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ASSESSING THE EFFICACY OF AUTOMATED DETECTION OF ESTRUS IN DAIRY CATTLE

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Animal Science in the
College of Agriculture, Food and Environment
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By

Lauren Maurice Mayo

Lexington, Kentucky

Co-Directors: Dr. Jeffrey M. Bewley, Associate Extension Professor of Animal Sciences
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Lexington, Kentucky

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ABSTRACT OF THESIS

ASSESSING THE EFFICACY OF AUTOMATED DETECTION OF ESTRUS IN DAIRY CATTLE

The detection of estrus continues to be a primary factor contributing to poor reproductive performance in modern dairy cattle. The objectives of this research were 1) to evaluate performance of automated detection of estrus using a reference standard of ovulation detection with temporal progesterone patterns 2) to evaluate the efficacy of parameters measured by automated detection of estrus systems 3) to evaluate the efficacy of alerts generated by several commercially available systems used for automated detection of estrus and 4) to determine the differences in these parameters among cows with or without poor health conditions at the time of estrus. Systems used for automated detection of estrus can perform better than the previous original reference standard, visual observation for standing behaviors. All systems used for automated detection of estrus tested were similar for estrus detection efficiency.

KEYWORDS: automated detection of estrus, precision dairy technology, behaviors of estrus

Lauren Maurice Mayo

July 30, 2015

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The moment when you discover what it is that you want to do for the rest of your life is the moment that many search for. I am still searching. However, I think that the moment you discover your passion in life is just as important. I have discovered my passion for dairy cattle and helping the dairy industry face its challenges. The discovery of my passion for the dairy industry is one that I have achieved as a result of my experiences in life thus far and the wonderful people in my life. I have been blessed with an opportunity that I only once dreamed of. Prior to attending the University of Kentucky for a master’s degree in the dairy systems management program, I did not think I was smart enough for research. I knew that I didn’t know as much as I wanted to about dairy cattle and thought it was the next step to expanding my knowledge. Within a month of being in Kentucky, I realized I had a passion for research. The discussions among our group, the never ending list of questions to be answered, and application of knowledge gained from research to dairy farms is what intrigues me the most.

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FREQUENTLY USED ABBREVIATIONS

AED = automated estrous detection

CL = corpus luteum

FN = false negative

FP = false positive

GnRH = gonadotropin releasing hormone

h = hour

kg = kilogram

l = liter

ml = milliliter

ng = nanogram

P4 = progesterone

PGF2α = prostaglandin F2α

TN = true negative

TP = true positive

THI = temperature humidity index

US = United States

CHAPTER 1

Review of Literature

Overview of Dairy Cattle Reproductive Performance and Fertility

In dairy cows, estrus is defined as the period of sexual receptivity during which a cow will accept being mounted by a bull (Senger, 2005). Sexual receptivity is defined as behavioral changes that occur for a period as few as 3 to 16 h with varying estrus expression intensity (Dransfield et al., 1998). Identifying time of estrus is necessary for timing of dairy cattle artificial insemination for optimal conception rates (Trimberger, 1948). Ovulation rate (**OR**), estrus detection rate (**EDR**), days open (**DO**), calving interval (**CI**), pregnancy rate (**PR**) and conception rate (**CR**) are used to measure reproductive management efficiency (Inchaisri et al., 2010). Poor reproductive management in detection of estrus, breeding, record keeping, and health before sexual receptivity lead to low pregnancy rates (Lucy, 2001).

Poor reproductive management efficiency and health management can lead to low estrus detection rates, an indicator of infertility (Aungier et al., 2012). Cows with low fertility, cows not pregnant 150 days postpartum, was prevalent in 83.5% ± 1.1% of all U.S. dairy operations (NAHMS, 2007). Dairy cattle infertility is a multifactorial dilemma among conditions with high economic and negative production impacts including mastitis and lameness (Spielman and Jones, 1939). Reproductive problems including metritis, dystocia, retained placenta, were prevalent in 38.8 ± 1.3% of U.S. dairy cows (NAHMS, 2007). Immunosuppressed animals can have poorly functioning reproductive systems, which affects estrous cyclicity and estrous expression (Senger, 2005) regardless of parity, body condition score, or milk yield. Many hypotheses exist for the declined ability of dairy cattle to conceive and maintain pregnancies (Coleman et al., 1985). Infertility leads to 23.3% ± 0.7% cows in the U.S. being culled for reproductive problems.

Infertility persists for various reasons, yet not all agree upon the primary or sole reason. Relationships with high milk yield and low fertility is the most common assumption due the decline in estradiol-17β (Sangsritavong et al., 2002; Lucy, 2001; Royal et al., 2000). The correlation of genetics related to milk production and fertility is nearly zero since fertility is highly variable (Raheja et al., 1989). Raheja et al. (1989) concluded that fertility, with a heritability of 0.03 to 0.06, is more dependent on management than genetics. Recent studies have reported variation in correlation of milk production and fertility from $r = 0.18$ to $r = 0.64$ (Windig et al., 2006; Veerkamp et al., 2000) indicating environment has a large impact on fertility. Sangsritavong et al., (2002) reported that higher yielding cows experienced higher metabolism of estradiol-17β, which leads to decreased expression of estrus. Decreased expression of estrus can lead to lower estrous detection efficiency with visual observation. Post-partum diseases can also lead to infertility (Wathes et al., 2007).

Measures of fertility can be biased or skewed depending on the management practice leading to conception or calculations used for the fertility measure (Royal et al., 2000). Cows observed for estrus without timed artificial insemination (**TAI**) tend to have lower conception rates in 21 day intervals used to calculate conception rate and pregnancy rate due to larger groups of cows on TAI than cows in spontaneous estrus (Pryce et. al, 2004). Conception rates for studies on commercial farms versus controlled research farms often differ due to varying levels of record keeping (Pryce et al., 1997).

Pryce et al. (1997) found a 64% CR in a controlled research farm versus a 66% CR in a commercial farm with the same grouping of cows and methods used for breeding.

Low dairy cow reproductive performance can result in more days open, which can average $3 to 5 per cow per day open (French and Nebel, 2003). Costs of days open can include the labor required for visual observation of estrus (Esselmont and Peeler, 1993; Galvao et al., 2013). Optimal EDR is 85% (De Rensis et al., 2003). Herds with 85% EDR in addition to TAI have the opportunity to increase the profit per cow per year $64.20 to $99.40 by improving detection of estrus. Improving estrus detection efficiency from ≤50% (Senger, 1994; Barr, 1975; Esselmont, 1976) can result in overall improved reproductive performance.

Dairy Cattle Reproductive Physiology & Endocrinology

Resumption of the estrous cycle is critical for dairy cattle reproductive performance and fertility. The focal event of the dairy cattle estrous cycle is estrus. Estrus is the presence of the ovulatory phase including sexual receptivity, a peak in estrogen, and LH surge before ovulation (Senger, 2005).Expression of estrus does not occur in all animals (Roelofs et al., 2006). Ovulation occurs approximately 31 ± 8 h after the onset of estrus.

Dairy cows are polyestrous animals, meaning estrous cycles are uniform and regular throughout the year (Senger, 2005). However, as spontaneous ovulators, several factors can affect resumption of the estrous cycle or length of follicular or luteal phases within the estrous cycle (Lucy, 1998; Ouweltjes et al, 1996). Anestrus, the time between two estrus events, occurs in 33% of cows (Peter et al., 2009; Wiltbank et al., 2006; Hall, 1959). Thus, continuous monitoring of estrous cycles for individual cows is necessary for predicting optimal insemination time relative to ovulation time (Roelofs et al., 2006).

Silent ovulation is one of most common reproductive dysfunctions in high yielding dairy cows and occurs more frequently in the first 60 DIM (Ranasinghe et al., 2009). Silent ovulations can easily affect dairy cow reproductive performance and decrease estrus detection rates (Roelofs et al., 2005).

Traditional Reproductive Management Strategies

Estrus detection efficiency (**EDE**) can only be determined by having recorded visual observations of estrus. Estrus detection efficiency is calculated as the total number of cows recorded in estrus divided by the number of estrus events that should have occurred over the time period multiplied by 100 (Heersche and Nebel, 1994). Estrus detection efficiencies greater than 60% are required to decrease calving intervals. Physiological factors can affect an animal’s ability to express estrus visually. However, the EDE can help determine a level of success for an estrus detection method. Often, human shortcomings are the root of lowered EDE. Estrus detection efficiency for visual observation is commonly less than 50% (Senger, 1994; Barr, 1975; Esselmont, 1974).Estrus detection method accuracy is often estimated with conception rate or records of interestrual intervals from progesterone level diagnosis or palpation (Heersche and Nebel, 1994).

The ultimate goal of continuous monitoring with automated systems is to detect animals in estrus to predict ovulation time. Predictors of ovulation time should have high sensitivity (89%) for detecting estrus behaviors within 18 h before ovulation (Trimberger, 1948). Intervals between detection of estrus, insemination, and ovulation are often longer when using visual observation for detection of estrus. Standing heat is an imperfect reference standard used for confirmation of estrus for breeding cattle. The first observed standing heat is often noted as the onset of estrus. Standing heat is not expressed by all animals, thus is not the best standard for detection of estrus (Roelofs, 2004).

Traditional methods used to detect estrous behavioral changes include visual observation for an uninterrupted period, tail painting, tail chalking, androgynous females, mounting pressure devices, or creating sexually active groups with Synchronization programs (Nebel et al., 2002). Most U.S. dairy producers, 93%, used visual observation, 40.3% use bulls and 34.7% use tail chalk or paint for estrus detection (NAHMS, 2007). The decreased efficiency, typically less than 40% (Senger, 1994; Barr, 1975; Esselmont, 1974) of traditional estrus detection methods, decreases the ability to identify cattle for breeding. Not only has the efficiency of detection of estrus decreased but the length of estrus has decreased from 15 h to 5 h (Dobson et al., 2008). The percentage of cows standing for mounts in the last 50 years also decreased from 80% to 50% due to the decline in fertility (Dobson et al., 2008). The use of pedometers and other automatic activity monitoring systems has increased estrus detection rates to 80% to 100% (Roelofs et al., 2010) but declined for visual observation as the sole method of detection of estrus.

Standing Heat

The most common methods used for detection of estrus as early as 1918 (Nebel, 1998) include visual observation for cows standing to be mounted. In a recent census, 93% of U.S. dairy producers visually observed for estrous behaviors (NAHMS, 2007). Standing heat times are used for timing of artificial insemination following the AM-PM guideline suggested by Trimberger and Cornell colleagues (1948). The 12-hour period was the peak of CR of 80% of the 6 to 24 hours before ovulation resulting in highest conception rates by Trimberger and Davis in 1943 and 1948.The guideline uses standing heat as an indicator of onset of estrus with insemination occurring 12 hours later (Trimberger, 1948). Thus, if an animal was seen standing in the morning, AI in the evening is suggested and if seen standing in the evening AI is suggested for the next morning.

Synchronization

Timed artificial insemination is often accomplished with the original Ovsynch, two injections of GnRH and an injection of $PGF_{2\alpha}$ (Pursley et al., 1995). Ovsynch is a synchronization protocol that is commonly used in the U.S. dairy industry (Caraviello, 2006). This may be due to having the ability to breed a group of cows at once instead of breeding off of natural heats. Variations of time to breeding after the final injection and additions to Ovsynch (Tucker et al., 2011; Giordano et al., 2012; Santos et al., 2004). Nebel et al. (1994) reported no differences in twice a day service with the AM-PM guideline compared to once daily AI, but this was contingent on optimum estrus detection. Synchronization with visual estrus detection is an opportunity to cluster animals (Nebel et al., 2000) but does not always result in desired conception rates greater than 65%.

Effects of early postpartum diseases, body condition change, lameness, subclinical mastitis, season, and parity on fertility and estrus

Immunosuppressed animals can have poor functioning reproductive systems, which affects estrous cyclicity (Senger, 2005) regardless of parity, body condition score, or milk yield. Controlling dairy cattle infertility begins with overall health and management efficiency (Aungier et al., 2012). Dairy cow reproductive performance can decline due to early postpartum diseases, environment, rapid changes in body condition, lameness, and other health conditions (Senger, 2005; Aungier et al., 2012). Optimum dairy cow reproductive performance begins with detection of estrus. Estrus detection efficiency is often less than 50% for dairy cows, possibly related to immunosuppression (Esselmont, 1974; Senger, 1994; Lucy, 2001). Immunosuppressed dairy cows are less likely to express estrous behaviors, especially standing for mounting by other cows (Lopez et al., 2004; Sangsritavong et al., 2002; Aungier et al., 2012).

Metabolic Diseases

Dairy cow negative energy balance (**NEB**) is the result of higher energy requirements of milk production and maintenance than energy provided in the diet and consumed (Bauman and Currie, 1980; Drackley, 1999). Metabolic diseases arise during the first 28 to 56 days postpartum from cows entering NEB (Collard et al., 2000). High yielding cows producing 13 kg/day of milk took more than 150 days to conceive (Wathes et al., 2007). Higher yielding cows have more fluctuations of metabolic hormones, including growth hormone (GH), growth hormone receptor, and IGF-1 that affect reproductive hormones (Wathes et al., 2007). Low levels of IGF-1and insulin can lead to decreased ovarian response to gonadotropins, including LH and FSH (Lucy, 2008) necessary for estrous cycle resumption and follicular development postpartum. The response of gonadotropins to low insulin and IGF-1 can affect the timing of estrus and ovulation (Lucy, 2008).

Thus, follicle development and growth and first ovulation are highly affected by NEB in dairy cows (Beam and Butler, 1999; Butler, 2003; Diskin, 2003). Cows in NEB have longer intervals to first ovulation (Butler, 2003). Cows with metabolic disorders are often high milk yield cows (Sangsritavong et al., 2002). High yielding cows tended to have 283 L/h more blood transferred though the liver decreasing the concentration of progesterone and subsequently higher metabolism of estradiol 17-β ($P < 0.0001$) (Sangsritavong et al., 2002). Higher metabolism of estradiol 17-β, estrogen, can lead to shortened periods of estrus, making it more difficult to detect estrus (Wiltbank et al., 2006). Thus, monitoring of metabolic diseases is critical for subsequent dairy cow reproductive performance.

Body Condition Changes

Changes in BCS can be a result of higher milk yields (Pryce et al., 2001). Days to first service decreased −5.2 ± 1.6 d for cows with a decrease in BCS greater than 1.0, 10 weeks postpartum ($P < 0.0001$) (Pryce et al., 2001). The genetic heritability of BCS and days to first service are low to moderate, 0.21 to 0.43, meaning BCS and the environment and management (Veerkamp et al., 2001) affect its effects on fertility more. The correlation between genetics of BCS loss with days to first service was 0.29 to 0.6, thus a lowered reproductive performance due to the genetics of BCs as well (Dechow, 2003).

Lameness

Lameness can affect the expression of estrus due to the pain of lameness reducing normal cow activity (Collick et al., 1989). Morris et al. (2011) reported 21% of lame cows failing to express estrus or ovulate due to low levels of estrogen. Collick et al. (1989) reported an 8-day increase in days to first service among 427 cases of lameness varying in cause. Lame cows are also more likely to have shorter periods of estrus earlier in the day (Morris et al., 2011) decreasing the chance for dairy producers to detect lame cows in estrus. Increased services per conception and a 52% less conception risk in 254 lame cows compared to 583 healthy cows was reported as significant ($P < 0.05$)

(Hernandez et al., 2001). Therefore, continuous monitoring of lame cows is necessary to improve their reproductive performance. However, monitoring of lame cows can lead to increased false positives due to restlessness because of cow discomfort and parameters other than activity should be evaluated (Roelofs, 2006).

Mastitis, Season and Parity

Subclinical mastitis is often missed by dairy producers (Schukken et al., 2008). Clinical mastitis is often a heightened response to subclinical mastitis (Viguier et al., 2009). Jersey cows with clinical mastitis were reported to have 93.6 days to first AI service compared to healthy cows with only 71.0 days to first AI service (Barker et al., 1998). Expression of estrus is similar among high and low SCC cows, but high SCC cows have a lower intensity and delayed expression of estrous ($P = 0.06$) (Morris et al., 2013).

Longer estrous intervals occur in heat stressed cattle causing decreased breeding efficiency (Scott and Williams, 1962). High temperatures in Arizona (Scott and Williams, 1962) and Florida (Cavestany et al., 1985) in June to September resulted in decreased CR and PR, and increased days open and services per conception. Cows during high temperature and humidity had estrus detection rates of 33% (Younas et al., 1993; De Rennis et al., 2003). Cows in natural estrus and hormonally induced estrus were 50% less likely to stand for mounting in the summer as the colder months (Pennington et al., 1985).

Primiparous cows take longer to first ovulation than multiparous cows (Lucy et al., 1992; Tananka, 2008) but more multiparous cows have negative energy balance delaying resumption of the estrous cycle.

Precision Dairy Farming & Automated Estrous Detection

Precision dairy farming (**PDF**) technologies can measure physiological, behavioral, and production indicators of individual animals that will help dairy farmers improve management strategies and overall efficiency (Bewley, 2010). Automated estrous detection systems are the predominant form of PDF technologies in the U.S. dairy industry. Among estrus detection methods used in the U.S., 5.7% of farmers use HeatWatch and 1.4% use pedometers (NAHMS, 2007). Synchronization programs combined with automated estrous detection have also been explored (Fricke et al., 2014; Neves et al., 2012). Fricke et al. (2014) reported using hormones for initial grouping and then using automated estrous detection with a collar-based system or using automated estrous detection then hormonal intervention for problem cows yielded similar days open and conception rates.

Automated detection of estrus is not a new concept as described by Boyd (1984) and Senger (1994) for an aid that was automatic, continuously monitoring individual animals, and highly accurate in identifying behavioral and physiological changes relative to ovulation. This aid would also last the time of a cow’s productive life, and possibly include measure several parameters. Most of these factors are viable in successful commercialized PDF technologies.

Lopez-Gautius (2005) and Van Eerdenberg (2008) used pedometers and reported increased walking activity at estrus similar to novel research beginning in the mid-1970 by Esselmont (1976) and Liu et al. (1993). Dohi et al. (1993) found that using pressure sensors could be used for continuous monitoring of estrous behaviors in agreement with Trimberger (1948) for recordings of standing mounts. Standing mounts were strongly (r =

0.86) indicative of estrus when pressure sensors are positioned correctly on the rump (Esselmont, 1980). However, increased activity and standing mounts are only the beginning of parameters measured by precision dairy technologies.

Secondary behaviors including feeding behaviors and lying time can change on the day of and day after estrus. Rumination time decreased in 94% of 265 estrus events and decreased as much as 247 minutes per day on the day of estrus (Reith et al., 2012). Lying time decreased by about 10% on the day of estrus and increases by 20% the day following estrus (McGowan et al., 2007).

Physiologically related parameters have been studied including various temperature measurement locations and milk progesterone. McArthur et al. (1992) found increased milk temperatures at estrus in research and commercial farms that were often skewed and only lasted for short periods as few as 9 h. Gil et al. (1997) reported a strong correlation in body temperature and increased milk temperature ($r = 0.90$) in 78.9% of 38 silent ovulations. Vaginal and ear skin (Redden et al., 1993), tympanic (Scott et al., 1983), temperatures increased at estrus and were similar to visual observation but all had false positive alerts.

Quantifying behavioral and physiological parameters with automated estrous detection may improve estrus detection rates as shown in previous research (Rorie et al., 2002; Michaelis et al., 2014) compared to visual observation. As early as 1948, Trimberger reported that methods for continuous monitoring of behavioral changes were necessary for improving the time to insemination and increasing estrus detection rate (Stevenson et al., 2014). The literature reviewed in automated estrous detection (**AED**) was often on one or a few systems on the same group of cows, reference standards, algorithms if known, or varying sample sizes (Rutten et al., 2013; Ginther, 2013; Rorie et al., 2002).

Activity Monitoring

Activity monitoring is another common use of automated estrous detection systems. Pedometers measuring number of steps is a common method used for activity monitoring (Stevenson, 2001). Activity monitors attached around the neck such as Alpro (DeLeval, Sweden), Heatime (SCR Engineers Ltd., Netanya, Israel), HeatPhone (Medria, Châteaubourg, France), MooMonitor (DairyMaster, Tralee, Ireland), and standing and lying time monitors attached to the leg similar to IceTag3D (IceRobotics Ltd, Edinburgh, Scotland), AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel), CowScout S Leg (Gea Farm Technologies GmbH, Bönen, Germany), and IceQube (IceRobotics Ltd, Edinburgh, Scotland) are the main categories of today's activity monitoring systems (Jonsson et al. 2011). The interval between current activity from previous activity which can be in seconds, minutes, hours, or days depending on the system's algorithm, collection frequency and storage strategy (Lopez-Gautius et al., 2005). The average ovulation occurs 29 to 33 hours after onset of increased activity and 17 to 19 h after the end of that increased activity (Stevenson et al., 2014; Roelofs et al., 2006).

Activity monitoring is the most common automated estrous detection system tested in research and used commercially (Firk et al., 2003; van Eerdenburg et al., 2008; Stevenson et al., 2014). Pedometers can improve reproductive performance, even in detecting up to 54% of silent heats (Galon, 2010). Galon (2010) reported an increase in herd undetected heat rate from 8.6% to 10% in Israel over a 5 year period. Thus the use of systems that can continuously monitor activity is necessary for improvement of reproductive performance (Lovendahl et al., 2010). Accelerometers are becoming more popular in place of pedometers in order to capture activity in multiple directions (Valenza et al., 2012).

Electronic Pressure Sensors

The use of non-electronic or electronic pressure sensors indicating an animal was mounted while standing is common (Gwazdauskas et al., 1990; Saumande, 2002; Johnson et al., 2012). Standing for mounting by another animal occurs sporadically but is the most indicative of an animal in estrus (Homer et al., 2013). HeatWatch (DDx Inc., Denver, CO) or HeatWatch II (CowChips LLC, Manalapan, NJ) are often used in beef and dairy cattle for detection of estrus in research settings to determine number of mounts, duration of mounts in seconds and duration of estrus based on first and last mounts (Perry et al., 2008; Walker et al., 1996). Systems originally used radiotelemetry to relay mounting data but new novel systems use ultra wideband technology (Homer et al.,2013).

Body Temperature

Body temperature is also used to monitor estrus in dairy cattle. Body temperature can decline 1.6°C up to 2 days before estrus and then up to a 1.0°C increase at the time of the LH peak (Firk et al. 2002; Fisher et al. 2008). The average increase in temperature is 0.48 degrees Celsius with a range of 0.40 to 3.22 degrees Celsius at the peak of LH. Possible effects on these temperatures to alter heat detection can include outside temperature, disease related hyperthermia, and local inflammation.

Redden et al. (1993) analyzed vaginal temperature, ear skin temperature, activity by pedometer compared to behaviors of estrus to determine detection accuracy. Factors that could affect the accuracy of their measurements included technique, frequency, and duration. Body temperature was monitored 32 to 51 d postpartum and 77 to 125 d postpartum. Radio transmitters were new during the time of this study. Modifications were made to adapt the technology to stay on the animal and correctly measure the parameters needed. The mean vaginal temperature at estrus increased by 0.65 ± 0.3°C. Milk samples were used to measure progesterone levels with radioimmunoassay. Estrus was defined with progesterone level of <1ng/ml and at or following ovulation a progesterone level >1ng/ml. Milk samples were taken on the day of suspected estrus, then 5 and 10 days after suspected estrus. The mean cow activity according to the pedometers was determined in order to see that activity at estrus was 2.3 times (on average) more than the mean activity.

Milk and Blood Progesterone Levels

The measurement of progesterone in milk is a reference standard for detecting cyclicity and estrus in lactating dairy cattle. At 80 h before ovulation, the average P4 concentration is < 5ng/ml and < 2ng/ml 71 h before ovulation with large ranges. Inline progesterone sensors are not common in the U.S. yet, due to regulation and economics. Researchers found that an area of concern for this PDF technology is milk fat concentrations. Larger fluctuations in progesterone levels were significant different ($P < 0.05$) among higher milk fat concentrations (Delwiche, 2001). Even in small differences in milk fat concentration, large differences in progesterone profiles existed. Correlations between levels of in-line progesterone and fertility relating to luteal activity thus ovulation after estrus and the phenotype of genetics relating to fertility is low, 0.01 to 0.07 (Tenghe et al., 2015).

Rumination Time

Research using rumination time as an indicator of ideal rumen health, stress, or disease were conducted using rumination and chewing halters as early as the 1980's (Penning, 1983). Rumination is critical for optimum rumen health because of increased saliva production (Welch, 1982). Penning (1983) reported importance of continuously record chewing and grazing behaviors for research purposes but also noted that the technologies were not sold commercially at the time (Penning, 1983). Halter based chewing and rumination monitors were validated by visual and video observation for chewing, eating, and rumination behavior (Luginbuhl et al., 1987). Halter based systems measured jaw movements, which could sometimes change depending on the animal's reaction to wearing the halter (Beauchemin et al., 1989).

Earlier versions of chewing and rumination halters were strictly for research purposes and required cables and frequent battery changes (Luginbuhl et al., 1987; Beauchemin et al., 1989). The accuracy of these halters was 1 to 5% greater than visual observations (Beauchemin et al., 1989). Cows in this study ruminated 396 minutes per day. Based on the data recorded, only 19.5% of the rumination time was visually observed but a greater amount of eating was observed, 46%. Computers were moderately correlated ($r = 0.67$) with visual observation and two chewing halters validated at the time (Beauchemin et al., 1989).

The jaw recorder validated in 1994 was a more robust and compact system that could record and keep more data than previous research by Matsui et al. (1994) with sheep, goats, and cattle. In using this device, Matsui found that cattle had a similar pause in regurgitation of 4 to 6 seconds compared to sheep of 5 seconds. This became important in using these devices across species and determining the sensitivity of 3 s min- 1 for the jaw recorder. Rutter et al. (1997) also validated another free-range halter and back based system for sheep that could be used for cattle to monitor rumination and eating activity. This system was 91% accurate (correspondence) using visual observation as the standard (Rutter et al., 1997).

The Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel), commercial since 2007, was validated (Schirmann et al., 2009; Burfiend, 2011) primarily for its efficacy in rumination monitoring as a microphone, microprocessor, and transponder based neck collar system, different than the previous devices used. A high interobserver correlation ($r = 0.99$, $P < 0.001$) was used as the standard in validating the system. The system recorded data in 2 h intervals with data offloads occurring when an animal walked past a reader or read manually with a handheld reader. High correlations remained in two validation trials between $r = 0.92$ and $r = 0.96$ for visual observation and the Hi-Tag. Variation (6.1%) between visual and the Hi-Tag was still less than previous research with 9.2% (Kononoff et al., 2002) and similar correlation to the jaw recorder ($r=0.91$ to 0.98) (Beauchemin et al., 1989).

Since this validation, the H-Tag monitoring system (SCR Engineers Ltd.) was developed to combine previous knowledge of obvious changes during visual observation like activity or mounting behavior with common knowledge that cows in estrus eat less (Maltz et al., 1997). Recording of rumination changes could assist in estrous detection (Reith and Hoy, 2012). Rumination time at estrus was significantly decreased on a daily average according to Reith and Hoy (2012). This reported the rumination time of days prior and after day of estrus finding an average 17% decrease in rumination time on day of estrus. The day before and after estrus were also significantly decreased ($p < 0.05$) from the average of 429 ± 107 minutes per day ruminating. This change in rumination is similar to findings of onset of estrus occurring in varying estrous lengths and behaviors (van Eerdenburgh, 1996). Differences were found in average rumination times across the 4 herds in this study but reported differences in feed management and ration composition may affect herd rumination times. Parity differences were noticed with primiparous cows with 29 minutes per day more in decrease of rumination time than multiparous cows (Reith and Hoy, 2012). The H-Tag validation for estrous detection was indicative of increased activity in primiparous and multiparous cows during estrus (Roelofs et al., 2005).

Comparisons of Detection Methods

Comparisons of reproductive management strategies leading to breeding with artificial insemination are frequent in the literature. Synchronization program variations in comparison to automated activity monitoring (**AAM**) resulted in similar time to pregnancy, for some farms (Neves et al., 2012) with a median of 99 d. Time to first service is logically delayed about 15 d using AAM because it requires expression of estrus (Dolecheck et al., unpublished data). Timed artificial insemination ultimately masks the issues associated with cow factors such as environment or health history (Firk et al., 2002). In comparison to visual observation, use of AAM or other AED systems results in shorter estrus to insemination and insemination to ovulation intervals and higher conception rates (Stevenson et al., 2014; Nebel et al., 2000).

CONCLUSIONS

Among an array of challenges dairy producers face, improving estrus detection efficiency is critical for improving dairy cattle reproductive performance. Commercially available precision dairy technologies are capable of automated detection of estrus. Management, environmental, and health factors may affect the efficacy of systems used for automated detection of estrus. Settings used to generate estrus alerts should be dairy herd management group specific. Activity monitors are the most common form of automated detection of estrus. However, other parameters measured by precision dairy technologies may improve the efficacy of automated alerts of estrus

CHAPTER TWO

Automated detection of estrus using multiple commercial precision dairy farming technologies in synchronized dairy cows

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INTRODUCTION

Dairy farmers strive to achieve economic and production goals using fewer, higher producing cattle, resources (feed, facilities, and investment capital), and cash reserves than in the past (Lucy 2001). Dairy cow infertility is among conditions with high economic and production impacts including mastitis and lameness (Spielman and Jones 1939). Maintaining an acceptable level of fertility begins with overall health and management efficiency (Aungier et al., 2012). Immunosuppressed animals can have poor estrous cyclicity (Senger, 2005) regardless of parity, body condition score, or milk yield. One factor contributing to the overall reproductive management efficiency of a dairy operation is the ability to inseminate dairy cattle in a timely and cost effective manner. Estrus detection rate is a common reproductive performance measure indicating efficiency of strategies used for detection of estrus (Inchaisri et al., 2010).

Resumption of the estrous cycle is critical for dairy cattle fertility. The focal event of the dairy cattle estrous cycle is estrus. In dairy cows, estrus is defined as the period of sexual receptivity during which a cow will accept being mounted by a bull (Senger, 2005). Sexual receptivity is defined as behavioral changes that occur for a period as few as 3 h to 16 h with varying estrus expression intensity (Dransfield et al., 1998). Common methods used to detect these behavioral changes include visual observation for an period without other distractions, tail painting or chalking, androgynous females, rump based pressure or scratch off systems, or creating sexually active groups with Synchronization programs (Nebel and Jones, 2002). The ability of dairy farm personnel to detect estrus with visual observation has declined over the past 40 years (Senger, 1994; Barr, 1975; Esselmont, 1974).

Precision dairy farming (**PDF**) technologies measure physiological or behavioral or production indicators or all indicators of individual animals to help dairy farmers improve management strategies and overall efficiency (Bewley, 2010). Precision dairy farming technologies are commonly used for detection of estrus (Nebel et al. 2000) because of their ability to monitor and measure behavioral and physiological changes that typically occur during estrus.

Novel research beginning in the mid-1970's by Esselmont (1980) and Liu et al. (1993) used pedometers to monitor activity at estrus finding an increase in activity. Recent research continues to find similar results in increases of activity regardless of neck or leg location of the device (Lopez-Gautius, 2005; Van Eerdenberg, 2008). Dohi et al. (1993) found that using pressure sensors could be used for continuous monitoring of estrous behaviors in agreement with Trimberger's view for the need for continuous recordings of standing mounts (1948). Standing mounts were strongly correlated with correct placement of pressure sensors on the rump (r = 0.86) indicative of high detection of estrus (Esselmont, 1980). However, increased activity and standing mounts are only the beginning of parameters measured by precision dairy technologies.

Secondary behaviors, including feeding behaviors and lying time, change on the day of and day after estrus. Rumination time decreased in 94% of 265 estrus events and decreased as much as 247 minutes per day on the day of estrus (Reith et al., 2012). Lying time decreased by about 10% on the day of estrus and increases by 20% the day following estrus (McGowan et al., 2007).

Physiologically related parameters have been studied including temperature in different locations and concentration of progesterone in milk. McArthur et al. (1992)

found that milk temperature increased at estrus. Temperatures were often highly variable and lasted for short periods with a mean of 9 h. Gil et al. (1997) reported a strong correlation in body temperature and increased milk temperature (r = 0.90) in 78.9% of 38 silent ovulations based on visual observation of standing to be mounted. Vaginal and ear skin (Redden et al., 1993), tympanic (Scott et al., 1983), temperatures increased at estrus. The effectiveness of these parameters to generate an alert for estrus were similar to visual observation, but all had false positives.

Quantifying behavioral and physiological parameters with automated estrous detection improves estrus detection rates (Rorie et al., 2002; Michaelis et al., 2014) compared to visual observation. As early as 1948, Trimberger reported that methods for continuous monitoring of behavioral changes were necessary for improving the time to insemination and increasing estrus detection rate. Several studies have evaluated the ability to improve reproductive performance and fertility using automated detection of estrus (Stevenson et al., 2014). The literature reviewed in automated estrous detection (**AED**) was often on one or a few systems on the same group of cows, reference standards, algorithms if known, or varying sample sizes (Rutten et al., 2013; Ginther, 2013; Rorie et al., 2002).

Therefore, the first objective of this study was to evaluate the efficacy of 6 commercially available AED systems using alerts generated by each system on the same cows. The second objective was to determine the value of parameters in addition to standing for mount behavior and increased activity to detect estrus in 9 automated estrous detection devices.

MATERIALS AND METHODS

This experiment was part of a larger study designed to quantify physiological and behavioral changes, using multiple precision dairy farming technologies, associated with mastitis, lameness, estrus, and metabolic diseases. All studies were performed with approval of the University of Kentucky Institutional Animal Care and Use Committee (IACUC protocol number: 2013-1199).

Animals, Feeding, and Housing

One hundred and nine lactating Holstein cows at the University of Kentucky Coldstream Dairy (Lexington, KY, USA) were enrolled in this study between January 2014 and May 2015. Cows were enrolled in the protocol in groups of 6 to 10 cows between 45 to 85 DIM. Lactating cows were housed in two freestall barns, one barn with 54 dual chamber waterbeds (Advanced Comfort technology, Inc., Reedsburg, WI) and the other equipped with 54 rubber-filled mattresses, both surfaces covered with sawdust.

Before and throughout the study, cows were balanced between barns by DIM and parity. Calving dates, breeding dates, and DIM were obtained from PCDART management software (Dairy Records Management Systems, Raleigh, NC). Parity ranged from 1 to 7. Mean cow parity was 1.99 ± 1.30. The average milk yield of enrolled cows during the protocol was 37.7 ± 9.8 kg. Mean DIM at enrollment was 66.5 ± 11.4 d. Mean DIM at estrus was 85.5 ± 11.4 d.

A weather station (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger - U23-002, Onset, Bourne, MA) was located inside each freestall barn that measured relative humidity and temperature every 15 minutes. Temperature humidity index was computed using the following formula (NOAA and Administration, 1976): THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8]. The estrual max THI was calculated by averaging the max THI for each barn on days 2 to 5 of the protocol for each study group of cows. The estrual max THI was used to assess the effect of max THI on the efficacy of detection of estrus and number of cows that stood for mounting.

Cows had ad libitum access to water in each barn and shared a feedbunk between barns. Lactating cows were fed the same ration at 0600 and 1330 daily. The lactating cow ration was balanced for level of milk production and cow size. The diet consisted of corn silage, alfalfa hay, mineral and vitamin supplement, concentrate mix, whole cottonseed, and alfalfa haylage. Cows were milked 2X at 0430 and 1530.

Synchronization Protocol

A modified G7G-Ovsynch (Figure 2.1) was used to synchronize cows into sexually active groups in order to visually observe estrous behaviors in groups of 6 to 10 cows at a time. Cows were pre-synchronized on protocol day -16 using the G7G protocol, starting with an injection of prostaglandin (**$PGF_{2\alpha}$;** 25 mg, Lutalyse, Pfizer Animal Health, New York, NY). Two days later, protocol day -14, cows received an injection of GnRH (100ug, Cystorelin, Merial Limited, Duluth, GA). Seven days after the GnRH injection, protocol day -7, the Ovsynch protocol, excluding the final shot of GnRH to allow for observation of estrous expression (Pursley et al., 1995), was initiated giving cows an injection of GnRH. Cows received a $PGF_{2\alpha}$ injection 7 days later, designated protocol day 0. An additional $PGF_{2\alpha}$ shot was administered, 6 hours later, on the same day of the first $PGF_{2\alpha}$ injection of Ovsynch.

Ultrasonography and Sampling

Transrectal ultrasonography was performed on days -16, 0, 5, and 11 in the protocol using an Ibex Pro Portable Ultrasound (E.I. Medical Imaging, Colorado, USA). Transrectal ultrasonography was performed by two of the authors, a research technician with 15 years of experience and graduate research assistant with 5 months of ultrasound experience prior to the start of the study. Ovarian cyclicity resumption at enrollment was verified by the presence of a corpus luteum (CL) on protocol day -16. On the final day of $PGF_{2\alpha}$ injection day (designated experiment day 0), presence of a CL, and preovulatory follicle verified cyclicity and response to synchronization. Regression of this CL and ovulation of the preovulatory follicle were recorded on day 5. Presence of a new CL on day 11 concluded verification of ovulation and served as the reference standard for ovulation in comparison to detection of estrus.

Blood samples were collected on days -2, -1, 0, 1, 2, 5, 7, 9, and 11 to quantify progesterone for verification of luteal regression and ovulation. Potential periods of estrus before ovulation were defined by the temporal progesterone pattern (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). A reference standard for ovulation, using temporal progesterone patterns, was the primary standard used in comparing the efficacy of automated estrous detection systems and parameters measured on the day of estrus. Cows that met the requirements for progesterone concentrations on the designated protocol days were classified as positive for having ovulated on days 9 or 11. Cows that failed to ovulate according to progesterone concentrations on days 9 or 11 were classified as negative for ovulation. Cows that did not have progesterone concentrations >1.0 ng/ml on days -2 or -1 but did on days 9 or 11 were considered positive for ovulation. All cows were included in the final analyses regardless of CL presence on protocol day -16 due to cows (Table 2.2 starting the protocol with a wide range of DIM. Transrectal ultrasonography results were only used in the final analyses for final verification of ovulation on protocol day 9 or 11 when cows were expected to have developed a new CL after ovulation.

Automated Estrous Detection Systems

Each cow was equipped with AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel), CowScout S Leg (Gea Farm Technologies GmbH, Bönen, Germany), DVM Bolus (DVM Systems, LLC, Greeley, CO), HR Tag (SCR Engineers Ltd, Netanya, Israel), CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), IceQube (IceRobotics Ltd, Edinburgh, Scotland), and Track a Cow (ENGS, Hampshire, UK) devices (Table 2.1) before study enrollment to allow for an adjustment period of at least two weeks. Heifers were equipped with all devices at least 10 to 14 days before their predicted calving date. Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) were placed in intravaginal devices, similar to a CIDR but lacking progesterone supplement, inserted into cows 7 days before the final injection of $PGF_{2\alpha}$.

Devices were placed according to recommendations of each company (Table 2.1). Leg devices were placed on the same leg for each technology for every cow. DVM boluses, active boluses, were inserted into the reticulorumen orally, using a bolus gun. Ear tags were positioned using an ear tagger, provided by each technology company to fit the respective device.

The Afimilk Milking Point Controller (Afimilk, Kibbutz Afikim, Israel) was used to collect individual milk yield and milking time for each milking. Body weights were recorded by AfiWeigh (Afimilk, Kibbutz Afikim, Israel), placed in a common exit alley. Cows were sorted into their respective groups using AfiSort (Afimilk, Kibbutz Afikim, Israel) after each milking.

All computer clocks were set to synchronize with NIST Internet Time Service (NIST, Gaithersburg, MD, USA) automatically, and time was checked on all computers manually on a weekly basis. Raw data, including measurements and recordings of behavioral and physiological parameters, and estrus alerts generated by each AED software program were downloaded daily. Default settings for report and alert generation within each system were used during the study. Proprietary algorithms and individual animal thresholds for each system were used to generate estrus alerts.

Visual Observation for Estrus

Cows were observed for behaviors of estrus over a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ at 4 times each day (0330, 1000, 1430, and 2200) for 30 minutes each observation period or until all cows stood to be mounted. In replicate 12 (Table 2.2), inclement weather presented observers from watching cows for estrus for 3 periods. Cows in replicate 12 were not observed at all. Originally, these cows were removed from the analyses but because all cows displayed other behaviors including sniffing and chin resting during the 1000 observation period the day before the snowstorm they were included in the final analyses.

These 3 cows were removed from final analysis of cows standing to be mounted. Barn lights were turned on for the 0330 and 2200 observation periods and turned off at the end of each observation period. Cows were adjusted to this routine before the study started to avoid differences in routine behavior. Cows were released to an exercise lot divided by barn for 1 hour each day during the 1000 observation period.

Cows were identified with neck strap digits and numbers spray-painted on each side of the body. The van Eerdenburg et al. (1996) scoring scale for observed estrous signs, including modifications used by Roelofs (2005) and additional modifications was used to quantify intensity of estrus. Behaviors of estrus were assigned points according the original system including: 100 points for standing heat, 45 points for mounting head side of other cows, 35 points for attempting or mounting other cows, 15 points for chin resting on the rump of other cows, 10 points for sniffing the vagina of another cow or being mounted but not standing, 5 points for restlessness (increased activity or pacing), and 3 points for clear mucous vaginal discharge (van Eerdenburg et al., 1996). When a cow reached a score of 100 points the animal is considered in estrus. Additional modifications included considering in estrus once a cow received greater than or equal to 100 points, instead of two consecutive periods required for definition of estrus. One observer per side watched for behaviors during each observation period. Each observer recorded behaviors by hand and recorded all standing heat times using a satellite powered watch (WV58A-1AV Atomic Digital Watch, Casio, Shibuya, Tokyo, Japan) synchronized with the AED system computers. Estrus periods were designated as periods when the score exceeded 100 points.

Data Handling of AED system alerts

Each AED system software, except Thermochron iButtons and DVM boluses, generated alerts for both 1) cows that should be inseminated and 2) cows that are suspect of estrus. Cows for insemination have met the threshold of a specific parameter or parameters as specified in the AED system software. Threshold s or alert requirements are typically regarded as confidential and proprietary by the AED system manufacturers. When indicated, suspect cows are ones which achieved a less stringent threshold, but not meeting the threshold s required for breeding with an acceptable probability of fertility. These slight changes in parameters used for alerts of estrus could have been because of group changes, hoof trimming, and treatment of animals. Suspected estrus alerts were not used for analyses due to different algorithms used within each system. Manufacturers of AED systems specified which report and alerts to use before final analysis.

Potential estrus periods (reference standard) were defined using the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Cows that ovulated according to the temporal pattern of progesterone were considered to have been in estrus regardless of visual observation. Two analyses were completed for comparison of alerts for estrus to the reference standard for ovulation and standing mounts as a standard of estrus. Cows in analysis 1 with less than 80% (19 h of 24 h) of raw data for the day before estrus and day of estrus for the parameters identified by each company as necessary for estrus alerts were removed. Analysis 2 only included a subset of cows (n = 35) that had all AED devices working at the same time without any data cleaning. The following analyses were performed for analyses 1 and 2.

Estrus alerts were categorized by the comparison of the alert provided by each AED system to verification of estrus. Estrus was verified by temporal progesterone patterns indicative of ovulation. Comparisons of reference standards of temporal progesterone patterns for ovulation and standing behavior for estrus, included calculating AED performance with each standard for analyses 1 and 2. True positives (**TP**) were estrous alerts generated for cows that were confirmed in estrus. False positives (**FP**) were estrous alerts generated for cows confirmed not in estrus. True negatives (**TN**) were estrous alerts not generated for cows confirmed not in estrus. False negatives (**FN**) were non-alerted confirmed estrus events.

Statistical Analysis

Automated estrous detection system alerts

The FREQUENCY procedure of SAS (SAS Institute, Inc., Cary, NC) was used to determine the frequency of TP, FP, TN, and FP for each AED system alerts. Sensitivity, the proportion of cows that ovulated or were in estrus who were correctly given an AED system alert for estrus, was calculated by TP/ (TP + FN) x 100. Specificity, the proportion of cows that did not ovulate and were correctly not alerted by the AED system, was calculated by TN / (TN + FP) x 100. The accuracy, the proportion of cows who were correctly identified in estrus or not in estrus, was calculated by (TP + TN) / (TP + TN + FP + FN) x 100. The positive predicted value, the proportion of cows with an alert and are in estrus or ovulated was calculated by TP / (TP + FP) x 100. The negative predictive value, the proportion of cows who were not alerted of the cows not alerted, was calculated by TN / (TN + FP) x 100.

Parameter changes for estrus vs. non-estrus

The GLM procedure of SAS® 9.3 was used to analyze the independent effects of estrus status on 26 parameters recorded by 9 AED devices (Table 2.1). The following model was used:

$$Y_i = \mu + S_i + \mathcal{E}_{ij}$$

Y_{ij} = parameter measured (Table 2.1)

S_i = effect of the *i*th estrus state defined by progesterone patterns

$\mathcal{E}_{ij}$ = residual error

Parameter percent changes at estrus

Cows confirmed by progesterone patterns and ultrasound that were not in estrus and did not ovulate were removed from the final analysis. The EXPAND procedure of SAS® 9.3 was used to create a baseline using the backward moving average of the 7 days before the day of estrus for 26 parameters (Table 2.1) measured by all AED devices. The percent change in each parameter on the day of estrus and each protocol day compared to the 7d baseline was calculated as follows:

(protocol day measurement – baseline measurement) / baseline measurement × 100

(estrus day measurement – baseline measurement) / baseline measurement × 100

RESULTS AND DISCUSSION

Automated estrous detection system alerts

***Analysis 1*:** All cows were included in the original analysis classified by progesterone pattern and standing mounts. Ninety-four cows (86.2%) of the 109 cows followed the temporal progesterone pattern. The remaining 15 cows did not follow the same pattern and were classified as negatives. Only 51 cows of the 109 cows stood to be mounted during visual observation of four times a day, 30 min each, for four days. The first analysis included all cows that were enrolled in the study excluding groups or individual cows in study groups defined as having a broken device or system computer, changing the total number of cows for each technology. The total number of cows with working AED devices or systems for AfiAct Pedometer Plus, CowScout, IceQube, HR Tag, SensoOr, and Track a)) Cow were 109, 107, 91, 24, 65, and 61, respectively (Table 2.3).

The sensitivities for AfiAct Pedometer Plus, CowScout, IceQube, HR Tag, SensoOr, and Track a)) Cow were 81%, 77%, 57%, 96%, 90%, and 70%, respectively. Comparatively using standing estrus and the estrous behavioral scoring system as methods of detection resulted in 54% and 66% sensitivity. Higher sensitivities were a result of fewer false negatives, systems not alerting cows confirmed to have ovulated using the reference standard of progesterone concentrations. The specificity for devices AfiAct Pedometer Plus, IceQube, and Track a)) Cow were 87%, 83%, and 91%, which were lower than the optimal 100% for the other detection methods. Higher specificities would indicate that the AED system does not create alerts for anestrus cattle. Higher specificities are a result of high true negatives and low false positives. The overall accuracy calculated for all devices took into account these effects of high false negatives and low false positives. The accuracy for AfiAct Pedometer Plus, CowScout, IceQube, HR Tag, SensoOr, and Track a)) Cow was 82%, 80%, 60%, 96%, 91%, and 74%, respectively.

Following analyses, each cow and alert was manually examined for explanation of variation among devices sensitivity, specificity, and accuracy among all cows (N = 109). Previous research with multiple AED devices has found common sensitivities and specificities as high as 89% and 100%, respectively (Firk, 2002).

IceQube was an internet-based system that was capable of storing the data on each cow's device for four days. The authors were informed after analyses that a malfunction was found in most devices. IceQube remained in the final analyses because the specific malfunctioning devices could not be identified. McGowan et al. (2007) reported IceTag, an earlier product of IceRobotics, with sensitivities of 92.9%, 83.6%, and 76.4% using different alert algorithms and cow sample sizes. IceTag3D was also tested for estrus detection efficiency resulting in 88.9% with either combination of lying and number of steps or number of steps alone (Jonsson et al., 2011). The algorithm for the IceTag3D using lying time only resulted in a 50% sensitivity. The results of the current study, regardless of sample size, exemplify the importance of early identification of malfunctioning devices.

The HR Tag has an unexpectedly high sensitivity. The authors note this high sensitivity maybe due to a small sample size of cows without broken tags or on the protocol during a down system. When the sample size was 109 cows in analysis 1, the sensitivity decreased from 95% to 77% analysis 2 with the subset of 35 cows. The HR

Tag was subject to the most system failure due to human error and lightning completely damaging the computer twice on June 8, 2014 and October 26, 2014. The time required to get this system working again took longer than expected. We suspect the raccoons may have damaged the antennas required to read in the data every two hours as designed.

When standing estrus was used as a reference standard instead of progesterone patterns for this group of cows, the sensitivity was much higher with four of the devices greater than 90%. The standards used for determination of estrus detection efficacy in previous research vary (Firk et al., 2002). However, the specificity in analysis 2 for all devices decreased 50% compared to the specificity of all devices using progesterone as the reference standard.

Analysis 2: Similar results to the first analysis among all device alerts in the second analysis exemplify that all AED devices are capable of detecting estrus in dairy cows. More importantly, the reference standard used for verifying estrus affects the efficiency results. All AED devices increased in sensitivity, using standing mounts as a reference standard, when comparing the same cows and period. The small differences in sensitivity results among devices may be due to differences in algorithms, location of device, and what parameters are included in the algorithm for an alert of estrus. All of the information regarding algorithms used to create alerts for estrus was proprietary.

The results indicate that increased activity may be included in all AED system algorithms tested. However, combinations of parameters used for improved detection of estrus is possible. Jonsson et al. (2011) reported algorithms with decreased lying time and increased number of steps were similar to AED systems with number of steps only but had fewer false alerts. Brehme et al. (2008) found similar results with lying and activity parameters combined to detect estrus. Illnesses such as lameness can lead to increased false alerts. Therefore, combining a behavior measure of lying time may remove cows that are lame and not in estrus (Brehme et al., 2008; de Mol et al., 1997).

Historic information, such as previous alerts or behavior changes, may also improve the sensitivity and specificity of alerts generated by AED. Firk et al. (2003) improved estrus detection rates by including previous measurements of activity. Since algorithms were proprietary, the authors were not aware of inclusion of previous measurements for estrus alert algorithms among all AED systems.

Parameter percent changes at estrus

The parameters measured by AED devices quantify activity, feeding, and lying behaviors and temperature (Table 2.1). Previous research reported a significant ($p < 0.05$) increase in walking activity on the day of estrus with pedometers or accelerometers (Liu et al, 1993; Roelofs et al, 2005; Michaelis et al, 2014). The current study shows similar increases in activity for all AED devices (Figure 2.2). The percent change of daily steps for AfiAct Pedometer Plus, CowScout, IceQube, and Track a)) Cow ranged between 87.8% and 229% increase from the individual cow threshold on the day of estrus. Lopez et al. (2005) reported an increase of 75% to 500% increase in activity on the day of estrus in 5883 services on two commercial dairy farms. Lopez et al. (2005) calculated the increase using day of estrus number of steps divided by the threshold determined by the AfiFarm system, different from percent change calculated in the current study. However, regardless of the method used in our study reported large increases in steps per day. A significantly ($P < 0.05$) large increase in any behavioral parameter used for alerts of estrus may decrease the number of false positives (Table 2.3) and algorithm noise associated with daily behavior monitoring. Activity was also measured in the form of neck activity using the HR Tag and head movement using the CowManager SensoOr. The percent changes in activity were not as large but still significantly ($P < 0.05$) different on the day of estrus. The HR Tag and CowManager SensoOr measured an increase of 53.5% and 31.7% respectively. Neck collar activity was reported with a strong correlation with number of steps from the IceQube ($r = 0.75$) indicating a possibility of similar capabilities in detecting estrus (Elischer et al., 2013). The CowManager SensoOr also recorded the difference in high activity, which increased 228.7% on the day of estrus. Published literature with the CowManager SensoOr is limited at this time to conclude anything regarding the difference in percent change of a leg-based, neck based, or ear based AED device. A motion index, created with proprietary information by IceRobotics also increased 158.3% on the day of estrus. Previous literature with versions of the IceTag (Jonsson et al. 2011; McGowan et al., 2007) does not mention the incorporation of a motion index for comparison to the current study.

Dairy cow core body temperature is often in agreement with other temperatures including the reticulorumen (Rose-Dye et al., 2011; Bewley et al., 2008), ear skin (Redden et al., 1993), and vagina ($r = 0.92$, $P < 0.001$) (Suthar et al., 2013; Burdick et al, 2012; Redden et al, 1993). Detecting estrus and predicting ovulation with estrual rises in temperature is not a novel concept (Wrenn et al., 1958). Wrenn et al. (1958) and Redden et al. (1993) reported a 1.0˚C to 1.6˚C decrease in vaginal temperatures the day before estrus and a similar increase the day of estrus and the day after ovulation. Temperature rhythmicity was reported significantly different on the day of estrus ($P < 0.001$) but mainly due to increased temperatures of 1.3 °C in the summer months (Piccione et al., 2003). In the current study, temperature for the reticulorumen, vagina, and ear skin were not significantly different ($P > 0.05$) than days before or after estrus. An unexplained decrease in ear skin temperature 4 days before estrus may be due to replacement of the CowManager SensoOr. The difference in mean temperatures between CowManager SensoOr, 22°C and the other temperatures recorded may be due to the effect of ambient temperature or consistent placement of the ear tag. Cows often lost these ear tags due to the plastic type easily breaking on metal bars and cow brushes thus were replaced within the week before observation of estrus.

Rumination time measured by the SCR HR Tag is commonly used in research for disease (Soriani et al., 2012), dry matter intake (Clement et al., 2014), and recently estrus (Kamphuis et al., 2012; Reith and Hoy, 2012; Elischer et al., 2013). Reith and Hoy (2012) reported 5.92 h spent ruminating averaging a 17% decrease on the day of estrus. The current study reports only a 4.22% decrease in daily rumination time. This may be due to cow variation as seen in Reith and Hoy (2012) with the range of change in rumination time −71% to +16%. Published studies for CowManager SensoOr detection of estrus do not yet exist. However, recent validations (Borchers et al., unpublished data) show strong correlations ($r = 0.93$) of rumination time with visual observation (Bikker et al., 2014) and moderate correlations of the same possibly due to the difficulty involved with visually quantifying a regurgitation and swallowing (Borchers et al., unpublished data). Literature on eating time during estrus is limited. Eating time in this study increased 52.7% on the day of estrus (Figure 2.4).

More frequent visits to the feedbunk spread throughout the day may explain this increase in eating time (Figure 2.4). Daily time at the feedbunk decreased on the day of estrus by 17.1% yet the cows made 62.9% more visits to the feedbunk (Figure 2.4). In visual observation, more cows stood near the feedbunk to access sprinklers on warmer days. The reading radius for the feedbunk line recorded with the leg-based tag Track a)) Cow was limited to the space directly in front of the raised feedbunk. However, time spent around the feedbunk may not equate to eating time.

Daily lying times characteristically decreased to 24.6% on the day of estrus for IceQube (Figure 2.5). In contrast, lying time increased 15.5% and 33.1% for AfiAct Pedometer Plus and Track a)) Cow, respectively. These differences may be due to different determinations of a day. The AfiAct Pedometer Plus only reported data twice daily when the cows entered the parlor for milking, giving a sum of 11 to 15 hours between parlor visits. The remaining devices reported data hourly. A day was defined as the time periods of 1200 to 2400 instead of 0500 to 1700 for the AfiAct Pedometer Plus. Further analysis is necessary for changes in all parameters by hour surrounding observed estrus. Lying time alone in algorithms for detection of estrus resulted in 50% sensitivity (McGowan et al, 2007). However, when combined with number of steps sensitivity was 88.9% with 20 cows (Jonsson et al, 2011). Time not active recorded by CowManager SensoOr decreased 33.5% on the day of estrus, similar to IceQube. However, time not active also includes time standing still or null head movement. As hypothesized, lying bouts increased 45.6% and 35.9% on the day of estrus for AfiAct Pedometer Plus and Track a)) Cow respectively then decreased the day after estrus (Figure 2.6). However, lying bouts decreased 11.2% and lying bout duration was shorter by 24.6% for IceQube

(Figure 2.6). A decrease in lying bouts may be explained by proprietary algorithms to determine what behavior patter counts as a lying bout. Overall, lying behaviors may be helpful as historic information for future estrus alert generation since lying behavior decreases the day after estrus (Rorie et al., 2002).

Parameter changes for estrus vs. non-estrus

Evaluation of percent changes in all parameters between cows in estrus and non-estrus exemplify the importance of basing alerts on individual cow threshold s instead of a herd or management group percent change (Table 2.7). Cows not in estrus during the study were used instead of comparing the individual cow's previous 7 days since environmental conditions were often different a week before visual observation. The number of steps significantly increased ($P < 0.05$) for CowScout, IceQube and Track a)) Cow for cows in estrus versus a decrease in daily number of steps for cows not in estrus. Motion index recorded by IceQube also increased significantly ($P = 0.01$) for cows in estrus when compared to those not in estrus (Table 2.7). Neck activity recorded by the HR Tag and high head movement activity recorded by the CowManager SensoOr were also significantly increased for cows in estrus ($P < 0.05$). Only rumination time for CowManager SensoOr was significantly decreased ($P < 0.05$) for cows in estrus. This may be due to variation in daily rumination time regardless of estrus. Eating time measured by CowManager SensoOr was significantly greater ($P = 0.02$) for cows in estrus than cows not in estrus.

CONCLUSIONS

All AED devices except for IceQube were better than visual observation in detecting estrus. All measures of activity significantly increased on the day of estrus in agreeance with previous literature. Independently, lying time, lying bout duration, rumination time, and eating time were all significantly different on the day of estrus at varying levels of change from the cow's baseline. These parameters may have potential for incorporation into new AED system algorithms. Future multivariate analyses are needed to evaluate the effects of all parameters in various combinations.

Reliability of AED systems and devices are critical in thorough evaluation of efficacy. Automated estrous detection devices are highly sensitive to environment and various cow health effects resulting in higher false negatives. Silent ovulation is still a challenge for automated estrous detection systems monitoring intense behavior changes. Sensitivity previously reported by automated estrous detection systems can be higher due to using standing behavior as a reference standard. Verification of ovulation may be a more useful reference standard in future studies.

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Table 2.1. Automated detection devices used in evaluation of alert efficacy and parameter usefulness for alerts of estrus for dairy cows synchronized with a modified G7G - Ovsynch protocol and visually observed for estrous behaviors. Alerts from automated estrous detection devices were compared among cows verified in estrus and anestrus.[1, 2]

Automated Estrous Detection Device	Parameters Measured	Frequency of measurements	Frequency of reporting data
AfiAct Pedometer Plus, Afimilk, Kibbutz Afikim, Israel	Activity (steps) Lying time (min) Lying bouts	Continuously	Per hour
Afimilk MPC Analyzer Afimilk, Kibbutz Afikim, Israel	Milk yield (lbs) Milk flow Milk conductivity	Each milking	End of milking
CowManager SensoOr, Agis Automatisering, Harmelen, Netherlands	Rumination time (min) Eating time (min) Time not active (min) Time active (min) Time high active (min)	Every minute	Every hour
CowScout S Leg, GEA Farm Technologies GmbH, Bönen, Germany	Activity (number of steps)	Continuously	15 minute intervals
DVM bolus, DVM Systems, LLC, Greeley, CO	Reticulorumen temperature (◦C)	Every 5 minutes	Hourly
HR Tag, SCR Engineers Ltd., Netanya, Israel	Neck activity Rumination time (min)	Continuously	Every 2 hours
IceQube, IceRobotics Ltd., Edinburgh, Scotland	Lying time (min) Steps Motion index Lying bouts Bout duration (min)	Continuously	15 minute intervals
Thermochron iButton, Embedded Data Systems, Kentucky, USA	Temperature[3]	Every 5 minutes	Every 5 minutes
Track a)) Cow, ENGS Systems Innovative Dairy Solutions, Israel	Activity unit Lying time (min) Lying bouts Bout duration (min) Time spent at feed bunk	Continuously	Every 5 minutes

[1]Only alerts from AfiAct Pedometer Plus, CowManager SensoOr, CowScout S Leg, HR Tag, IceQube, and Track a)) Cow were used in assessing efficacy of systems for automated estrous detection.
[2]Estrus was verified by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) indicating ovulation and estrus (reference standard). Progesterone radioimmunoassay were completed with blood plasma.
[3]Thermochron iButtons were attached to an intravaginal device to continuously collect vaginal temperature a week before and a week after estrus in cows

Table 2.2. Means of parameters measured by automated estrous detection systems for cows synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N = 94).[1, 2]

Automated estrous detection device parameters	Automated estrous detection System3	Mean ± SD
Activity (steps/d)	AfiAct Pedometer Plus	3827.10 ± 2901.71
Activity (steps/d)	CowScout S Leg	4410.24 ± 1815.18
Activity (steps/d)	Track a)) Cow	2269.55 ± 990.79
Activity (steps/d)	IceQube	1137.71 ± 612.63
Motion index	IceQube	42.93 ± 22.72
Active time (min/d)	SensoOr	56.82 ± 27.24
High activity	SensoOr	52.32 ± 39.74
Neck activity	HR Tag	414.79 ± 136.85
Lying time (h/d)	AfiAct Pedometer Plus	8.90 ± 2.86
Lying bouts	AfiAct Pedometer Plus	9.17 ± 4.01
Lying time (h/d)	IceQube	9.11 ± 2.76
Lying bouts	IceQube	16.22 ± 7.48
Bout duration (min/ bout)	IceQube	39.77 ± 31.08
Time not active (h/d)	SensoOr	6.69 ± 2.22
Lying time (min/d)	Track a)) Cow	562.97 ± 178.53
Lying bouts	Track a)) Cow	10.65 ± 5.59
Rumination time (h/d)	SensoOr	9.18 ± 1.93
Rumination time (h/d)	HR Tag	7.81 ± 1.39
Eating time (h/d)	SensoOr	3.48 ± 1.55
Intake visits	Track a)) Cow	8.59 ± 4.32
Time at feedbunk (min/d)	Track a)) Cow	173.46 ± 90.99
Mean vaginal temperature °C	Thermochron iButton	38.98 ± 0.47
Max vaginal temperature °C	Thermochron iButton	39.73 ± 1.34
Ear skin temperature °C	SensoOr	22.22 ± 6.57
Reticulorumen temperature °C	DVM bolus	39.02 ± 0.38
Milk yield (kg/d)	Afimilk MPC Analyzer	37.73 ± 9.79

[1]Means of parameters using all 28 days of study protocol
[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.
[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solution)

Table 2.3. Assessing the efficacy of alerts generated by 6 automated estrous detection systems in comparison to visual observation of standing mounts and estrous behavioral scoring system using temporal progesterone patterns as the standard of reference for ovulation. Cows (N=109) were synchronized with a modified G7G Ovsynch protocol.[1, 2, 3, 4, 5, 6]

Estrus detection Method	True Positives	False Positives	True Negatives	False Negatives	Total Cows (n)[7]	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
AfiAct Pedometer Plus	76	2	13	18	109	80.9%	86.7%	81.7%	97.4%	41.9%
CowScout S Leg Tag	72	0	14	21	107	77.4%	100.0%	80.4%	100.0%	40.0%
IceQube	45	2	10	34	91	57.0%	83.3%	60.4%	95.7%	22.7%
HR Tag	21	0	2	1	24	95.5%	100.0%	95.8%	100.0%	66.7%
CowManager SensoOr	51	0	8	6	65	89.5%	100.0%	90.8%	100.0%	57.1%
Track a)) Cow	35	1	10	15	61	70.0%	90.9%	73.8%	97.2%	40.0%
Standing	51	0	15	43	109	54.3%	100.0%	60.6%	100.0%	25.9%
Behavioral score	62	0	15	32	109	66.0%	100.0%	70.6%	100.0%	31.9%

[1]Sensitivity = TP/ (TP + FN) x 100, specificity = TN/ (TN + FP) x 100, accuracy = (TP + TN)/ (TP + TN + FP + FN) x 100, positive predictive value = TP/ (TP + FP) x 100, and negative predictive value = TN/ (TN + FN)100; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative

[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solution

[3] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4]Scoring system as defined by van Eerdenburg et al. (1996) and modified to detect a cow in estrus once total points for one observation period ≥ 100 points.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[7]109 lactating Holstein cows 45-85 DIM were enrolled in the study. However, only cows who had functioning devices are reflected for each device. Devices were considered broken for cows with less than 80% (19/24h) of raw data for the day before estrus and day of estrus for the parameters identified by each company as necessary for estrus alerts.

Table 2.4. Assessing the efficacy of alerts generated by 6 automated estrous detection systems in comparison to visual observation of standing mounts and estrous behavioral scoring system using standing mounts as the reference standard of estrus. Cows (N=109) were synchronized with a modified G7G Ovsynch protocol.[1, 2, 3, 4, 5, 6]

Estrus detection Method	True Positives	False Positives	True Negatives	False Negatives	Total Cows (n)[7]	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
AfiAct Pedometer Plus	47	31	27	4	109	92.2%	46.6%	67.9%	60.3%	87.1%
CowScout S Leg Tag	47	25	31	4	107	92.2%	55.4%	72.9%	65.3%	88.6%
IceQube	29	18	32	12	91	70.7%	64.0%	67.0%	61.7%	72.7%
HR Tag	15	6	3	0	24	100.0%	33.3%	75.0%	71.4%	100.0%
CowManager SensoOr	30	21	14	0	65	100.0%	40.0%	67.7%	58.8%	100.0%
Track a)) Cow	22	14	18	7	61	75.9%	56.3%	65.6%	61.1%	72.0%

[1]Sensitivity = TP/ (TP + FN) x 100, specificity = TN/ (TN + FP) x 100, accuracy = (TP + TN)/ (TP + TN + FP + FN) x 100, positive predictive value = TP/ (TP + FP) x 100, and negative predictive value = TN/ (TN + FN) x 100; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative

[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solution

[3] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4]Scoring system as defined by van Eerdenburg et al. (1996) and modified to detect a cow in estrus once total points for one observation period ≥ 100 points.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[7]109 lactating Holstein cows 45-85 DIM were enrolled in the study. However, only cows who had functioning devices are reflected for each device. Devices were considered broken for cows with less than 80% (19/24h) of raw data for the day before estrus and day of estrus for the parameters identified by each company as necessary for estrus alerts.

Table 2.5. Assessing the efficacy of alerts generated by 6 automated estrous detection systems in comparison to visual observation of standing mounts and estrous behavioral scoring system using temporal progesterone patterns as the reference standard of estrus. Cows (N=35) were synchronized with a modified G7G Ovsynch protocol.[1, 2, 3, 4, 5, 6]

Estrus detection Method	True Positives	False Positives	True Negatives	False Negatives	Total Cows (n)[7]	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
AfiAct Pedometer Plus	27	1	3	4	35	87.1%	75.0%	85.7%	96.4%	42.9%
CowScout S Leg Tag	25	1	3	6	35	80.6%	75.0%	80.0%	96.2%	33.3%
IceQube	19	1	3	12	35	61.3%	75.0%	62.9%	95.0%	20.0%
HR Tag	24	1	3	7	35	77.4%	75.0%	77.1%	96.0%	30.0%
CowManager SensoOr	28	0	4	3	35	90.3%	100.0%	91.4%	100.0%	57.1%
Track a)) Cow	26	1	3	5	35	83.9%	75.0%	82.9%	96.3%	37.5%
Standing	18	0	4	13	35	58.1%	100.0%	62.9%	100.0%	23.5%
Score	19	0	4	12	35	61.3%	100.0%	65.7%	100.0%	25.0%

[1]Sensitivity = TP/ (TP + FN)100, specificity = TN/ (TN + FP)100, accuracy = (TP + TN)/ (TP + TN + FP + FN)100, positive predictive value = TP/ (TP + FP)100, and negative predictive value = TN/ (TN + FN)100; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative

[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solution

[3] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4]Scoring system as defined by van Eerdenburg et al. (1996) and modified to detect a cow in estrus once total points for one observation period ≥ 100 points.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[7]All cows enrolled in the protocol between December 2014 and March 2015 were included in a separate analysis to determine the efficacy comparing the same cows and time periods. No cows were removed from this analysis regardless of missing raw data.

Table 2.6. Assessing the efficacy of alerts generated by 6 automated estrous detection systems in comparison to visual observation of standing mounts and estrous behavioral scoring system using standing mounts as the reference standard of estrus. Cows (N=35) were synchronized with a modified G7G Ovsynch protocol.[1, 2, 3, 4, 5, 6]

Estrus detection Method	True Positives	False Positives	True Negatives	False Negatives	Total Cows (n)[7]	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
AfiAct Pedometer Plus	16	12	5	2	35	88.9%	29.4%	60.0%	57.1%	71.4%
CowScout S Leg Tag	16	10	7	2	35	88.9%	41.2%	65.7%	61.5%	77.8%
IceQube	11	9	8	7	35	61.1%	47.1%	54.3%	55.0%	53.3%
HR Tag	15	10	7	3	35	83.3%	41.2%	62.9%	60.0%	70.0%
CowManager SensoOr	18	10	7	0	35	100.0%	41.2%	71.4%	64.3%	100.0%
Track a)) Cow	16	11	6	2	35	88.9%	35.3%	62.9%	59.3%	75.0%
Score	16	10	7	2	35	88.9%	41.2%	65.7%	61.5%	77.8%

[1]Sensitivity = TP/ (TP + FN)100, specificity = TN/ (TN + FP)100, accuracy = (TP + TN)/ (TP + TN + FP + FN)100, positive predictive value = TP/ (TP + FP)100, and negative predictive value = TN/ (TN + FN)100; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative

[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solution

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[4]Scoring system as defined by van Eerdenburg et al. (1996) and modified to detect a cow in estrus once total points for one observation period ≥ 100 points.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[7]All cows enrolled in the protocol between December 2014 and March 2015 were included in a separate analysis to determine the efficacy comparing the same cows and periods. No cows were removed from this analysis regardless of missing raw data.

Table 2.7. Differences in automated estrous detection device activity and lying behavior parameters between cows in estrus and cows not in estrus on the predicted day of estrous expression synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N=109). [1, 2]

Automated estrous detection device parameter	Automated estrous detection device[3]	n	Estrus Mean % change ± SD	Non-Estrus Mean % change ± SD	P - value
Activity (steps/d)	AfiAct Pedometer Plus	84	82.66% ± 40.57%	-5.67% ± 94.81%	0.39
Activity (steps/d)	CowScout S Leg	87	69.80% ± 9.48%	-13.3% ± 23.71%	0.00
Activity (steps/d)	Track a)) Cow	75	77.66% ± 14.88%	-0.98% ± 34.09%	0.04
Activity (steps/d)	IceQube	86	117.98% ± 17.44%	-6.83% ± 43.31%	0.01
Motion index	IceQube	86	103.89% ± 14.29%	-6.15% ± 35.5%	0.01
Active time	SensoOr	50	21.28% ± 7.12%	-12.51% ± 17.64%	0.08
High activity	SensoOr	50	169.62% ± 22.25%	-3.59% ± 55.15%	0.01
Neck activity	HR Tag	55	40.17% ± 562.02%	5.42% ± 1471.72%	0.03
Lying time	AfiAct Pedometer Plus	109	19.52% ± 7.95%	24.17% ± 19.89%	0.83
Lying bouts	AfiAct Pedometer Plus	109	33.31% ± 15.47%	15.83% ± 38.73%	0.68
Lying time (h/d)	IceQube	86	-13.31% ± 3.77%	-3.79% ± 9.35%	0.35
Lying bouts	IceQube	86	-4.19% ± 6.17%	9.16% ± 15.32%	0.42
Bout duration (min/ bout)	IceQube	86	-13.31% ± 3.77%	-3.79% ± 9.35%	0.35
Time not active	SensoOr	50	-23.49% ± 6.97%	-3.24% ± 17.26%	0.28
Lying time	Track a)) Cow	70	18.06% ± 19.03%	-3.09% ± 41.84%	0.65
Lying bouts	Track a)) Cow	69	29.71% ± 7.18%	7.49% ± 15.66%	0.20
Lying percent	Track a)) Cow	51	26.11% ± 21.2%	0.34% ± 49.14%	0.63

[1] Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100 for the day of observed standing mount for cows in estrus and the second day of visual observation for non-estrus cows

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions

Table 2.8. Differences in automated estrous detection device feeding behavior, temperature, and milk yield parameters between cows in estrus and cows not in estrus on the predicted day of estrous expression synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N=109).[1, 2]

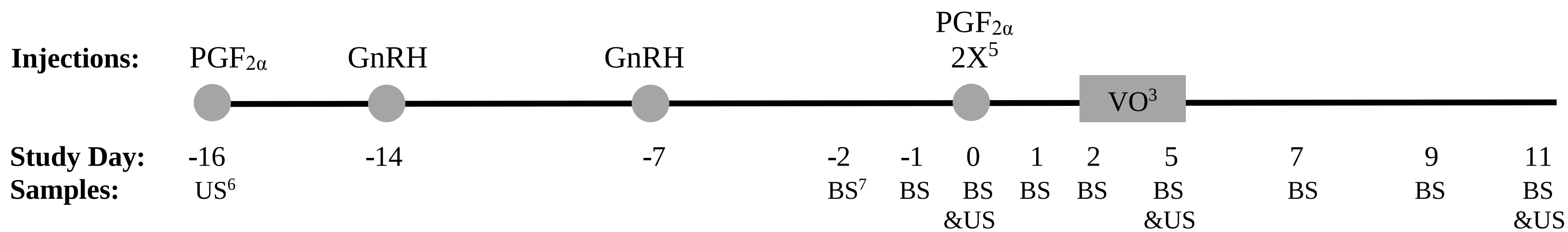
Automated estrous detection device parameter	Automated estrous detection device[3]	n	Estrus Mean % change ± SD	Non-Estrus Mean % change ± SD	P - value
Rumination time (min/d)	SensoOr	50	-15.86% ± 3.12%	3.71% ± 7.72%	0.02
Rumination time (min/d)	HR Tag	55	-2.6% ± 330.76%	15.89% ± 866.12%	0.05
Eating time (min/d)	SensoOr	50	45.21% ± 8.03%	-5.25% ± 19.9%	0.02
Intake visits	Track a)) Cow	50	36.95% ± 19.06%	-10.22% ± 38.14%	0.27
Time at feedbunk (min/d)	Track a)) Cow	50	-24.32% ± 4.98%	-4.96% ± 9.96%	0.09
Mean vaginal temperature °C	Thermochron iButton	76	0.28% ± 0.07%	-0.0042% ± 0.15%	0.09
Max vaginal temperature °C	Thermochron iButton	76	0.47% ± 0.12%	-0.04% ± 0.26%	0.07
Ear skin temperature °C	SensoOr	45	6.81% ± 8.39%	10.73% ± 19.54%	0.85
Reticulorumen temperature °C	DVM bolus	47	0.35% ± 0.08%	0.35% ± 0.17%	1.00
Milk yield (kg/d)	Afimilk MPC Analyzer	109	-1.92% ± 1.88%	3.3% ± 4.71%	0.31

[1] Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100 for the day of observed standing mount for cows in estrus and the second day of visual observation for non-estrus cows

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions)

Figure 2.1. Protocol (28 days) used to assess the efficacy of 8 automated estrous detection systems for cows synchronized with a modified G7G - Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4]



[1]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[2]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[3]Visual observation (**VO**) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

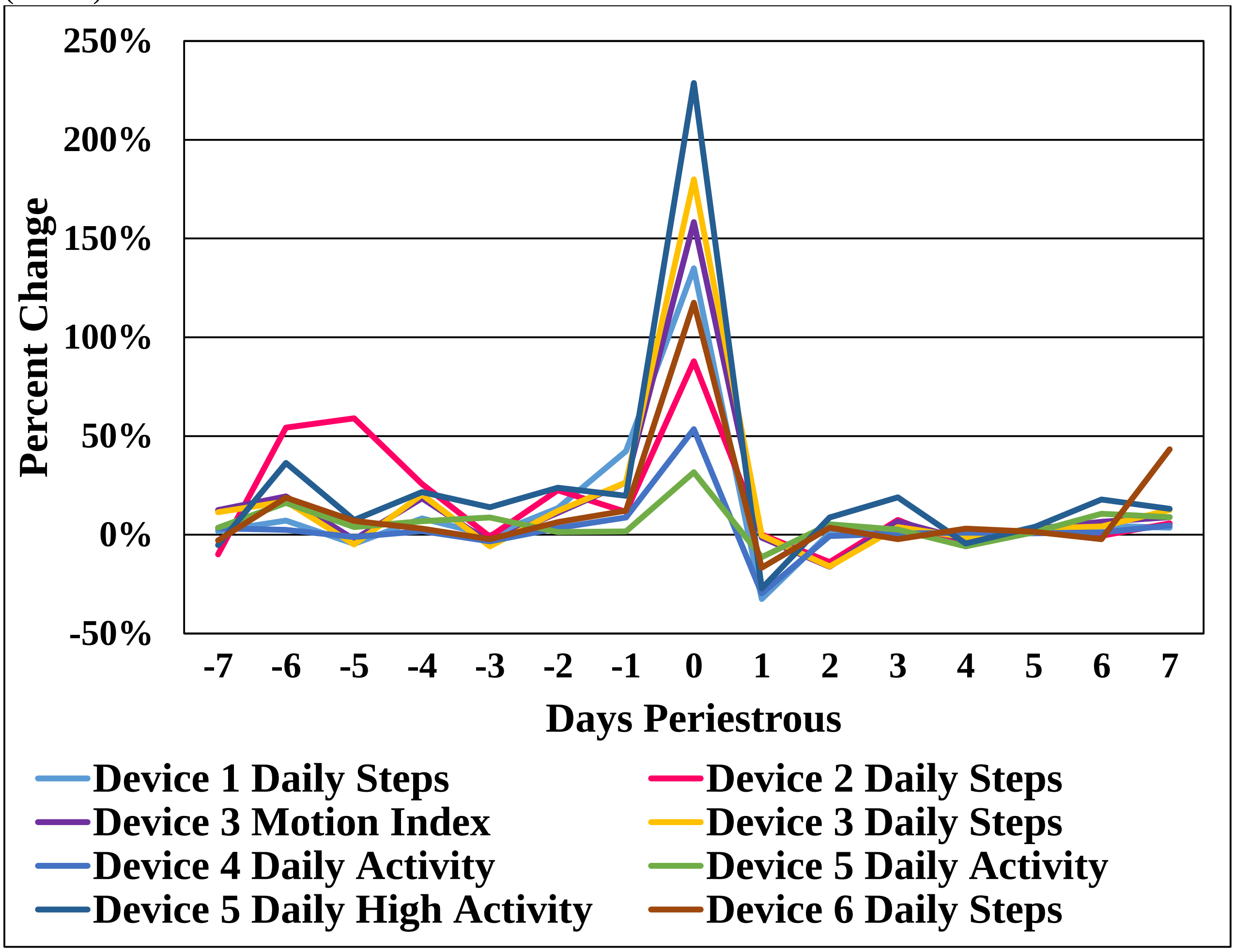
[4]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11).

[5]$PGF_{2\alpha}$ was administered twice on day 0, 6 hours apart at 0800 and 1400.

[6]Transrectal ultrasonography (**US**) was performed at 0800 to verify resumption of ovarian cyclicity at enrollment (d -16), presence of a corpus luteum (**CL**) on the day of the final injection (designated experimental day 0), regression of the CL by day 5, and presence of a new CL on day 11

[7]Blood samples (**BS**) were taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.2. Percent change in activity parameters measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

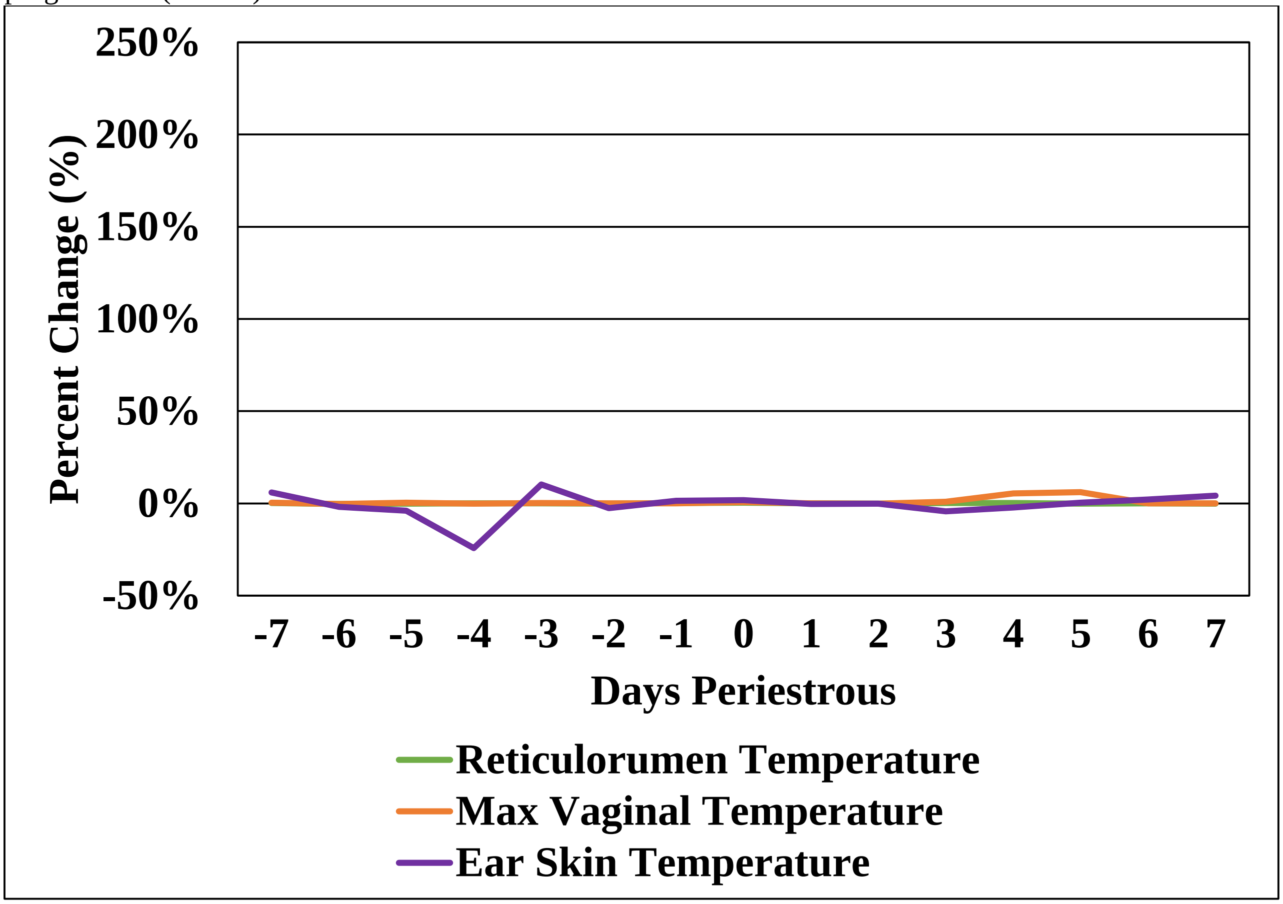
[2]Device 1 was the AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); Device 5 was the CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); Device 2 was the CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); Device 4 was the HR Tag (SCR Engineers Ltd., Netanya, Israel); Device 3 was the IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Device 6 was the Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.3. Percent change in temperature parameters measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

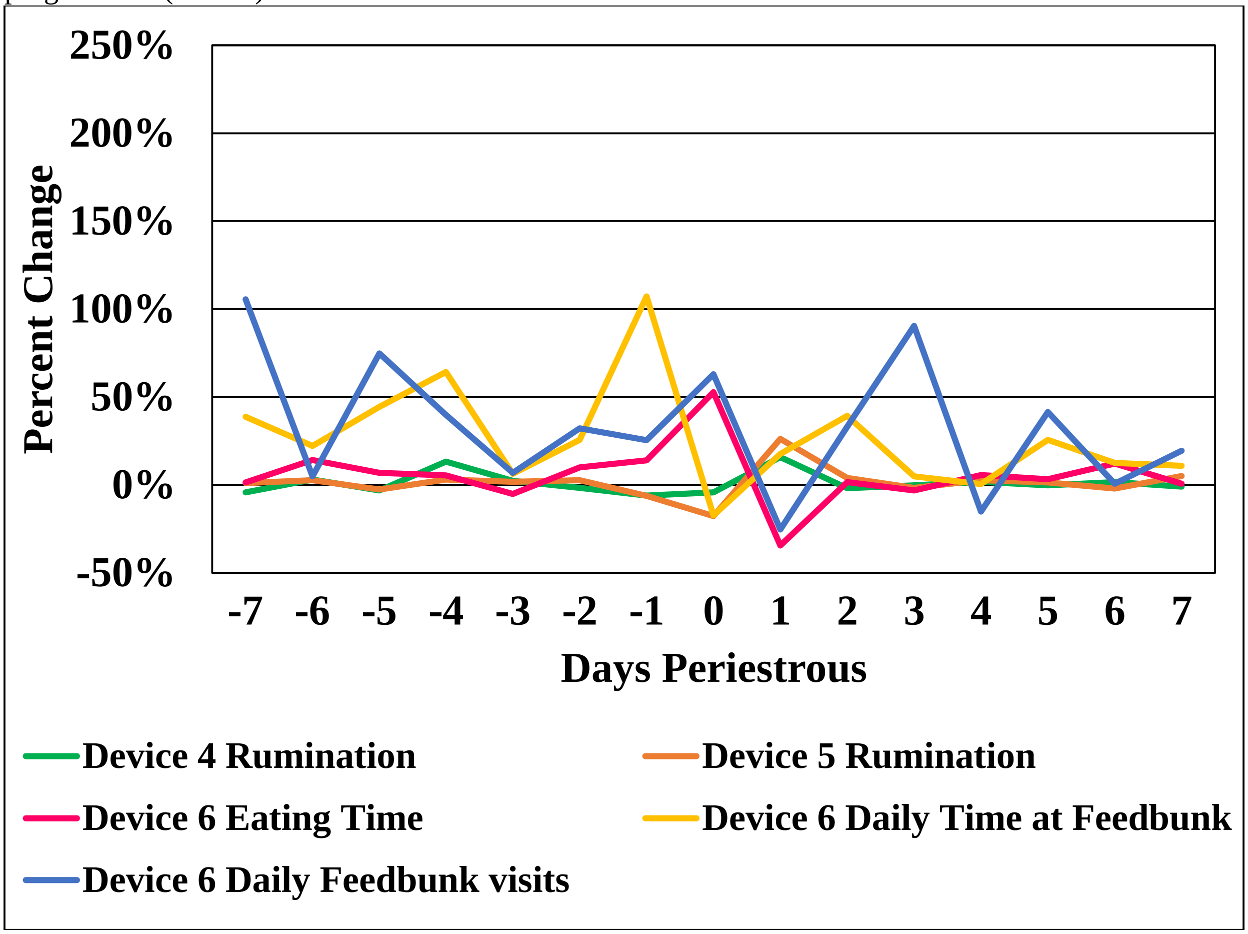
[2]CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands) measured ear skin temperature; DVM bolus (DVM Systems, LLC, Greeley, CO) measured reticulorumen temperature; Thermochron iButton, (Embedded Data Systems, Kentucky, USA) was inserted into an intravaginal device 7 days before protocol day 0 (final injection of prostaglandin) and removed 7 days after day 0

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.4. Percent change in feeding behavior parameters measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

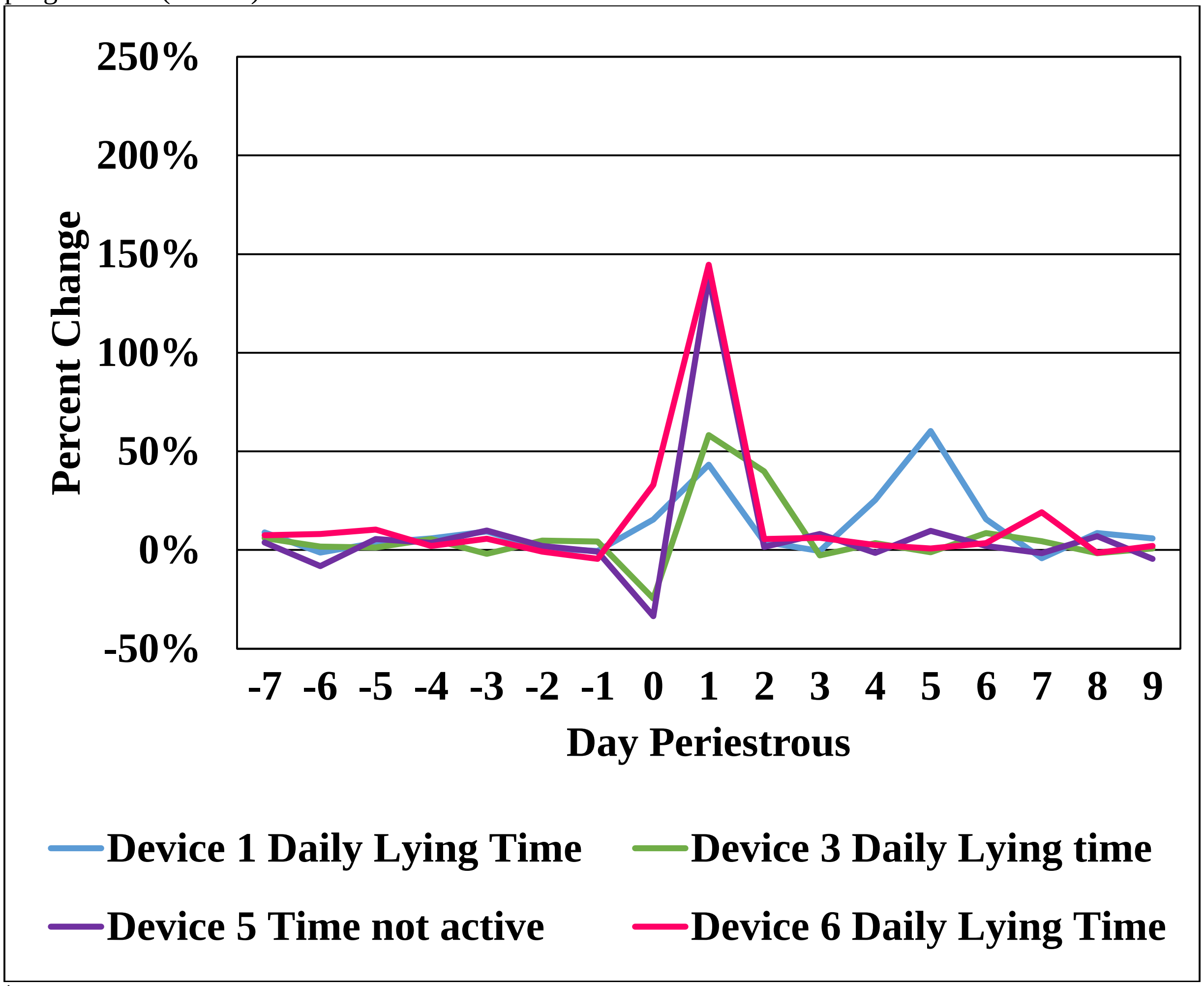
2Device 5 was the CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands) Device 4 was the HR Tag (SCR Engineers Ltd., Netanya, Israel); Device 6 was the Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.5. Percent change in lying time and time not active measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

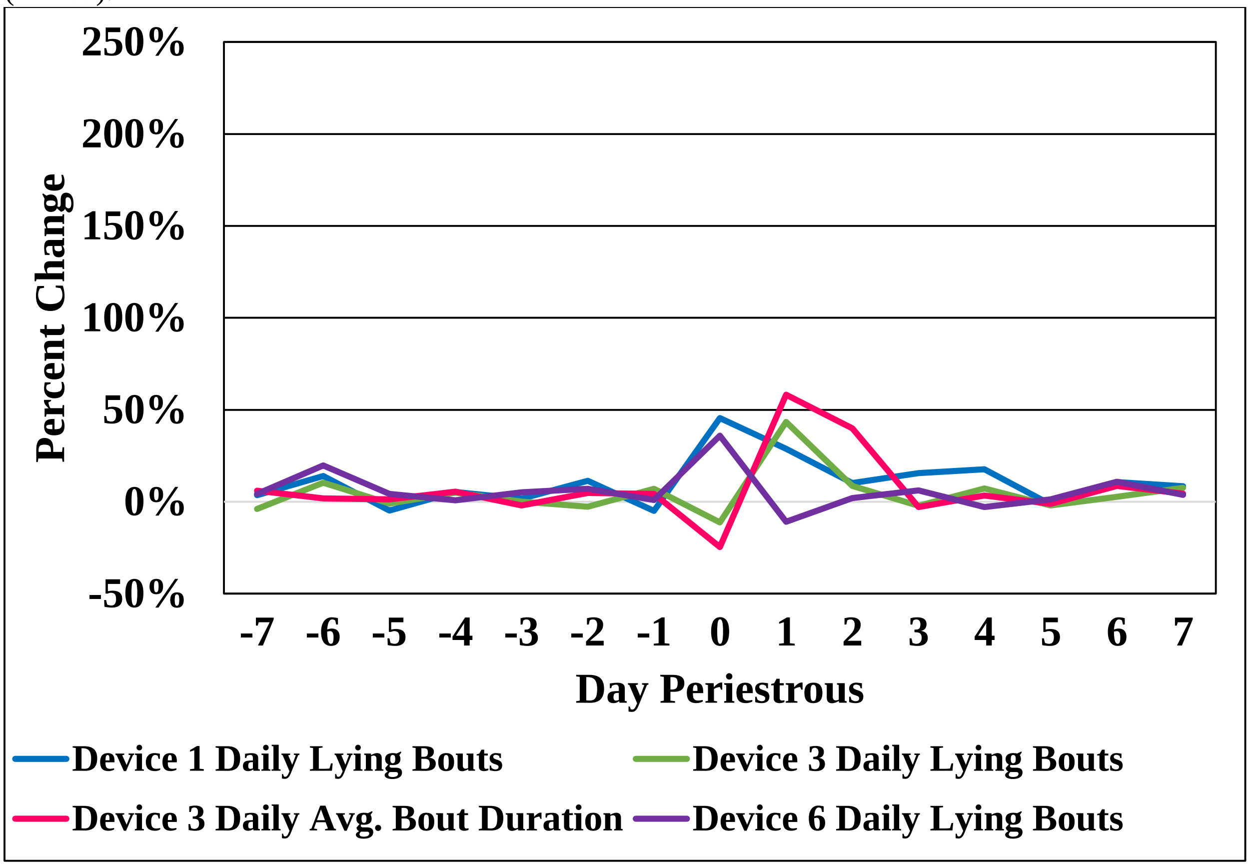
2Device 1 was the AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); Device 5 was the CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); Device 3 was the IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Device 6 was the Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.6. Percent change in lying behaviors measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

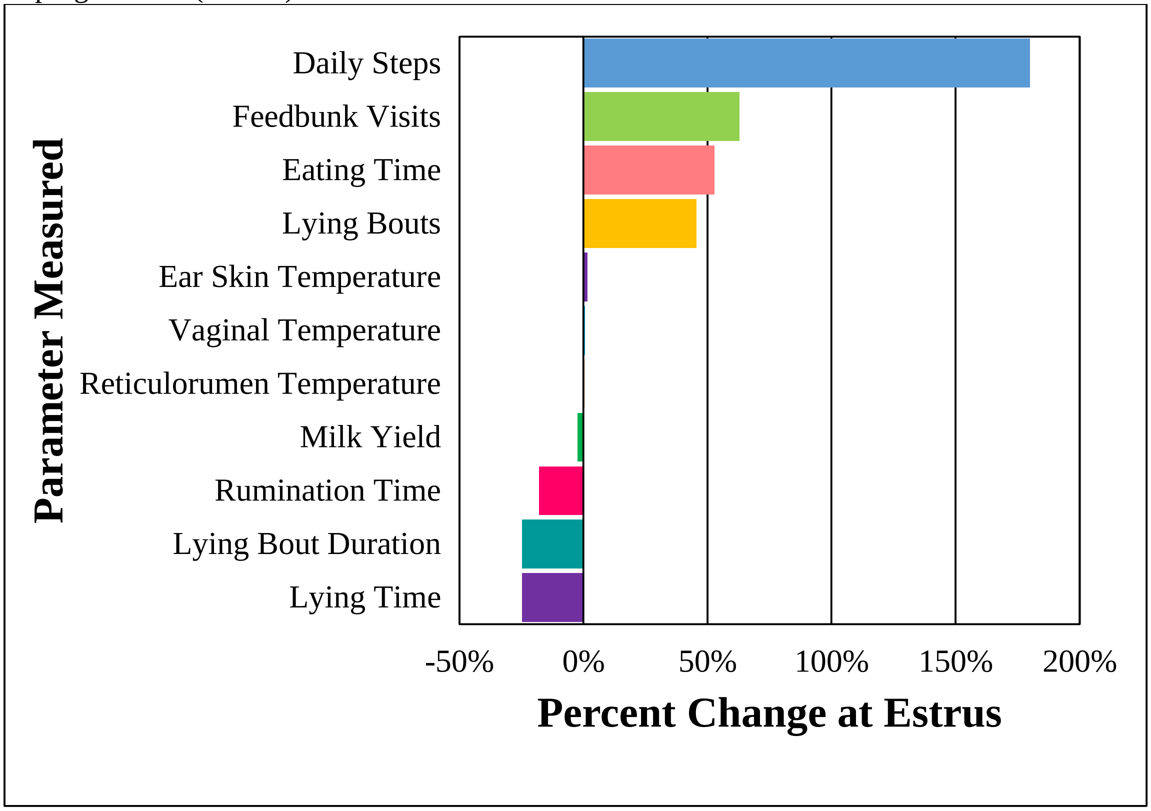
2Device 1 was the AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); Device 3 was the IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Device 6 was the Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

Figure 2.7. Percent change in behaviors and biological changes measured and recorded by multiple automated estrous detection devices on cows synchronized with a modified G7G-Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4, 5]



[1]Percent change was determined for each day of the protocol using a backward 7 day average not including the protocol day then calculated by ((protocol day measurement – baseline measurement) / baseline measurement) × 100

[2]Lying bouts and milk yield were recorded by AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); DVM bolus (DVM Systems, LLC, Greeley, CO) measured reticulorumen temperature; Thermochron iButton, (Embedded Data Systems, Kentucky, USA) was inserted into an intravaginal device 7 days before protocol day 0 (final injection of prostaglandin) and removed 7 days after day 0; Lying time, steps per day, and average lying bout duration were measured by the IceQube, (IceRobotics Ltd., Edinburgh, Scotland); rumination time, eating time, and ear skin temperature were measured by the CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands)

[3]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[4]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[5]Potential periods of estrus (gold standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11) from blood samples taken at 0800to obtain plasma for progesterone radioimmunoassay

CHAPTER THREE

Differences in early postpartum disease status, body condition score, locomotion score, SCC, season, and parity on automated detection of estrus and behavioral and physiological parameters measured in synchronized dairy cows

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INTRODUCTION

Dairy cow reproductive performance can decline because of early postpartum diseases, environment, rapid changes in body condition, lameness, and other health ailments (Senger, 2005; Aungier et al., 2012). Optimum dairy cow reproductive performance begins with detection of estrus. Estrus detection efficiency is often less than 50% for dairy cows possibly related to immunosuppression (Esselmont, 1974; Senger, 1994; Lucy, 2001). Immunosuppressed dairy cows are less likely to express estrous behaviors, especially standing for mounting by other cows (Lopez et al., 2004; Sangsritavong et al., 2002; Aungier et al., 2012). Cows with metabolic disorders yield more milk than cows without metabolic disorders (Sangsritavong et al., 2002). High yielding cows tended to have 283 L/h more blood transferred though the liver decreasing the concentration of progesterone and subsequently higher metabolism of estradiol 17-β ($P < 0.0001$) (Sangsritavong et al., 2002). Higher metabolism of estradiol 17-β, can lead to shortened periods of estrus due to lower levels of estradiol 17-β, making it more difficult to detect estrus (Wiltbank et al., 2006).

Higher yielding cows have more incidences of inadequate metabolic hormones, including growth hormone (GH), growth hormone receptor, and IGF-1 that affect reproductive hormones (Wathes et al., 2007). Changes in BCS can be related to higher milk yields (Pryce et al., 2001). Days to first service was reported to decrease -5.2 ± 1.6 d for cows with a decrease in BCS 10 weeks postpartum ($P < 0.0001$) (Pryce et al., 2001). The genetic heritability of BCS and days to first service are low, 0.21 to 0.43, meaning BCS and the environment and management than genetics (Koenen and Veerkamp, 1999; Veerkamp et al., 2001) affect its effects on fertility more.

Lameness can affect the expression of estrus due to the pain of lameness (Collick et al., 1989). Morris et al. (2011) reported 21% of lame cows failed to express estrus or ovulate due to low levels of estrogen. Collick et al. (1989) reported an 8-day increase in days to first service among 427 cases of lameness. Lame cows were also more likely to have shorter periods of estrus earlier in the day (Morris et al., 2011) decreasing the chance for dairy producers to detect lame cows in estrus. Hernandez et al., (2001) reported increased services per conception and a 52% less conception risk in 254 lame cows compared to 583 healthy cows ($P < 0.05$) Therefore, continuous monitoring of lame cows may be helpful in improving reproductive performance. However, monitoring of lame cows can lead to increased false positives. Restlessness because of cow discomfort can lead to irregular measures of parameters other than activity (Roelofs, 2006).

2008).Dairy producers (Schukken et al., 2008) often miss subclinical mastitis. Clinical mastitis is often a heightened response to subclinical mastitis (Viguier et al., 2009). Jersey cows with clinical mastitis were reported to have 93.6 days to first AI service compared to healthy cows with only 71.0 days to first AI service (Barker et al., 1998). Expression of estrus is similar among high and low SCC cows, but high SCC cows have a lower intensity and delayed expression of estrous ($P = 0.06$) (Morris et al., 2013).

Longer estrous intervals occur in heat stressed cattle causing decreased breeding efficiency (Scott and Williams, 1962). High temperatures in Arizona (Scott and Williams, 1962) and Florida (Cavestany et al., 1985) in June to September resulted in decreased CR and PR, and increased days open and services per conception. Cows had estrus detection rates of 33% during high temperature and humidity (Younas et al., 1993; De Rennis et al., 2003). Cows in natural estrus and hormonally induced estrus were 50% less likely to stand for mounting in the summer than in the colder months (Pennington et al., 1985).

Primiparous cows take longer to first ovulation than multiparous cows (Lucy et al., 1992; Tananka, 2008) but more multiparous cows have negative energy balance delaying resumption of the estrous cycle. Parity may affect expression of estrus since primiparous cows have not had multiple lactations to develop as many diseases or experience the stress of multiple gestations and parturitions. Therefore, the objectives of the current study were to determine the differences between classifications of cows in expression of parameters measured by several commercial precision dairy technologies and standing behavior that could lead to decreased estrus detection efficiency.

MATERIALS AND METHODS

This experiment was part of a larger study designed to quantify physiological and behavioral changes, using multiple precision dairy farming technologies, associated with mastitis, lameness, estrus, and metabolic diseases. All studies were performed with approval of the University of Kentucky Institutional Animal Care and Use Committee (IACUC protocol number: 2013-1199).

Animals, Feeding, and Housing

One hundred and nine lactating Holstein cows at the University of Kentucky Coldstream Dairy (Lexington, KY, USA) were enrolled in this study between January 2014 and May 2015. Cows were enrolled in the protocol in groups of 6 to 10 cows between 45 to 85 DIM. Lactating cows were housed in two freestall barns with one barn of 54 dual chamber waterbeds (Advanced Comfort technology, Inc., Reedsburg, WI) and the other equipped with 54 rubber-filled mattresses, all covered with sawdust.

Before and throughout the study, cows were balanced between barns by DIM and parity. Calving dates, breeding dates, and DIM were obtained from PCDART management software (Dairy Records Management Systems, Raleigh, NC). Parity ranged from 1 to 7. The average milk yield of cows during the protocol was 37.7 ± 9.8 kg. Mean cow parity was 1.99 ± 1.30. Mean DIM at enrollment was 66.5 ± 11.4 d. Mean DIM at estrus was 85.5 ± 11.4 d.

A weather station (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger - U23-002, Onset, Bourne, MA) was located inside each freestall barn that measured relative humidity and temperature every 15 minutes. Temperature humidity index was computed using the following formula (NOAA and Administration, 1976): THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8]. The estrual max THI was calculated by averaging the max THI for each barn on days 2 to 5 of the protocol for each study group of cows. The estrual max THI was used to assess the effect of max THI on automated estrous detection rates and number of cows with standing mounts.

Cows had ad libitum access to water in each barn and shared a feedbunk between barns. Lactating cows were fed the same ration at 0600 and 1330 daily. The lactating cow ration consisted of corn silage, alfalfa hay, mineral and vitamin supplement, concentrate mix, whole cottonseed, and alfalfa haylage. Cows were milked 2X at 0430 and 1530.

Synchronization Protocol

A modified G7G-Ovsynch (Figure 3.1) was used to synchronize cows into sexually active groups in order to visually observe estrous behaviors in groups of 6 to 10 cows at a time. Cows were pre-synchronized using the G7G protocol, starting with an injection of prostaglandin (**$PGF_{2\alpha}$;** 25 mg, Lutalyse, Pfizer Animal Health, New York, NY). Two days later, cows received an injection of GnRH (100 ug, Cystorelin, Merial Limited, Duluth, GA). Seven days after the GnRH injection, the Ovsynch protocol, excluding the final shot of GnRH to allow for observation of estrous expression (Pursley et al., 1995), was initiated. Cows received a GnRH injection followed by a $PGF_{2\alpha}$ injection 7 days later. An additional $PGF_{2\alpha}$ shot was administered on the same day of the first $PGF_{2\alpha}$ injection of Ovsynch, 6 hours later. The day of the last $PGF_{2\alpha}$ injection was designated day 0 of the experimental protocol.

Ultrasonography and Sampling

Transrectal ultrasonography was performed on days -16, 0, 5, and 11 in the protocol using an Ibex Pro Portable Ultrasound (E.I. Medical Imaging, Colorado, USA). Ovarian cyclicity resumption at enrollment was verified by corpus luteum (CL) presence. On the final $PGF_{2\alpha}$ injection day (designated experiment day 0) presence of a newly formed CL and preovulatory follicle verified response to the initial $PGF_{2\alpha}$ and GnRH injections. Regression of this CL and ovulation of the preovulatory follicle were recorded on day 5. Presence of a new CL on day 11 concluded verification of ovulation and served as the reference standard for ovulation detected by automated estrous detection systems.

Blood samples were collected on days -2, -1, 0, 1, 2, 5, 7, 9, and 11 to quantify progesterone for verification of luteal regression and ovulation. Potential estrous periods (reference standard) were defined by the progesterone temporal pattern (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11).

Automated Estrous Detection Systems

Each cow was equipped with AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel), CowScout S Leg (Gea Farm Technologies GmbH, Bönen, Germany), DVM Bolus (DVM Systems, LLC, Greeley, CO), HR Tag (SCR Engineers Ltd, Netanya, Israel), CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), IceQube (IceRobotics Ltd, Edinburgh, Scotland), and Track a Cow (ENGS, Hampshire, UK) devices (Table 3.1) before study enrollment to allow for an adjustment period of at least two weeks. Heifers were equipped with all devices at least 10 to 14 days before their predicted calving date. Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) were placed in intravaginal devices inserted into cows 7 days before the final injection of $PGF_{2\alpha}$.

Device locations were determined by device previous use or company experience for each device (Table 3.1). Leg devices were placed on the same leg for each technology for every cow. DVM boluses were inserted into the reticulorumen orally, using a bolus gun. Ear tags were positioned using an ear tagger, provided by each technology company to fit the respective device.

Afimilk Milking Point Controllers (Afimilk, Kibbutz Afikim, Israel) were used to collect individual milk yield and milking time for each milking. Body weights were recorded by AfiWeigh (Afimilk, Kibbutz Afikim, Israel), placed in a common exit alley. Cows were sorted into their respective groups using AfiSort (Afimilk, Kibbutz Afikim, Israel) after each milking.

All computer clocks were set to synchronize with NIST Internet Time Service (NIST, Gaithersburg, MD, USA) automatically, and time was checked on all computers manually on a weekly basis. Raw data, including measurements and recordings of behavioral and physiological parameters, and estrus alerts generated by each AED software program were downloaded daily. Default settings for report and alert generation within each system were used during the study. Proprietary algorithms and individual animal threshold s for each system were used to generate estrus alerts.

Visual Observation for Estrus

Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period, until all cows stood to be mounted. Cancelation due to inclement weather only occurred for one study group of cows for THREE observation periods with THREE cows that had not yet expressed estrus, due to a severe snowstorm restricting access to cows. The 3 cows were removed from final analysis of cows standing to be mounted. Barn lights were turned on for the 0330 and 2200 observation periods and turned off at the end of each observation period. Cows were adjusted to this routine before the study started to avoid differences in routine behavior. Cows were released to an exercise lot divided by pen for 1 hour each day during the 1000 observation period.

Cows were identified with neck strap digits and numbers spray-painted on each side of the body. The van Eerdenburg et al. (1996) scoring scale for observed estrous signs, including modifications used by Roelofs (2005) and additional modifications was used to quantify intensity of estrus. Behaviors of estrus were assigned points according the original system including: 100 points for standing heat, 45 points for mounting head side of other cows, 35 points for attempting or mounting other cows, 15 points for chin resting on the rump of other cows, 10 points for sniffing the vagina of another cow or being mounted but not standing, 5 points for restlessness (increased activity or pacing), and 3 points for clear mucous vaginal discharge (van Eerdenburg et al., 1996). When a cow reached a score of 100 points the animal is considered in estrus. Additional modifications included considering in estrus once a cow received greater than or equal to 100 points, instead of two consecutive periods required for definition of estrus. One observer per side watched for behaviors during each observation period. Each observer recorded behaviors by hand and recorded all standing heat times using a satellite powered watch (WV58A-1AV Atomic Digital Watch, Casio, Shibuya, Tokyo, Japan) synchronized with the AED system computers. Estrus periods were designated as periods when the score exceeded 100 points.

Early Lactation Metabolic Disease Monitoring

Starting June 2014, physical exams were performed for each cow at 0730 ± 1 h for the first 21 days of lactation. Behavioral scoring (Sterrett et al., 2013) was completed daily for each cow for the first 21 DIM. The 4 point behavioral scoring system included: score 1: no systemic signs of ill health (looks normal), eyes bright and alert, perky ears; score 2: additional signs of illness, looked mildly depressed, droopy ears, dull eyes; score 3: looked moderately depressed, droopy ears, dull and sunken eyes, lethargic; and score 4: looked extremely depressed, droopy ears, dull and very sunken eyes, lethargic, anorexic, often refuses to stand, uninterested in surrounding environment. Rectal temperature was collected with a GLA thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) daily at 0730 ± 1 h for the first 21 DIM.

Uterine discharge sample scores, blood samples for Ca, and blood samples for level of ketones using Precision Xtra (Abbott Laboratories, Abbott Park, Illinois) were collected on days 3, 5, 7, 9, 11, 14, and 21. A Metricheck (Simcro Tech Ltd, Hamilton, New Zealand) device (50-cm-long stainless steel rod with a 4-cm hemisphere of silicon at the end for vaginal insertion) was used to obtain a uterine discharge sample. A uterine discharge scoring system (Sterrett et al., 2013) was used, based on visual appearance of sample; score 1: thick, viscous discharge, clear, opaque or red to brown in color, no odor or mild, non-offensive odor; score 2: white or yellow pus, moderate to thick discharge, no odor or mild, non-offensive odor; score 3: pink, red, dark red, or black watery discharge, detectable offensive odor, possibly intolerable. Cows with at least one uterine discharge score ≥2 were classified as clinical metritis cases.

The first blood sample for Ca diagnosis was collected in a 10 ml red-top VACUTAINER® tube containing no anticoagulant. Samples were spun down in a centrifuge to obtain the serum. Serum was sent to the University of Kentucky Veterinary Diagnostic Lab (Lexington, KY) for evaluation of calcium. Cows with Ca levels lower than 8 mg/dL of at least one sample (Goff, 2008) were classified as subclinical hypocalcemia cases.

One drop of blood from a 1mL syringe was deposited on the end of a ketone test strip for Precision Xtra BHBA analysis. Cows with a Precision Xtra™ BHBA measurement greater than 1.4 mmol/L of at least one sample were classified as subclinical ketosis cases (Duffield, 1997; Geishauser et al., 2001; Oetzel, 2004).

Other Classifications

Gait scoring (Olmos et al., 2009; O'Callaghan et al., 2003) was performed weekly by the same observer throughout the entire study. Cows were released individually to walk past the observer in an open alley on the way to an exercise lot at approximately 1000. The observer watched the cow walk from a front, side, and hind view. Scores for each gait aspect: abduction and adduction, tracking, spine curvature, head bobbing, speed, and general symmetry were recorded. An average of all gait aspects was calculated. Cows scored the week of estrus, as 3 or higher for: abduction and adduction, tracking, general symmetry, or gait score average was classified as lame. Cows scored less than 3 for abduction and adduction, tracking, general symmetry, or gait score average were classified as sound. Abduction and adduction is the rotation of feet from the direction of travel. Each gait aspect was analyzed as a separate effect on estrous expression. A professional trimmer performed routine hoof trims every 6 months to prevent lameness and to ensure that lesions were properly treated.

The same observer performed body condition scoring (Ferguson et al., 1994) weekly during the full study. Body condition scores were determined upon evaluation of the following body regions: ischial tuberosity, illeal tuberosity, loin edges, coccygeal ligament, thurl region, sacral ligament, and spine were classified to result in a BCS. Body condition scores during the week of calving and predicted estrus were used to calculate the change in BCS from calving to estrus.

The test day closest to observation days for individual cow somatic cell counts from DHI (Dairy Records Management Systems, Raleigh, NC) were used to classify SCC the week of predicted estrus. Cows with less than 200,000 somatic cells were classified as low. Cows 200,000 somatic cells or more were classified as high.

Statistical Analysis

The MEANS procedure of SAS® 9.3 (SAS® Institute, Inc., Cary, NC) was used to determine the frequency of all recorded parameters (Table 3.1) on a daily basis. Cows and dates with less than 80% of the data for the day before estrus and day of standing behavior were removed from the final analysis by each device parameter. The day of estrus for each cow was classified within the same calendar day as standing or visual observation. Cows that did not stand during visual observation were given the date of the second day of visual observation, predicted day of estrus. Cows confirmed by progesterone patterns and ultrasound that were not in estrus and did not ovulate were removed from the final analysis.

The EXPAND procedure of SAS® 9.3 was used to create a baseline using the backward average of the 7 days before the day of estrus for all parameters (Table 3.1) measured by all AED devices. The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows:

$$(\text{estrus measurement} - \text{baseline measurement}) / \text{baseline measurement} \times 100$$

A one-way ANOVA and the LSMEANS of percent changes for each parameters using the GLM procedure of SAS® 9.3 was used to analyze the independent effects on expression of estrus including: first 21 d disease status (subclinical ketosis, clinical metritis, subclinical hypocalcemia, number of diseases, and any disease); locomotion (abduction and adduction, general symmetry, tracking, and gait score average); body condition (score at estrus and change in BCS from calving to estrus); SCC (low or high);

season (cool or warm); and parity (primiparous or multiparous). Each parameter percent change from the AED devices was used as a dependent variable of each independent effect. The LSMEANS of each parameter percent change and ANOVA p-value were used to determine significance between classifications of each effect.

Standing to be mounted and expression of visual estrous behavior, was analyzed for association with each independent effect: first 21 d disease status (subclinical ketosis, clinical metritis, subclinical hypocalcemia, number of diseases, and any disease); gait (abduction and adduction, general symmetry, tracking, and gait score average); BCS (score at estrus and change in BCS from calving to estrus); SCC (low or high); season (cool defined < 68 THI or warm defined ≥ 68 THI); and parity (primiparous or multiparous) using the FREQ procedure of SAS® 9.3 and Fisher's exact test.

RESULTS AND DISCUSSION

Eighty-five cows (86%, n = 99) were classified in estrus identified using temporal progesterone patterns (Figure 1). The number of cows with each parameter measured by a precision dairy technology varies due to broken tags or system failure (Tables 3.4 to3.19). Seventeen cows (n = 56) were classified with subclinical ketosis. Forty-five cows were classified with clinical metritis. None of the parameters measured by AED devices were significantly different (P > 0.05) among cows with subclinical ketosis or clinical metritis and without either disease (Tables 3.4 to 3.7). Both subclinical ketosis and clinical metritis cows had numerically significant differences from healthy cows of 10% to 15% in most parameters (Tables 3.4 to 3.7). A large numeric difference in percent change on day of estrus may result in a false negative when determining efficacy of a system. Fourichon et al. (2000) reported effects of metritis on reproductive performance with 7 more days to first service. These effects may affect conception rates more than estrus detection rates. In the same meta-analysis, cows with clinical metritis had 2 to 3 more days to first service than healthy cows.

Only 12 cows (n = 56) were classified with subclinical hypocalcemia. Seventy papers in a meta-analysis on the effects of disease on reproduction, effects of subclinical milk fever were not significant for any fertility measures (Fourichon et al., 2000). Percent change in maximum vaginal temperature (P = 0.03) and ear skin temperature (P = 0.03) were the only parameters statistically different among cows with and without subclinical hypocalcemia. Cows with subclinical hypocalcemia had a 1.07% ± 0.33% increase in maximum vaginal temperature and 4.04% ± 7.3% decrease in ear skin temperature on the day of estrus. Comparatively, cows without hypocalcemia had a 0.23% ± 0.18% and 15.38% ± 4.51% increase for maximum vaginal temperature and ear skin temperature respectively on the day of estrus. Similar numerical differences were found in activity measures and rumination time as subclinical ketosis and clinical metritis (Tables 3.8 and 3.9).

Percent change in milk yield was the only parameter measured with a significant difference of 7.92% ± 2.89% ($P = 0.04$) percent change at estrus between cows without any early postpartum diseases and cows with at least one early postpartum disease. Milk yields can decrease due to early postpartum diseases (Collard et al., 2000). Decreases in milk yields are common among cows with negative effects of negative energy balance (Collard et al., 2000; de Vries et al., 2000). No parameters were statistically different among cows with any disease or no disease.

All measures of activity and lying behaviors were significantly lesser ($P < 0.05$) among cows with different numbers of early postpartum diseases (Table 3.12). All percent changes in number of steps per day were significantly ($P < 0.05$) lower for cows with 3 early postpartum diseases. Cows often have more than one early postpartum disease at once causing decrease in conception rate and days to first service (Lopez-Gautius et al., 2005; LeBlanc et al., 2002). Rumination time, eating time, and all temperature percent changes on the day of estrus were also significantly less for cows with 3 early postpartum diseases (Table 3.13).

Lame cows were not significantly different but numerically different in all parameters at estrus, regardless of the gait aspect used to classify cows as lame or sound (Tables 3.14 to 3.19). Lame cows walk with an arched back and irregular steps (Maertens et al., 2011) which may explain the numerical difference in number of steps per day for lame cows (Tables 3.14, 3.16, 3.18). Twenty four cows of 99 cows total were classified as lame using abduction and adduction or tracking (Table 3.20). Only 23 cows were classified as lame using general symmetry (Table 3.20). No significant differences ($P > 0.05$) in the number of cows standing or not standing exist among any classifications of effects on estrus. Cows were not balanced on classification of disease, locomotion, season, SCC, or BCS to determine specific cause of percent changes. Cows could have had multiple effects that lead to their decrease or increase in percent change of any parameter on the day of estrus.

CONCLUSIONS

Cow variation in health status explain false negative alerts from automated estrous detection systems if a certain level of percent change is required to create an alert for estrus. Parameters measured were not conclusive for significant differences among immunosuppressed cows and healthy cows. Significant differences found may be due to unequal sample sizes among classifications of disease, BCS, SCC, and locomotion. Further research is needed to determine adjustments to algorithms for cows of less than desired health status or parity.

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Table 3.1. Parameters measured and recorded by automated estrous detection devices for cows synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N=109).[1]

Automated Estrous Detection System	Parameters Measured	Frequency of measurements	Frequency of reporting data
AfiAct Pedometer Plus, Afimilk, Kibbutz Afikim, Israel	Activity (steps) Lying time (min) Lying bouts	Continuously	Per hour
Afimilk MPC Analyzer Afimilk, Kibbutz Afikim, Israel	Milk yield (lbs) Milk flow Milk conductivity	Each milking	End of milking
CowManager SensoOr, Agis Automatisering, Harmelen, Netherlands	Rumination time (min) Eating time (min) Time not active (min) Time active (min) Time high active (min)	Every minute	Every hour
CowScout S Leg, GEA Farm Technologies GmbH, Bönen, Germany	Activity (steps)	Continuously	15 minute intervals
DVM bolus, DVM Systems, LLC, Greeley, CO	Reticulorumen temperature (◦C)	Every 5 minutes	Hourly
HR Tag, SCR Engineers Ltd., Netanya, Israel	Neck Activity Rumination time (min)	Continuously	Every 2 hours
IceQube, IceRobotics Ltd., Edinburgh, Scotland	Lying time (min) Steps Motion index Lying bouts Bout duration (min)	Continuously	15 minute intervals
Thermochron iButton, Embedded Data Systems, Kentucky, USA	Temperature[2]	Every 5 minutes	Every 5 minutes
Track a)) Cow, ENGS Systems Innovative Dairy Solutions, Israel	Activity unit Lying time (min) Lying bouts Bout duration (min) Time spent at feed bunk	Continuously	Every 5 minutes

[1]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.

[2]Thermochron iButtons were attached to an intravaginal device to continuously take vaginal temperature a week before and a week after estrus in cows

Table 3.2. Means of parameters measured by automated estrous detection systems for cows synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N = 94).[1, 2]

Automated estrous detection device parameters	Automated estrous detection System[3]	Mean ± SD
Activity (steps/d)	AfiAct Pedometer Plus	3827.10 ± 2901.71
Activity (steps/d)	CowScout S Leg	4410.24 ± 1815.18
Activity (steps/d)	Track a)) Cow	2269.55 ± 990.79
Activity (steps/d)	IceQube	1137.71 ± 612.63
Motion index	IceQube	42.93 ± 22.72
Active time (min/d)	SensoOr	56.82 ± 27.24
High activity	SensoOr	52.32 ± 39.74
Neck activity	HR Tag	414.79 ± 136.85
Lying time (h/d)	AfiAct Pedometer Plus	8.90 ± 2.86
Lying bouts	AfiAct Pedometer Plus	9.17 ± 4.01
Lying time (h/d)	IceQube	9.11 ± 2.76
Lying bouts	IceQube	16.22 ± 7.48
Bout duration (min/ bout)	IceQube	39.77 ± 31.08
Time not active (h/d)	SensoOr	6.69 ± 2.22
Lying time (min/d)	Track a)) Cow	562.97 ± 178.53
Lying bouts	Track a)) Cow	10.65 ± 5.59
Rumination time (h/d)	SensoOr	9.18 ± 1.93
Rumination time (h/d)	HR Tag	7.81 ± 1.39
Eating time (h/d)	SensoOr	3.48 ± 1.55
Intake visits	Track a)) Cow	8.59 ± 4.32
Time at feedbunk (min/d)	Track a)) Cow	173.46 ± 90.99
Mean vaginal temperature °C	Thermochron iButton	38.98 ± 0.47
Max vaginal temperature °C	Thermochron iButton	39.73 ± 1.34
Ear skin temperature °C	SensoOr	22.22 ± 6.57
Reticulorumen temperature °C	DVM bolus	39.02 ± 0.38
Milk yield (kg/d)	Afimilk MPC Analyzer	37.73 ± 9.79

[1]Means of parameters using all 28 days of study protocol

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.

[2]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solution)

Table 3.3. Means of parameters recorded for protocol period (28 days) by precision automated estrous detection systems for cows synchronized with a modified G7G Ovsynch protocol that ovulated and in estrus verified by temporal progesterone patterns (N=84).[1]

Parameter	Mean % change ± SD	Number of Cows
DIM at estrus	85.5 ± 11.4	99
Somatic cell count (cells/mL)	236434.34 ± 557733.63	99
Estrus BCS	2.71 ± 0.28	99
Change[5] in BCS	-0.14 ± 0.38	99
Abduction and adduction score	1.78 ± 0.84	97
General symmetry score	1.81 ± 0.85	97
Tracking score	2.2 ± 0.90	97
Max THI at estrus	56.25 ± 15.15	91

[1]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Progesterone radioimmunoassay were completed with blood plasma.

Table 3.4. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows with subclinical ketosis and no subclinical ketosis for first 21DIM.[1, 2]

Activity

AED device parameters	AED system[3]	Number of cows	Subclinical ketosis Mean % change ± SD	No subclinical ketosis Mean % change ± SD	P-value
Steps per day	AfiAct Pedometer Plus	48	65.12 ± 22.88	90.89 ± 14.68	0.35
Steps per day	CowScout S Leg	38	14.24 ± 4.14	14.17 ± 2.48	0.99
Steps per day	Track a)) Cow	41	98.43 ± 22.93	102.08 ± 15.63	0.90
Motion index	IceQube	41	84.38 ± 35.92	162.58 ± 21.75	0.16
Active time (min/d)	SensoOr	37	23.35 ± 14.51	32.71 ± 10.05	0.07
High activity	SensoOr	37	194.07 ± 58.67	261.07 ± 40.64	0.60
Intake visits per day	Track a)) Cow	32	55.4 ± 46.58	71.61 ± 29.14	0.35
Neck activity	HR Tag	15	15.05 ± 12.56	43.59 ± 10.26	0.77

Lying

AED device parameters	AED system[3]	Number of cows	Subclinical ketosis Mean % change ± SD	No subclinical ketosis Mean % change ± SD	P-value
Lying time (min/d)	AfiAct Pedometer Plus	48	-4.94 ± 10.37	-23.68 ± 6.65	0.13
Lying bouts per day	AfiAct Pedometer Plus	48	-25.45 ± 14.04	-12.14 ± 9.01	0.43
Lying time (min/d)	IceQube	41	-3.66 ± 9.82	-27.33 ± 5.95	0.05
Lying bouts per day	IceQube	41	-20.2 ± 8.69	-21.76 ± 5.26	0.88
Bout duration (min/bout)	IceQube	41	-3.66 ± 9.82	-27.33 ± 5.95	0.05
Time not active (min/d)	SensoOr	37	-19.11 ± 10.21	-36.33 ± 7.08	0.17

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Estrus was synchronized in lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.5. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows with subclinical ketosis and no subclinical ketosis for first 21DIM.[1, 2]

AED device parameters	AED system[3]	Number of cows	Subclinical ketosis Mean % change ± SD	No subclinical ketosis Mean % change ± SD	P-value
Rumination					
Rumination time (min/d)	SensoOr	37	-18.97 ± 4.84	-24.74 ± 0.03	0.33
Rumination time (min/d)	HR Tag	15	-10.83 ± 7.49	-14.17 ± 6.11	0.74
Temperature					
AED device parameters	**AED system[3]**	**Number of cows**	**Subclinical ketosis Mean % change ± SD**	**No subclinical ketosis Mean % change ± SD**	**P-value**
Average vaginal temperature	Thermochron iButton	32	0.31 ± 0.15	0.31 ± 0.09	1.00
Max vaginal temperature	Thermochron iButton	32	0.43 ± 0.34	0.41 ± 0.19	0.96
Ear skin temperature	SensoOr	29	14.25 ± 7.91	8.42 ± 4.88	0.54
Reticulorumen temperature	DVM bolus	28	22.16 ± 16.82	30.14 ± 9.71	0.68
Other					
AED device parameters	**AED system[3]**	**Number of cows**	**Subclinical ketosis Mean % change ± SD**	**No subclinical ketosis Mean % change ± SD**	**P-value**
Milk yield (kg/d)	Afimilk MPC Analyzer	48	-7.92 ± 2.89	-0.7 ± 1.85	0.04
Eating time (min/d)	SensoOr	37	57.97 ± 18.34	62.06 ± 12.7	0.86

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.6. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (AED) systems for synchronized dairy cows with clinical metritis and no clinical metritis for first 21DIM.[1, 2]

Activity

AED device parameters	AED system[3]	Number of cows	Clinical metritis Mean % change ± SD	No clinical metritis Mean % change ± SD	P-value
Steps per day	AfiAct Pedometer Plus	48	80.7% ± 14%	93.52% ± 27.28%	0.68
Steps per day	CowScout S Leg	38	14.18% ± 2.48%	14.21% ± 4.14%	1.00
Steps per day	Track a)) Cow	41	105.36% ± 14.08%	79.35% ± 31.03%	0.45
Motion index	IceQube	41	138.81% ± 21.29%	155.14% ± 46.92%	0.75
Active time (min/d)	SensoOr	37	29.44% ± 9.06%	30.92% ± 20.6%	0.95
High activity	SensoOr	37	259.5% ± 35.97%	135.17% ± 81.77%	0.17
Intake visits per day	Track a)) Cow	32	46.25% ± 25.16%	179.4% ± 58.47%	0.05
Neck activity	HR Tag	15	30.55% ± 9.83%	38.71% ± 19.67%	0.72

Lying

AED device parameters	AED system[3]	Number of cows	Clinical metritis Mean % change ± SD	No clinical metritis Mean % change ± SD	P-value
Lying time (min/d)	AfiAct Pedometer Plus	48	-14.88% ± 6.36%	-30.89% ± 12.39%	0.26
Lying bouts per day	AfiAct Pedometer Plus	48	-16.14% ± 8.58%	-15.59% ± 16.72%	0.98
Lying time (min/d)	IceQube	41	-18.66% ± 5.81%	-32.23% ± 12.81%	0.34
Lying bouts per day	IceQube	41	15.2% ± 4.93%	-25.3% ± 10.87%	0.69
Bout duration (min/bout)	IceQube	41	138.81% ± 5.81%	-32.23% ± 12.81%	0.34
Time not active (min/d)	SensoOr	37	-20.53% ± 6.2%	-5.66% ± 14.09%	0.06
Lying time (min/d)	Track a)) Cow	38	-18.66% ± 5.33%	-3.98% ± 12.31%	0.39
Lying bouts per day	Track a)) Cow	38	-35.6% ± 6.85%	17.78% ± 15.82%	0.49
Lying percent	Track a)) Cow	31	-23.31% ± 5.58%	-20.23% ± 17.05%	0.70

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.7. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows with clinical metritis and no clinical metritis for first 21DIM.[1, 2]

Rumination

AED device parameters	AED system[4]	Number of cows	Clinical metritis Mean % change ± SD	No clinical metritis Mean % change ± SD	P-value
Rumination time (min/d)	SensoOr	37	-23.31% ± 3.05%	-20.62% ± 6.93%	0.72
Rumination time (min/d)	HR Tag	15	-9.09% ± 4.78%	-27.82% ± 9.57%	0.10

Temperature

AED device parameters	AED system[4]	Number of cows	Clinical metritis Mean % change ± SD	No clinical metritis Mean % change ± SD	P-value
Average vaginal temperature	Thermochron iButton	32	0.25% ± 0.09%	0.44% ± 0.14%	0.29
Max vaginal temperature	Thermochron iButton	32	0.26% ± 0.19%	0.82% ± 0.3%	0.12
Ear skin temperature	SensoOr	29	12.47% ± 4.58%	0.66% ± 8.97%	0.25
Reticulorumen temperature	DVM bolus	28	0.27% ± 0.09%	0.34% ± 0.18%	0.72

Other

AED device parameters	AED system[4]	Number of cows	Clinical metritis Mean % change ± SD	No clinical metritis Mean % change ± SD	P-value
Milk yield (kg/day)	Afimilk MPC Analyzer	48	-3.21% ± 1.83%	-1.3% ± 3.57%	0.64
Eating time (min/d)	SensoOr	37	64.36% ± 11.31%	42% ± 25.71%	0.43

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.8. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows with subclinical hypocalcemia and no hypocalcemia for first 21DIM.[1, 2]

Activity					
AED device parameters	**AED system[3]**	**Number of cows**	**Subclinical hypocalcemia Mean % change ± SD**	**No subclinical hypocalcemia Mean % change ± SD**	**P-value**
Steps	Pedometer Plus	48	77.95% ± 27.32%	84.8% ± 14.01%	0.82
Steps	CowScout S Leg	38	11.17% ± 4.1%	15.27% ± 2.45%	0.40
Steps	Track a)) Cow	41	13.26% ± 7.88%	18.12% ± 4.18%	0.59
Motion index	IceQube	41	147.47% ± 41.42%	139.95% ± 21.97%	0.87
Active time (min/d)	SensoOr	37	37.43% ± 16.76%	27.18% ± 9.5%	0.60
High activity	SensoOr	37	201.81% ± 68.2%	251.4% ± 38.67%	0.53
Intake visits	Track a)) Cow	32	167.1% ± 53.42%	43.96% ± 25.66%	0.05
Neck activity	HR Tag	15	89.38% ± 29.17%	103.72% ± 14.36%	0.66

Lying					
AED device parameters	**AED system[3]**	**Number of cows**	**Subclinical hypocalcemia Mean % change ± SD**	**No subclinical hypocalcemia Mean % change ± SD**	**P-value**
Lying time (min/d)	Pedometer Plus	48	-19.13% ± 12.57%	-17.97% ± 6.45%	0.93
Lying bouts	Pedometer Plus	48	-20.07% ± 16.71%	-14.96% ± 8.57%	0.79
Lying time (min/d)	IceQube	41	-19.57% ± 11.43%	-21.37% ± 6.06%	0.89
Lying bouts	IceQube	41	-22.82% ± 9.6%	-20.93% ± 5.09%	0.86
Bout duration (min/bout)	IceQube	41	-19.57% ± 11.43%	-21.37% ± 6.06%	0.89
Time not active Bout duration (min/d)	SensoOr	37	-19.37% ± 11.91%	-34.4% ± 6.75%	0.28
Lying time Bout duration (min/bout)	Track a)) Cow	38	-4.73% ± 11.39%	-15.8% ± 5.41%	0.39
Lying bouts per day	Track a)) Cow	38	29.52% ± 14.74%	27.52% ± 7.01%	0.90

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.9. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows with subclinical hypocalcemia and no subclinical hypocalcemia for first 21DIM.[1, 2]

Rumination

AED device parameters	AED system[3]	Number of cows	Subclinical hypocalcemia Mean % change ± SD	No subclinical hypocalcemia Mean % change ± SD	P-value
Rumination time (min/d)	SensoOr	37	-18.29% ± 5.6%	-24.34% ± 3.17%	0.35
Rumination time (min/d)	HR Tag	15	-14.06% ± 8.23%	-12.22% ± 5.82%	0.86

Temperature

AED device parameters	AED system[3]	Number of cows	Subclinical hypocalcemia Mean % change ± SD	No subclinical hypocalcemia Mean % change ± SD	P-value
Average vaginal temperature	Thermochron iButton	32	0.54% ± 0.16%	0.24% ± 0.08%	0.11
Max vaginal temperature	Thermochron iButton	32	1.07% ± 0.33%	0.23% ± 0.18%	0.03
Ear skin temperature	SensoOr	29	-4.04% ± 7.3%	15.38% ± 4.51%	0.03
Reticulorumen temperature	DVM bolus	28	0.49% ± 44.29%	29.17% ± 8.52%	0.53

Other

AED device parameters	AED system[3]	Number of cows	Subclinical hypocalcemia Mean % change ± SD	No subclinical hypocalcemia Mean % change ± SD	P-value
Milk yield (kg/d)	Afimilk MPC Analyzer	48	-1.09% ± 3.56%	-3.26% ± 1.83%	0.59
Eating time (min/d)	SensoOr	37	35.1% ± 20.59%	68.97% ± 11.67%	0.16

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.10. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows with any early postpartum disease and no early postpartum disease for first 21DIM.[1, 2, 3]

Activity

AED device parameters	AED system[4]	Number of cows	Early Postpartum Disease Mean % change ± SD	No early postpartum disease Mean % change ± SD	P-value
Steps per day	AfiAct Pedometer Plus	48	65.12 ± 22.88	90.89 ± 14.68	0.35
Steps per day	CowScout S Leg	38	14.24 ± 4.14	14.17 ± 2.48	0.99
Steps per day	Track a)) Cow	41	98.43 ± 22.93	102.08 ± 15.63	0.90
Motion index	IceQube	41	84.38 ± 35.92	162.58 ± 21.75	0.16
Active time (min/d)	SensoOr	37	23.35 ± 14.51	32.71 ± 10.05	0.07
High activity	SensoOr	37	194.07 ± 58.67	261.07 ± 40.64	0.60
Intake visits oer day	Track a)) Cow	32	55.4 ± 46.58	71.61 ± 29.14	0.35
Neck activity	HR Tag	15	15.05 ± 12.56	43.59 ± 10.26	0.77

Lying

AED device parameters	AED system[4]	Number of cows	Early Postpartum Disease Mean % change ± SD	No early postpartum disease Mean % change ± SD	P-value
Lying time (min/d)	AfiAct Pedometer Plus	48	-4.94 ± 10.37	-23.68 ± 6.65	0.13
Lying bouts per day	AfiAct Pedometer Plus	48	-25.45 ± 14.04	-12.14 ± 9.01	0.43
Lying time (min/d)	IceQube	41	-3.66 ± 9.82	-27.33 ± 5.95	0.05
Lying bouts per day	IceQube	41	-20.2 ± 8.69	-21.76 ± 5.26	0.88
Bout duration (min/bout)	IceQube	41	-3.66 ± 9.82	-27.33 ± 5.95	0.05
Time not active (min/d)	SensoOr	37	-19.11 ± 10.21	-36.33 ± 7.08	0.17

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[3]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[4]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.11. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows with any early postpartum disease and no early postpartum disease for first 21DIM.[1, 2, 3]

AED device parameters	AED system[4]	Number of cows	Early Postpartum Disease Mean % change ± SD	No early postpartum disease Mean % change ± SD	P-value
Rumination					
Rumination time (min/d)	SensoOr	37	-18.97 ± 4.84	-24.74 ± 0.03	0.33
Rumination time (min/d)	HR Tag	15	-10.83 ± 7.49	-14.17 ± 6.11	0.74
Temperature					
Average vaginal temperature	Thermochron iButton	32	0.31 ± 0.15	0.31 ± 0.09	1.00
Max vaginal temperature	Thermochron iButton	32	0.43 ± 0.34	0.41 ± 0.19	0.96
Ear skin temperature	SensoOr	29	14.25 ± 7.91	8.42 ± 4.88	0.54
Reticulorumen temperature	DVM bolus	28	22.16 ± 16.82	30.14 ± 9.71	0.68
Other					
Milk yield	Afimilk MPC Analyzer	48	-7.92 ± 2.89	-0.7 ± 1.85	0.04
Eating time (min/d)	SensoOr	37	57.97 ± 18.34	62.06 ± 12.7	0.86

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.12. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows classified with any early postpartum disease and no early postpartum disease for first 21DIM.[1, 2, 3]

Activity

AED device parameters	AED system[4]	Number of cows	No Disease Mean % change ± SD	1 Disease Mean % change ± SD	2 Diseases Mean % change ± SD	3 Diseases Mean % change ± SD
Steps	Pedometer Plus	48	112.92% ± 32.83%	77.49% ± 17.37%	91.09% ± 26.19%	54.45% ± 38.84%
Steps	CowScout S Leg	38	-35.08% ± 14.99%	-19.54% ± 7.93%	-7.55% ± 11.95%	-11.41% ± 17.73%
Steps	Track a)) Cow	41	-5.63% ± 20.34%	-15.57% ± 10.76%	-21.87% ± 16.23%	-19.99% ± 24.07%
Motion index	IceQube	41	172.08% ± 56.32%	149.86% ± 26.26%	120.84% ± 44.52%	106.32% ± 56.32%
Active time (min/d)	SensoOr	37	-21.08% ± 13.2%	-22.69% ± 6.15%	-17.46% ± 10.43%	-21.61% ± 13.2%
High activity	SensoOr	37	-38.01% ± 14.88%	-24.93% ± 6.94%	-1.52% ± 11.76%	-16.87% ± 14.88%
Intake visits	Track a)) Cow	32	-11.69% ± 17.84%	-40.45% ± 8.19%	-25.33% ± 11.89%	-18.85% ± 15.96%
Neck activity	HR Tag	15	-16.97% ± 8.23%	-26% ± 3.78%	-25.81% ± 5.49%	-10.41% ± 7.36%

Lying

AED device parameters	AED system[4]	Number of cows	No Disease Mean % change ± SD	1 Disease Mean % change ± SD	2 Diseases Mean % change ± SD	3 Diseases Mean % change ± SD
Lying time (min/d)	Pedometer Plus	48	-35.08% ± 14.99%	-19.54% ± 7.93%	-7.55% ± 11.95%	-11.41% ± 17.73%
Lying bouts	Pedometer Plus	48	-5.63% ± 20.34%	-15.57% ± 10.76%	-21.87% ± 16.23%	-19.99% ± 24.07%
Lying time (min/d)	IceQube	41	-2.39% ± 4.06%	0.02% ± 2.15%	-10.16% ± 3.24%	-1.37% ± 4.8%
Lying bouts	IceQube	41	17.05% ± 5.05%	13.16% ± 3.07%	15.5% ± 5.05%	12.28% ± 5.98%
Bout duration (min)	IceQube	41	0.38% ± 0.18%	0.22% ± 0.1%	0.51% ± 0.18%	0.25% ± 0.25%
Lying time (min/d)	Track a)) Cow	41	-5.44% ± 15.1%	-21.21% ± 6.75%	-6.87% ± 9.55%	-2.12% ± 15.1%
Lying bouts	Track a)) Cow	41	18.32% ± 19.94%	28.12% ± 8.92%	27.51% ± 12.61%	37.22% ± 19.94%
Time not active (min)	SensoOr	37	0.77% ± 0.38%	0.21% ± 0.23%	0.37% ± 0.38%	0.98% ± 0.54%

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[3]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[4]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.13. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows classified with any early postpartum disease and no early postpartum disease for first 21DIM.[1, 2, 3]

AED device parameters	AED system[4]	Number of cows	No Disease Mean % change ± SD	1 Disease Mean % change ± SD	2 Diseases Mean % change ± SD	3 Diseases Mean % change ± SD
Rumination						
Rumination time (min/d)	SensoOr	37	-16.97% ± 8.23%	-26% ± 3.78%	-25.81% ± 5.49%	-10.41% ± 7.36%
Rumination time (min/d)	HR Tag	15	60.68% ± 31.97%	61.04% ± 14.67%	76.38% ± 21.31%	31.48% ± 28.59%
Temperature						
Average vaginal temperature ˚C	Thermochron iButton	32	0.38% ± 0.18%	0.22% ± 0.1%	0.51% ± 0.18%	0.25% ± 0.25%
Max vaginal temperature ˚C	Thermochron iButton	32	0.77% ± 0.38%	0.21% ± 0.23%	0.37% ± 0.38%	0.98% ± 0.54%
Ear skin temperature ˚C	SensoOr	29	-38.01% ± 14.88%	-24.93% ± 6.94%	-1.52% ± 11.76%	-16.87% ± 14.88%
Reticulorumen temperature ˚C	DVM bolus	28	30.63% ± 10.34%	18.87% ± 4.82%	5.08% ± 8.18%	14.22% ± 10.34%
Other						
Milk yield (kg/d)	MPC Analyzer	48	-2.39% ± 4.06%	0.02% ± 2.15%	-10.16% ± 3.24%[a]	-1.37% ± 4.8%
Eating time (min/d)	SensoOr	37	60.68% ± 31.97%	61.04% ± 14.67%	76.38% ± 21.31%	31.48% ± 28.59%

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.14. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows classified **lame or sound** using the general symmetry aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

AED device parameters	AED system[4]	Number of cows	Lame Mean % change ± SD	Sound Mean % change ± SD	P-value
Activity					
Steps	AfiAct Pedometer Plus	85	66.72% ± 20.57%	77.57% ± 9.59%	0.63
Steps	CowScout S Leg	73	-30.05% ± 8.58%	-17.2% ± 4%	0.18
Steps	Track a)) Cow	70	103.4% ± 26.7%	91.27% ± 12.25%	0.68
Motion index	IceQube	73	102.23% ± 34.53%	122.34% ± 15.44%	0.60
Active time	SensoOr	49	-17.08% ± 8.04%	-15.95% ± 3.6%	0.90
High activity	SensoOr	49	-33.92% ± 8.49%	-18.6% ± 3.79%	0.10
Intake visits	Track a)) Cow	47	-35.77% ± 10.09%	-27.53% ± 5.43%	0.48
Neck activity	HR Tag	28	1542.1% ± 2052.14%	3225.45% ± 725.54%	0.45
Lying					
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Lying time	AfiAct Pedometer Plus	85	-30.05% ± 8.58%	-17.2% ± 4%	0.18
Lying bouts	AfiAct Pedometer Plus	85	-22.21% ± 11.05%	-15.1% ± 5.15%	0.56
Lying time	IceQube	73	-33.89% ± 8.48%	-18.6% ± 3.79%	0.10
Lying bouts	IceQube	73	25.12% ± 5.74%	13.05% ± 2.57%	0.06
Bout duration	IceQube	73	102.23% ± 34.53%	122.34% ± 15.44%	0.60
Lying time	Track a)) Cow	65	-28.15% ± 8.47%	-12.46% ± 4.03%	0.10
Lying bouts	Track a)) Cow	65	5.61% ± 11.05%	23.57% ± 5.26%	0.15
Time not active	SensoOr	49	-35.77% ± 10.09%	-27.53% ± 5.43%	0.48

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[3]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[4]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.15. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows classified **lame or sound** using the general symmetry aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

AED device parameters	AED system[4]	Number of cows	Lame Mean % change ± SD	Sound Mean % change ± SD	P-value
Rumination					
Rumination time (min/d)	SensoOr	49	-19.30% ± 4.97%	-21.57% ± 2.68%	0.69
Rumination time (min/d)	HR Tag	28	74.86% ± 17.96%	55.81% ± 9.66%	0.35
Temperature					
Average vaginal temperature	Thermochron iButton	59	0.49% ± 0.17%	0.25% ± 0.07%	0.21
Max vaginal temperature	Thermochron iButton	59	0.92% ± 0.35%	0.45% ± 0.15%	0.22
Ear skin temperature	SensoOr	41	-33.89% ± 8.48%	-18.6% ± 3.79%	0.10
Reticulorumen temperature	DVM bolus	50	25.12% ± 5.74%	13.05% ± 2.57%	0.06
Other					
Milk yield (kg/d)	Afimilk MPC Analyzer	85	-9.34% ± 2.83%	-2.48% ± 1.32%	0.03
Eating time (min/d)	SensoOr	49	74.86% ± 17.96%	55.81% ± 9.66%	0.35

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.16. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows classified **lame or sound** using the tracking aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

AED device parameters	AED system[4]	Number of cows	Lame Mean % change ± SD	Sound Mean % change ± SD	P-value
Activity					
Steps per day	AfiAct Pedometer Plus	85	63.9% ± 18.24%	79.07% ± 9.86%	0.47
Steps per day	CowScout S Leg	73	-15.86% ± 7.69%	-20.55% ± 4.16%	0.59
Steps per day	Track a)) Cow	70	-5.58% ± 9.74%	-19.53% ± 5.27%	0.21
Motion index	IceQube	73	135.65% ± 28.98%	113.84% ± 16.11%	0.51
Active time (min/d)	SensoOr	49	27.84% ± 13.92%	25.56% ± 7.93%	0.89
High activity	SensoOr	49	295.67% ± 58.09%	205.37% ± 33.08%	0.18
Intake visits per day	Track a)) Cow	47	1.7% ± 6.22%	10.22% ± 3.53%	0.24
Neck activity	HR Tag	28	15.31% ± 37.3%	71.6% ± 19.39%	0.19
Lying					
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Lying time (min/d)	AfiAct Pedometer Plus	85	-15.86% ± 7.69%	-20.55% ± 4.16%	0.59
Lying bouts per day	AfiAct Pedometer Plus	85	-5.58% ± 9.74%	-19.53% ± 5.27%	0.21
Lying time (min/d)	IceQube	73	-4.5% ± 2.58%	-3.47% ± 1.4%	0.72
Lying bouts	IceQube	73	9.05% ± 2.89%	12.88% ± 1.61%	0.25
Bout duration (min/bout)	IceQube	73	0.39% ± 0.14%	0.26% ± 0.08%	0.45
Lying time (min/d)	Track a)) Cow	65	0.75% ± 0.3%	0.46% ± 0.16%	0.38
Lying bouts per day	Track a)) Cow	65	-25.71% ± 7.24%	-19.74% ± 4.02%	0.47
Time not active (min/d)	SensoOr	49	-38.58% ± 9.59%	-26.4% ± 5.46%	0.28

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[3]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[4]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.17. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows classified **lame or sound** using the tracking aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

			Rumination		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Rumination time (min/d)	SensoOr	49	-23.56% ± 4.75%	-20.25% ± 2.71%	0.55
Rumination time (min/d)	HR Tag	28	-1440.13% ± 634.16%	-1004.88% ± 338.97%	0.55
			Temperature		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Average vaginal temperature	Thermochron iButton	59	0.39% ± 0.14%	0.26% ± 0.08%	0.45
Max vaginal temperature	Thermochron iButton	59	0.75% ± 0.3%	0.46% ± 0.16%	0.38
Ear skin temperature	SensoOr	41	-25.71% ± 7.24%	-19.74% ± 4.02%	0.47
Reticulorumen temperature	DVM bolus	50	18.8% ± 4.92%	13.9% ± 2.73%	0.39
			Other		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Milk yield (kg/d)	Afimilk MPC Analyzer	85	-4.5% ± 2.58%	-3.47% ± 1.4%	0.72
Eating time (min/d)	SensoOr	49	70.72% ± 17.26%	56.64% ± 9.83%	0.48

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.18. Mean percent changes for day of estrus compared to 7d backward average (baseline) for activity and lying parameters measured by automated estrous detection (**AED**) systems for synchronized dairy cows classified **lame or sound** using the abduction and adduction aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

Activity

AED device parameters	AED system[4]	Number of cows	Lame Mean % change ± SD	Sound Mean % change ± SD	P-value
Steps per day	AfiAct Pedometer Plus	85	63.9% ± 18.24%	79.07% ± 9.86%	0.47
Steps per day	CowScout S Leg	73	-15.86% ± 7.69%	-20.55% ± 4.16%	0.59
Steps per day	Track a)) Cow	70	-5.58% ± 9.74%	-19.53% ± 5.27%	0.21
Motion index	IceQube	73	135.65% ± 28.98%	113.84% ± 16.11%	0.51
Active time (min/d)	SensoOr	49	27.84% ± 13.92%	25.56% ± 7.93%	0.89
High activity	SensoOr	49	295.67% ± 58.09%	205.37% ± 33.08%	0.18
Intake visits per day	Track a)) Cow	47	15.31% ± 37.3%	71.6% ± 19.39%	0.19
Neck activity	HR Tag	28	-22.4% ± 7.68%	-13.25% ± 4.21%	0.30

Lying

AED device parameters	AED system[4]	Number of cows	Lame Mean % change ± SD	Sound Mean % change ± SD	P-value
Lying time (min/d)	AfiAct Pedometer Plus	85	-15.86% ± 7.69%	-20.55% ± 4.16%	0.59
Lying bouts per day	AfiAct Pedometer Plus	85	-5.58% ± 9.74%	-19.53% ± 5.27%	0.21
Lying time (min/d)	IceQube	73	-4.5% ± 2.58%	-3.47% ± 1.4%	0.72
Lying bouts per day	IceQube	73	9.05% ± 2.89%	12.88% ± 1.61%	0.25
Bout duration (min/bout)	IceQube	73	0.39% ± 0.14%	0.26% ± 0.08%	0.45
Lying time (min/d)	Track a)) Cow	65	0.75% ± 0.3%	0.46% ± 0.16%	0.38
Lying bouts per day	Track a)) Cow	65	-25.71% ± 7.24%	-19.74% ± 4.02%	0.47
Time not active (min/d)	SensoOr	49	-38.58% ± 9.59%	-26.4% ± 5.46%	0.28

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[3]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[4]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.19. Mean percent changes for day of estrus compared to 7d backward average (baseline) for rumination, temperature, milk yield, and eating time parameters measured by automated estrous detection systems for synchronized dairy cows classified **lame or sound** using the abduction and adduction aspect of the Olmos et al. (2008) gait scoring system during estrus.[1, 2, 3]

			Rumination		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Rumination time (min/d)	SensoOr	49	-23.56% ± 4.75%	-20.25% ± 2.71%	0.55
Rumination time (min/d)	HR Tag	28	70.72% ± 17.26%	56.64% ± 9.83%	0.48
			Temperature		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Average vaginal temperature	Thermochron iButton	59	0.39% ± 0.14%	0.26% ± 0.08%	0.45
Max vaginal temperature	Thermochron iButton	59	0.75% ± 0.3%	0.46% ± 0.16%	0.38
Ear skin temperature	SensoOr	41	-25.71% ± 7.24%	-19.74% ± 4.02%	0.47
Reticulorumen temperature	DVM bolus	50	18.8% ± 4.92%	13.9% ± 2.73%	0.39
			Other		
AED device parameters	**AED system[4]**	**Number of cows**	**Lame Mean % change ± SD**	**Sound Mean % change ± SD**	**P-value**
Milk yield (kg/d)	Afimilk MPC Analyzer	85	-4.5% ± 2.58%	-3.47% ± 1.4%	0.72
Eating time (min/d)	SensoOr	49	70.72% ± 17.26%	56.64% ± 9.83%	0.48

[1]The percent change in each parameter on the day of estrus compared to the 7d baseline was calculated as follows: (estrus measurement – baseline measurement) / baseline measurement ×100

[2]Estrus was synchronized in 24 lactating Holstein dairy cows using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus).

[3]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11)

[4]Afimilk MPC Analyzer (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

Table 3.20. Effect of differences in early postpartum disease status and gait classification on visual observation of standing for mounting in dairy cows synchronized with a modified G7G-Ovsynch protocol for detection of estrus.[1, 2, 3, 4, 5]

	Stood		Did not stand			
Effect	Number of cows negative for disease	Number of cows positive for disease	Number of cows negative for disease	Number of cows positive for disease	n	*P-value*
Subclinical ketosis[6]	25	6	14	11	56	0.08
Subclinical hypocalcemia[7]	24	7	20	5	56	1.00
Clinical metritis[8]	6	25	5	20	56	1.00
≥ 1 postpartum disease	4	27	4	21	56	1.00
Effect	Number of cows classified sound	Number of cows classified lame	Number of cows classified sound	Number of cows classified lame	n	*P-value*
General Symmetry	40	9	36	14	99	0.34
Tracking	37	12	38	12	99	1.00
Abduction and Adduction	37	12	38	12	99	1.00

[1] Starting June 2014, physical exams were performed for each cow in the morning at 0730 ± 1h for the first 21 days of lactation. Uterine discharge sample scores, blood samples for Ca, and blood samples for Precision Xtra (Abbott Laboratories, Abbott Park, Illinois) were collected.

[2]Gait scoring (Olmos et al., 2009; O'Callaghan et al., 2003) was performed weekly by the same observer throughout the entire study. Cows were released individually to walk past the observer in an open alley on the way to an exercise lot at approximately 1000. Scores for each gait aspect: abduction and adduction, tracking, spine curvature, head bobbing, speed, and general symmetry were recorded. Cows scored the week of estrus, as 3 or higher for: abduction and adduction, tracking, general symmetry, or gait score average were classified as lame. Cows scored less than 3 for abduction and adduction, tracking, general symmetry, or gait score average were classified as sound.

[3] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4] The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]Metricheck (Simcro Tech Ltd, Hamilton, New Zealand) is a 50-cm-long stainless steel rod with a 4-cm hemisphere of silicon at the end for vaginal insertion to obtain a uterine discharge sample. The uterine discharge scoring system (Sterrett et al., 2013) used was based on visual appearance of sample; score 1: thick, viscous discharge, clear, opaque or red to brown in color, no odor or mild, non-offensive odor; score 2: white or yellow pus, moderate to thick discharge, no odor or mild, non-offensive odor; score 3: pink, red, dark red, or black watery discharge, detectable offensive odor, possibly intolerable. Cows with at least one uterine discharge score ≥2 were classified as clinical metritis cases.

[7]The first blood sample for Ca diagnosis was collected in a 10 ml red-top VACUTAINER® tube containing no anticoagulant. Cows with Ca levels lower than 8 mg/dL of at least one sample (Goff, 2008) were classified as subclinical hypocalcemia cases.

[8]One drop of blood from a 1mL syringe was deposited on the end of a ketone test strip for Precision Xtra BHBA analysis. Cows with a Precision Xtra™ BHBA measurement greater than 1.4 mmol/L of at least one sample were classified as subclinical ketosis cases (Duffield, 1997; Geishauser et al., 2001; Oetzel, 2004).

Table 3.21. Effect of differences in Max THI, parity, and SCC on visual observation of standing for mounting in dairy cows synchronized with a modified G7G-Ovsynch protocol for detection of estrus.[1, 2, 3, 4, 5]

	Stood		Did not stand			
Effect	Number of cool cows	Number of warm cows	Number of cool cows	Number of warm cows	n	*P-value*[6]
Max THI	34	11	27	19	91	0.12
Effect	Number of primiparous cows	Number of multiparous cows	Number of primiparous cows	Number of multiparous cows	n	*P-value*[6]
Parity	21	28	29	21	99	0.16
Effect	Number of cows with SCC ≤ 200,000	Number of cows with SCC > 200,000	Number of cows with SCC ≤ 200,000	Number of cows with SCC > 200,000	n	*P-value*[6]
SCC	42	7	40	10	99	0.60

[1] Temperature humidity index was computed using the following formula (NOAA and Administration, 1976): THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8]. The estrual max THI was calculated by averaging the max THI for each barn on days 2 to 5 of the protocol for each study group of cows. The estrual max THI was used to assess the effect of max THI on automated estrous detection rates and number of cows with standing mounts.

[2] The test day closest to observation days for individual cow somatic cell counts from DHI (Dairy Records Management Systems, Raleigh, NC) were used to classify SCC the week of predicted estrus. Cows with less than 200,000 somatic cells were classified as low. Cows 200,000 somatic cells or more were classified as high.

[3] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4] The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[5]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[6]The FREQUENCY procedure of SAS 9.3 (Cary, NC) using the Chi-Square analysis determined the number of cows who stood for mounting and cows that did not stand for mounting and level of significance for differences among effects that stood and did not stand for mounting

Table 3.22. Effect of differences in BCS at estrus on visual observation of standing for mounting in dairy cows synchronized with a modified G7G-Ovsynch protocol for detection of estrus.[1, 2, 3, 4]

BCS at estrus	Stood	Did not stand	n	P-Value[5]
2.25	7	7	-	-
2.5	7	9	-	-
2.75	27	21	-	-
3	2	9	-	-
3.25	6	4	99	0.21

[1] Body condition scoring (Ferguson et al., 1994) was performed weekly by the same observer during the full study. Body condition scores were determined upon evaluation of the following body regions: ischial tuberosity, illeal tuberosity, loin edges, coccygeal ligament, thurl region, sacral ligament, and spine were classified to result in a BCS. Body condition scores during the week of calving and predicted estrus were used to calculate the change in BCS from calving to estrus.

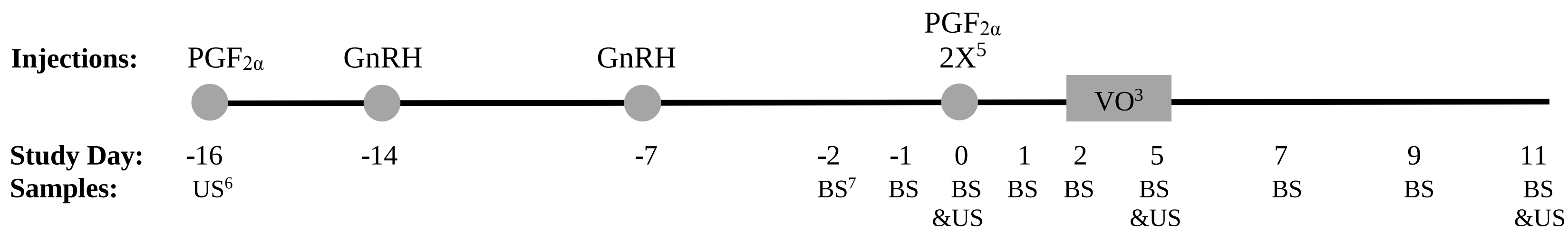
[2] Visual observation for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha)}$ for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[3] The last GnRH injection of a traditional Ovsynch protocol (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the protocol.

[4]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11). Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

[5]The FREQUENCY procedure of SAS 9.3 (Cary, NC) using the Chi-Square analysis determined the number of cows who stood for mounting and cows that did not stand for mounting and level of significance for differences among effects that stood and did not stand for mounting

Figure 3.1. Protocol (28 days) in assessing the efficacy of 8 automated estrous detection systems for cows synchronized with a modified G7G - Ovsynch protocol for visual observation of estrus and verification of estrus with temporal patterns in progesterone (N=109). [1, 2, 3, 4]



[1]AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands); CowScout S Leg (GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems, LLC,Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube, (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton, (Embedded Data Systems, Kentucky, USA); Track a)) Cow (ENGS Systems Innovative Dairy Solutions, Israel)

[2]The last GnRH injection of a traditional Ovsynch protocol was withheld to permit expression of estrus.

[3]Visual observation (VO) for estrous behaviors occurred during a 4 day period (days 2 to 5 after the final $PGF_{2\alpha}$) for 4 times a day (0330, 1000, 1430, and 2200) for 30 minutes each observation period

[4]Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/ml on days -2, -1 and 0, <1.0 ng/ml on day 2 and >1.0 ng/ml on days 9 and 11).

[5]$PGF_{2\alpha}$ was administered twice on day 0, 6 hours apart at 0800 and 1400.

[6]Transrectal ultrasonography was performed at 0800 to verify resumption of ovarian cyclicity at enrollment (d -16), presence of a corpus luteum (CL) on the day of the final injection (designated experimental day 0), regression of the CL by day 5, and presence of a new CL on day 11

[7]Blood samples were taken at 0800to obtain plasma for progesterone radioimmunoassay

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VITA

Lauren Mayo is a Riverview, FL native with a strong passion for the dairy industry. She is a 2013 graduate of the University of Florida with a B.S. degree in Animal Sciences specializing in Dairy Industry and a minor in Agricultural Communications. She developed this passion for the dairy industry at a young age, despite not growing up on a dairy farm. Lauren was an active member in 4H and FFA with poultry, swine, and dairy projects along with dairy judging and dairy quiz bowl. Her involvement in college was extensive including leadership roles in the UF College of Agricultural and Life Sciences Ambassadors, National ADSA Student Affiliate Division Executive Team, UF Dairy Science Club, Block n Bridle, and Collegiate Farm Bureau. She gained her interest for a potential career in extension from competing in Dairy Challenge on the regional and national level. She also completed an internship at the W.H. Miner Agricultural Research Institute in dairy farm management and the Southern Great Plains Dairy Consortium to further her knowledge of dairy cattle science and husbandry.

Lauren began at the University of Kentucky in August 2013. She was part of the dairy systems management program under the guidance of Drs. Jeffrey Bewley and William Silvia. Her research focus there was evaluating the efficacy of parameters and estrus alerts measured and recorded by automated estrous detection systems. Lauren has given presentations on her work at several Kentucky extension events and the 2015 ADSA-ASAS Joint Annual Meeting in Orlando, FL. She served a professional development chair for the UK Animal and Food Sciences Graduate Association. Lauren is a member of the American Dairy Science Association and Dairy Cattle Reproduction Council. Her goals are to work in research, extension, and someday own a dairy farm.

Scientific Abstracts:

L.M. Mayo, W.J. Silvia, G. Heersche Jr., I.C. Tsai, B.A. Wadsworth, A.E. Stone, and J.M. Bewley. 2015. Automated detection of estrus using multiple commercial precision dairy farming technologies in synchronized dairy cows. Abstract 65820. 2015 ADSA-ASAS Joint Annual Meeting, Orlando, FL.

I.C. Tsai, **L.M. Mayo**, A.E. Stone, B.A. Wadsworth, D.L. Ray, J.D. Clark and J.M. Bewley. 2015. Differences in rumination time, lying time, and rectal temperature between cows with and without metritis, ketosis, and subclinical hypocalcemia. Abstract 64898. 2015 ADSA-ASAS Joint Annual Meeting, Orlando, FL.

B.A. Wadsworth, **L.M. Mayo**, N.I. Tsai, A.E. Stone, D.L. Ray, J.D. Clark, J.M. Bewley. 2015. Comparison of lying times of lame versus sound dairy cattle using a leg-based accelerometer. Abstract 63378. 2015 ADSA-ASAS Joint Annual Meeting, Orlando, FL.

L.M. Mayo, W.J. Silvia, G. Heersche Jr., A.E. Sterrett, B.A. Wadsworth, I.C. Tsai, and J.M. Bewley. 2014. Automated estrus detection using vaginal temperature, ear skin and tympanic temperature, activity, standing behavior and rumination in synchronized dairy cows. 2014 University of Kentucky Animal and Food Science Graduate Student Association Poster Symposium. Lexington, KY.