which 139 had a negative interval from time of onset of estrus based on activity to insemination (i.e., the AI was before the activity signal) and 55 had an interval longer than 24h. These inseminations were likely based on visual observation of signs of estrus (e.g. the cow was seen mounting or being mounted before the activity data were transferred at milking and the signal processed). Of the inseminations that occurred before an activity alert and therefore likely based on visual detection 25% resulted in a pregnancy and for the inseminations that occurred past 24 h after activity alert 29% resulted in pregnancies. Figure 3.1 shows the distribution of the remaining 1399 inseminations that occurred 0 to 8 h, 8 to 16 h and 16 to 24 h after the activity alert, which were used in the final model. The variable for activity monitor system (which was confounded with frequency of milking) was not significant in the model. In preliminary analyses, farm had a significant effect on the odds of pregnancy. However, there was no interaction between farm and interval to insemination. The variables that remained in the model were interval to insemination, parity, and the interaction between interval to insemination and parity, with farm and cow as random effects. For multiparous cows the mean (± SE) probability of pregnancy per AI was 31.3 ± 3.1% and was not different (P = 0.7) among intervals from onset of estrus to AI. For primiparous cows, AI within 8 h of onset of estrus was associated with 1.75 and 2.12 greater odds of pregnancy than AI 8 to 12 or 16 to 24 h after onset, respectively. The predicted P/AI for primiparous cows for each interval were 49 ± 5.0%, 36 ± 5.2% and 32 ± 5.5%, respectively. Figure 3.2 depicts the association between predicted pregnancy risk and the interaction between the time intervals after activity monitor alert and parity.

DISCUSSION

The finding that primiparous cows should be inseminated earlier after AAM alert compared to multiparous cows is novel. This result differs from Stevenson *et al.* (2014) who found that pregnancy was maximized for primiparous cows when inseminated between 13 and 16 hours after activity alert, but among multiparous cows pregnancy risk did not differ between the 0 and 12 h intervals. However, their observation of the tendency for primiparous cows to ovulate slightly earlier than multiparous cows generally fits with our data. Our optimal timing of insemination for primiparous cows fits into the wide optimal range that Maatje *et al.* (1997) found of 6 to 17 h after increased pedometer activity. Our result that the conception risk for multiparous cows did not change up to 24 hours after an activity monitor alert agrees with the findings of Foote (1978) and Nebel *et al.* (1994) who found that once a day insemination could achieve similar P/AI as AI twice a day.

Though there are differences in the fertility of the various bulls that were used on these farms this factor was not evaluated. The herds use a large variety of bulls each using between 22 and 90 different bulls throughout the trial. There could be an impact of bull fertility on optimal timing of insemination but this relation would need a larger sample size to evaluate this.

Cows that were inseminated in the later intervals after activity alert (8 to 16h or 16 to 24h) could potentially have been more likely to have high progesterone at insemination as the estrus period could be coming to an end. However, of the subsample of cows that had progesterone measured at the time of insemination from Chapter 2 only a small percentage (4%) of the 445 samples collected from cows inseminated based on AAM had high progesterone and were considered not in estrus. Therefore, the sample size was not large enough to evaluate this.

Because the objective was not to investigate individual farm effects, farm was considered a random effect. Some cows had multiple inseminations recorded and others contribution only one insemination to the study. Therefore, a random effect of cow was included to account for this. In this study, milking frequency was confounded with AAM system: the farms that had the SCR activity monitors milked 2x and those with AfiAct activity monitor systems milked 3x. When screening variables, activity monitor system was not significant. The interaction between the system and the interval to insemination was also not significant supporting that our findings regarding timing of AI and parity do not depend on the type of activity monitor system.

A limitation to this study is that cows were not systematically confirmed as in estrus when they were signalled by the activity monitor and inseminated. However, for a subset of these AI (n=445) only 3% of cows that were flagged by the activity monitor and inseminated had high P4 at insemination and therefore were not truly in estrus (Chapter 2).

Though the planned sample size of 990 AI for each pairwise comparison of 0 to 8 h, 8 to 16 h and 16 to 24 h was not achieved, a significant and larger-than-expected difference between the intervals was seen for primiparous cows. The P/AI for multiparous were nearly identical, so the chance of a type II error is small. The activity monitoring systems had many differences in how they detected cows in estrus and the raw activity data that they produced. The data output from the 2 different SCR systems was made consistent by running both the data sets through the Excel formulas to recreate *Activity Change* and then the macro, as using the commercial algorithm for onset of estrus was not an option. Though Afi and SCR produced activity data in 8 h and 2 h blocks, respectively, our interest was when the AAM system actually flagged cows in estrus and the manager could take action based on that alert. This difference was overcome since we were evaluating the interval from when the cow was flagged in estrus by the AAM system and not from the exact moment signs of estrus were displayed. Future research may consider whether newer AAM systems that provide data and alarms in near-real time change or narrow the optimal timing of AI. Optimized timing of AI would have to be balanced against the practicality of performing AI several times throughout the day and night.

CONCLUSION

Our data suggest that primiparous cows should be inseminated between 0 to 8 hours after they are signalled in estrus by the activity monitor to maximize the probability of pregnancy. Conversely, we found no difference in the odds of pregnancy for the multiparous cows inseminated up to 24 hours after the activity monitor alert. Prioritizing the insemination of first lactation cows to achieve intervals of < 8h from the AAMs signal has the potential to increase the probability of pregnancy. However, with divergent results between this study and another large field study, further research is needed to confirm the optimal timing of AI for primiparous cows, or the factors that influence the optimal timing. For multiparous cows, there appears to be more flexibility to inseminate up to 24h after the onset of estrus based on AAM. Therefore, we suggest that performing AI once per day is acceptable for multiparous cows, but that inseminating based on activity data at least twice (if not three times) per day may improve pregnancy per AI in primiparous lactating dairy cows.

Table 3.1 The threshold values used by 2 farms for the AfiAct activity monitors to classify onset of estrus

Baseline Average Steps Per Hour	% Increase above baseline to be classified as in estrus[1]
0-49	80
50-99	80
100-149	75
150-199	75
200-249	70
250-299	65
300-349	60
350-399	55
400-499	50
500-599	45
600-699	40
700+	35

[1] Current 8 h period relative to the 10-d baseline average

Figure 3.1 Distribution of intervals (h) from the activity monitor alert to time of insemination (n=1399 inseminations from 757 cows on 4 farms)

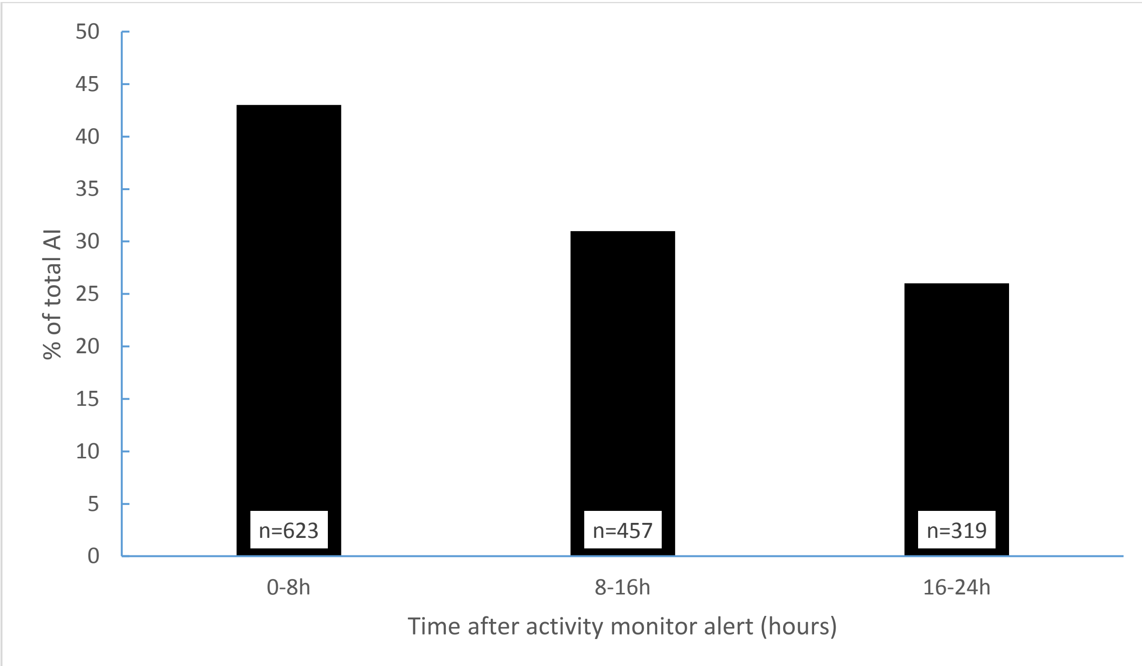
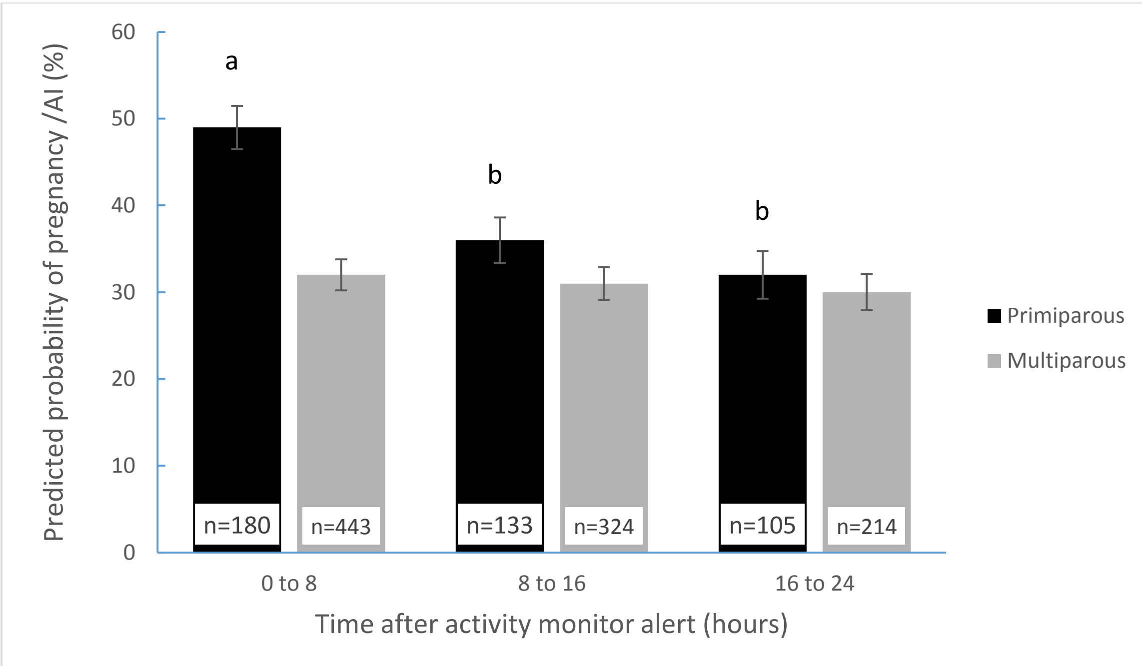


Figure 3.2 Predicted pregnancy risk (± SE) for intervals from activity monitor alert to AI for primiparous and multiparous cows from a model accounting for the random effects of farm and cow



a,b P = 0.006

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Chapter 4: General Discussion

Previous studies had found that automated activity monitors can accurately detect estrus. Timely first AI is a critical first step to achieving a profitable herd pregnancy rate. Many Canadian dairy producers express a desire to manage reproduction through detection of estrus if possible (Denis-Robichaud, unpublished data).

In our study, 83% of cows were detected in estrus and inseminated by 80 DIM. Factors that affected whether cows would be identified in estrus by the AAM system included being anovular, multiparous, positive for PVD and being lame with a BCS ≤ 2.5. Among inseminated cows, there were less than 4% with high P4 (not in estrus) on the day of insemination and there was no difference between inseminations based on AAM and those based on TAI. However, 17% of cows that were flagged by the activity monitor were not inseminated by the producer showing that there was some selection occurring. Though signs of estrus data were not collected on the cows that were not inseminated, for the cows that were inseminated, we found that signs of estrus at insemination had no significant impact on pregnancy risk. Producers may have been selecting not to inseminate a cow on the basis of an abnormal length of cycle or on the occurrence of a pen change or hoof trimming that may have caused an activity peak.

Our finding that there was no effect of observed signs of estrus on pregnancy risk was contrary to other studies. However, our signs of estrus were chance observations as the herd managers only recorded the signs that they happened to see and there was no systematic method of observations in place. However, consistently capturing all the signs of estrus in a large trial is quite challenging. A possibility of how to make the observations more consistent would be if cows were observed by a technician on the way to the milking parlour for the same amount of time at each farm or if video cameras were used.

The optimal timing of insemination has been researched for over 70 years relative to the onset of signs of estrus (Trimberger, 1943). There has been limited research looking at the optimal time of insemination relative to an alert from an activity monitor. We found that relative to the activity monitor alert, primiparous cows should be inseminated between 0 to 8 hours after being flagged. The pregnancy risk for multiparous cows did not change for time intervals from 0 to 8 h, 8 to 16 h or 16 to 24 h.

For a future study, a larger sample size would allow for the evaluation of what interval after AMM alert that has the highest conception down to a window smaller than 8 hours. If the interval for the highest conception was discovered down to a few hour interval the overall conception rate of the herd could be improved. However, to make it economically feasible, the increase in conception rate would have to be of significant magnitude to account for the multiple times that the producer would have to inseminate cows throughout the day and night.

Having a record of the reason for not inseminating a cow when the AAM system alerts that a cow is in estrus would be a benefit as it would allow the classification of reasons not to inseminate when using AAM system. Keeping a record of events such as pen changes and non-daily events such as footbaths or hoof trimming could also help explain some of the false positive alerts.

Having P4 measurements throughout the entire lactation on all the cows would allow the classification of not only false positives but also show how many estrus events were missed by the activity monitor throughout the entire lactation. To have a full P4 profile, samples should be taken a minimum of 2 times a week from every cow that could be coming into estrus and therefore using milk samples is likely the most feasible method. By having this information, all estrus events that occur would be captured and therefore the number that the activity monitor misses could be determined. Also, silent estruses could be identified even though the activity monitor had no opportunity to detect that estrus event.

Further research looking at the time of insemination related to activity alert from AAM system is required to identify any differences in the optimal time of insemination using various activity monitor systems. Though there was no differences found between the two systems used in this trial, other AAM systems should be evaluated. If an AAM system happens to alert estruses earlier or later and the optimal timing of insemination from this study is used there is potential that cows will not be inseminated at a time that would optimize conception; especially for primiparous cows.

Looking into the effect of parity on the optimal time to inseminate should be further evaluated as our finding that the optimal time to inseminate primiparous cows was earlier than multiparous contradicts the findings of Stevenson *et al.*, 2014. This difference between the studies may be caused by the AAM systems having different algorithms. It is possible that to be flagged by the AAM system cows had to achieve a higher threshold and therefore cows were flagged later into their estrus. Both studies found that primiparous cows have a more defined period that has increased risk of pregnancy and that multiparous cows are less sensitive to what time interval that they are inseminated in.

Finding that a cow being anovular, having PVD or being lame with a BCS ≤ 2.5 has a negative impact on the ability of activity monitors to detect a cow in estrus and be inseminated by 80 DIM supports the importance of managing these cow level issues. To improve the ability of AAM systems to detect cows by 80 DIM, cows should be monitored, treated and prevented from developing these issues.

Conducting a trial that involved a variety of activity monitors and a sample size capable of detecting an increase of conception within a shorter interval would be an interesting future study. Having a variety of activity systems could discover if all systems have the same optimal interval. Having a large sample size would determine if there is a more specific time interval that results in a higher conception rate or that the 8 hour range that was used in this trial was truly the optimal interval.

Our findings that there was no effect of observed signs of estrus on pregnancy rate is consistent with finds of previous studies (Madureira *et al.*, 2015). However, our study was not designed to look at this question. Conducting a large scale study where all signs of estrus were recorded consistently by possibly video camera footage or by consistent daily observation could provide insight to what signs of estrus when accompanied with an activity alert would increase accuracy of insemination.

In summary, we found that activity monitors could identify the majority of cows by 80 DIM. There is still a group of cows that will require intervention, such as cows that are anovular. Of the cows that were identified in estrus by AAM systems and inseminated, only 3% had high P4 and were considered not in estrus. When the cows that were flagged by the activity monitor but not inseminated were included, 9% of samples had high P4 at insemination. Breeding primiparous cows by 8 hours after being flagged in estrus by the AAM system provided the highest conception risk however, multiparous cows had no change in the risk of conception up to

24 h after the AAM alert. Determining what 8 hour interval after AAM alert has the highest conception is a practical time interval as producers are usually in the barn and could inseminate a cow up to three times a day. However, conducting another study with a larger sample size and including a larger variety of AAM systems would provide the opportunity to find if there is a more precise optimal timing of insemination or if the 8 hour window is optimal and to see if the systems determine the same optimal interval when compared to each other.

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APPENDIX

Standardized form producers used to record inseminations

Date	**Cow #**	**Breeder**	**Time**	**Timed AI**	**Signs of Estrus**	**Ease of Breeding**	**Notes**
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
			AM or PM	Yes or No	☐ Activity monitor alert ☐ Increased visual activity ☐ Mounting ☐ Mounted ☐ ↓ Milk Yield ☐ Mucus Discharge	☐ Normal ☐ Difficult ☐ Not Through	
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