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ASSESSMENT OF THE TECHNICAL AND ECONOMIC POTENTIAL OF
AUTOMATED ESTRUS DETECTION TECHNOLOGIES FOR DAIRY CATTLE

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
at the University of Kentucky

By

Karmella Ann Dolecheck

Lexington, Kentucky

Director: Dr. Jeffrey M. Bewley, Associate Extension Professor of Animal Sciences

Lexington, Kentucky

2015

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ABSTRACT OF THESIS

ASSESSMENT OF THE TECHNICAL AND ECONOMIC POTENTIAL OF AUTOMATED ESTRUS DETECTION TECHNOLOGIES FOR DAIRY CATTLE

Poor estrus detection can limit the reproductive performance of a dairy herd. One objective of this research was to evaluate an alternative method to traditional estrus detection in the form of automated monitoring technologies. To accomplish this, the first study considered the ability of automatically monitored parameters (activity, number of steps, lying bouts, lying time, feeding time, rumination time, and temperature) to detect estrus. A second study compared automated activity monitoring to timed artificial insemination as reproductive management strategies on commercial herds. The other objective of this research was to evaluate the economic potential of automated estrus detection technologies. This was accomplished by creating and evaluating a farm specific decision support tool to determine the net present value of adopting an automated estrus detection technology.

KEYWORDS: Estrus Detection, Automated Estrus Detection, Precision Dairy Farming, Automated Activity Monitoring, Decision Support Tool

Karmella Ann Dolecheck
March 9, 2015

ASSESSMENT OF THE TECHNICAL AND ECONOMIC POTENTIAL OF AUTOMATED ESTRUS
DETECTION TECHNOLOGIES

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March 9, 2015
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FREQUENTLY USED ABBREVIATIONS

AAM = automated activity monitoring

AED = automated estrus detection

AI = artificial insemination

CL = corpus luteum

CI = calving interval

CR = conception rate

d = day

DHI = Dairy Herd Improvement

DIM = days in milk

DO = days open

E2 = estradiol

EDR = estrus detection rate

FN = false negative

FP = false positive

FSH = follicle stimulating hormone

g = gram

GnRH = gonadotropin releasing hormone

h = hour

kg = kilogram

l = liter

LH = luteinizing hormone

min = minute

ml = milliliter

ng = nanogram

NPV = net present value

P4 = progesterone

pg = picogram

PGF_{2α} = prostaglandin F_{2α}

RPO = retention pay-off

TAI = timed artificial insemination

TN = true negative

TP = true positive

PR = pregnancy rate

THI = temperature humidity index

VO = visual observation

VWP = voluntary waiting period

US = United States

CHAPTER ONE

Review of Literature

THE ESTROUS CYCLE

The estrous cycle is a cyclical pattern of ovarian activity that causes a period of sexual receptivity (behavioral estrus) in female animals starting at puberty (Forde et al., 2011). Cattle are polyestrous (ovulating several times throughout the year), having an 18 to 24 d estrous cycle and displaying behavioral estrus on average every 21 d (Hammond, 1927). The cycle includes a 14 to 18 d luteal phase when formation of the corpus luteum (**CL**) occurs, followed by a 4 to 6 d follicular phase which includes final maturation and ovulation of the dominate follicle. Hormones released from the hypothalamus (gonadotropin releasing hormone; **GnRH**), anterior pituitary (follicle stimulating hormone; **FSH** and luteinizing hormone; **LH**), ovaries (progesterone; **P4** and estradiol; **E2**), and uterus (prostaglandin $F_{2\alpha}$; **PGF_{2\alpha}**) work through a series of positive and negative feedback loops to regulate the estrous cycle.

During fetal development, the ovaries develop approximately 150,000 primordial follicles (Erickson, 1966), each composed of a single oocyte surrounded by a squamous follicular epithelium (Hansel and Convey, 1983). After puberty, increases in FSH, which follicles depend on for development, initiate 2 or 3 waves of follicle growth occurring per estrous cycle (Adams et al., 1992; Sunderland et al., 1994). Through the process of selection and dominance, each wave results in a single large, healthy dominant follicle (Senger, 1999). The wall of the dominant follicle is composed of 2 steroidogenic cell types: granulosa cells proliferated from the squamous follicular cells of the primordial follicles and theca cells differentiated from surrounding connective tissue (Hansel and

Convey, 1983). As the dominant follicle continues to grow, E2 synthesis increases through the 2 cell, 2 gonadotropin model that Fortune and Quirk (1988) first described in cows. In short, binding of LH to receptors on the theca cells of the follicle drives the conversion of cholesterol to testosterone. Testosterone moves to the granulosa cells where conversion to E2 occurs. High E2 concentrations stimulate behavioral estrus in the absence of P4 (Allrich, 1994). Therefore, behavioral estrus can only occur if no P4-producing CL is present on the ovaries. The high E2 level that causes behavioral estrus is also responsible for suppression of FSH, which the original follicle wave depends on for growth (Sunderland et al., 1994; Ginther et al., 2000a; b). Unlike the smaller follicles, which regress, the dominant follicle can function independent of FSH because of LH receptors developed on the granulosa cells (Xu et al., 1995). Ovulation occurs around 27 h after the onset of behavioral estrus when E2 reaches threshold, creating an LH surge (Roche, 1996; Walker et al., 1996).

After ovulation, LH (the major luteotropic hormone) mediates CL formation from the granulosa and theca cells of the ovulated dominant follicle (Forde et al., 2011). The CL produces high P4 concentrations that aid in maintenance of pregnancy, if established (Senger, 1999). The persistent P4 concentration during the luteal phase prevents frequent enough LH pulses to result in dominant follicle ovulation (Senger, 1999). Dominant follicles that develop during this period undergo atresia rather than ovulation (Forde et al., 2011). If no maternal recognition of pregnancy signal (interferon tau) is detected by cycle Day 16, CL luteolysis occurs via countercurrent exchange of uterine PGF_{2α} from the uterine vein to the ovarian artery (Senger, 1999; Forde et al., 2011). When the CL

regresses, P4 concentrations decrease and the resulting increase in GnRH allows the cow to enter the follicular phase of the cycle again (Senger, 1999; Forde et al., 2011).

If pregnancy is established, follicular waves occur regularly during the first two trimesters, but stop about 21 d prepartum (Ginther et al., 1996). Upon resumption of basal levels of PGF_{2α} and E2 after parturition, FSH increases and follicular waves resume allowing the first dominant follicle to develop between 7 and 10 d postpartum (Murphy et al., 1990; Savio et al., 1990; Crowe et al., 1993; Crowe et al., 1998). Whether the first dominant follicle ovulates or undergoes atresia depends on the follicle's ability to produce enough E2 to result in a GnRH and, in turn, an LH surge (Austin et al., 2001). Most cows resume ovulation between 15 and 45 d postpartum, averaging 27 to 35 d (Murphy et al., 1990; Savio et al., 1990; Crowe et al., 1993; Crowe et al., 1998; Forde et al., 2011).

REPRODUCTIVE PERFORMANCE OF THE DAIRY COW

The most common reproductive parameters used to evaluate a dairy herd include estrus detection rate (**EDR**), conception rate (**CR**), pregnancy rate (**PR**), days open (**DO**), and calving interval (**CI**; USDA, 2007). The number of cows detected in behavioral estrus divided by the number of cows eligible to come into behavioral estrus throughout a 21d period, multiplied by 100 is EDR. Conception rate is the number of cows that become pregnant divided by the number of cows bred during a 21 d period, multiplied by 100. Pregnancy rate is the percent of cows eligible to become pregnant during a 21 d period that actually do, as a percentage (Niles et al., 2001). A herd's DO represents the mean number of days from calving to conception and CI is the mean number of days between calvings in a herd. Through evaluation of these reproductive parameters over

time, it is clear that reproductive performance has declined since the 1970s (Washburn et al., 2002; De Vries and Risco, 2005; Hare et al., 2006).

Washburn et al. (2002) emphasized the regional reproductive efficiency decline in the Southeast United States (**US**) through a 10-state study of 532 Holstein and 29 Jersey herds. Between 1976 and 1999, both breeds experienced an 18.5% CR decrease and an increase in services per conception of 1.75. De Vries and Risco (2005) conducted another study that analyzed almost 3 million records from Florida and Georgia Holstein herds, finding a 46 d increase in DO, a 9.6% decrease in PR, and a 30 d increase in CI between 1982 and 1998.

On a national level, Hare et al. (2006) evaluated changes in CI between 1980 and 2004 for roughly 130,000 Ayrshire, 210,500 Brown Swiss, 277,000 Guernsey, 18,900,000 Holstein, and 1,000,000 Jersey Dairy Herd Improvement (**DHI**) enrolled cows. All breeds experienced a 0.90 to 1.07 d increase in CI per year, except Jerseys (0.49 d per year). Norman et al. (2009) completed a similar study that examined reproductive decline in over 5 million DHI enrolled Holstein and Jersey cows between 1996 and 2006. For both breeds, mean services per lactation increased (2.1 to 2.5 in Holsteins and 2.0 to 2.3 in Jerseys) over the 9 years of data. During the same time, service interval (the time between services) increased 18 d in Holsteins and 11 d in Jerseys. In contrast, both first service CR and CI declined only until 2001 and have since increased. Data collected by the US Department of Agriculture Animal Improvement Programs Laboratory agreed with the recent upward trend in reproductive performance (USDA, 2011). When analyzing all DHI enrolled Holstein cows, most reproductive traits improved between 2003 and 2011, including DO (147 to 129 d), CR (0.30 to 0.31), and

CI (426 to 409 d). However, enrollment in DHI only represented 47% of US dairy cows in 2013 (USDA, 2013b).

In a retrospective study considering data from 1991 to 2000 on Spanish dairies, López-Gatius (2003) found a 9.2% decrease in PR. Gonzalez-Recio et al. (2004) conducted another Spanish study spanning 1988 to 2001 and found DO increased from 106 to 122 d and first service CR decreased from 56 to 40%. The United Kingdom has also experienced fertility challenges, including a 16% decline in first service CR and a 20 d increase in CI between 1975 and 1998 (Royal et al., 2000). Therefore, consistent reproductive decline has occurred in other countries outside of the US.

Multiple theories surrounding the reproductive performance decline exist. One established explanation is that the genetic correlation between milk yield and reproductive traits is unfavorable (Dematawewa and Berger, 1998; Stevenson, 2001b; VanRaden et al., 2004). VanRaden et al. (2004) estimated genetic correlations between first lactation DO and first lactation milk, fat, and protein yield at 0.38, 0.33, and 0.32, respectively, meaning selection for milk yield has resulted in selection against reproductive performance. Annual milk yield per cow increased 3.3 times between 1950 and 2000 (Stevenson, 2001b).

Increased milk production has also influenced other factors that can affect fertility. For example, the additional feed intake required to sustain high levels of milk production increases metabolism, resulting in faster metabolism of hormones required for estrus expression (E2) and pregnancy maintenance (P4; Sangsritavong et al., 2002). Sangsritavong et al. (2002) found lactating cows experienced greater liver blood flow (1183 vs. 757 l/h), lower circulating P4 (2.43 vs. 3.53 ng/ml), and lower circulating E2

(265 vs. 351 pg/ml) when compared to non-lactating cows. Both P4 and E2 are essential to reproduction and low levels may interrupt reproductive cyclicity.

Another genetic factor that might have contributed to observations of reduced reproductive performance is inbreeding in the US Holstein population, which was estimated at 6.05% in 2013 (USDA, 2013a). Bjelland et al. (2013) evaluated homozygosity in 5,800 Holstein cows and discovered that for every 1% increase, DO increased 1.76 d. Additionally, VanRaden et al. (2011a) revealed the existence of multiple recessive defects, which occur at greater rates in inbred animals, that can cause embryo loss. Fortunately, recent research suggests that inbreeding may be declining, especially with increased utilization of genomic evaluations (VanRaden et al., 2011b).

Environmental changes could also have played a role in the declining reproductive performance of dairy cows (Wolfenson et al., 2000; Rensis and Scaramuzzi, 2003; De Vries and Risco, 2005; López-Gatius et al., 2005; Schüller et al., 2014). The 1990s was the warmest decade of the 20th century (Bradley, 2000), resulting in increased heat stress levels and a greater percent of the US experiencing heat stress (Lucy, 2001). Rensis and Scaramuzzi (2003) identified reduced estrus expression, lower reproductive hormone secretions, inferior follicle growth, and inhibited embryo formation as negative effects of heat stress on reproduction. In a commercial German herd, Schüller et al. (2014) discovered that when mean THI was 73 or greater between 21 and 1 d pre-breeding, CR dropped from 31 to 12%. In addition to immediate effects, summer heat stress has a long-term effect on reproduction that can last into October and November (Wolfenson et al., 2000).

ESTRUS DETECTION

Behavioral estrus is a period of sexual receptivity in cows, that allows for identification of ovulation (Roelofs et al., 2010). As the pre-ovulatory follicle grows, E2 production increases until reaching a threshold, resulting in stimulation of the brain that triggers behavioral responses (estrus). Estrus detection is the ability to detect behavioral estrus and breed cows at the proper time. Importance of estrus detection has increased since the replacement of bulls with artificial insemination (**AI**) starting in the 1940s (Foote, 1975; Foote, 1996) and has been identified as a major limiting factor in reproductive performance (Rounsaville et al., 1979; Heersche and Nebel, 1994; Nebel and Jobst, 1998; Ferguson and Skidmore, 2013). Both PR and CR depend on accurate estrus detection to ensure that breeding occurs at the appropriate time relative to ovulation (Roelofs et al., 2010). If insemination occurs too early, sperm quality is reduced before ovulation (Pursley, 1998). When insemination occurs too late, oocyte quality lowers as it awaits the sperm.

Traditionally, estrus detection involves visual observation (**VO**) with the most definitive sign of estrus being a cow that stands and allows another cow to mount her (standing estrus). The mean duration of standing estrus is only 7.1 h with 8.5 standing events (Dransfield et al., 1998). Total mounting activity during estrus has been estimated as only 24.1 s (Walker et al., 1996), making secondary signs of behavioral estrus equally important. Secondary signs of estrus include increased activity, restlessness, nudging, sniffing others, flehmen response, resting the chin on another cow, mounting others, licking, rubbing, head butting, raising of tail, pink and swollen vulva, clear mucus discharge from the vulva, and decreases in both milk production and feed intake (Foote,

1975; Van Eerdenburg et al., 1996; Kerbrat and Disenhaus, 2004). Emphasizing their importance, Roelofs et al. (2005b) identified only 58% of behavioral estrus events when observing cows for standing estrus only, but 90% when also considering mounting other cows.

Breed, genetics, age, milk production, and other individual cow characteristics influence behavioral estrus expression (De Silva et al., 1981; Van Vliet and Van Eerdenburg, 1996; Orihuela, 2000; López-Gatius et al., 2005; Roelofs et al., 2005b; Cavestany et al., 2008). López-Gatius et al. (2005) showed that an increase of 1 lactation number or 1 kg milk production resulted in a 21.4% or 1.6% decrease in estrus-related walking activity, respectively. Other research has shown primiparous animals have a significantly longer duration of estrus (13.6 vs. 10.8 h) and a more intense estrus when all signs of estrus were included (Roelofs et al., 2005b). In addition to individual cow factors, many management and environmental factors can influence estrus expression.

Seasonal variation in estrus expression is evident, largely because of heat stress (Gangwar et al., 1965; Pennington et al., 1985; López-Gatius et al., 2005). A climate controlled housing experiment conducted by Gangwar et al. (1965) found that length of behavioral estrus was reduced from 20 h in spring and air conditioned situations to 11 h under simulated hot conditions. López-Gatius et al. (2005) further demonstrated that walking activity during estrus was significantly lower in warm weather than cool weather (369 vs. 384% increase, respectively), emphasizing the importance of heat abatement.

Lameness is the aversion to place weight on a foot because of pain (Scott, 1989) and occurs in 12.5% of cows in the US (USDA, 2007). A cow experiencing lameness may have physical limitations that result in reduced expression of behavioral estrus

(Walker et al., 2008a). Sood and Nanda (2006) discovered lower frequencies of standing events during estrus in lame cows (2.4 vs. 8.0 events, respectively). Walker et al. (2008b) found that lame cows experience up to a 37% reduction in estrus expression, likely because of reduced standing and walking times.

Housing or access to pasture could also play a significant role in estrus expression (Cutullic et al., 2009; Palmer et al., 2010). Cutullic et al. (2009) found that access to pasture significantly increased the chance (2.28 odds ratio) of standing estrus compared to cows housed indoors. Palmer et al. (2010) also compared cows in housing to those on pasture, finding that a greater portion of pastured cows expressed standing estrus as recorded by an automated mounting detector (91 vs. 52%), but that estrus duration and intensity did not differ.

Flooring, especially concrete, can reduce mounting activity (Vailes and Britt, 1990; Rodtian et al., 1996; Platz et al., 2008). Vailes and Britt (1990) showed a cow's preference to mount another cow was up to 15 times greater on dirt than on concrete floors. Rodtian et al. (1996) also found that cows confined to concrete displayed significantly lower mounts per estrus period compared to cows free to move between cement and dirt (11.4 vs. 26.9). Grooving of concrete or rubber mats over sub-ideal flooring can help to reduce these problems (van der Tol et al.; Platz et al., 2008).

The number of social interactions a cow experiences, influenced by herd size, also affects estrus expression (Hurnik et al., 1975; Van Vliet and Van Eerdenburg, 1996; Roelofs et al., 2005b; Yaniz et al., 2006; Cutullic et al., 2009). Yaniz et al. (2006) showed that every additional cow in estrus increased walking activity 6.1% in cows experiencing estrus. Another study conducted by Cutullic et al. (2009) found that the

chance of observing standing estrus in a cow increased 4 times when in the presence of a herdmate in estrus compared to cows not in the presence of a herdmate in estrus.

Observation timing, length, and frequency play a role in estrus detection when VO is used (Hurnik et al., 1975; Van Vliet and Van Eerdenburg, 1996; Cavestany et al., 2008). Van Vliet and Van Eerdenburg (1996) found that estrus behavior decreased before feeding and milking times and increased during the night. When compared to ultrasonography, 3, 60-minute observations of cows for estrus throughout the day resulted in 94% EDR whereas only a 41% EDR was reached when 2, 30-minute observations were conducted (Cavestany et al., 2008).

Estrus detection rate varies between herds, but is estimated at only 50 to 60% (Chanvallon et al., 2014). Additionally, 19% of AI events occur at infertile times or in pregnant animals, resulting in 17% embryonic loss (Sturman et al., 2000). A need for alternatives to traditional visual estrus detection, which is difficult because of the number of influencing factors discussed above, is evident. One alternative, which 46.8% of US producers use, is non-automated estrus detection aids such as tailhead patches and tail chalk (USDA, 2007). Other alternative methods include efforts to eliminate estrus detection (through synchronized of estrus or ovulation) or the use of automated estrus detection (**AED**) technologies.

SYNCHRONIZATION

Synchronizing estrus or ovulation is a common reproductive management strategy to facilitate AI. Combinations of hormones can be used for two basic forms of synchronization: 1) treatment with a progestational compound to prevent estrus for

enough time that when removed estrus will follow and 2) use of an agent to remove the corpora luteum and stimulate estrus (Hansel and Convey, 1983).

Synchronization Development

During the early 1960s, progestational compounds, in the form of feed and water additives, implants, topical treatments, and vaginal inserts were examined (Hansel and Convey, 1983). This strategy resulted in 80 to 90% of animals showing estrus over a 4 d period beginning 2 d after withdrawal, but a reduction in CR from 10 to 15%.

Combinations of P4 with E2 or gonadotropins were tested in hopes of gaining better control over the timing of estrus and ovulation, but were unsuccessful. Modern synchronization protocols that still use this method administer P4 trans-vaginal via controlled internal drug release.

Most contemporary synchronization protocols depend on lysing the CL, promoting premature occurrence of estrus, and eliminating the need or extent of estrus detection (Stevenson, 2001b). Prostaglandin $F_{2\alpha}$ and GnRH, administered as intramuscular injections, are the two synthetic hormones commonly used to accomplish this. Administering $PGF_{2\alpha}$ will regress a CL if present on the ovaries (Pursley et al., 1995). Ideally, GnRH will induce ovulation of a dominant follicle by triggering release of LH (Pursley et al., 1995). Two less common alternatives to GnRH exist for luteinization of the dominant follicle by initiating an LH surge: E2 and human chorionic gonadotrophin (De Rensis et al., 1999; Wiltbank and Pursley, 2014). Three $PGF_{2\alpha}$ and GnRH protocols commonly used in the US are targeted breeding, modified targeted breeding, and Ovsynch.

A targeted breeding program consists of two or three injections of $\text{PGF}_{2\alpha}$ administered 11 to 14 d apart (Nebel and Jobst, 1998; Stevenson, 2001b). The specific interval of 11 to 14 d is used to allow enough time for cows that respond to the first injection to have a new CL before the second injection is administered (Stevenson, 2001b). If a cow did not respond to the first injection, she should be at a point in her estrous cycle that allows her to respond at the time of the second injection. Cows not observed in estrus after the second injection receive a third $\text{PGF}_{2\alpha}$ and TAI 80 h later.

A modified targeted breeding program begins with $\text{PGF}_{2\alpha}$, followed by GnRH two weeks later (Stevenson, 2001b). The protocol ends with a second $\text{PGF}_{2\alpha}$ injection seven days after the GnRH is given and AI occurs at visually observed estrus or 72 to 80 h later. The advantage of including GnRH compared to simple targeted breeding is that it induces ovulation and result in a new or additional CL, allowing more cows to respond to the second $\text{PGF}_{2\alpha}$ and enter estrus.

Ovsynch

Pursley et al. (1995) developed Ovsynch as a way to synchronize ovulation rather than estrus. This protocol begins with a GnRH injection and $\text{PGF}_{2\alpha}$ is given 1 week later (Figure 1.1) . An additional GnRH injection 48 to 56 h after the $\text{PGF}_{2\alpha}$ prompts ovulation 24 to 34 h later. Timed AI occurs 0 to 32 h after the second GnRH injection (Stevenson, 2001b). The ideal time of AI is 16 h after the GnRH injection because of low sperm viability when inseminated too early and low oocyte viability when inseminated too late (Pursley, 1998).

Ovsynch has been the most widely adopted of synchronization protocols. In a recent survey conducted by Caraviello et al. (2006b), over 50% 103 large commercial US

dairy farms indicated that they used Ovsynch for synchronized breeding. National surveys indicate that at least 16% of operations use Ovsynch on cows before the first AI (USDA, 2007).

One of the first studies that compared Ovsynch to typical reproductive management occurred in 1997 on 3 commercial farms in Wisconsin (Pursley et al., 1997a). The comparison involved control cows managed using visual estrus detection with periodic PGF_{2α} and Ovsynch synchronized cows. In the Ovsynch group, days to first service (54 vs. 83) and DO (99 vs. 118) declined compared to the control group, whereas PR was similar (37% in Ovsynch vs. 39% in controls). Keister et al. (1999) also found that DO decreased (114 to 102 d) on two commercial herds when using Ovsynch compared to no hormonal intervention.

When comparing PR in lactating herds using targeted breeding or Ovsynch, results were similar (38.9 vs. 37.8%, respectively), but the same study in heifers found Ovsynch to produce a lower PR (35.1 vs. 74.4%) for reasons that are not fully understood (Pursley et al., 1997b). A 2005 meta-analysis further investigated fertility differences in cows bred to Ovsynch and other reproductive management strategies, determining no differences in CR or PR between Ovsynch and bull breeding, VO, or PGF_{2α} based protocols (Rabiee et al., 2005). Thus the reduced DO observed in Ovsynch synchronized cows is not because of better fertility, but because of increased service rates (Wiltbank and Pursley, 2014).

Ovsynch Presynchronization

The point in the cycle when the cow begins Ovsynch is important because her responsiveness to GnRH dictates follicle size at time of AI (Vasconcelos et al., 1999).

Souza et al. (2007) discovered that medium sized follicles, between 15 and 19 mm, produced better fertility (52.6% pregnant at 60 d) than small or large follicles (38.2 and 34.3%, respectively) when supplemented with E2 8 h before the second GnRH injection. As a result, when Ovsynch was started on Days 1 to 4 or 14 to 21, lower ovulation rates and lower PR (25%) were observed compared to cows initiating the protocol between Days 5 and 13 (36% PR). The development of presynchronization protocols has increased the number of cows beginning Ovsynch between the ideal Day 5 and 13 of the estrous cycle.

The first presynchronization strategy developed specifically for Ovsynch was Presynch-12 (Moreira et al., 2001). Using Presynch-12, cows receive 2 PGF_{2α} injections 14 d apart, with the second administered 12 d before beginning Ovsynch (Figure 1.1). Presynchronization increased fertility (42.8 vs. 29.4% 74-d pregnancy diagnosis) compared to cows undergoing only Ovsynch (Moreira et al., 2001). A similar response was observed in subsequent studies (El-Zarkouny et al., 2004; Navanukraw et al., 2004). However, a limitation of a presynchronization protocol that only includes PGF_{2α} is that it does not address anovular cows (Wiltbank and Pursley, 2014).

Another common presynchronization protocol is G6G or G7G. This protocol consists of one PGF_{2α} injection followed by GnRH 2 d later and Ovsynch beginning 6 (G6G) or 7 (G7G) d after (Figure 1.1; Peters and Pursley, 2002). Original research found that G6G increased the number of cows with luteal function at the beginning of Ovsynch, but showed no improvement in PR when compared to Ovsynch alone (Peters and Pursley, 2002). Further research by Bello et al. (2006) also identified improved synchronization when using G6G compared to Ovsynch alone and showed a numeric, but

not statistically significant, difference in PR between treatments (G6G Ovsynch: 50% vs. Ovsynch only: 27%).

Double-Ovsynch is a third presynchronization protocol that involves completing a full Ovsynch without AI, waiting 7 d, and then completing another Ovsynch with AI (Figure 1.1; Souza et al., 2008). This process should result in all cows beginning the second Ovsynch on Day 7 of the estrous cycle, which is within the optimal Day 5 to 13 period (Vasconcelos et al., 1999; Wiltbank and Pursley, 2014). When compared to Ovsynch alone, Double-Ovsynch can increase CR (39% vs. 30%; Giordano et al., 2012). A comparison to Presynch-12 found increased CR in only primiparous (65.2 vs. 45.2%) and not multiparous (37.5 vs. 39.3%) animals (Souza et al., 2008). Herlihy (2012) also showed increased fertility in primiparous, but not multiparous cows subjected to Double-Ovsynch compared to Presynch-12. When Double-Ovsynch was compared to G6G, no difference in overall CR (36.3 vs. 34.8%) was found but primiparous Double-Ovsynch cows again reported a greater CR (44.3%) than multiparous Double-Ovsynch cows (31%; Astiz and Fargas, 2013).

Synchronization Adoption

According to a national survey published in 2007, 58.2% of operations used some form of PGF_{2α} or GnRH based synchronization programs for at least a portion of their heifers or cows (USDA, 2007). A larger percent of medium operations use TAI (70.8%) than small operations (53.2%). Some argue that the increased reproductive performance in the last 15 years is related to the increased use of synchronization (Wiltbank and Pursley, 2014). Indeed, the reduced time to first service seen since the early 2000s has occurred almost exclusively in herds using TAI (Norman et al., 2009). Overall, Norman

et al. (2009) found that synchronized herds experienced 18 fewer days to first service and a 7 d shorter CI, though this was accompanied by a 12% lower 70 d non-return rate, a 2% lower CR, and 0.4 more services per pregnancy.

Regardless, many economic, management, and sociological aspects are important to consider before implementing any synchronization protocol (Wiltbank and Pursley, 2014). Although some protocols tend to produce better results than others, the best protocol will be easy to manage and simple to comply with which may vary by farm and management situation (Stevenson, 2001b). Perhaps the next step in synchronization is to simplify protocols to make them more useful in commercial operations (Wiltbank and Pursley, 2014).

AUTOMATED ESTRUS DETECTION

An alternative to TAI is AED technologies (Lehrer et al., 1992). These monitors supplement a producer's ability to collect information about their cows without increasing cow stress through disturbances or handling (Wathes et al., 2008). Examples of automatically measured parameters related to behavioral estrus include mounting events, activity, rumination, body temperature, and P4 levels (Senger, 1994; Saint-Dizier and Chastant-Maillard, 2012; Fricke et al., 2014b).

Algorithms

A common problem with all information, except mounting behavior, collected using automated technologies is that some changes in cow behavior and physiology are not exclusive to estrus. As a result, software specific algorithms (sets of rules to follow during calculations) must be used to compare an animal's current behavior with a cow-specific reference period, creating an estrus alert when a set threshold is exceeded (Saint-

Dizier and Chastant-Maillard, 2012). To determine usefulness of a technology, comparison are made between estrus events identified by the technology and a gold standard such as VO, ultrasonography, blood or milk P4 levels, or a combination of these. Correctly identified estrus events are considered true positives (**TP**), non-alerted estrus events are false negatives (**FN**), non-alerted non-estrus events are true negatives (**TN**), and alerted non-estrus events are false positives (**FP**; Firk et al., 2002). Detection is a balance of sensitivity and specificity. Sensitivity, the probability that an event is alerted, is equal to $TP/(TP+FN)*100$ (Hogeveen et al., 2010). Specificity, the probability that when an event does not occur no alert is generated, is equal to $TN/(TN+FP)*100$. Because neither sensitivity nor specificity account for the prevalence of the event, other comparative measurements are also useful. These include positive predictive value $[TP/(TP+FP)*100]$ and negative predictive value $[TN/(TN+FN)*100]$. Other common measures of detection ability include error rate $[FP/(TP+FP)*100]$ and accuracy $[(TP+TN)/(TP+TN+FP+FN)]$.

The algorithms used for any technology alert will greatly influence success (Saint-Dizier and Chastant-Maillard, 2012). An Australian study testing 5 different algorithms for AED using automated activity monitoring (**AAM**) found a variation in sensitivity ranging from 79.4 to 94.1% and a variation in specificity between 90.0 and 98.2% (Hockey et al., 2010). Similar studies testing different algorithms for AED using AAM reported sensitivities from 51 to 87% (Roelofs et al., 2005a; Lovendahl and Chagunda, 2010).

Mounting

Standing estrus is the most definitive sign of estrus because it occurs almost exclusively in animals experiencing estrus. This behavior has automatically been monitored using pressure-sensitive technologies glued to the tailhead of the cow (Xu et al., 1998; At-Taras and Spahr, 2001; Cavalieri et al., 2003a; Saint-Dizier and Chastant-Maillard, 2012). When activated by a standing event, cow ID, date, time, and duration of mount are sent to a computer to be reviewed (Saint-Dizier and Chastant-Maillard, 2012). Standing events per estrus and length of standing estrus have been recorded using these devices in multiple studies (Stevenson et al., 1996; Dransfield et al., 1998; Xu et al., 1998; At-Taras and Spahr, 2001; Cavalieri et al., 2003b). The most recent study, conducted by Johnson et al. (2012), found 18.4 ± 8.9 standing events per 6.0 ± 4.9 h estrus period. Each standing event can last 2.3 to 3.8 s (Xu et al., 1998; At-Taras and Spahr, 2001). Season can affect results, with hot weather decreasing duration of estrus but not number or duration of individual mounts (At-Taras and Spahr, 2001). Number of mounts can be affected by both parity and days in milk (**DIM**), with primiparous cows and cows <80 DIM having increased occurrence (Xu et al., 1998; At-Taras and Spahr, 2001; Peralta et al., 2005).

Cavalieri et al. (2003b) compared VO of estrus length and number and duration of mounts to rump-mounted pressure-sensitive technologies and found low correlations in synchronized cows. Still, both methods were successful at detecting estrus with sensitivity rates of 97.5% and 93.8% for VO and rump-mounted pressure-sensitive technologies, respectively (Cavalieri et al., 2003b). Additional studies agree that rump-mounted pressure-sensitive technology sensitivity is comparable to or better than VO in

both cows (Xu et al., 1998; At-Taras and Spahr, 2001; Saumande, 2002; Peralta et al., 2005) and heifers (Stevenson et al., 1996), with sensitivity and positive predictive value as high as 91.7 and 100%, respectively, using milk P4 as a comparison. Rump-mounted pressure-sensitive technologies have also shown results comparable to tail paint and pedometers (Cavalieri et al., 2003a).

One limitation of rump-mounted pressure-sensitive technologies is the labor required to attach and remove them because they are not left on the animal for an entire lactation like other automated technologies can be (Rorie et al., 2002). Additionally, some studies have reported estrus detection trouble because of lost or displaced monitors (Dohi et al., 1993; Xu et al., 1998). Researchers have considered a subcutaneous implantable device for measuring pressure from mounting, but concerns of animal welfare, consumer perception, and potential residue issues have limited development (Senger, 1994).

Recently, an alternative method of automated mounting detection has shown potential (Homer et al., 2013). An ultra-wideband radio technology captured 3-dimensional positioning of animals to determine height changes associated with cows mounting others or standing to be mounted. The ultra-wideband radio system identified 9 of 10 cows in estrus and 6 of 6 cows not in estrus compared to milk P4, VO, and activity monitoring. Although promising, further commercial demonstration of this method is necessary.

A restraint of both of these mounting behavior monitors is that mounting behavior must occur for them to work (Saint-Dizier and Chastant-Maillard, 2012). Multiple studies have reported standing estrus occurrence in fewer than 50% of estrus events (Van

Eerdenburg et al., 1996; Heres et al., 2000). Modern facilities, especially concrete, limit mounting behavior (De Silva et al., 1981; Britt et al., 1986). Additionally, most pressure sensitive systems only detect mounts lasting ≥ 2 s, but 40% of mounts may last < 2 s (Walker et al., 1996).

Activity

An increase in activity associated with estrus was first observed in rats in 1923 (Wang, 1923). Additional research showed this response in other female mammals, including humans, swine, and cattle (Altmann, 1941; Farris, 1944; Farris, 1954). One of the first activity monitoring studies in cattle found that number of steps per h increased 2 to 4 times in cows experiencing estrus when compared to cows not in estrus (Kiddy, 1977). Duration of the activity increase associated with estrus is 16.1 ± 4.7 h (Valenza et al., 2012) and multiple studies from a recent review estimated current AAM systems can accurately detect 70% of cows in estrus (Fricke et al., 2014b). Two types of AAM systems are currently available: 1) pedometers, usually attached to the leg and 2) accelerometers, which have been attached to the neck, leg, or ear (Saint-Dizier and Chastant-Maillard, 2012). Pedometers measure the number of steps taken and accelerometers measure three-dimensional movement, estimating overall activity (Fricke et al., 2014b).

In a recent comparison between AAM and VO conducted by Michaelis et al. (2014), no difference in EDR existed (42.1 vs. 37.3%, respectively). The sensitivity and positive predictive value of AAM (35.6 and 83.3%, respectively) was numerically, but not significantly, greater than VO (34.3 and 75.1%). The ability of AAM to produce similar or better results than VO has also been shown in other research (Peter and Bosu,

1986; Liu and Spahr, 1993; At-Taras and Spahr, 2001). Automated activity monitoring can also be useful in heifers under a variety of housing systems, including pasture, dry lot, and tiestall (Sakaguchi et al., 2007). Comparisons between AAM and other estrus detection methods also exist. Cavalieri et al. (2003a) compared estrus detection of a pedometer, a rump-mounted pressure-sensitive mounting detector, and tail paint using milk P4 levels and pregnancy diagnosis and found no differences in sensitivities (81.4, 88.4, and 91.3%, respectively).

Reports concerning the percent of estrus events identified using AAM vary between 51 and 84% in both confinement and pasture situations (Lewis and Newman, 1984; Redden et al., 1993; Roelofs et al., 2005a; McGowan et al., 2007; Hockey et al., 2010; Kamphuis et al., 2012; Valenza et al., 2012). Yaniz et al. (2006) stated that a reduction in physical activity occurs with increased milk production, parity, and temperature humidity index (**THI**). Holman et al. (2011) agreed that high milk yield and low BCS may negatively affect AAM sensitivity, additionally adding that lameness can affect results from leg mounted technologies. However, synchronization, parity, cow age, milk yield, season, DIM, and weather have been found in other studies to have no effect on physical activity (At-Taras and Spahr, 2001; Yaniz et al., 2006).

Recently, studies have focused on comparing AAM to TAI. In 2010, Galon (2010) found no difference in first service CR between Ovsynch (17.6%) and pedometers (22.6%). A more comprehensive study compared TAI to AAM using over 900 animals from 3 herds (Neves et al., 2012). Time to pregnancy was shorter (82 vs. 125 d) for cows bred using the AAM.

Rumination

Automated rumination monitoring can use a microphone system that lies on the cow's neck to identify the regurgitation and re-chewing of cud (Burfeind et al., 2011) or an accelerometer to identify motions associated with rumination (Bikker et al., 2014). Schirmann et al. (2009) validated a commercial, microphone-based rumination monitoring device, finding high correlations to VO of 51 cows ($r = 0.93$). Because of the decrease in feed intake during estrus (Maltz et al., 1997), the resulting decrease in rumination provides another possible method for AED (Reith and Hoy, 2012). Reith and Hoy (2012) showed a reduction in rumination on the day of estrus from a baseline of 429 min/d to 355 min/d. Overall, mean decrease in rumination during 265 estrus events was 17% (74 min), but with high variation (-71 to +16%). In a follow-up study that looked at 453 estrous cycles, rumination time decreased 19.6% (83 min/d) on the day of estrus (Reith et al., 2014). Pahl et al. (2015) also found a decrease in rumination on the day of (19.3%) and the day before (19.8%) inseminations leading to pregnancy.

Temperature

Cow temperature fluctuates throughout the estrous cycle, being lowest just before estrus, high on the day of estrus, and low again at the time of ovulation in comparison to the high temperatures seen throughout the luteal phase of the cycle (Wrenn et al., 1958; Lewis and Newman, 1984; Suthar et al., 2011). The decrease before estrus may result from lowered P4 levels after luteolysis (Wrenn et al., 1958; Kyle et al., 1998), though Suthar et al. (2011) identified no correlation between body temperature and serum P4 concentrations ($r = 0.018$). The increase in temperature during estrus could be associated with the increase in activity during behavioral estrus (Walton and King, 1986; Redden et

al., 1993). Yet tie stall cows, whose movement is constricted, have also experienced increases in vaginal temperature during estrus (Suthar et al., 2011). Other hypothesis for increased vaginal temperature surrounding estrus are enhanced blood flow to the area (Suthar et al., 2011) and correlation with the LH surge (Clapper et al., 1990).

Regardless of reasoning, reticulorumen boluses, vaginal inserts, temperature monitoring ear tags, and milk temperature sensing technologies originally designed for disease detection could provide an additional method of estrus detection. Vaginal temperature increases between 0.10 and 1.02 °C (Lewis and Newman, 1984; Redden et al., 1993; Kyle et al., 1998; Fisher et al., 2008; Suthar et al., 2011) and milk temperature increases of 0.3 °C (Maatje and Rossing, 1976; McArthur et al., 1992) have been recorded during estrus. Rectal temperatures, though non-automated, have even greater reported increases during estrus (1.3 °C; Piccione et al., 2003). These temperature increases last for 6.8 ± 4.6 h in dairy cows and 6.5 ± 2.7 h in beef cows (Redden et al., 1993; Kyle et al., 1998).

Maatje and Rossing (1976) found 84% of visually observed estrus events were identifiable using twice-daily milk temperature monitoring. A follow-up study by McArthur et al. (1992) introduced skepticism after only 50% of estrus events were identified via milk temperature monitoring compared to P4 concentrations in the milk. Other studies have focused on vaginal temperature monitoring, finding sensitivities ranging from 69 to 86% when compared to P4 concentrations, making them similar to VO (Redden et al., 1993; Kyle et al., 1998). Overall, temperature monitoring as a tool for estrus detection has both potential and difficulties (Ball et al., 1978; Schlünsen et al., 1987; Fordham et al., 1988; Cooper-Prado et al., 2011; Culmer, 2012). Past challenges

have included large daily fluctuations in temperature, variability in temperature rises, seasonal variation, and problems with data recovery from reticulorumen temperature boluses. Many studies agree that temperature alone may not be specific enough to use for estrus detection because of the variety of factors (sickness, ambient temperature, water intake, etc.) that may also affect it (Walton and King, 1986; Fordham et al., 1988)

A newly proposed tool for automated temperature monitoring is measurements of body surface temperature using infrared technology (Talukder et al., 2014). Although originally discredited for high rates of FP and FN (Hurnik et al., 1985), new technology has been developed that is much more promising. Talukder et al. (2014) measured surface temperature on the vulva and muzzle of 20 cows and identified a significant decrease in temperature 48 h before, increase 24 h before, and another decrease at ovulation as determined by ultrasound evaluation. The sensitivity and specificity of this method for estrus detection compared to plasma P4 varied from 58 to 92% and 29 to 57%, respectively, depending on the algorithm used. Creation of an accurate algorithm and automation of vulval temperature monitoring is challenging because of fecal contamination and tail placement. Alternative locations for infrared temperature monitoring such as the eye and back of the ear may be more appropriate (Hoffmann et al., 2013).

Progesterone

Progesterone measurements can be estimated through both blood and milk sampling and are often used as the gold standard comparison when testing other estrus detection methods (Firk et al., 2002). Roelofs et al. (2006) demonstrated that milk P4 concentrations decline to < 5 ng/ml 80 h before and < 2 ng/ml 71 h before ovulation, with

blood P4 following a similar pattern. Multiple reproductive parameters can be gained from measuring P4, including identification of estrus and estrus detection errors, likelihood of insemination success, pregnancy diagnosis or loss, ovarian cyst diagnosis, anestrus identification, and evaluation of responses to hormone intervention (Nebel, 1988; Blom and Ridder, 2010; Mazeris, 2010; Saint-Dizier and Chastant-Maillard, 2012). On-farm, individual milk P4 tests have been developed (Marcus and Hackett, 1986; Worsfold et al., 1987; Nebel, 1988), but are not automated.

An alternative is automated detection through inline milk sampling systems (Pemberton et al., 2001; Gillis et al., 2002; Saint-Dizier and Chastant-Maillard, 2012). The only commercially available system of this kind is Herd Navigator (DeLaval, Tumba, Sweden), which collects milk at specific time points throughout the estrous cycle to determine a P4 curve for each cow (Friggens and Chagunda, 2005; Mazeris, 2010). An algorithm in the system then determines if the cow receives an estrus alert depending on her point in the estrous cycle. A group of Danish herds using the Herd Navigator system have reported a CR between 40 and 63% and a mean reduction in DO of 22 d since adoption (Blom and Ridder, 2010). A separate survey reported PR of Herd Navigator test farms changed from 22.8% pre-installation to 40% two years later (Durkin, 2010).

When compared to inseminations resulting in pregnancy, the high sensitivity (93.3%) and specificity (93.7%) for estrus detection has identified the usefulness of the Herd Navigators as an AED tool (Friggens et al., 2008). Furthermore, the Herd Navigator can also conduct measurements of lactate dehydrogenase, urea, and β

dehydroxybutyrate to detect metabolic diseases and mastitis in addition to estrus detection benefits. Regardless, high cost of the system has limited its adoption.

Others

Lewis and Newman (1984) found vaginal pH to be lowest on the day of estrus, decreased milk yield surrounding estrus, and heart rate to be slowest during estrus. However, these variations were small and repeated measurements (because of lack of automation) are not yet feasible for commercial dairies. Similar inability to automate has reduced interest in other areas, including monitoring electrical resistance of vaginal mucus, dry matter concentration and crystallization patterns of vaginal mucus, and blood P4 around estrus (Noonan et al., 1975; Leidl and Stolla, 1976; Heckman et al., 1979).

Technology Combinations

According to de Mol et al. (1997), the missing link in automated technology monitoring is merging all available data. Combinations of multiple parameters would improve EDR when certain conditions (environmental temperature, pen changes, etc.) interfere with one monitoring method (Firk et al., 2002). Maatje et al. (1997a) considered the combination of activity, milk yield, and milk temperature for estrus detection, finding sensitivity improvements of 10 to 20% over activity alone. Peralta et al. (2005) also tested three parameters, finding the sensitivity of VO, activity monitoring, and mounting detection alone was 49.3%, 37.2% and 48%, respectively. The combination of all three systems increased estrus detection sensitivity to 80.2%. Additional studies have shown the usefulness of combining multiple variables for estrus detection (Redden et al., 1993; de Mol and Woldt, 2001b; Brehme et al., 2008; O'Connell et al., 2011).

Merging automatically collected data (activity, rumination, etc.) with an individual cow's history can also improve estrus detection algorithms. Firk et al. (2003) demonstrated that including information about the length of time since a cow's last estrus period decreased sensitivity from 91.7 to 87.9% but improved error rate from 34.6 to 12.5%.

The potential for multiple parameter combinations in estrus detection requires improved data analysis compared to univariate scenarios. Some multivariate evaluation techniques include statistical process control, fuzzy logic, neural networks, and machine learning.

Statistical process control monitors and detects changes in data over time. Control limits are set through calculations of the mean variation between observations and when an observation goes outside of those control limits, an alert is triggered (De Vries and Conlin, 2003b). This allows the model to distinguish between natural variation and real change. Statistical process control has been used to manage mastitis (Niza-Ribeiro et al., 2004; Lukas et al., 2005) and reproductive performance (De Vries and Conlin, 2003a; b).

Fuzzy logic analysis involves 3 steps: fuzzification, fuzzy inference, and defuzzification (Firk et al., 2002). Fuzzification is the process of transforming real variables into linguistic variables. Fuzzy inference then applies rules to the transformed variables in a fashion similar to "if, then" statements to classify them. Defuzzification returns the values created by fuzzification and fuzzy inference back to readable values. In the dairy industry, fuzzy logic has been applied to mastitis (De Mol and Woldt, 2001a;

Cavero et al., 2006; Kramer et al., 2009), lameness (Kramer et al., 2009), and estrus detection (De Mol and Woldt, 2001a).

Neural networks do not require a specific algorithm to work (Grzesiak et al., 2006). Instead, they learn how to make associations and adapt when presented with new data. Although most commonly used in engineering, business, and medicine, some models can predict milk production (Sanzogni and Kerr, 2001; Grzesiak et al., 2006; Sharma et al., 2007) and mastitis occurrence (Ankinakatte et al., 2013) (Heald et al., 2000; Hassan et al., 2009).

Machine learning is another method of programming that allows for constant algorithm improvement through experience and data analysis (Alpaydin, 2004). Machine learning is applicable to retailers who track customer behavior, financial institutions when identifying risk, and manufacturing scenarios to help minimize resource consumption. Reproductive performance in the dairy industry has also been evaluated using machine learning (Mitchell et al., 1996; Caraviello et al., 2006a; Shahinfar et al., 2013).

Technology Effect on Timing of Insemination

Pregnancy outcome is dependent on timing of AI relative to ovulation (Nebel et al., 1994). Automated monitoring technologies' ability to predict ovulation may help maximize CR by determining ideal AI time (Senger, 1994). Dransfield et al. (1998) evaluated 2,661 inseminations in 17 herds and reported the highest CR when cows underwent AI 4 to 12 h after the onset of standing activity as measured by an automated rump-mounted pressure-sensitive technology. A similar study using pedometer readings showed AI 6 to 17 h after increased activity levels resulted in the highest CR, with no

effect of disease, inseminator, or bull on the results (Maatje et al., 1997b). Vaginal temperature has also shown a high correlation ($r = 0.74$) to ovulation (Rajamahendran et al., 1989), and strong relationships with the LH peak (Clapper et al., 1990; Mosher et al., 1990; Fisher et al., 2008).

Automated technologies' ability to measure intensity and duration of estrus may further improve CR. Dransfield et al. (1998) reported that the probability of pregnancy increased with an increased number of standing events. Cows that stood for mounting less than 3 times experienced a 41% lower chance of becoming pregnant compared to cows that stood to be mounted 3 or more times before AI. Stevenson et al. (1983) agreed that increased estrus intensity resulted in a significant positive effect on CR.

Technology Adoption

Technology adoption on dairy farms has been slow (Russell and Bewley, 2013). In 2007, the USDA estimated dairy herds using pedometers and pressure sensing technologies for estrus detection at 1.4 and 5.7%, respectively (USDA, 2007). Nevertheless, Borchers and Bewley (2014) recently conducted a producer survey and identified high adoption interest in mounting and cow activity monitoring technologies.

Reasons producers may consider adopting automated technologies include current reductions in availability of skilled labor, greater opportunities to meet production goals, and increased electronic record keeping opportunities (Wathes et al., 2008). Producers may reject automated technologies because of lack of confidence in technology and uncertainty in payback period. Russell and Bewley (2013) conducted a survey to identify reasons for slow technology adoption in Kentucky herds and 42% and 30% of producers identified undesirable cost to benefit ratio and no economic value, respectively. Borchers

and Bewley (2014) also identified economics (benefit to cost ratio and investment cost) as the two biggest factors influencing technology adoption. These results highlight the importance of evaluating economic feasibility of automated technologies.

ECONOMICS OF REPRODUCTION

Reproductive performance is one of the largest factors affecting dairy farm profitability because of its direct relationship to milk production, replacement availability, genetic progress, and culling (Britt, 1985; Plaizier et al., 1997; Olynk and Wolf, 2008). Dijkhuizen et al. (1985) estimated that reproductive failure accounted for 2% of gross production or 10% of an average farmer's income. In agreement, both Plaizier et al. (1997) and Kalantari and Cabrera (2012) have identified high correlations between reproductive performance and herd value.

The mean value of a new pregnancy is between US\$192 and \$278, depending on parity, point in lactation, milk production, probability of pregnancy, and replacement heifer price (De Vries, 2006b; Cabrera, 2012). A more common economic measurement of reproductive performance is the cost of extended DO. One extra DO represents a loss of US\$0.10 to \$5.41 per cow, depending on calculation strategy and time point used (Holmann et al., 1984; Groenendaal et al., 2004; Meadows et al., 2005; De Vries, 2006a). This cost decreases with an increase in feed cost and slaughter price, a shorter voluntary waiting period (**VWP**), and lower milk production (Bewley et al., 2010). The cost of DO increases as reproductive performance declines and replacement and milk prices increase (Meadows et al., 2005; Bewley et al., 2010).

Estrus detection methods differ in cost because of differences in EDR, CR, and inputs (Holmann et al., 1987; Olynk and Wolf, 2009). Inputs include labor for estrus

detection and estrus detection aids such as tail paint, synchronization hormones, and automated detection technologies (Holmann et al., 1987). Additional reproductive management inputs include cost of semen, insemination, and pregnancy diagnosis. Galvão et al. (2013) estimated cost of visual estrus detection at \$0.15/cow/d, assuming labor costs of \$15.00/h and the ability to monitor 100 cows/h. Mean cost per straw of semen, AI labor cost per insemination, and cost per pregnancy diagnosis have recently been valued at \$10.00, \$5.00, and \$3.00, respectively (Galvão et al., 2013).

Synchronization Economics

Recent research estimated commercial prices for GnRH and PGF_{2α} at \$2.40 and \$2.65/dose, respectively (Galvão et al., 2013). Cost of labor for an injection was \$0.25, assuming 60 injections/h by an employee earning \$15.00/h. Lima et al. (2010) estimated that 10.8 injections per cow per year were required in a TAI program. With injection prices accumulating quickly, the benefits of TAI may be offset by drug costs (Tenhagen et al., 2004). To determine this, studies have compared the economics of synchronization with other reproductive management strategies.

One study conducted a partial budget comparison of natural service (exclusive use of bulls) and a modified Presynch-Ovsynch with estrus detection, assuming a large, western U.S. Holstein dairy herd (Overton, 2005). On average, natural service costs totaled US\$10 more per cow per year. Lima et al. (2010) also compared natural service to a modified Ovsynch TAI protocol, finding that the cost for each program per cow per year was \$100.49 and \$67.80, respectively. However, after accounting for the difference in VWP and PR, TAI's advantage over natural service reduced to \$9.73/cow per year.

The advantage of TAI over natural service depended on the cost to feed bulls, semen price, and genetic merit of purchased semen.

Olynk and Wolf (2009) compared the economics of three reproductive management programs: VO for estrus detection, Ovsynch, and Cosynch, a variation of Ovsynch involving insemination at the same time as the last GnRH injection. They found that Ovsynch resulted in economic preference until nine additional minutes of labor (estimated at \$1.92) were required, at which point Cosynch was preferred. A net present value (**NPV**) analysis by Olynk and Wolf (2008) emphasized that when labor costs are low, visual estrus detection has a greater value but when labor costs are high, Ovsynch has a greater value. However, these conclusions were highly dependent on sensitivity of the visual estrus detection program.

Whereas some dairies use only TAI, many dairies combine TAI and visual estrus detection (Giordano et al., 2012a). Recently, an economic comparison of VO for estrus, TAI (Presynch Ovsynch), and a combination of both systems considered varying EDR, accuracy of estrus detection, TAI compliance, and milk price (Galvão et al., 2013). Individually, the VO groups with a high (60%) EDR, regardless of accuracy of estrus detection (85 or 95%), showed the greatest profit followed by the TAI group with high (95%) compliance. Combining TAI and VO increased profits in all situations regardless of increased input costs.

Giordano et al. (2011) created a similar, though more user friendly, comparison model. The tool calculates NPV per cow per year for different reproductive management programs and can be adapted to an individual farm's situation using production, reproduction, and economic inputs. Reproductive management options comparable by

the tool include 100% VO, 100% TAI, and any combination of TAI and VO. In an example presented by the authors using information from a commercial Wisconsin dairy herd, 100% TAI programs were more profitable than 100% VO. Combining TAI and VO was only valuable when the CR was low in the program. Giordano et al. (2012a) found that the net value of programs combining TAI and visual estrus detection was reliant on the number of cows receiving AI after visually detected and the CR.

Automated Estrus Detection Economics

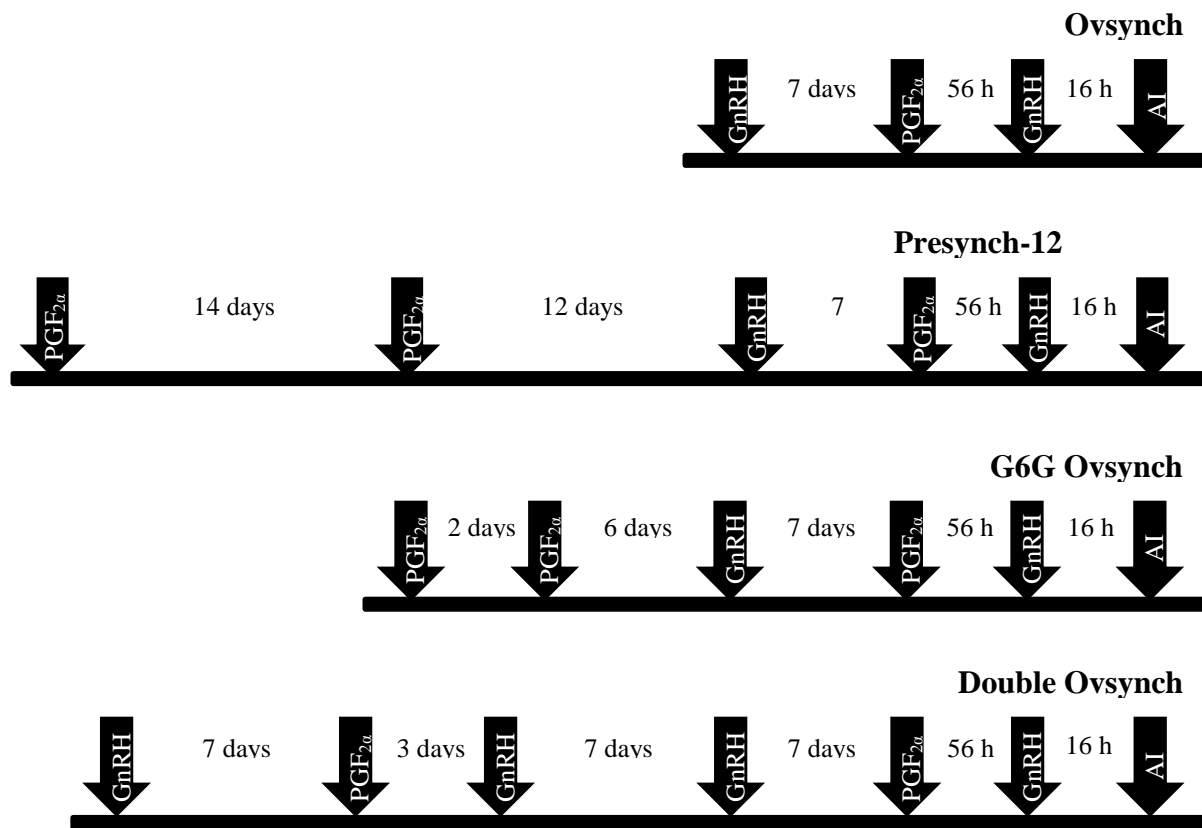
The full economic value of automated technologies is difficult to determine because most of them provide additional benefits (i.e. health monitoring, cow comfort evaluation, etc.) beyond estrus detection. One study has estimated the reproductive benefit of an inline P4 indicator at US\$63.50 per cow-year on a typical Danish herd, assuming that adoption would improve EDR, reduce time to first service, and reduce inaccurate AI (Ostergaard et al., 2005).

To the author's knowledge, publications of economic comparisons between AED technologies alone and other reproductive management programs do not exist. However, Fricke et al. (2014c) conducted an economic comparison between three reproductive management strategies: 1) cows inseminated using AAM and undergoing Ovsynch if not detected in estrus by 62 DIM, 2) cows inseminated using AAM after presynchronization and receiving Ovsynch if not detected in estrus by 62 DIM, and 3) cows inseminated after Presynch-Ovsynch. Their analysis resulted in a similar NPV for all three systems, indicating potential for the combination of activity monitoring and TAI.

CONCLUSIONS

Reproductive performance of dairy cows is below acceptable standards. One aspect of reproductive performance that we have substantial control over is estrus detection. Improvements to estrus detection may be obtainable through TAI or AED technologies. The economics of alternative reproductive management systems require evaluation before adoption.

Figure 1.1. Injection protocol for Ovsynch, Presynch-12 Ovsynch, G6G Ovsynch, and Double Ovsynch using gonadotrophin releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).



CHAPTER TWO

Estrus Detection using Multiple Automated Technologies Compared to Visual Observation

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INTRODUCTION

Detecting a high percentage of cows in estrus is essential to maintain reproductive performance in dairy herds using AI. Estrus detection rate is the most common measure used to determine estrus detection efficiency. Estrus detection rate is calculated as the number of cows detected in behavioral estrus, divided by the number of cows eligible to come into behavioral estrus throughout a 21 d period, multiplied by 100 (USDA, 2007). Traditionally, estrus detection is accomplished using VO. The most definitive sign of estrus is when a cow stands and allows another cow to mount her. Other observable signs of estrus include increased activity, restlessness, tail raising, clear mucus discharge from the vulva, a pink and swollen vulva, reduced milk yield and feed intake, displaying a flehmen response, and increased interactions with other cows (sniffing the vulva area, chin resting, mounting, licking, grooming, head butting, etc.; Foote, 1975; Van Eerdenburg et al., 1996; Kerbrat and Disenhaus, 2004).

Visual observation generally produces poor results, regardless of being the most common form of estrus detection (used by 93% of US dairy operations; USDA, 2007). The Dairy Records Management Systems reported mean yearly EDR on US Holstein herds (including all reproductive management strategies) as 44.9% in 2015 (DRMS, 2015). The low EDR may be a result of the extreme decline in Holstein cattle estrus duration (from 18 h to less than 8 h) over the last 50 years (Reames et al., 2011). Increasing age, milk production, and environmental factors (greater ambient temperature, uncomfortable housing, etc.) can also negatively affect length and intensity of estrus expression (Vailes and Britt, 1990; López-Gatius et al., 2005; Palmer et al., 2010).

Many farms have successfully implemented timed artificial insemination (**TAI**) as a replacement for visual estrus detection. In 2007, the USDA reported that 58.2% of dairy operations used some form of PGF_{2α} or GnRH based synchronization programs for at least a portion of their heifers or cows (USDA, 2007). However, protocol incompliance (i.e. missed injections) often reduces the success of TAI (Galvão et al., 2013). Additionally, consumer concerns surrounding hormone use could limit this management option in the future (Saint-Dizier and Chastant-Maillard, 2012; Nebel, 2013).

Automated estrus detection technologies are another available alternative to supplement or replace visual estrus detection. Parameters with potential for AED include mounting events, activity level, lying time, rumination events, plod or milk P4 levels, feeding time, body temperature, and more (Senger, 1994; Saint-Dizier and Chastant-Maillard, 2012; Fricke et al., 2014a). Estrus related changes in some of these parameters (mounting events, activity level, lying time, rumination events, and P4) have been quantified repeatedly. However, a lack of consistent data exists surrounding estrus related changes in feeding time and body temperature. Additionally, these parameters have not been measured on the same cows during the same estrus periods.

Although mounting events are nearly exclusive to estrus, the remaining secondary signs of estrus are not. Therefore, to create estrus alerts, technologies use algorithms or sets of rules to follow during calculations. Algorithms vary between technologies and are specific to each technology manufacturer. Most algorithms work by comparing an animal's current behavior with a cow-specific reference period. The reference period is

used to create a threshold and when that threshold is exceeded, an estrus alert is created (Saint-Dizier and Chastant-Maillard, 2012).

To determine usefulness of a specific technology, estrus events identified by the technology algorithm are compared to a gold standard such as VO, ultrasonography, blood or milk P4 levels, or a combination of these. Correctly identified estrus events are considered TP, non-alerted estrus events are FN, non-alerted non-estrus events are TN, and alerted non-estrus events are FP (Firk et al., 2002). Detecting estrus events is a balance of sensitivity and specificity. Sensitivity, the probability that an event is alerted, is equal to $TP/(TP+FN)*100$ (Hogeveen et al., 2010). Specificity, the probability that when an event does not occur no alert is generated, is equal to $TN/(TN+FP)*100$. Because neither sensitivity nor specificity account for the prevalence of the event, other comparative measurements are also useful. These include positive predictive value $[TP/(TP+FP)*100]$, negative predictive value $[TN/(TN+FN)*100]$, and accuracy $[(TP+TN)/(TP+TN+FP+FN)]$.

The “estrus alerts” created by individual technologies are a combination of the parameters measured by the technology and the technology manufacturer algorithm. Most technology manufacturer algorithms are considered proprietary, making it difficult to directly compare detection performance between technologies. Machine learning techniques can be used to replace the manufacturer alert algorithms and compare technologies to one another based solely on parameter data collected. Machine learning techniques work in two stages. First, the machine learning technique uses a training set of parameter data to identify a subset of features associated with the event of interest. Second, a testing data set is used to evaluate machine learning technique prediction

power , given the associations established using the training data (Breiman, 2001). Three common machine learning techniques are random forest, linear discriminant analysis, and neural network. Each of the three methods works in a different way. The random forest method develops a group of tree-structured classifications using the training data. Then, within each iteration of the testing series, each tree contributes an opinion of how the data should be classified (Breiman, 2001; Shahinfar et al., 2014). Linear discriminant analysis is used to determine relationships between one categorical variable and a set of independent variables (McLachlan, 2004), similar to logistic regression analysis. Neural networks are the most frequently used of machine learning techniques in animal sciences (Shahinfar et al., 2014). These models are designed to make connections similar to a human's nervous system (Lippmann, 1987). Neural networks are valued for their ability to learn independently and adapt quickly (Krieter, 2005).

Mitchell et al. (1996) and Krieter (2005) have previously described the use of machine learning techniques for estrus detection. However, both studies used the technique to identify the day of estrus rather than a more specific time frame. Additionally, no commercially available AED technology data was used in those analyses.

This study included two objectives. The first objective was to evaluate estrus related changes in activity level, number of steps, lying bouts, lying time, feeding time, rumination events, ear surface temperature, and reticulorumen temperature as measured using five AED technologies on the same cows. The second objective of this study was to apply machine learning techniques to parameters collected by AED technologies to see if improvements in estrus detection ability could be made.

MATERIALS AND METHODS

This study was conducted at the University of Kentucky Coldstream Dairy under Institutional Animal Care and Use Committee protocol number 2013-1069. All lactating cows ($n = 82$) were housed in two groups, separated by a shared, raised feedbunk. Both groups maintained open access to freestalls, one group with sawdust-covered rubber-filled mattresses (PastureMat; Promat, Ontario, Canada) and the other group with sawdust-covered Dual Chamber Cow Waterbeds (Advanced Comfort Technology, Inc., Reedburg, WI). Cows were allowed access to a grass seeded exercise lot for 1 h per d at 1000, weather permitting. All other surfaces accessible to cows (freestall area, feed bunk, holding pen, and alleys) contained grooved concrete. Delivery of a TMR ration containing corn silage, alfalfa silage, whole cottonseed, and grain mix occurred 2X at 0530 and 1330. Milking occurred 2X at 0430 and 1530.

This study enrolled 32 Holstein cows that had not been bred in their current lactation. Parity, DIM, and PTA milk (mean \pm SD) of these cows at the beginning of the study protocol was 2.0 ± 1.2 , 77.8 ± 20.5 d, and 639.7 ± 535.8 , respectively. Cow ovulations were synchronized in three groups of 14, 10, and 8 cows, starting on January 24, March 19, and May 14, respectively. The synchronization protocol was a modification of the standard Ovsynch (Pursley et al., 1995), preceded by G7G (Bello et al., 2006; Figure 2.1). In contrast to the standard Ovsynch, administration of the last injection of GnRH (gonadorelin diacetate tetrahydrate, Cystorelin; Merial Limited, Duluth, GA; 100 μ g intramuscular) did not occur to stimulate estrus expression. Additionally, to stimulate CL regression, two PGF_{2 α} injections (dinoprost tromethamine, Lutalyse; Zoetis, Florham Park, NJ; 25 mg intramuscular) were given on the last day of

the protocol (7 d after the first GnRH injection), 6 h apart (0800 and 1400). Day 0 was designated as the last day of the synchronization protocol in each group (Figure 2.1).

Estrus Confirmation

Blood samples (10 ml) were collected from cow coccygeal vein on d -2, -1, 0, 1, 2, 7, 9, and 11 (Figure 2.1). Plasma was separated from centrifuged samples and stored at -20 °C until the concentration of P4 was determined by radioimmunoassay (Coat-a-Count Progesterone, Siemens Medical Solutions USA, Inc., Malvern, PA). Response to the synchronization protocol was confirmed if P4 was greater than 1.0 ng/ml on d -2, -1, and 0, dropped to less than 1.0 ng/ml by d 1, and returned above 1.0 ng/ml by d 9. Ultrasonography identified ovarian structures on d -16, 0, 5, 7, and 11 (Figure 2.1), further confirming synchronization response.

Visual observation of cows for 4, 30-min periods at 0330, 1000, 1430, and 2200 occurred on d 2, 3, 4, and 5 (Figure 2.1). Two observers were present at each shift, with one assigned to each side of the separated housing area. Study cows were clearly identified using spray paint. Observers recorded the time of each standing estrus event.

Technologies Evaluated

Each cow was fitted with 5 automated monitoring technologies before beginning synchronization. The DVM bolus (DVM Systems, LLC, Greeley, CO), placed into the reticulorumen using a bolus gun, recorded reticulorumen temperature twice daily using a passive radio-frequency identification transponder. Data download occurred at the time of parlor entrance, where panel readers were located. The HR Tag (SCR Engineers Ltd., Netanya, Israel), held on the left side of the neck using a nylon collar, measured neck activity and rumination time in 2 h blocks using a 3-axis accelerometer and microphone

with a microprocessor, respectively. The rumination portion of the HR Tag was previously validated in dairy cattle (Schirmann et al., 2009; Burfeind et al., 2011). The IceQube (IceRobotics Ltd., Edinburgh, Scotland), attached to the left rear leg using a plastic strap, reported number of steps, lying bouts, and lying time every 15 minutes using a 3-axis accelerometer. The SensOor (Agis Automatisering, Harmelen, Netherlands), attached to the left ear, used a 3-axis accelerometer to classify each min into one of six behaviors (rumination, feeding, resting, low activity, regular activity, or high activity) and reported hourly percentage of time associated with each behavior. Additionally, the SensOor used a digital surface temperature monitor to evaluate mean hourly ear surface temperature. The behavioral portion of the SensOor, but not the temperature monitor, was previously validated on dairy cows (Bikker et al., 2014). The Track a Cow (Animart Inc., Beaver Dam, WI and ENGS, Rosh Pina, Israel), attached to the front right leg using a nylon strap, used a 3-axis accelerometer to measure hourly activity and lying time.

Statistical Analysis

Regardless of data reporting frequency method, all technology parameter data was summed by hour. Parameter data was then averaged by hour across 12 hour blocks of time. If observed in standing estrus during VO periods, a cow's estrus was classified as the 12 h period of time leading up to the first observed standing estrus event. The 28, 12 h periods (14 d) before the estrus period were classified as periods of non-estrus.

Estrus vs. Non-estrus. The MIXED procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) analyzed the effect of estrus status (estrus or non-estrus) on DVM bolus reticulorumen temperature; HR Tag neck activity and rumination; IceQube lying bouts,

lying time, and number of steps; SensOor ear surface temperature, feeding time, high ear activity, and rumination; and Track a Cow leg activity and lying time, considering cow as a random effect (c_i):

$$y_{ij} = \mu + ES_j + c_i + e_{ij}$$

Where y_{ij} is the parameter measurement (DVM bolus reticulorumen temperature; HR Tag neck activity or rumination; IceQube lying bouts, lying time, or number of steps; SensOor ear surface temperature, feeding time, high ear activity, or rumination; or Track a Cow leg activity or lying time) of the i th cow; μ is the intercept; ES_j is an indicator of estrus or non-estrus at time j ; and e_{ij} is residual error.

Correlations. The CORR procedure of SAS 9.3 calculated daily relationships between DVM bolus reticulorumen temperature; HR Tag neck activity and rumination; IceQube lying bouts, lying time, and number of steps; SensOor ear surface temperature, feeding time, high ear activity, and rumination; and Track a Cow leg activity and lying time for each cow. The MEANS procedure of SAS 9.3 averaged the 18 daily cow correlations and the median was used to represent cow level correlations.

Group level correlations were also analyzed. The MEANS procedure of SAS 9.3 averaged the 14 d of non-estrus data from all 18 cows by parameter and day. The CORR procedure of SAS then summarized relationships between DVM bolus reticulorumen temperature; HR Tag neck activity and rumination; IceQube lying bouts, lying time, and number of steps; SensOor ear surface temperature, feeding time, high ear activity, and rumination; and Track a Cow leg activity and lying time.

Machine learning. The package <caret> from R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to create a 4-fold cross-validation,

including 10 analysis per series, for each technology (DVM bolus, HR Tag, IceQube, SensOor, and Track a Cow) using three machine learning techniques (random forest, linear discriminant analysis, and neural network). The training data set contained 70% of visually observed estrus events ($n = 13$). The remaining 30% of observations were used in the testing data set ($n = 5$). The models were used to predict which time block (of the 29, 12 h periods defined earlier) each data line referred to. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of each technology and machine learning technique combination were calculated based on time block predictions.

RESULTS AND DISCUSSION

Progesterone analysis combined with ultrasonography indicated that 29 of the 32 cows (90.6%) ovulated after completing the synchronization protocol. Eighteen cows (62.1%) were observed standing to be mounted during the VO periods. Failure to detect the remaining 11 cows may have resulted from unexpressed estrus periods or short estrus lengths that went unobserved because of non-continuous observation.

A researcher error resulted in some data not being properly saved from the computer. Consequently, 4 cows observed in estrus were missing lying time data from the Track a Cow and were removed from affected analysis. Additionally, a technology malfunction resulted in no IceQube data recorded for 1 other cow, which was also removed from affected analysis. All remaining technology parameter analysis included all 18 cows observed in estrus.

Activity

All activity measures increased significantly during estrus compared to non-estrus ($P < 0.01$; Table 2.2.1). The percent activity increase between non-estrus and estrus for HR Tag neck activity, IceQube number of steps, SensOor high ear activity, and Track a Cow leg activity was 71.2%, 224.8%, 330.4%, and 177.1%, respectively. Similar estrus associated increases in numbers of steps (2 to 4 times) have been reported previously (Kiddy, 1977; Redden et al., 1993; Roelofs et al., 2005a). The range of increase in activity may have resulted from differing accelerometer attachment locations. The largest estrus related activity increase was recorded by the ear-mounted accelerometer, followed by the leg location, and then the neck (Table 2.2.1). Holman et al. (2011) compared the same neck activity monitor as used in this study to a leg activity monitor, finding no difference in sensitivity (58.9 vs. 63.3%) but noting that different factors (BCS, milk yield, and lameness) affected each system's ability to detect estrus.

Activity monitoring is the most widely marketed of automated measures used for estrus detection. The ability of AAM to detect estrus, as reported in multiple studies, ranges between 51.0 and 89.2% in both confinement and pasture situations (Roelofs et al., 2005a; Dela Rue et al., 2014). McGowan et al. (2007) evaluated the estrus detection ability of activity as measured by the IceTag, an earlier version of the IceQube, and found a range in sensitivity from 76 to 84% and a range in specificity from 12.5 to 22%. Kamphuis et al. (2012) evaluated the estrus detection ability of neck activity using the HR Tag, finding a 62.4% sensitivity, 99.3% specificity, and 76.6% positive predictive value.

Group level correlations between activity monitors in this study ranged from a low of 0.76 between HR Tag neck activity and Track a Cow leg activity and a high of 0.99 between all activity monitoring technologies (IceQube, SensOor, and Track a Cow) except the HR Tag (Table 2.2). At the cow level, correlations were reduced and ranged from 0.17 between the SensOor ear activity and Track a Cow leg activity and 0.55 between the number of steps recorded by the IceQube and the SensOor ear activity (Table 2.2). Activity monitor location affected activity level reading, resulting in the wide range of correlations. Differences between group level and cow level correlations were expected because daily variation at the cow level will be masked at the group level.

Lying Time and Lying Bouts

All lying measures were significantly less during estrus as compared to non-estrus ($P < 0.01$; Table 2.2.1). Percentage change between non-estrus and estrus for IceQube lying bouts, IceQube lying time, and Track a Cow lying time were similar at -50.0%, -56.0%, and -51.6%, respectively. Time spent lying decreases around estrus because of increased activity (Esslemont and Bryant, 1976; Livshin et al., 2005; Jonsson et al., 2011). However, lying time alone has rarely been used as a predictor for estrus. McGowan et al. (2007) found that lying time recorded by the IceTag produced poor results alone as an indicator of estrus (50% estrus detection). Similarly, Jonsson et al. (2011) reported a 50.0% sensitivity, 99.6% specificity, and 18.2% error rate [calculated as $FP/(TP+FP)*100$] using lying behavior reported by the IceQube to predict estrus. Compared to IceQube step count from the same study (sensitivity: 88.9%, specificity: 99.4%, and error rate: 15.8%), results were poor. However, by adding lying behavior to

step count, error rate was reduced to 5.9%. Therefore, there may be potential to use lying behavior in combination with other parameters to reduce false estrus alerts.

The correlation between lying time as measured by the IceQube and Track a Cow in this study was 0.85 at the group level and 0.44 at the cow level (Table 2.2).

Differences in lying time between technologies may be explained by technology location.

The IceQube was placed on a rear leg whereas the Track a Cow was placed on a front leg. At times, cows may lay so that their back legs are more perpendicular to the ground than their front legs, or vice versa.

Rumination and Feeding Time

Both measures of rumination time decreased significantly from non-estrus to estrus ($P < 0.01$; Table 2.2.1). Previous research analyzing the change in rumination relative to estrus is limited. Reith and Hoy (2012) evaluated 265 estrus events, finding that rumination on the d of estrus decreased 17% (74 min), but with large variation between herds (14 to 24%). In a follow-up study that looked at 453 estrous cycles, rumination time decreased 19.6% (83 min) on the d of estrus (Reith et al., 2014). Pahl et al. (2015) also found a decrease in rumination on the d of (19.3%) and the d before (19.8%) inseminations leading to pregnancy. In this study, the percent decrease in rumination time for the HR Tag and SensOor was 49.9% and 42.7%, respectively, during the 12 h period before estrus. These large decreases could be the result of a narrower “estrus” window (12 h) as compared to the previous studies (1 d).

The correlation between rumination time recorded by the HR tag and rumination time recorded by the SensOor was 0.78 at the group level and 0.36 at the cow level (Table 2.2). Differences between technologies (4.88 min/h during estrus and 6.42 min/h

during non-estrus) could be the result of differing recording methods. The HR Tag uses a microphone system that rests on the cow's neck to identify the regurgitation and re-chewing of cud. The SensOor uses an accelerometer to identify ear movement associated with rumination. Both systems have been validated with high correlations to VO (SensOor: $r = 0.93$ and HR Tag: $r = 0.93$; Bikker et al., 2014 and Schirmann et al., 2009). However, the SensOor validation was done on a per minute basis whereas the HR Tag validation was done on a 2-hour basis. Therefore, results are not directly comparable.

One explanation for decreased rumination around estrus is decreased feed intake (Maltz et al., 1997; Diskin and Sreenan, 2000). Contrarily, feeding time as recorded by the SensOor in this study increased significantly during estrus (15.55 min/h) as compared to non-estrus (8.88 min/h; $P < 0.01$; Table 2.2.1). In agreement, group level correlations between SensOor feeding time and HR Tag rumination ($r = -0.37$) and SensOor rumination ($r = -0.79$) were negative (Table 2.2). Other researchers agree that feed intake may not always decrease around estrus. De Silva et al. (1981) found no change in feed intake during the 3 d period surrounding estrus and Lukas et al. (2008) found DMI actually increased 0.61 kg/d during estrus. The method by which the SensOor measured feeding time in the current study depended on the ability of an accelerometer to distinguish ear movements related to feeding and is not a true measure of intake. Therefore, the reported increase in feeding time may not represent an actual increase in DMI but rather head movements similar to those occurring when a cow is feeding.

Temperature

Reticulorumen temperature as measured by the DVM bolus increased significantly during the 12 h period of estrus (39.36 ± 0.24 vs. 38.85 ± 0.21 ; $P < 0.0$;

Table 2.2.1). Ear surface temperature as recorded by the SensOor showed a numeric but non-significant increase during estrus (24.27 ± 1.21 vs. 23.00 ± 0.85 ; $P = 0.16$; Table 2.2.1). Ear surface temperature is influenced by both core body temperature and ambient temperatures (Mader and Kreikemeier, 2006). Therefore, ear surface temperature was expected to be less than and fluctuate more than reticulorumen temperature (a measure of core body temperature alone). The large variation in ear surface temperature was evident in the greater standard error as compared to reticulorumen temperature. SensOor temperature measurements are not advertised for estrus detection use, likely because of this variation.

Previously, vaginal temperature increases between 0.10 and 1.02 °C (Lewis and Newman, 1984; Kyle et al., 1998) and milk temperature increases of 0.3 °C (Maatje and Rossing, 1976; McArthur et al., 1992) have been reported during estrus. Rectal temperatures, though non-automated, have even greater reported increases during estrus (1.3 °C; Piccione et al., 2003). These estrus related temperature increases have been reported to last for 6.8 ± 4.6 h in dairy cows and 6.5 ± 2.7 h in beef cows (Redden et al., 1993; Kyle et al., 1998).

Regardless of reported estrus related changes in temperature, inconsistent results have been found when using temperature for estrus detection in previous studies. Maatje and Rossing (1976) found 84% of visually observed estrus events were identifiable using twice-daily milk temperature monitoring. However, McArthur et al. (1992) found only 50% of estrus events were identified via milk temperature monitoring as compared to P4 concentrations.

Correlation between the reticulorumen temperature measured by the DVM bolus and ear surface temperature measured by the SensOor was negative at both the group level ($r = -0.24$) and the cow level ($r = -0.04$; Table 2.2). The different temperature monitor locations and large measurement variations likely contributed to this result.

Behavioral Parameter Relationships

Table 2.2 includes all group level and cow level correlations between parameters. As discussed previously, group level correlations were expected to be greater than cow level correlations because daily variation at the cow level will be masked at the group level. Both types of relationships between parameters may be useful in the creation of multivariable estrus detection algorithms. Discussion below considers group level correlations.

As expected, activity measures showed moderate to strong negative correlation with lying measures ($r = -0.44$ to -0.96). Both rumination measures showed negative correlations with activity (group level: $r = -0.95$ to -0.15) and strong positive correlations with lying measures ($r = 0.63$ to 0.88), indicating that most rumination occurred when cows were lying down. As expected, feeding time showed strong negative correlations with lying measure ($r = -0.90$ to -0.68) and strong positive correlations with activity ($r = 0.86$ to 0.94). Temperature showed weak correlations ($r = -0.19$ to 0.19) with most parameters. The only exception was a moderately negative correlation between SensOor ear surface temperature and HR Tag rumination ($r = -0.57$), for reasons not fully understood.

Machine Learning

Because of the low number of observed estrus events in this study ($n = 18$), when 70% of the data was used for the training set, data from only 5 cows was left for the testing set. Consequently, results should be interpreted carefully, keeping in mind the low sample size. Table 2.3 shows the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy accomplished using different combinations of each of the five technologies and machine learning techniques (random forest, linear discriminant analysis, or neural network). The objective of this analysis was to compare estrus detection ability of automatically collected technology data, independent of alert algorithm.

Using random forest, the SensOor and IceQube produced the greatest accuracy (98.6%; Table 2.3). The SensOor also produced the greatest accuracy (100%) when using linear discriminant analysis whereas the IceQube produced the greatest accuracy (100%) when using neural networks (Table 2.3). The number and variety of parameters measured by both the SensOor and IceQube likely gave them an advantage in these analysis. Peralta et al. (2005) showed that although VO, activity monitoring, and mounting detection alone produced low estrus detection sensitivities (49.3%, 37.2% and 48.0%, respectively), combining all three produced an acceptable sensitivity of 80.2%. Redden et al. (1993) also found that by combining two parameters (activity and vaginal temperature) that alone each produced an 80% EDR, a 90% EDR was possible.

The DVM bolus produced the poorest results in the machine learning analysis. The model found few patterns in the DVM bolus data useful for predicting estrus events, as indicated by multiple cases of 0.0% sensitivity or 0.0% specificity. Overall accuracy was still acceptable for the DVM bolus (71.7 to 96.6%), but only because of the low number of

potential TP ($n = 5$) and the large number of potential TN ($n = 140$) in the analysis rather than actual predicting ability. The DVM bolus was the only tested technology that contained only one measured parameter. Machine learning techniques work by finding patterns between parameters and likely did not have enough data to predict estrus in this scenario.

Between the remaining technologies, the IceQube produced the second best accuracy (97.9 to 100.0%), followed by the HR Tag (96.6 to 97.9%), and then the Track a Cow (91.0 to 97.2%). However, all technology results were similar. Compared to other studies that have tested similar machine learning techniques for estrus detection, these results are high. Krieter (2005) applied the neural network technique, combining activity and time since last estrus to a testing set of 74 estrus events, and accomplished a sensitivity, specificity, and error rate of 77.5, 99.6, and 9.1%, respectively. Mitchell et al. (1996) used machine learning techniques to identify 69% of estrus events in a 44 cow testing set, but experienced a large number of FP (74%). That analysis included milk yield, milking order, and time since last estrus as predictors. Both of those analyses were used to predict the day of estrus, whereas the current study focused on predicting a 12 h period before estrus. Narrowing the estrus period may be more accurate as most researchers agree that estrus does not last a full 24 h. Another explanation for the improved results in this study is the low number of observations in the testing set, which could have resulted in the models overestimating predicting ability.

Size and structure of the dataset can influence the performance of different machine learning techniques (Shahinfar et al., 2014), making multiple analyses using different machine learning techniques important. Overall, differences between machine learning

techniques were small. In general, estrus detection ability of machine learning techniques were superior to VO. When VO was compared to P4 results of all 32 cows, a 62.1% sensitivity, 100% specificity, 100% positive predictive value, 21.4% negative predictive value, and a 65.6% accuracy of estrus detection were achieved. Non-continuous monitoring likely limited the ability of VO to detect short periods of estrus. Additionally, using secondary signs of estrus to define estrus rather than standing events alone likely would have increased detection rate. Roelofs et al. (2005b) identified 19% of behavioral estrus events when observing cows for standing estrus alone, but 90% when considering multiple secondary signs. The ability to continuously monitor cows using automated monitoring technologies, allowing detection of short or unexpressed estrus periods, likely contributed to improved performance over VO.

Machine learning techniques in this study also improved on previous reports of AED capabilities. The greatest EDR reported by an automated monitoring technology that the author's are aware of was 89.2% using a leg activity monitor (Dela Rue et al., 2014). The only automated monitoring technology and machine learning technique combinations that did not exceed 90% detection in the current study were the DVM bolus (regardless of machine learning technique) and the random forest technique combined with the HR Tag and the IceQube (Table 2.3). The improvements observed when using machine learning techniques indicate that this method may be useful for improving AED manufacturer algorithms.

CONCLUSIONS

Reticulorumen temperature, neck activity, rumination, lying bouts, lying time, step count, feeding time, ear activity, and leg activity may be useful as predictors of

estrus. Ear surface temperature, as monitored in this study, holds less potential for detecting differences between periods of estrus and non-estrus. When comparing five technologies using machine learning techniques, an ear-mounted accelerometer had a slight advantage in estrus detection ability. This could be because of the increased number of parameters the device was capable of measuring. Overall, estrus detection ability of machine learning techniques were superior to VO and improved on previous reports of automated monitoring technology capabilities. Based on these results, it was concluded that multiple measureable parameters may be useful for AED. Additionally, applying machine learning techniques to automatically collected parameters has the potential to improve estrus detection compared to labor intensive VO and current automated monitoring technology algorithms.

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Table 2.1. Comparison of automated monitoring technology¹ parameters (adjusted means \pm SE) during estrus (12 h before first observed standing event²) and non-estrus (the 14 d before estrus).

Parameter monitored	n	Estrus	Non-estrus	P-value
DVM bolus reticulorumen temperature (°C)	18	39.36 \pm 0.24	38.85 \pm 0.21	< 0.01
HR Tag neck activity (units/2 h)	18	48.34 \pm 1.99	28.24 \pm 0.85	< 0.01
HR Tag rumination (min/2 h)	18	16.65 \pm 2.45	33.26 \pm 0.46	< 0.01
IceQube lying bouts (per h)	17	0.36 \pm 0.10	0.72 \pm 0.07	< 0.01
IceQube lying time (min/h)	17	10.84 \pm 1.85	24.65 \pm 1.05	< 0.01
IceQube number of steps (per h)	17	258.61 \pm 11.13	79.63 \pm 5.36	< 0.01
SensOor ear surface temperature (°C)	18	24.27 \pm 1.21	23.00 \pm 0.85	0.16
SensOor feeding time (min/h)	18	15.55 \pm 0.95	8.88 \pm 0.69	< 0.01
SensOor high ear activity (min/h)	18	17.69 \pm 0.60	4.11 \pm 0.38	< 0.01
SensOor rumination time (min/h)	18	13.20 \pm 1.04	23.05 \pm 0.57	< 0.01
Track a Cow leg activity (units/h)	18	261.40 \pm 11.19	94.34 \pm 6.60	< 0.01
Track a Cow lying time (min/h)	14	8.75 \pm 2.94	18.07 \pm 2.39	< 0.01

¹DVM bolus, DVM Systems, LLC, Greeley, CO; HR Tag, SCR Engineers Ltd., Netanya, Israel; IceQube, IceRobotics Ltd., Edinburgh, Scotland; SensOor, Agis Automatisering, Harmelen, Netherlands; and Track a Cow, Animart Inc., Beaver Dam, WI and ENGS, Rosh Pina, Israel

²Observations for standing estrus occurred for 30 min periods at 0330, 1000, 1430, and 2200 daily

Table 2.2. Group level¹ and cow level² correlations between HR Tag activity (HRACT), IceQube number of steps (IQSTEP), SensOor high ear activity (SOACT), Track a Cow activity (TCACT), IceQube lying bouts (IQLB), IceQube lying time (IQLT), Track a Cow lying time (TCLY), HR Tag rumination (HRRUM), SensOor rumination (SORUM), SensOor feeding time (SOFD), DVM bolus reticulorumen temperature (DVMT), and SensOor ear surface temperature (SOT)³ as recorded on 18 cows⁴.

	HRACT	IQSTEP	SOAct	TCACT	IQLB	IQLT	TCLT	HRRUM	SORUM	SOFD	DVMT	SOT	
HRACT		0.39	0.39	0.23	-0.02	-0.11	-0.39	0.13	-0.23	0.25	0.14	0.11	
IQSTEP	0.78***		0.55*	0.47†	0.20	-0.20	-0.26	-0.24	-0.27	0.21	-0.12	0.29	
SOACT	0.81***	0.99***		0.17	0.21	-0.07	-0.36	-0.12	-0.46†	0.19	0.05	-0.15	
TCACT	0.76**	0.99***	0.99***		0.24	-0.12	-0.16	-0.05	0.11	-0.01	-0.05	0.04	
IQLB	-0.70**	-0.88***	-0.84***	-0.87***		0.38	0.21	0.27	-0.12	0.26	-0.23	-0.35	
IQLT	-0.73**	-0.94***	-0.93***	-0.96***	0.94***		0.44†	0.20	0.12	-0.10	-0.08	-0.09	
TCLT	-0.44	-0.72**	-0.69**	-0.74**	0.74**	0.85***		0.19	0.35	-0.28	0.05	-0.22	
HRRUM	-0.15	-0.68**	-0.60*	-0.68**	0.64**	0.65**	0.63*		0.36	0.04	0.00	0.09	
SORUM	-0.65**	-0.95***	-0.93***	-0.95***	0.83***	0.88***	0.66**	0.78***		-0.48†	0.13	0.22	
SOFEED	0.86***	0.91***	0.94***	0.92***	-0.79***	-0.90***	-0.68**	-0.37	-0.79***		-0.12	-0.15	
DVMTEMP	0.10	0.10	0.19	0.13	-0.03	-0.07	-0.03	0.07	-0.12	0.17		-0.04	
SOTEMP	-0.11	0.18	0.10	0.13	-0.15	-0.07	-0.07	-0.57	-0.19	-0.11	-0.24		
Group level													

Cow level

¹Correlations between the daily mean of all cows combined

²Median of daily individual cow correlations

³DVM bolus, DVM Systems, LLC, Greeley, CO; HR Tag, SCR Engineers Ltd., Netanya, Israel; IceQube, IceRobotics Ltd., Edinburgh, Scotland; SensOor, Agis Automatisering, Harmelen, Netherlands; and Track a Cow, Animart Inc., Beaver Dam, WI and ENGS, Rosh Pina, Israel

⁴Significance level indicated by † ($P < 0.10$), * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$)

Table 2.3. Estrus detection capability¹ of different automated monitoring technologies² and machine learning techniques (random forest, linear discriminant analysis, and neural network). Models attempted to identify the 12 h period of time before the first observed standing event³ from the 28, 12 h periods leading up to observed estrus. The analysis included 18 total cows, with 70% used for training and 30% used for testing.

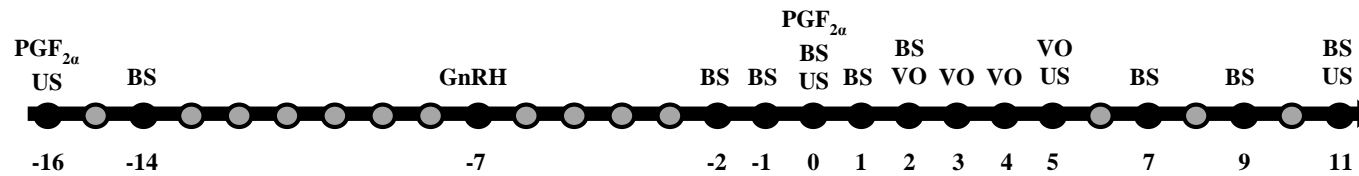
Technique	Technology	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Random forest	SensOor	100.00	98.6	71.4	100.0	98.6
	DVM bolus	0.0	94.3	0.0	96.4	91.0
	HR Tag	60.0	99.3	75.0	98.6	97.9
	IceQube	80.0	99.3	80.0	99.3	98.6
	Track a Cow	100.0	97.1	55.6	100.0	97.2
Linear discriminant analysis	SensOor	100.0	100.0	100.0	100.0	100.0
	DVM bolus	0.2	73.6	0.0	96.3	71.7
	HR Tag	100.0	97.9	62.5	100.0	97.9
	IceQube	100.0	97.9	62.5	100.0	97.9
	Track a Cow	100.0	96.4	50.0	100.0	96.6
Neural network	SensOor	100.0	98.6	71.4	100.0	98.6
	DVM bolus	0.0	100.0	0.0	96.6	96.6
	HR Tag	100.0	96.4	50.0	100.0	96.6
	IceQube	100.0	100.0	100.0	100.0	100.0
	Track a Cow	100.0	97.9	27.8	100.0	91.0

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

²DVM bolus, DVM Systems, LLC, Greeley, CO; HR Tag, SCR Engineers Ltd., Netanya, Israel; IceQube, IceRobotics Ltd., Edinburgh, Scotland; SensOor, Agis Automatisering, Harmelen, Netherlands; and Track a Cow, Animart Inc., Beaver Dam, WI and ENGS, Rosh Pina, Israel

³Observations for standing estrus occurred 4X for 30 min periods at 0330, 1000, 1430, and 2200 daily.

Figure 2.1. Timeline of synchronization injections, ultrasound (US), blood sampling (BS) and visual observation (VO) for cows used in a study testing five automated monitoring technologies' estrus detection capabilities. The synchronization protocol was a modified G7G Ovsynch with injections given at 0800. Two injections of PGF_{2α} (6 h apart; 0800 and 1400) were administered on d 0. Ultrasonography and blood sampling was done at 0800. Visual observation was conducted 4X for 30 min periods at 0330, 1000, 1430, and 2200.



CHAPTER THREE

Comparison of timed artificial insemination and automated activity monitoring as reproductive management strategies in three commercial dairy herds

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INTRODUCTION

Mean yearly EDR of US Holstein herds is 44.9% (DRMS, 2015).

Estrus detection efficiency has been identified as a major limiting factor in dairy reproductive performance (Heersche and Nebel, 1994; Nebel and Jobst, 1998; Ferguson and Skidmore, 2013). The effectiveness of traditional, visual estrus detection is reduced by short estrus periods and influenced by the timing, length, and frequency of observation (Van Vliet and Van Eerdenburg, 1996; Dransfield et al., 1998; Cavestany et al., 2008). The average estrus period lasts only 7.1 h with 8.5 standing events (Dransfield et al., 1998), which can easily be missed during twice per day observations. Timed artificial insemination, commonly accomplished through a synchronization protocol known as Ovsynch (Pursley et al., 1995), has successfully been implemented on many farms as a replacement for visual estrus detection. Unfortunately, lack of protocol compliance (i.e. missed injections) often reduces the success of TAI (Galvão et al., 2013). Additionally, consumer concerns surrounding hormone use could limit this management option in the future (Saint-Dizier and Chastant-Maillard, 2012; Nebel, 2013).

One alternative to TAI is AED technologies. Parameters with potential for AED include mounting events, activity levels, lying time, feeding time, rumination, body temperature, and P4 levels (Senger, 1994; Saint-Dizier and Chastant-Maillard, 2012; Fricke et al., 2014a). Challenges associated with AED include determining how and when to intervene with anovular cows and determining the best time to inseminate relative to behavioral changes (Fricke et al., 2014a). Variation among animals complicates both of these factors. Another drawback associated with AED, as identified

in producer surveys, is the large investment cost and uncertainty in payback period (Russell and Bewley, 2013; Borchers and Bewley, 2014).

Most AED technologies currently available to dairy farmers work via AAM. An increase in activity associated with estrus was first observed in rats in 1923 (Wang, 1923). A similar response was established in other female mammals, including swine and cattle (Altmann, 1941; Farris, 1944; Farris, 1954). In one of the first cattle activity monitoring studies, the authors found that the number of steps per hour increased two- to four-times in cows displaying estrus behavior compared to cows not (Kiddy, 1977). In an evaluation by Liu and Spahr (1993), 74% of estrus events were detected by AAM as compared to pregnancy diagnosis. In comparison, only 58% of estrus events were detected by direct VO. Cavalieri et al. (2003a) compared AAM to other estrus detection methods (a rump-mounted pressure-sensitive mounting detector and tail paint) using milk P4 levels and pregnancy diagnosis as verification. They found no differences in the percent of true estrus events detected (81.4, 88.4, and 91.3%, respectively). Estrus detection rate of AAM, as reported in other studies, ranges between 51.0 and 89.2% in both confinement and pasture situations (Roelofs et al., 2005a; Dela Rue et al., 2014).

Direct comparisons or reproductive performance in dairy cows bred by TAI versus AAM are limited. Galon (2010) found no difference in first service CR (17.6 vs. 22.6%, respectively) between cows on AAM versus those on a TAI protocol. Neves et al. (2012) compared TAI to AAM using over 900 animals from three herds. They found no differences between TAI and AAM groups in PR (15.9 vs. 14.6%) or CR (30.0 vs. 31.0%). However, the results of this experiment are difficult to interpret since breeding based on visual detection was permitted in both the TAI and AAM groups.

The objective of this study was to compare the reproductive performance of cows in three commercial dairy herds that were bred solely based on AAM versus cows from the same herds bred using only an intensive TAI.

MATERIALS AND METHODS

This study was conducted in three commercial Kentucky Holstein dairy herds. Farm location, herd size, and willingness to participate were considered during herd selection. Milking herd size and rolling herd average milk production of herds A, B, and C at the beginning of the study were 247 cows and 11,587 kg, 130 cows and 9,588 kg, and 419 cows and 8,341 kg. At the conclusion of the study, milk herd size and rolling herd average milk production was 261 cows and 12,230 kg, 190 cows and 11,040 kg, and 432 cows and 9,381 kg, for herds A, B, and C, respectively. Herds B and C housed cows exclusively on sand-bedded freestalls. Herd A housed cows on both sand-bedded freestalls and compost-bedded pack barns. Cows in herd A may have been housed exclusively on one or switched between the two housing systems during the study. Herd A and C's facilities contained grooved concrete, whereas Herd B's facilities contained ungrooved concrete.

Study Design

Each herd manager agreed to enroll cows in the study for one year, beginning in October 2012. At the conclusion of one year, cows from herds A and B continued to be enrolled for an additional three months. All cows in herd C completed the study by January 2014. The last cows in herds A and B completed the study in June 2014.

The VWP was 60 d for herds B and C, and 80 d for herd A. Cows were assigned to a treatment group when they reached 17 to 31 d before the end of the VWP. This

resulted in cows being assigned to groups at 14 d intervals. To be considered eligible for this experiment, cows had to meet specific health requirements. First, cows had to have a $BCS \geq 2.5$ (1 to 5 scale; Ferguson et al., 1994). Cows with a $BCS < 2.50$ (emaciated) were excluded from the study ($n = 27$). Remaining cows were grouped into two categories: 2.50 to 2.75 and 3.00 to 5.00. Second, each cow was subjected to a routine reproductive exam by the herd veterinarian to verify normal progression of uterine involution (free of any clinical signs of metritis or pyometra) and the commencement of ovarian cyclicity (indicated by the presence of a CL or follicle greater than 9 mm in diameter). Third, cows must have been free of clinical metabolic disorders since calving. Removal from the study occurred if a cow did not meet the reproductive tract criteria ($n = 149$) or if she had experienced any clinical, recorded metabolic disorders in the current lactation ($n = 11$). These criteria were set in an attempt to reduce the effect of factors known to affect cow fertility.

Eligible cows were classified by parity (primiparous or multiparous) and predicted milk yield (DHIA). Each cow's predicted milk yield was classified as greater than or less than herd mean based on PTA milk. If PTA milk was not available for a cow, estimated relative producing ability milk was used. If neither PTA milk nor estimated relative producing ability milk was available, first test day milk production was used. Assignment of cows to treatments (TAI or AAM) followed, alternating alike herdmates (determined by parity and predicted milk yield classification) between the treatments. This allocation procedure resulted in a balance in BCS, parity, and summit milk among treatment groups (Table 3.1). Forty-five cows were enrolled twice during

the study period. Because this number was too small to account for repeated measures, only the first completed enrollment for each cow was included in the statistical analysis.

Cows remained in their assigned treatment for 90 d after the end of the VWP.

The study was designed to compare two non-visual reproductive management strategies. Consequently, insemination according to VO of estrus throughout the study period resulted in removal from analysis (n = 55). Additionally, any cows that were reported by the herd manager as having missed TAI injections (n = 60), were eligible for breeding at the time of AAM system failure (n = 46), were not bred at the time of an AAM alert as reported by the herd manager (n = 6), were culled throughout their 90 day study period (n = 61), or were removed from the study by the herd manager (n = 53) were excluded from analysis.

TAI Treatment

Synchronization of ovulation in cows assigned to the TAI treatment involved a combination of protocols commonly referred to as G7G, Ovsynch, and Resynch (Figure 3.1). The G7G presynchronization consisted of PGF_{2α} (dinoprost tromethamine, Lutalyse; Zoetis, Florham Park, NJ; 25 mg intramuscular) and GnRH (gonadorelin diacetate tetrahydrate, Cystorelin; Merial Limited, Duluth, GA; 100 µg intramuscular) administered 9 and 7 days before beginning Ovsynch, respectively. The Ovsynch protocol consisted of GnRH on day 0, followed by PGF_{2α} (day 7), GnRH (day 8), and insemination (day 9; Pursley et al., 1995) . Resynch began with GnRH one week before pregnancy diagnosis. Cows received PGF_{2α} at the time of open diagnosis, followed by GnRH and AI 56 and 72 hours later, respectively. This method allowed up to three

breedings in TAI cows during the 90 day study period. Herd managers supervised the synchronization procedure and each had previous experience with TAI protocols.

AAM Treatment

Cows assigned to the AAM treatment received an ankle-mounted, mechanical activity monitor measuring 9.2 x 7.4 x 2.4 cm and weighing 92 g (AfiAct Pedometer Plus, Afimilk, Kibbutz Afikim, Israel). The monitor was attached to a rear leg at least 10 d before the end of the VWP to establish the normal activity level of each cow. The AAM system continuously recorded activity level using a 3-axis accelerometer that relayed information to a computer at each milking. Steps per hour since the last milking and a 10 d backwards moving mean steps per hour were calculated after each data download. An estrus alert was generated when the most recent measurement of a cow's steps per hour exceeded her 10 d backward moving mean as determined using a proprietary algorithm, consistent across herds. Herd managers were instructed to check the AAM alert list twice per day and breed AAM cows at every alert throughout the 90 day study period, unless specific management practices (i.e. pen changes, hoof trimming, etc.) indicated a false alert. All herds began using the AAM system in March 2012 (6 months before beginning the study) and were considered past the initial adjustment and learning stage.

If an open cow experienced no AAM alert generation for a 39 ± 7 d period beginning at the end of the VWP, hormone intervention ($\text{PGF}_{2\alpha}$ or GnRH) was permitted as directed by the herd veterinarian. Regardless of hormone intervention, cows remained in the AAM treatment for the analysis.

Pregnancy Diagnosis

The herd veterinarian or a trained researcher conducted pregnancy diagnosis via ultrasound at 39 (herd B and herd C) or 40 (herd A) d post insemination for TAI cows and 39 ± 7 (herd B and C) or 40 ± 7 (herd A) d post insemination for AAM cows.

Pregnancy loss was determined via a second ultrasound after 60 d pregnant.

Temperature and Humidity Data

County weather stations managed by the Kentucky Mesonet recorded temperature and humidity using a Platinum Resistance Thermometer (Thermometrics Corporation, Northridge, CA) and a HMP45C Temperature and Relative Humidity Probe (Campbell Scientific, Inc., Logan, UT), respectively, every 5 minutes. Weather stations were located 5, 4, and 18 nautical miles from herds A, B, and C, respectively. Daily THI was calculated using the maximum daily temperature and maximum daily relative humidity in the following equation (NOAA, 1976) : $THI = \text{temperature } (^{\circ}\text{F}) - [0.55 - (0.55 \times \text{relative humidity}/100)] \times [\text{temperature } (^{\circ}\text{F}) - 58.8]$. An individual THI for each cow was calculated using the mean daily THI over her 90 day study period.

Herd Records

PCDART (Dairy Records Management Systems, Raleigh, NC) records from each farm provided calving dates, predicted milk yield, insemination dates, pregnancy diagnosis, summit milk, and culling information. These records aided in the calculation of days to first service, first service CR, repeat service CR, service interval, DO, services per pregnancy, pregnancy loss, and the proportion of cows pregnant at 90 d past the VWP. Only services 2 and 3 were included in repeat service CR because TAI cows did not have the opportunity to be bred more than three times. Additionally, the number of

AAM services greater than 3 was low ($n = 12$). Because of the herd differences in VWP, DO was calculated as days past the VWP.

Statistical Analysis

To account for both continuous and binomial response variables, construction of multiple models was required. All main effects remained in each model, regardless of significance. Two-way interactions determined to have potential biological significance remained in models when $P < 0.05$, as determined through stepwise backward elimination.

The MIXED procedure of SAS (Version 9.3 SAS Institute, Inc., Cary, NC) analyzed the effects of treatment, herd, parity, BCS, THI, summit milk, and selected interactions on days to first service, service interval, services per pregnancy, and DO:

$$y_{ijklm} = \mu + Treatment_i + Herd_j + Parity_k + BCS_l + THI_m + Summit_m + (Treatment_i \times Herd_j) + (Treatment_i \times Summit_m) + (Summit_m \times Herd_j) + (Treatment_i \times THI_m) + (Summit_m \times THI_m) + (Herd_j \times THI_m) + (Treatment_i \times Parity_k) + (Summit_m \times Parity_k) + (THI_m \times Parity_k) + e_{ijklm}$$

where y_{ijklm} is the outcome variable of the m th cow, in the l BCS category, in the k parity category, in the j th herd, with treatment i ; μ is the intercept; i is TAI or AAM; j is A, B, or C; k is primiparous or multiparous; l is 2.50 to 2.75 or 3.00 to 5.00; THI_m is the 90 day mean THI for the m th cow; $Summit_m$ is the summit milk production of the m th cow; and e_{ijklm} is residual error.

To analyze first service CR, pregnancy loss, and proportion of cows pregnant at 90 d past the VWP, binomial datasets were created using “1” to identify the occurrence of each event (pregnancy or pregnancy loss) and “0” to identify no event occurrence. The

GENMOD procedure of SAS was used to analyze the effects of treatment, herd, parity, BCS, THI, summit milk, and selected interactions on first service CR, pregnancy loss, and proportion of cows pregnant at 90 d past the VWP:

$$\begin{aligned}
y_{ijklm} = & \mu + Treatment_i + Herd_j + Parity_k + BCS_l + THI_m + Summit_m + \\
& (Treatment_i \times Herd_j) + (Treatment_i \times Summit_m) + (Summit_m \times Herd_j) + \\
& (Treatment_i \times THI_m) + (Summit_m \times THI_m) + (Herd_j \times THI_m) + (Treatment_i \times \\
& Parity_k) + (Summit_m \times Parity_k) + (THI_m \times Parity_k) + e_{ijklm}
\end{aligned}$$

where y_{ijklm} is the outcome variable of the m th cow, in the l BCS category, in the k parity category, in the j th herd, with treatment i ; μ is the intercept; i is TAI or AAM; j is A, B, or C; k is primiparous or multiparous; l is 2.50 to 2.75 or 3.00 to 5.00; THI_m is the 90 day mean THI for the m th cow; $Summit_m$ is the summit milk production of the m th cow; and e_{ijklm} is residual error.

A similar binomial model evaluated repeat service CR, which differed only in the inclusion of service number (2 or 3) as a fixed effect:

$$\begin{aligned}
y_{ijklmn} = & \mu + Treatment_i + Herd_j + Parity_k + BCS_l + Service_m + THI_n + Summit_n + \\
& (Treatment_i \times Herd_j) + (Treatment_i \times Summit_n) + (Summit_n \times Herd_j) + (Treatment_i \\
& \times THI_n) + (Summit_n \times THI_n) + (Herd_j \times THI_n) + (Treatment_i \times Parity_k) + \\
& (Summit_n \times Parity_k) + (THI_n \times Parity_k) + e_{ijklmn}
\end{aligned}$$

where y_{ijklmn} is repeat service CR of the n th cow, in the l BCS category, in the k parity category, in the j th herd, with treatment i n th cow; μ is the intercept; i is TAI or AAM; j is A, B, or C; k is primiparous or multiparous; l is 2.50 to 2.75 or 3.00 to 5.00; m is service 2 or 3; THI_n is the 90 day mean THI for the n th cow; $Summit_n$ is the summit milk production of the n th cow; and e_{ijklmn} is residual error.

Survival analysis was used to evaluate the effect of treatment on risk of pregnancy establishment using the PHREG procedure of SAS. The outcome variable was the DO at which a cow conceived. The model included treatment as an effect.

RESULTS AND DISCUSSION

Herd A, B, and C had 214, 141, and 187 cows complete the study, respectively. Percent of cows removed from the study was 34.2%, but differed between herds. Of all cows that started the study, herd A, B, and C removed 17.1% (56.8% TAI, 43.2% AAM), 18.5% (37.5% TAI, 62.5% AAM), and 52.4% (57.8% TAI, 42.2% AAM), respectively. Herd C experienced greater than expected removal rates. A slow manager reaction to an AAM system failure on herd C after a severe thunderstorm accounted for most of the AAM cows dropped from that herd ($n = 42$). This highlights the need for a backup reproductive management plan when using AAM in the case of system failure. Herd C also reported multiple instances where breeding of TAI cows occurred after observed estrus ($n = 45$) and where TAI protocols were not followed properly ($n = 48$). Compliance with TAI protocols is often overlooked, even though it has been shown to have a greater negative effect on reproductive performance than poor accuracy of estrus detection (Galvão et al., 2013).

Of the cows that completed the study, 51.5% were TAI ($n = 279$) and 48.5% were AAM ($n = 263$). Both treatment and herd groups were evenly balanced for parity, BCS, and summit milk production (Table 3.1). In the AAM group, 24.7% of all cows (in herd A: 23.2%, herd B: 31.4%, and herd C: 21.0%) received hormonal intervention (81.7% PGF_{2α}, 18.3% GnRH) upon veterinarian recommendation after no alert generation for 39 ± 7 days. Some of the interventions occurred before first service, likely because of

delayed cyclicity. Petersson et al. (2008) reported that delayed cyclicity occurs in 13.8 to 18.2% of lactations. Early embryo loss may also have extended length of time between estrus events in AAM cows, resulting in intervention. Santos et al. (2004) summarized that 50% of lactating cows have viable embryos 5 to 6 days post-insemination, but CR on days 27 to 31 are usually only between 35 and 45%. An alternative explanation could be that the AAM threshold was set too high to create alerts for some cows with low increases in activity during estrus. Setting a threshold for alerts can be challenging because it requires a balance between FP (if set too low) and FN (if set too high). Of the AAM cows that received hormonal intervention ($n = 65$), 92.3% were re-inseminated before the end of the study. Regardless of intervention, analyses included all AAM cows.

Cows that were never bred were only included in the analysis of proportion of cows pregnant at 90 d past the VWP and the survival analysis to determine risk of pregnancy establishment. Cows that never became pregnant were excluded from the pregnancy loss analysis. Only cows that became pregnant and did not experience pregnancy loss were included in the analysis of services per pregnancy and DO. A summary of reproductive performance between treatments, herds, parity, BCS category, THI, and summit milk production is included in Table 3.2 and discussed further in the following paragraphs.

Time to First Service

Time to first service (mean \pm SE) was 6.7 ± 0.7 and 21.9 ± 0.8 d after the VWP for TAI and AAM, respectively ($P < 0.01$). This resulted from cows in the AAM treatment requiring natural estrus expression before the first insemination whereas insemination of TAI cows occurred at a predetermined time. The 15.2 d difference in

this study is similar to that reported by Norman et al. (2009) who found cows managed using TAI experienced 18 fewer days to first service compared to unsynchronized cows. Herd ($P < 0.01$) and the interaction of treatment and herd ($P < 0.01$) also affected time to first service. This indicates that even when the same management strategies are used, herd compliance and attention to detail may affect results of both TAI and AAM. Neves et al. (2012) similarly reported an effect of herd on reproductive performance when comparing TAI and AAM, although greater differences between herds (i.e. TAI protocol) existed in that case. Regardless, herd management can affect reproductive performance (Lucy, 2001). For example, a management strategy focused on reducing negative energy balance in the fresh period could expect cows to return to cyclicity faster (Butler, 2000; Butler, 2003; Van Knegsel et al., 2007). Although all herds experienced a longer time to first service in AAM cows, that difference was greater in herd C (20.6 d) than either herd A or B (14.0 and 11.1 d, respectively). This may be the result of delayed cyclicity in cows or missed AAM alerts in herd C.

Temperature humidity index, parity, and the interaction between THI and parity ($P = 0.04$, $P = 0.04$, $P = 0.04$, respectively) also affected time to first service. As mean THI increased, time to first service increased in multiparous cows, but not primiparous cows. This suggests that multiparous cows in this study were more susceptible to heat stress than primiparous cows. Using production levels, Aguilar et al. (2009) stated that the effect of heat stress increased with increasing parities, doubling between first and second parity and again between second and third parity.

Time to first service was not affected by BCS category ($P = 0.38$). Delaying BCS evaluation until after the transition period and excluding cows below a score of 2.5 likely

affected this result. Summit milk production also did not affect time to first service ($P = 0.58$) or any other measure of reproductive performance in this study. Although an established link exists between increased milk production and reduced reproductive performance (Dematawewa and Berger, 1998; Stevenson, 2001a; VanRaden et al., 2004), study enrollment criteria (BCS and cyclicity) likely offset some of these effects.

First Service CR

First service CR (mean \pm SEM) was $41.1 \pm 3.1\%$ and $41.1 \pm 3.1\%$ for TAI and AAM, respectively ($P = 0.99$). Cows undergoing TAI in this study were subject to presynchronization (G7G) before first service, which should have enhanced fertility by reducing variation in time to ovulation. Therefore, the expectation was that CR would be greater of TAI cows would be greater than AAM cows. The ability to use hormonal intervention on AAM cows not displaying increased activity may have offset some of the presynchronization advantage. However, hormone intervention was only used on 24.7% of AAM cows.

Herd and BCS did affect first service CR ($P < 0.01$ and $P < 0.01$, respectively). The differences between herds was the result of a low first service CR in herd C ($29.5 \pm 3.5\%$) and a high first service CR in herd A ($54.4 \pm 3.5\%$), whereas herd B fell in the middle ($40.6 \pm 4.4\%$). Reproductive performance before the study began was lowest in herd C and continued inferior performance was expected. Herd A's 20 day longer VWP may have been an advantage by giving cows longer to begin cycling before consideration for breeding. An estimated 20 to 30% of high-producing lactating Holsteins are anovular at 60 to 75 DIM (Gumen et al., 2003; Lopez et al., 2005). In agreement, Stevenson and Phatak (2005) found that delaying first service three weeks (from 56 to 77 DIM)

improved CR by 20.1%. Cows with a BCS between 3.00 and 5.00 experienced a greater first service CR ($47.4 \pm 3.1\%$) than cows with a BCS between 2.50 and 2.75 ($35.2 \pm 3.3\%$). In agreement with this study, Roche et al. (2007) found that first service CR decreased 5% as BCS at first service declined by one unit on a 10 point scale. Reduced energy balance may play a role in these observations (Roche et al., 2009).

First service CR was not affected by THI, parity, or summit milk ($P = 0.35$, $P = 0.57$, and $P = 0.13$, respectively). No parity effect was expected and the lack of summit milk effect was consistent in all analysis.

Repeat Service CR

Repeat service CR (mean \pm SE) was 34.9 ± 3.8 and $45.8 \pm 4.7\%$ for TAI and AAM, respectively ($P = 0.04$). The effect of herd ($P = 0.01$), the interaction between treatment and herd ($P = 0.04$), and the interaction between treatment and parity ($P = 0.04$) affected these results. The herd by treatment effect was the result of a low ($17.9 \pm 3.7\%$) repeat service CR in herd C's TAI group compared to all other treatment within herd combinations. Although an attempt was made to remove cows with TAI protocol errors, this result indicates that some errors may not have been reported. The treatment by parity effect occurred because of the primiparous AAM group having a significantly greater repeat service CR ($53.2 \pm 7.2\%$) than the primiparous TAI group ($31.4 \pm 5.0\%$). This difference indicates that first lactation cows bred to natural estrus may have a greater repeat service CR than those bred to synchronized ovulation.

Insemination number, BCS category, herd, and the interaction between herd and THI ($P = 0.04$, $P = 0.04$, $P = 0.01$, and $P = 0.02$, respectively) did affect repeat service CR. Second service inseminations produced a greater CR ($45.8 \pm 3.5\%$) than third

service inseminations ($34.9 \pm 4.7\%$). Chebel et al. (2004) also found decreasing CR with increasing services. With each increased service, CR decreased 7.0%, similar to the 10.9% found between second and third service in this study. Cows with a BCS between 3.00 and 5.00 pre-breeding experienced a greater repeat service CR ($45.9 \pm 4.4\%$) than cows with a BCS between 2.50 and 2.75 ($34.8 \pm 4.0\%$). As previously discussed, reduced energy balance may play a role in these observations (Roche et al., 2009).

As THI increased, herd A displayed no difference in repeat service CR. This likely resulted from the use of heat abatement that included natural ventilation, fan placement, and sprinkler usage. Herds B and C experienced surprisingly greater CR as THI increased. In herd B, this is likely because fewer inseminations occurred in the warm months ($n = 10$) as compared to the cool months ($n = 112$). In herd C, the long AAM system failure during the summer months limited the number of cows classified with a high THI. Therefore, these results may not be completely representative of actual farm conditions.

Repeat service CR was not affected by THI, parity, or summit milk ($P = 0.07$, $P = 0.54$, and $P = 0.20$, respectively). These results are consistent with those for first service CR.

Service Interval

Service interval (mean \pm SE) was 42.2 ± 0.7 and 28.6 ± 0.8 d for TAI and AAM, respectively ($P < 0.01$). The effect of parity ($P < 0.01$) and the interaction between treatment and parity ($P < 0.01$) affected these results. Service interval of both primiparous AAM cows (25.5 ± 1.3) and multiparous AAM cows (31.7 ± 1.0) was less than both primiparous TAI cows (42.0 ± 1.1) and multiparous TAI cows (42.3 ± 0.9).

This resulted from the ability to rebreed open AAM cows as soon as the AAM system detected them in estrus again. Conversely, TAI cows could not be rebred until after pregnancy diagnosis, regardless of observed estrus activity. In practice, rebreeding TAI cows observed in estrus before pregnancy diagnosis is common. Therefore, our study design may have affected these results. In addition to the difference between treatments, primiparous AAM cows experienced a 6 d shorter service interval than multiparous AAM cows ($P < 0.01$).

Service interval was not effected by herd, THI, BCS, or summit milk ($P = 0.35$, $P = 0.91$, $P = 0.87$, and $P = 0.93$, respectively).

Services per Pregnancy

Services per pregnancy (mean \pm SE) was 1.59 ± 0.05 and 1.56 ± 0.05 for TAI and AAM, respectively ($P = 0.74$). Norman et al. (2009) reported greater services per pregnancy for both synchronized (2.8) and unsynchronized cows (2.4) and a significant difference between the two breeding systems ($P < 0.01$). Contrary to this study, Norman et al. (2009) did not have specific criteria (i.e. BCS ≥ 2.5 , no clinical, recorded metabolic diseases, resumed cyclicity, etc.) that a cow must meet to be included. Additionally, the current study design only allowed for a 90 d breeding period, therefore limiting the number of insemination possibilities.

Herd ($P < 0.01$) did affect services per pregnancy. The effect of herd resulted from a reduced services per pregnancy in herd A (1.42 ± 0.06) compared to herds B (1.66 ± 0.07) and C (1.65 ± 0.07). The increased first service CR in herd A because of the longer VWP likely influenced this result.

Services per pregnancy was not affected by THI, parity, BCS, or summit milk ($P = 0.63$, $P = 0.19$, $P = 0.16$, and $P = 0.53$, respectively).

Pregnancy Loss

Pregnancy loss (mean \pm SEM) was $14.1 \pm 2.8\%$ and $9.0 \pm 2.3\%$ for TAI and AAM, respectively ($P = 0.14$). Lee and Kim (2007) also found no difference in pregnancy loss between synchronized animals (9.0%) and non-synchronized animals (6.3%). This study is in line with previous reports of 7.2% embryonic loss between 28 and 84 d (Silke et al., 2002) and 12.5% pregnancy loss between 31 and 45 d (Chebel et al., 2004).

Herd and the interaction between herd and THI did affect pregnancy loss ($P < 0.01$ and $P = 0.01$, respectively). As THI increased, pregnancy loss increased in Herd B but slightly decreased in Herds A and C. Chebel et al. (2004) reported no effect of heat stress before or after insemination on pregnancy loss. Again, a low number of cows completing the study under warm conditions may have affected the accuracy of these results.

Pregnancy loss was not affected by THI, parity, BCS, or summit milk ($P = 0.46$, $P = 0.09$, $P = 0.84$, and $P = 0.92$, respectively).

Days Open and Proportion of Pregnant Cows at 90 d Past the VWP

Days open (30.7 ± 1.9 vs 34.8 ± 1.9 d past the VWP for TAI and AAM, respectively) and the proportion of pregnant cows at 90 d past the VWP (61.9 ± 3.4 vs. $64.1 \pm 3.4\%$ for TAI and AAM, respectively) did not differ between treatments. The lack of difference between treatments shows that AAM cows recovered from the 15.2 d difference in days to first service. This is likely the result of cows in the AAM treatment

having experienced a 10.9% greater repeat service CR and a 13.6 d shorter service interval, as previously discussed.

Herd did affect DO ($P < 0.01$). The effect of herd resulted from an enhanced performance in herd A (25.7 ± 2.0 d past the VWP) over herds B and C (35.8 ± 2.6 and 36.7 ± 2.6 d past the VWP, respectively). Herd A's high first service CR, resulting from the longer VWP, likely played a role in these results.

Days open was not affected by THI, parity, BCS, or summit milk ($P = 0.18$, $P = 0.11$, $P = 0.12$, and $P = 0.57$, respectively).

The proportion of pregnant cows at 90 d past the VWP was affected by BCS, herd, and the interaction of herd and THI ($P = 0.01$, $P < 0.01$, and $P = 0.01$, respectively). The proportion of pregnant cows at 90 d past the VWP favored cows with a BCS between 3.00 and 5.00 over cows with a BCS between 2.50 and 2.75 (68.8 ± 3.1 vs. $56.7 \pm 3.8\%$, respectively). Roche et al. (2007) noted that a one unit greater than median BCS at nadir (BCS 3.8 vs. 2.8) increased 42 d pregnancy establishment by 7%, similar to the 12.1% difference reported in this study.

As THI increased, the proportion of cows pregnant at 90 d past the VWP remained consistent in Herd A. This was likely the result of sufficient heat abatement, as discussed earlier. At the same time, the proportion of cows pregnant at 90 d past the VWP decreased in Herd B, but increased in Herd C.

The proportion of pregnant cows at 90 d past the VWP was not affected by THI, parity, or summit milk ($P = 0.16$, $P = 0.30$, and $P = 0.24$; respectively).

Risk of Pregnancy Establishment

Results of the survival analysis that considered the effect of treatment on the percent of cows not pregnant by DO past the VWP appear in Figure 3.2. The risk of pregnancy establishment was 1.7% less for AAM cows (hazard ratio = 0.98, 95% CI = 0.80 to 1.21), but that was not significantly different from TAI cows throughout the 90 day study period ($P = 0.87$). The lack of difference found between TAI and AAM when considering both DO and the proportion of pregnant cows at 90 d past the VWP supports this result.

Further Discussion

Timed artificial insemination has successfully been implemented on many farms as a replacement for visual estrus detection. However, TAI requires strict compliance and dedicated labor for success. Based on the results of this study, a reproductive management program based on AAM can achieve similar effectiveness with minimal hormonal intervention. Another solution could be a combination of the two systems. Recently, Fricke et al. (2014c) evaluated the effectiveness of TAI, with or without AAM supplementation at first service. They found that supplementing TAI with AAM reduced time to first service by 7.5 to 12.4 d and decreased CR by 8.0% as compared to TAI alone. In another study, Stevenson et al. (2014) compared PR in cows bred to a Presynch-Ovsynch-Resynch, TAI protocol with cows bred using AAM exclusively from 40 to 75 DIM. Cows in the AAM group received an injection of PGF_{2α} at 54 DIM if they were not bred by that point in time. Mean DO of cows in the AAM group was 24 days less. Further research comparing different TAI protocols, AAM systems with various sensitivity settings, or a combination of these two systems may be warranted.

Although both reproductive management strategies produced similar results in this study, the economics of each system should be analyzed before adoption on a commercial herd. The initial investment in AAM is large, but may be offset by reoccurring injection and labor costs associated with TAI. Fricke et al. (2014c) found that NPV per cow per year of the different reproductive management strategies differed by only \$4.00 to \$8.00. This indicates that multiple reproductive management strategies can be economically feasible, depending on individual herd scenarios.

CONCLUSIONS

When compared as reproductive management strategies, TAI and AAM resulted in similar performance on three commercial herds. Timed artificial insemination cows experienced a 15.2 d shorter time to first service. Automated activity monitored cows experienced a 10.9% greater CR at repeat services and a 13.6 d shorter service interval. No treatment difference in DO or risk of pregnancy establishment existed. Herd and parity did affect AAM performance in some situations.

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Table 3.1. Characteristics of cows (mean \pm SD) in a study comparing timed artificial insemination (TAI) and automatic activity monitoring (AAM) as reproductive management strategies on three commercial dairy herds.

Parameter	Treatment		Herd		
	TAI	AAM	A	B	C
BCS ^{1,2}	2.96 \pm 0.33	2.93 \pm 0.33	2.92 \pm 0.31	2.90 \pm 0.25	3.01 \pm 0.39
Parity	1.93 \pm 1.12	2.10 \pm 1.34	2.01 \pm 1.20	2.09 \pm 1.29	1.95 \pm 1.24
Summit milk ³	39.38 \pm 8.43	40.33 \pm 9.26	39.19 \pm 8.89	39.76 \pm 8.21	40.65 \pm 9.23

¹Body condition score at enrollment: 24 \pm 7 d before the end of the VWP (herd A: 80 DIM, herd B: 60 DIM, herd C: 60 DIM).

²As evaluated using the 1 to 5, 0.25 increment system developed by Ferguson et al. (1994)

³Data presented was collected from Dairy Records Management Systems (Raleigh, NC)

Table 3.2. Summary of time to first service in days after the voluntary waiting period (TFS), first service conception rate (CR1), repeat (2nd and 3rd) service conception rate (CR2), service interval (SI), days open (DO), services per pregnancy (SP), pregnancy loss (PL), and the proportion of cows pregnant at 90 d after the voluntary waiting period (P90) for three commercial herds in a study comparing two reproductive management strategies.

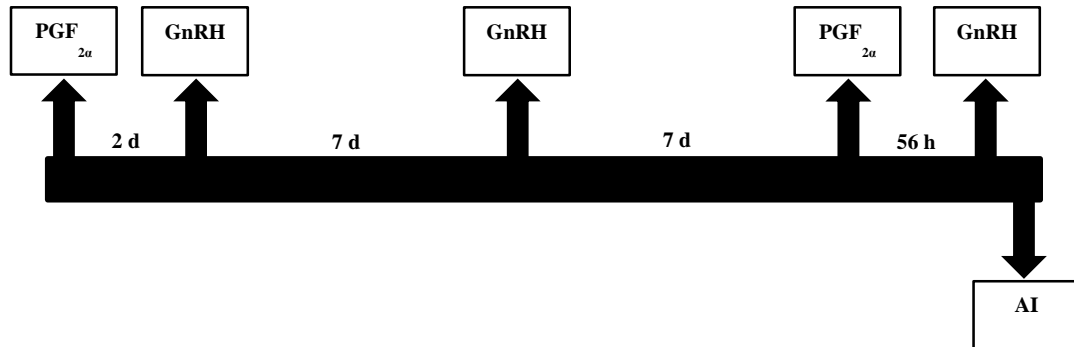
		TFS ¹	CR1 ²	CR2 ²	SI ¹	DO ¹	SP ¹	PL ²	P90 ²
Effect		(n = 538)	(n = 538)	(n = 437)	(n = 293)	(n = 353)	(n = 353)	(n = 395)	(n = 542)
Treatment	TAI ³	6.67 ± 0.7 ^a	41.11 ± 3.1	34.93 ± 3.8 ^b	42.16 ± 0.7 ^b	30.66 ± 1.9	1.59 ± 0.05	14.07 ± 2.8	61.85 ± 3.4
	AAM ⁴	21.91 ± 0.8 ^b	41.12 ± 3.1	45.75 ± 4.7 ^a	28.63 ± 0.8 ^a	34.77 ± 1.9	1.56 ± 0.05	9.03 ± 2.3	64.07 ± 3.4
Herd	A	12.74 ± 0.8 ^a	54.42 ± 3.5 ^a	42.17 ± 4.6 ^a	35.36 ± 0.9	25.70 ± 2.0 ^a	1.42 ± 0.06 ^a	9.03 ± 2.2	73.89 ± 3.1 ^a
	B	12.61 ± 1.1 ^a	40.61 ± 4.4 ^b	51.11 ± 6.9 ^a	36.37 ± 1.0	35.76 ± 2.6 ^b	1.66 ± 0.07 ^b	12.19 ± 3.9	64.68 ± 5.5 ^a
	C	17.50 ± 0.9 ^b	29.50 ± 3.5 ^c	28.56 ± 4.0 ^b	34.45 ± 0.8	36.68 ± 2.6 ^b	1.65 ± 0.07 ^b	13.07 ± 3.4	48.69 ± 3.9 ^b
Parity	Primiparous	14.51 ± 0.8	42.44 ± 3.5	41.90 ± 4.8	33.79 ± 0.9 ^a	30.51 ± 2.1	1.52 ± 0.06	8.51 ± 2.4	65.37 ± 3.7
	Multiparous	14.05 ± 0.7	39.85 ± 2.9	38.57 ± 3.7	37.00 ± 0.8 ^b	34.92 ± 1.8	1.63 ± 0.05	14.87 ± 2.7	60.50 ± 3.2
BCS	2.50 to 2.75	14.77 ± 0.8	35.16 ± 3.3 ^b	34.77 ± 4.0 ^b	35.31 ± 0.8	34.94 ± 2.2	1.63 ± 0.06	11.67 ± 2.9	56.72 ± 3.8 ^a
	3.00 to 5.00	13.79 ± 0.7	47.39 ± 3.1 ^a	45.93 ± 4.4 ^a	35.49 ± 0.8	30.49 ± 1.8	1.52 ± 0.05	10.95 ± 2.4	68.81 ± 3.1 ^b
THI ^{5,6}		0.01 ± 0.07	-0.01 ± 0.01	0.04 ± 0.02	-0.01 ± 0.05	0.17 ± 0.13	0.00 ± 0.00	-0.03 ± 0.02	0.02 ± 0.01
Summit milk ⁶		0.02 ± 0.03	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.03	-0.04 ± 0.07	0.00 ± 0.00	0.00 ± 0.01	0.01 ± 0.00

Lowercase letters indicate significant differences within effect and column ($P < 0.05$)

¹Reported as mean ± SE; ²Reported as mean ± SEM; ³Timed artificial insemination; ⁴Automated activity monitoring; ⁵Temperature humidity index; ⁶Reported as model estimate

Figure 3.1. Timed artificial insemination G7G, Ovsynch, and Resynch protocols using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and gonadotrophin releasing hormone (GnRH). Artificial insemination (AI) occurred 16 h after the last GnRH injection.

G7G-Ovsynch



Resynch

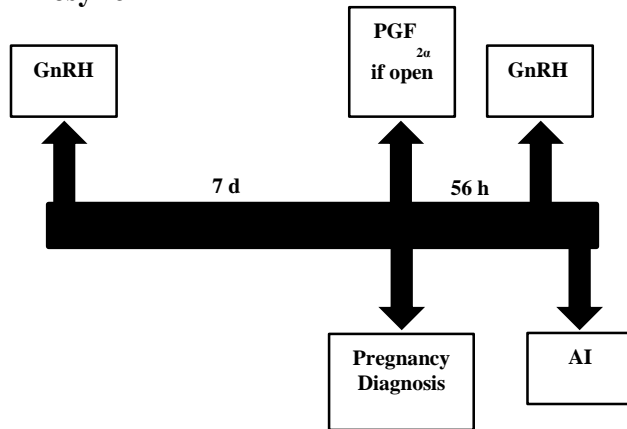
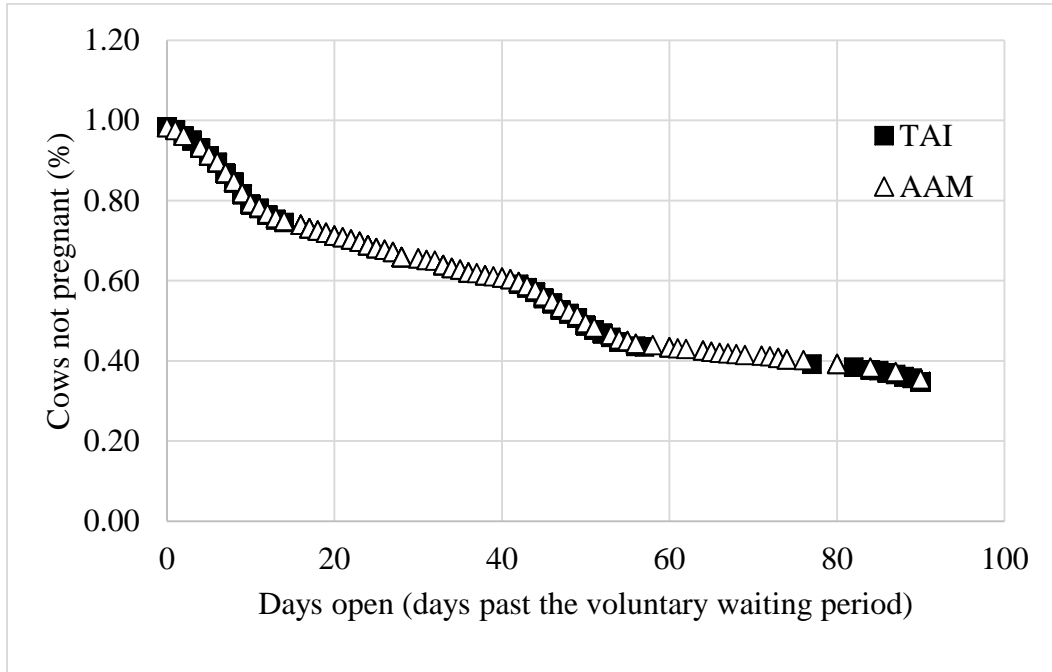


Figure 3.2. Survival curve representing the proportion of cows not pregnant by days open past the voluntary waiting period for cows bred according to timed artificial insemination (TAI) or automated activity monitoring (AAM). The risk of pregnancy throughout the 90 day study period was not different (hazard ratio = 0.97; $P = 0.77$).



CHAPTER FOUR

Investment analysis of automated estrus detection technologies as compared to alternative breeding strategies

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INTRODUCTION

Reproductive performance is one of the largest factors affecting dairy farm profitability because of its direct relationship to milk production, replacement animal availability, genetic progress, and culling (Britt, 1985; Plaizier et al., 1997; Olynk and Wolf, 2008). Dijkhuizen et al. (1985) estimated that reproductive failure accounted for 2% of a farm's gross production value. Plaizier et al. (1997) found that adjusted CI (calculated as the projected CI divided by the percent of cows not culled for reproductive purposes) and net revenue were strongly correlated ($R^2 = 0.72$).

The mean value of a new pregnancy has been estimated between \$192 and \$278, depending on parity, DIM, milk production, probability of pregnancy, cull price, and replacement heifer price (De Vries, 2006b; Cabrera, 2012). A more commonly discussed economic measurement of reproductive performance is cost of extended DO. One extra DO represents a loss of -\$1.54 to \$5.41 per cow, unadjusted for inflation (Holmann et al., 1984; Groenendaal et al., 2004; Meadows et al., 2005; De Vries, 2006a; Liang, 2013). The cost per DO is reduced when feed cost or slaughter price increase or when the VWP or milk production are reduced (Bewley et al., 2010). The cost per DO increases when reproductive performance declines or replacement price or milk price increases (Meadows et al., 2005; Bewley et al., 2010).

Many reproductive management programs exist, including bull breeding, VO for estrus, timed artificial insemination, and AED. Reproductive management program costs differ with different EDR, CR, labor requirements, and associated costs (Holmann et al., 1987; Olynk and Wolf, 2009). Associated costs may include estrus detection aids or replacements in the form of tail paint, hormones, or AED technologies (Holmann et al.,

1987). Additional reproductive management costs include those associated with semen purchases, insemination costs, and pregnancy diagnosis.

Economic differences between reproductive management programs have been compared previously. Both Overton (2005) and Lima et al. (2010) estimated the cost of TAI using Ovsynch was around \$10 less per cow per year compared to natural service (bull breeding). The difference in cost depended on bull feed costs, semen price, and the observed improvement in genetic merit when using semen over a bull. Other researchers have stated that Ovsynch produces an economic preference over VO, depending on labor costs and efficiency of VO (Olynk and Wolf, 2008; 2009).

Recently, economic comparisons have moved towards analyzing the value of combining VO and TAI. Galvão et al. (2013) compared VO, TAI (Presynch Ovsynch), and a combination of both systems, account for varying EDR, estrus detection accuracy, TAI compliance, and milk price. Individually, the VO groups with a high EDR (60%), regardless of accuracy of estrus detection (85 or 95%), resulted in the greatest profits followed by the TAI group with high compliance (95%). Combining TAI and VO increased profits in all situations, regardless of increased input costs.

Giordano et al. (2011) created a similar, but more user friendly, comparison model. The tool calculates NPV per cow per year for different reproductive management programs and can be adapted to an individual farm's situation using production, reproduction, and economic inputs. In an example presented by the authors using information from a commercial Wisconsin dairy herd, 100% TAI programs were more profitable than 100% VO. Because of a high TAI service rate, combining TAI and VO was only valuable when CR was low.

To the authors' knowledge, publication of economic comparisons between AED technologies and other reproductive management programs do not exist. However, Fricke et al. (2014c) used the tool created by Giordano et al. (2011) to conduct an economic comparison between three first service reproductive management methods: 1) cows inseminated using AED and undergoing Ovsynch if not detected in estrus by 62 DIM, 2) cows inseminated using AED after presynchronization and receiving Ovsynch if not detected in estrus by 62 DIM, and 3) cows inseminated after Presynch-Ovsynch. Their analysis resulted in a similar NPV (\$2,728, \$2,732, and 2,736 per cow per year) for all three methods when investment cost of the system was assumed as \$10,000 with a \$100 tag price.

The objective of this project was to create a producer-friendly, farm-specific decision support tool dashboard specifically for NPV investment analysis of AED technologies. As AED interest and use increases, such a tool would fill an industry need. A secondary objective of this study was to develop a new cost per DO equation using stochastically simulated variables and retention pay-off (**RPO**) values.

MATERIALS AND METHODS

A model to investigate the economics of transitioning to AED from an alternative reproductive management program (bull breeding, VO, synchronization, visual detection aids, or a technician service) was developed using Excel 2013 (Microsoft, Redmond, WA). The model estimated the DO costs, semen costs, and pregnancy diagnosis costs associated with both pre- and post-AED investment. Differences in costs were used to calculate NPV of the investment. Additionally, the model was turned into a user-friendly, farm-specific decision support tool dashboard.

Equations

Cost of days open. Days open was calculated using the process described by Pecsok et al. (1994). Equation 4.1 first calculated the number of eligible breeding cycles:

$$N = \frac{(DNB - VWP - 10.5)}{21} \quad (\text{Eq. 4.1})$$

Where N is the number of eligible breeding cycles, DNB is the DIM to stop breeding a cow if she has not yet become pregnant, VWP is voluntary waiting period, 10.5 days is the mean length of time after the VWP before a cow will express estrus, and 21 is the mean length of the estrous cycle in cattle. Equation 4.2 calculated DO:

$$DO_i = \left(\left((VWP + 10.5) + 21 \times \left(\left(\frac{(1-PR_i) - ((1-PR_i)^N)}{PR_i} \right) - N - 1 \right) \times ((1-PR_i)^N) \right) + ((DNB - VWP - 21) \times (1-PR_i)^N) \right) \quad (\text{Eq. 4.2})$$

Where DO is days open, *i* is pre- or post- AED investment, VWP is voluntary waiting period, 10.5 is the mean length of time after the VWP before a cow will express estrus, 21 is the mean length of the estrous cycle in cattle, PR is pregnancy rate as calculated by the multiplication of EDR and first service CR, N is the number of eligible breeding cycles (Equation 4.1), and DNB is the DIM to stop breeding a cow if she has not yet become pregnant.

Equations 4.3 (Meadows et al., 2005) and 4.4 calculated CI and the number of cows calving each year, respectively:

$$CI_i = \frac{(DO_i + 280)}{30.42} \quad (\text{Eq. 4.3})$$

$$CLVY_i = (HS - (HS \times CULL)) \times \left(\frac{365.25}{(CI_i \times 30.42)} \right) \quad (\text{Eq. 4.4})$$

Where CI is the calving interval, i is pre- or post- AED investment, DO is days open (Equation 4.12), 280 is the mean Holstein gestation length, 30.42 is the mean number of days in one month, CLVY is the number of cows calving each year, HS is the lactating herd size, CULL is the culling rate, and 365.25 is the mean number of days in a year.

To determine cost per DO, the whole farm stochastic simulation model previously described by Bewley et al. (2010) and Liang (2013) was used. This model uses the RPO value of a cow to determine cost per DO, accomplished by subtracting the RPO value of a cow at the DIM she conceived from the RPO value of a cow in the same lactation at 60 DIM. Using the model, 10,000 iterations were run for each lactation (1 to ≥ 5) with cost per DO as an output and variables expected to have potential effects on cost per DO (rolling herd average milk production, age at first calving, mature cow live weight, heifer calf value, bull calf value, semen cost, DIM dictating an open cow as a reproductive cull, milk production level dictating an open cow as a production cull, veterinarian costs, discount rate, milk price, feed price, replacement price, cull cow price, VWP, EDR, and CR) as inputs. The simulation allowed for a large number of variable combinations to be created, which would be difficult or impossible to collect in real life conditions. Using the simulation results, the GLMSELECT procedure of SAS (Version 9.3 SAS Institute, Inc., Cary, NC) analyzed the effects of each input and their two-way interactions on the cost per DO, using $P < 0.05$ as the inclusion criteria for the model. The resulting

estimates were used to develop lactation specific equations for cost per DO. Main effects of each variable on all lactations are shown in Table 4.2.

Equation 4.5 calculated total yearly economic losses resulting from DO:

$$TYCDO_{ij} = (CLVY_i \times Lact_j) \times ((DO_i - VWP) \times CDOL_j) \quad (\text{Eq. 4.5})$$

Where TYCDO is total yearly cost of DO in lactation j , i is pre- or post- AED investment, CLVY is the number of cows calving each year (Equation 4.4), Lact is the percent of cows in lactation j , DO is mean days open (Equation 4.2), VWP is voluntary waiting period, and $CDOL_j$ is the cost per DO for lactation j (Table 4.2).

Semen and pregnancy diagnosis cost. Conception rates for the first 12 services were calculated using the first service CR (model input) and assuming a reduction of 2.6 percent at each insemination (Galvão et al., 2013). The weighted mean CR was then determined by weighting each service CR by the number of inseminations occurring at each breeding, assuming that all cows that calved were bred at each service until they became pregnant or reached DNB status. To determine timing of inseminations and when cows reached DNB status, Equations 4.6 (Pecsok et al., 1994) and 4.7 (Heersche and Nebel, 1994) calculated DIM at first breeding and breeding interval, respectively:

$$DIM1_i = \left(\frac{21}{EDR_i} \right) + VWP - N \quad (\text{Eq. 4.6})$$

$$BI_i = \frac{(DO_i - DIM1_i)}{\left(\left(\frac{1}{WCR_i} \right) - 1 \right)} \quad (\text{Eq. 4.7})$$

Where DIM1 is days in milk at first service, i is pre- or post- AED investment, EDR is estrus detection rate, VWP is voluntary waiting period, N is the number of eligible breeding cycles (Equation 4.11), BI is breeding interval, DO is mean days open (Equation 4.12), and WCR is weighted mean CR.

Total yearly semen cost was determined by multiplying semen cost (model input) by the total number of inseminations in one year. Total yearly pregnancy diagnosis costs were determined assuming that all cows that conceived underwent pregnancy diagnosis, along with those not redetected in estrus before the time of the pregnancy diagnosis (as determined using EDR).

Pre-AED estrus detection method costs. Model versatility allowed selection of bull breeding, VO, synchronization, visual detection aids, or a technician service as an input for pre-AED investment reproductive management program. Regardless of chosen program, weekly labor costs associated with the program is required as an input. Other variable inputs, depending on selected reproductive management method, include cost and number of hormone injections per service, cost and number of detection aids used per service, breeding fee per service, yearly cost to maintain a bull, and other weekly reproductive management costs. Variable input costs associated with each service were multiplied by the total number of inseminations in one year to determine yearly costs. Total yearly variable costs associated with the pre-AED estrus detection method were calculated as labor costs plus other yearly variable costs.

The model allows either elimination or a percent reduction of the use of the pre-AED investment reproductive management program, therefore eliminating or reducing associated variable costs by the same extent. For example, if the pre-AED program was associated with a variable cost of \$15 per cow per year and a producer decided to keep using this program 25% of the time post-AED investment, variable costs were reduced to \$3.75 per cow per year, not including costs associated with the AED system. Additionally, when the selection is made to continue using a portion of the pre-AED

reproductive management program, post-AED investment EDR is calculated as a weighted mean of the pre- and post- AED investment EDR. For example, if pre-AED program EDR was 60% and was still used 25% of the time after AED adoption, with AED EDR at 80%, post-AED EDR would be 75%.

AED costs. Yearly variable costs associated with AED included labor cost, the cost to replace lost or broken tags, and the maintenance costs charged by the AED company. Equation 4.8 calculated the total fixed investment cost of the AED system:

$$TIC = II + (MCOST \times (MCOW \times HS)) \quad (\text{Eq. 4.8})$$

Where TIC is total fixed investment cost of the AED system, II is initial fixed investment cost of the AED system, MCOST is the individual AED system tag price, MCOW is the percent of the lactating herd receiving an AED system tag, and HS is lactating herd size.

Cash Flow. Differences between pre- and post- AED investment TYCDO, semen costs, pregnancy diagnosis costs, and estrus detection method costs were used to calculate yearly change in cash flow (Equation 4.9):

$$FLOW_n = (PostCDO + PostSemen + PostDiagnosis + PostED) - (PreCDO + PreSemen + PreDiagnosis + PreED) \quad (\text{Eq. 4.9})$$

Where FLOW is cash flow in year n , PostCDO is the year n CDO after AED investment, PostSemen is the year n semen cost after AED investment, PostDiagnosis is the year n pregnancy diagnosis cost after AED investment, Post ED is the year n estrus detection method cost after AED investment, PreCDO is the year n CDO before AED investment, PreSemen is the year n semen cost before AED investment, PreDiagnosis is

the year n pregnancy diagnosis cost before AED investment, and PreED is the year n estrus detection method cost before AED investment.

Net Present Value. Equation 4.10 calculated NPV of the AED over a 10-year investment period, assuming no terminal value at the conclusion:

$$NPV = \sum_{n=1}^N \frac{FLOW_n}{(1+DR)^n} - TIC \quad (\text{Eq. 4.10})$$

Where NPV is the net present value of the AED system over the 10 y investment period, n is the year of investment, FLOW is the change in cash flow for each year n (Equation 4.9), DR is the discount rate, and TIC is the total investment cost of the AED system (Equation 4.8).

Breakeven point. Equation 4.11 calculated breakeven point, in years:

$$BE = L_n / \frac{CC_n}{CC_{n+1}} \quad (\text{Eq. 4.11})$$

Where BE is breakeven point, L_n is the last year (n) of negative cumulative cash flow, CC_n is the absolute value of cumulative cash flow in year n , and CA_{n+1} is the actual cash flow in the year after n .

Interface

Figure 4.1 shows the interface of the model, after development into a user-friendly decision support tool dashboard using Xcelsius 4.0 (SAP BusinessObjects, Newtown Square, PA). A user will enter herd specific information about their current reproductive management program and information about the proposed AED technology system in order to receive farm-specific AED adoption results. User output includes current and estimated new (using the AED system) mean DO and reproductive cull percent, calculated as the percent of cows reaching DNB status in one year. Investment

analysis results are also included in the form of NPV and BE. The decision support tool is available online at <http://www2.ca.uky.edu/afsdairy/HeatDetectionTechnologies>.

Investment Analysis Demonstration

To demonstrate model utility, an investment analysis demonstration was conducted. Herd input assumptions were gathered from DairyMetrics (Dairy Records Management Systems, Raleigh, NC), Food and Agricultural Policy Research Institute (FAPRI; Columbia, MO), and published literature to represent an average US Holstein dairy herd. Parameters gathered from DairyMetrics used the limitations of only Holstein herds with more than 100 cows and EDR between 10 and 70%, to attempt elimination of herds miss-reporting EDR. Ten-year (2014 to 2023) predicted means were used for milk price, feed cost, replacement cost, and cull cow value to reduce the effects of high or low prices at one specific time point. Resulting mean lactating herd size, rolling herd average milk production, milk price, and feed cost were 316, 10,533 kg, \$0.40/kg, and \$0.17/kg DM. The remaining herd assumptions used in the investment demonstration are shown in Table 4.1.

Four investment scenarios were considered: 1) 100% VO to 100% AED, 2) 100% VO to 75% AED and 25% VO, 3) 100% TAI to 100% AED, and 4) 100% TAI to 75% AED and 25% TAI. In situations where VO was used pre-AED investment, the assumption was that 0.60 min per cow per day was required for labor (Galvão et al., 2013), accomplishing a 48.6% EDR (Dairy Records Management Systems, Raleigh, NC). When TAI was used pre-AED investment, an Ovsynch protocol was assumed (2 doses GnRH and 1 dose PGF_{2α} per service) with a 95% service rate (Galvão et al., 2013) and a strict 38 d pregnancy diagnosis with no re-inseminations occurring before pregnancy

diagnosis. Doses of GnRH and PGF_{2α} were estimated at \$2.40 and \$2.65, respectively, and labor required for TAI was estimated at 1.00 min/injection. Labor costs and first service CR were held consistent at \$15.00/h (Galvão et al., 2013) and 36.8% (Dairy Records Management Systems, Raleigh, NC), regardless of reproductive management program.

AED technologies with varying initial fixed investment cost (low: \$5,000 vs. high: \$10,000), tag price (low: \$50 vs. high \$100), and EDR (low: 60% vs. high: 80%) were compared, resulting in eight AED combinations. Percent of cows to receive a tag (50%), percent of tags to replace per year (5%), system maintenance cost per year (\$0), labor required per week (3.5 h), labor costs (\$15.00/h; Galvão, 2013), and expected first service CR (36.8%; Dairy Records Management Systems, Raleigh, NC) were held constant in all AED combinations. A summary of pre- and post- AED investment reproductive management scenario assumptions is included in Table 4.3.

RESULTS AND DISCUSSION

Cost of Days Open

Two factors differentiate the new cost per DO equations produced in this study from previous models: stochastic simulation of market conditions and the use of RPO to quantify the value of the cow. Stochastic simulation allows for randomness within outcomes, unlike deterministic simulation, which will always produce the same outcome. By using stochastic simulations, we are able to consider a variety of variable combinations and determine how our value of interest (cost per DO) changes with changing scenarios. The RPO method determines the difference in projected future profit achievable by a cow and the projected future profit achievable by her replacement

(Groenendaal et al., 2004; Liang, 2013). The resulting RPO value represent the maximum amount of money that can be spent in order to attempt to keep that cow (Van Arendonk, 1984; Huirne et al., 1997; Groenendaal et al., 2004). When RPO is not accounted for, culling costs are not considering the potential future profits associated with either the current cow or her replacement (Giordano et al., 2011). Groenendaal et al. (2004) also used the RPO method to estimate the cost per DO, but did not use stochastic simulation. Additionally, that model differed from this one by including dry cow feed costs, herd discount rate, and weight of the animal at birth as variables, but not including the DIM dictating an open cow as a reproductive cull, the milk production level dictating an open cow as a production cull, or the difference between bull and heifer calf value.

Using the assumptions in Table 4.1, the resulting cost per DO for each lactation (1 to ≥ 5) considering varying EDR (40, 60, or 80%) are shown in Table 4.4. Cost per DO ranged from \$1.79 to \$3.27 in lactation 1, \$2.66 to \$3.88 in lactation 2, \$4.71 to \$7.19 in lactation 3, \$2.43 to \$7.95 in lactation 4, and \$1.70 to \$3.03 in lactation ≥ 5 . Results of this study and previous studies are similar, with other estimates of cost per DO (unadjusted for inflation) being \$0 to \$3.00 (Groenendaal et al., 2004), \$1.37 (Meadows et al., 2005), \$3.19 to 5.41 (De Vries, 2006a), and -\$1.54 to 2.78 (Liang, 2013). Previous studies have also noted an effect of lactation on cost per DO (Olds et al.; Groenendaal et al., 2004; Liang, 2013). Olds et al. (1979) estimated a \$0.47 lower cost per DO in first lactation animals over later parities (\$0.71 vs. 1.18, unadjusted for inflation). Liang (2013) also reported reduced costs per DO in first lactation animals, noting a possible effect of lactation persistency.

Investment Analysis Demonstration

Net present value helps determine the profitability of an investment by considering the differences between returns and costs, including the time value of money (Butler, 1996). When NPV is less than zero, an investment is not profitable. A NPV equal to or greater than zero encourages investment. Net present value per cow per year for the four investment scenarios (100% VO to 100% AED, 00% VO to 75% AED and 25% VO, 100% TAI to 100% AED, and 100% TAI to 75% AED and 25% TAI), considering varying AED EDR, system cost, and tag price combinations is shown in Table 4.5. The NPV was always least in the AED EDR 60%, \$10,000 initial fixed investment cost, \$100 tag price scenario and greatest in the 80% AED EDR, \$5,000 initial fixed investment cost, and \$50 tag price scenario. This was expected because of the combined extremes of fixed costs and EDR.

Of the three varying factors analyzed in this study, AED EDR (60 vs. 80%) had the greatest effect on NPV, followed by the tag price (\$50 vs. 100), and lastly the initial fixed investment cost (\$10,000 vs. 5,000). This highlights the importance of reproductive performance over cost of a reproductive management program, as also noted by Galvão et al. (2013). Additionally, the fact that the variable costs (tag price) were more influential than the fixed costs (initial investment cost) shows that herd size will influence investment results. Further discussion of each investment scenario is included below.

VO vs. AED. When pre-AED estrus detection was VO alone, mean DO and TYCDO per cow were 153 d and \$84.58, respectively. When the post AED-investment EDR was 60%, mean DO dropped to 138 d and mean TYCDO per cow dropped to \$73.96. When the post AED-investment EDR was 80%, mean DO dropped to

118 d and mean TYCDO per cow dropped to \$47.53. As expected, improving EDR decreased DO, thereby reducing the cost of DO.

The range in NPV per cow per year and payback period for investment in AED after VO was \$27.06 to \$48.81 and 0.55 to 1.71 y, respectively. This represents \$2,706 to \$4,881 per year on a 100 cow dairy or \$27,060 to \$48,810 per year on a 1,000 cow dairy. Regardless of both AED system cost and AED EDR, NPV was always positive and indicated a positive investment situation.

The two factors contributing to the positive NPV in this scenario were the reduced labor costs and the improved EDR. Olynk and Wolf (2008) also stated that the major factors affecting the economics of reproductive management are EDR and labor costs. Labor costs associated with VO in this study were estimated at \$54.60 per cow per year, using the assumption of 0.60 min required per cow per d (Galvão et al., 2013). Labor costs associated with the AED system were \$15.23 per cow per year, resulting in a reduction of \$39.37 per cow per year. This would be the equivalent of \$3,937 per year in a 100 cow herd or \$39,370 per year in a 1,000 cow herd. Labor costs associated with VO may be reduced if a farm spends less time on estrus detection each day or if cheaper labor is available. Reducing labor costs would reduce NPV and could eventually lead to an unprofitable AED investment situation.

Estrus detection rate improved 11.4 to 31.4% after AED investment, which reduced DO and associated costs. These results were greatly affected by what the VO EDR was defined as. For example, when VO EDR was increased from 48.6 to 91% (the 99th percentile of US Holstein herds, DRMS), all combinations of the AED system were no longer profitable investments (-\$18.85 to -\$2.34 NPV per cow per year). Therefore,

although the average farm may improve EDR using AED, individual farm situations will vary.

VO vs. 75% AED and 25% VO. When the post-AED investment estrus detection method used a combination of 75% AED and 25% VO, the weighted EDR was 57.2 and 72.2%, for the 60 and 80% AED EDR systems, respectively. The combination of the 60% AED EDR system and VO reduced mean DO and TYCDO per cow by 12 d and \$7.58, respectively. The combination of the 80% AED EDR system and VO reduced mean DO and TYCDO per cow by 28 d and \$25.81, respectively. By combining AED and VO, reproductive performance was improved, although not to the same extent as when using AED alone because of the relatively low EDR when using VO. If VO produced a greater EDR, reproductive performance in this scenario would be improved.

The range in NPV per cow per year and payback period for investment in AED in this scenario was \$15.90 to \$32.41 and 0.80 to 2.56 y, respectively. When compared to the previous scenario, EDR was not increased as greatly (8.6 to 23.6%), nor was labor cost decreased as much (\$32.31 per cow per year), explaining the reduced NPV. However, AED investment was still profitable and improved reproductive performance using any combination of system cost, tag price, and AED EDR.

TAI vs. AED. When TAI was used pre-AED investment, mean DO and mean TYCDO per cow were 137 d and \$108.98, respectively. When the post AED-investment EDR was 60%, mean DO remained similar at 138 d while mean TYCDO per cow decreased to \$73.96. When the post AED-investment EDR was 80%, mean DO decreased to 118 d and mean TYCDO per cow decreased to \$47.532.

The range in NPV per cow per year and payback period for investment in AED after TAI was \$26.13 to \$47.89 and 1.75 to 0.56 y, respectively. This represents \$2,613 to \$4,789 per year on a 100 cow dairy or \$26,130 to \$47,890 per year on a 1,000 cow dairy. Regardless of both AED system cost and AED EDR, NPV was always positive and indicated a positive investment situation.

The shorter service interval of AED (21 d) contributed most to the positive NPV of investing. An assumption was made that TAI cows would not be rebred until after pregnancy diagnosis (38 d), regardless of observed estrus activity. In practice, rebreeding TAI cows observed in estrus before pregnancy diagnosis is common. Therefore, our scenario assumptions may have affected these results. Reducing the TAI service interval would change NPV but would not necessarily lead to a negative AED investment scenario.

TAI vs. 75% AED and 25% TAI. When the post-AED investment estrus detection method used a combination of 75% AED and 25% TAI, the weighted EDR was 68.8 and 83.8%, for the 60 and 80% AED EDR systems, respectively. The combination of the 60% AED EDR system and TAI decreased mean DO and TYCDO per cow by 9 d and \$45.65, respectively. The combination of the 80% AED EDR system and TAI decreased mean DO and TYCDO per cow by 22d and \$67.11, respectively. Because of the shorter service interval using AED, combining AED and TAI improved reproductive performance compared to TAI alone.

The range in NPV per cow per year and payback period for investment in AED in this scenario was \$28.42 to \$47.23 and 0.57 to 1.64 y, respectively. Combining reproductive management strategies had two benefits. First, labor of the combined TAI

and AED system was \$11.91 per cow per year, which was lower than either TAI (\$13.08/c/y) or AED (\$15.23/c/y) alone. Second, the service interval was reduced when using AED (21 d) as compared to TAI (38 d).

Model Limitations

The goal of this study was to develop a user-friendly, farm-specific decision support tool. Therefore, inputs and calculations were kept simple. Although simplification may reduce accuracy and reliability in some cases, the ability to quantify results is an improvement over a producer's guess (Delorenzo and Thomas, 1996). Additionally, the relative expected consequences are more important than exact numbers (Lien, 2003). Some factors that were not accounted for in this model include additional benefits from the AED system (i.e. health status monitoring, lameness detection, etc.), the effect of AED on the quality of a producer's life, and the change in heifer calf inventory resulting from changes in reproductive performance. Although these limitations exist, the flexibility of the model to handle multiple situations and provide farm-specific results is beneficial to producers considering investing in AED, especially given that no other decision support tools of this kind currently exist.

CONCLUSIONS

A new equation for calculating cost per DO, using stochastically simulated variables and RPO, was used in an investment analysis of AED technologies. On an average US Holstein dairy, investment in an AED system produced a positive NPV when switching from either VO or TAI. Combinations of AED and VO or TAI produced similar results to using AED alone. Investment analysis results were highly dependent on the assumptions used, especially VO and AED EDR, TAI service rate and interval, and

labor costs. Producers can use farm-specific inputs with the decision support tool dashboard to determine individual results.

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Table 4.1 Herd assumptions used in an automated estrus detection technology investment analysis demonstration.

Input	Assumption	Source ¹
Milking herd size	316	DairyMetrics
Percent of herd in 1 st lactation (%)	36.1	Dhuyvetter et al. (2007)
Percent of herd in 2 nd lactation (%)	26.0	Dhuyvetter et al. (2007)
Percent of herd in 3 rd lactation (%)	17.7	Dhuyvetter et al. (2007)
Percent of herd in 4 th lactation (%)	11.0	Dhuyvetter et al. (2007)
Percent of herd in 5 th and later lactations (%)	9.2	Dhuyvetter et al. (2007)
Rolling herd average milk production (kg)	10,533	DairyMetrics
Milk price (\$/kg) ²	0.40	FAPRI, 2014
Feed cost (\$/kg DM) ²	0.17	FAPRI, 2014
Culling rate (%)	37.7	DairyMetrics
Cull cow value (\$/kg) ²	1.43	FAPRI, 2014
Mature cow live weight (kg)	723	Liang (2013)
Days in milk do not breed (d)	300	Bewley et al. (2010)
Cull milk yield (kg/d)	15.9	Bewley et al. (2010)
Replacement heifer price (\$) ²	1825	Liang (2013)
Heifer calf value (\$)	400.00	Dhuyvetter et al. (2007)
Bull calf value (\$)	100.00	Dhuyvetter et al. (2007)
Age at first calving (m)	25.6	DairyMetrics
Voluntary waiting period (DIM)	58.5	DairyMetrics
First service CR (%)	36.8	DairyMetrics
Semen cost (\$/straw)	10.00	Galvão et al. (2013)
Pregnancy diagnosis (\$/c)	3.00	Galvão et al. (2013)
Veterinary costs (\$/c/y)	61.61	Groenendaal et al. (2004)
Metritis incidence (%)	7.8	Wilson et al. (2004)
Retained placenta incidence (%)	10.4	Wilson et al. (2004)
Labor cost (\$/h)	15.00	(Galvão et al., 2013)
Discount rate (%)	8.0	Hyde and Engel (2002)

¹DairyMetrics information was collected in August 2014 from Dairy Records Management Systems, (Raleigh, NC) and FAPRI is Food and Agricultural Policy Research Institute (Columbia, MO)

²Ten-year predicted means (2014 to 2023)

Table 4.2. Lactation specific equations for cost per day open were derived from the model described by Bewley, 2010 and Liang, 2014. Relationships¹ between rolling herd average milk production (RHA), age at first calving (AFC), mature cow live weight (weight), heifer calf value (heifer value), bull calf value (bull value), semen cost, days in milk dictating an open cow as a reproductive cull (DIM DNB), milk production level dictating an open cow as a production cull (Cull MY), veterinarian costs (vet costs), discount rate (DR), milk price, feed price, replacement price (replace price), cull cow price (cull price), voluntary waiting period (VWP), estrus detection rate (EDR), and conception rate (CR) as determine by the model are shown for each lactation (1 to ≥ 5).

Variable	Lactation				
	1	2	3	4	≥ 5
Intercept	-74.2215	-67.8471	-44.9264	21.3131	54.4406
RHA	0.0020	0.0018	0.0014	-0.0005	-0.0008
AFC		-0.1535	-0.2318		-0.2510
Weight	0.0258	0.0205	0.0106		
Heifer value	0.0038				
Bull value			0.1146		-0.0553
Semen cost	-0.0790				
DIM DNB				0.0239	
Cull MY					-0.2920
Vet costs		-0.0014			
DR					
Milk price	-0.0001	3.2775	1.4262		
Feed price					-0.9481
Replace	-0.0545	-0.0436	-0.0420	-0.0206	-0.0130
Cull price	60.7782	41.0304	53.9354	39.1645	30.9424
VWP	0.2807	0.2688	0.2762		-0.1708
EDR	21.1000	29.9581	6.8555	-39.7913	-55.3717
CR	50.3472	60.6795	48.5017	-60.4479	-93.2207

¹Coefficients were calculated using the imperial system rather than the metrics system.

Table 4.3. Assumptions used in investment analysis demonstration analyzing the net present value of switching from visual observation (VO) or timed artificial insemination (TAI) to automated estrus detection (AED). The AED systems displayed represent two combinations of extreme costs and estrus detection rate (EDR). All costs assume a 100 c herd investing in 100 AED tags.

Breeding system	Variable ¹			
	EDR (%)	First service conception rate (%)	Total fixed investment cost (\$)	Variable costs (\$/c/y)
VO	48.6	36.8	0	54.60
TAI	95.0	36.8	0	13.08
Low AED	60.0	36.8	20,000 ³	32.30
High AED	80.0	36.8	10,000 ⁴	29.80

¹Determined using previously published literature and national statistics (Dairy Records Management Systems, Raleigh, NC)

²Includes labor for each breeding system plus cost of hormones in TAI and cost to replace 5% of tags in AED.

³\$10,000 initial fixed investment cost plus \$100 tag price

⁴\$5,000 initial fixed investment cost plus \$50 tag price

Table 4.4. Cost per day open for lactations 1, 2, 3, 4, and ≥ 5 assuming an average United States Holstein herd¹ and ten year predicted estimates for milk price, feed price, slaughter price², and replacement price³. Varying estrus detection rates (40, 60, and 80 100%) were compared.

Lactation	Estrus detection rate (%)		
	40	60	80
1	1.79	2.53	3.27
2	2.66	3.27	3.88
3	4.71	5.95	7.19
4	2.43	5.19	7.95
5	1.70	2.37	3.03

¹Determined using August 2014 DairyMetrics (Dairy Records Management Systems, Raleigh, NC) and published literature

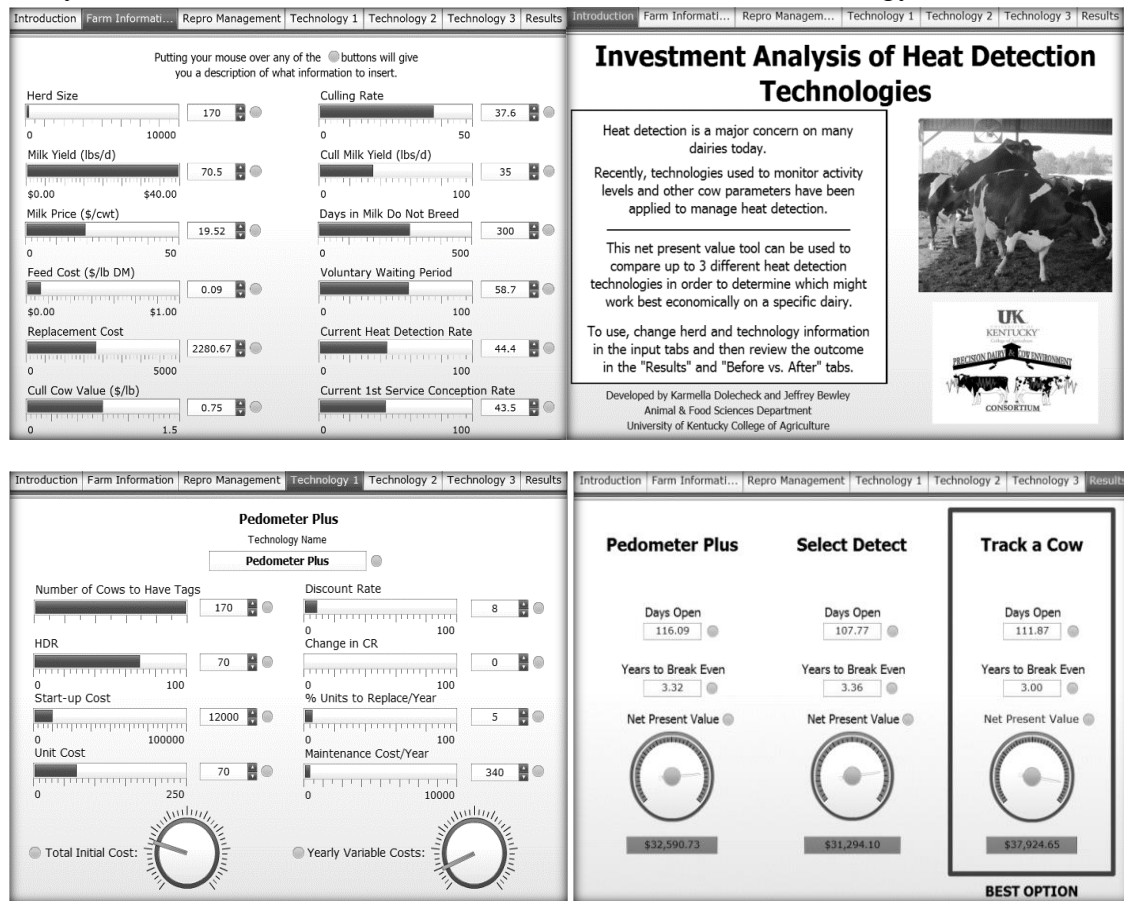
²Milk price, feed price, and slaughter price estimates were collected from the 2014 Food and Agricultural Policy Research Institute Report (Columbia, MO)

³Liang (2013)

Table 4.5. Net present value per cow per year associated with switching from either visual observation (VO) or timed artificial insemination (TAI) as a reproductive management program to either automated estrus detection (AED) or a combination of AED and VO or TAI. Variation in AED estrus detection rate (60 vs. 80%), system cost (\$10,000 or 5,000), and tag price (\$50 or 100) were evaluated.

Investment situation	Net present value (\$/c/y)							
	AED estrus detection rate (%)							
	60				80			
	AED system cost (\$)				AED system cost (\$)			
	5,000		10,000		5,000		10,000	
	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)	AED tag price (\$)
	50	100	50	100	50	100	50	100
VO to AED	32.04	28.64	30.45	27.06	48.81	45.42	47.23	43.84
VO to 75% AED and 25% VO	20.88	17.49	19.30	15.90	32.41	29.01	30.83	27.43
TAI to AED	31.11	27.72	29.53	26.13	47.89	44.50	46.31	42.91
TAI to 75% AED and 25% TAI	33.40	30.00	31.82	28.42	47.23	43.83	45.64	42.25

Figure 4.1. Interface of a decision support tool dashboard available for net present value analysis of the investment in an automated estrus detection technology.



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VITA

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Scientific Publications:

Lawson, B.D., A.H. Shahzad, **K.A. Dolecheck**, E.L. Martel, K.A. Velek, D.L. Ray, J.C. Lawrence, W.J. Silvia. 2014. A pregnancy detection assay using milk samples: Evaluation and considerations. *J. Dairy Sci.* 97 (10): 6316-6325

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Dolecheck, K.A., W.J. Silvia, G. Heersche Jr., A.E. Sterrett, B.A. Wadsworth, and J.M. Bewley. 2014. Changes in behavioral and physiological parameters around estrus in partially synchronized cows. Abstract 1491. American Dairy Science Association Annual Meeting. Kansas City, MO.

Dolecheck, K.A., W.J. Silvia, G. Heersche Jr., and J.M. Bewley. 2014. Reproductive performance of timed artificial insemination and activity-based estrus detection. Abstract 344. American Dairy Science Association Annual Meeting. Kansas City, MO.

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Dolecheck, K. A., J. M. Vera, A. J. Young, A. H. Smith, V. Fellner, and J.-S. Eun. 2012. Effects of Supplementing propionibacteria in lactation dairy diets on ruminal fermentation in continuous cultures. Abstract 199. American Dairy Science Association Annual Meeting. Phoenix, AZ.