

# Comparison of three estrus detection systems during summer heat stress in a large commercial dairy herd

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## ABSTRACT

The objective of the study was to compare three systems for detection of estrus and combinations of these systems on a large commercial dairy (1000 lactating cows) during stress of summer heat. At 37 to 45 days in milk (DIM), 266 cows were fitted with a HeatWatch (HW) device (HeatWatch; DDx Inc., Boulder, CO), an activity (A) sensor (ALPRO; DeLaval Inc., Kansas City, MO), and observed visually (V) twice daily. Pregnancy status was determined by uterine palpation 35 to 49 d following artificial insemination (AI). The effects of DIM, parity, physical activity, standing events, months, AI technician, and interval between onset of estrus and AI on conception rate were determined using linear contrasts and logistic regression. Efficiencies for detection of estrus, determined by comparing detected periods of estrus with a theoretical total of 707 periods, were 45.8% (V), 33.2% (A), 40.3% (HW), and 71.6% for all three systems simultaneously. Conception rates (LSM  $\pm$  SE) by method of detection were  $16.7 \pm 4.9$  for HW,  $19.8 \pm 5.5$  for A,  $7.9 \pm 3.4$  for V,  $16.3 \pm 6.0$  for V + A,  $27.6 \pm 4.6$  for V + HW,  $21.1 \pm 4.9$  for A + HW, and  $21.9 \pm 4.5$  for V + A + HW. Conception rate and number of mounts decreased for cows in first versus second and third parity ( $P < 0.05$ ). For periods of estrus detected by A, the lowest conception rate ( $P < 0.05$ ) occurred  $>18$  h after the onset of estrus ( $16.7 \pm 7.9$ ). The highest conception rate occurred with the combination of V + HW, which confirms the premise that combination of multiple systems enhances both the efficiency and accuracy of detection.

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## Introduction

Net income for dairy farmers needs to be raised in the modern dairy farm because of the inflated costs with which farmers must operate. They have to battle with potential loss due to reductions from fewer calving, culls and fluctuating milk prices (Esslemont and Peeler, 1993). One of the major factors influencing the profitability of a dairy herd is reproductive performance. Pelissier (1982) estimated that infertility resulted in a net loss of about \$116 per dairy cow in the US. Collectively, this loss totaled nearly \$1.3 billion in the US dairy industry. About 37% of the loss was associated with additional costs for herd replacements and losses of calves not produced because infertile cows were culled. Most of the loss estimated by Pelissier (1982) could be reduced by improvements of rate of detection of estrus, improvements in conception rates, and reductions of losses as a result of reproductive disorders.

Following mastitis, failure in detection of estrus is the second largest cause of economic losses to dairy farmers (Maatje et al., 1997). Losses due to erroneous detection of estrus have been calculated as \$300 million annually for the dairy industry (Senger, 1994). Oltenacu et al. (1981) simulated effects on profit from improvements in rates of detection of estrus and conception. They reported that when conception rates were constant (55%), improvement in heat detection rate from 35 to 55% resulted in an additional return of \$60 per cow per year. However, improving heat detection rate by another 20% (from 55 to 75%) resulted in only \$8 additional return if conception rate was 58%. When heat detection was held constant (55%), changing conception from 50 to 58% resulted in an additional return of \$39 and a loss of \$7, respectively. Thus, their study seems to indicate that a point of diminishing returns is reached beyond a heat

detection rate of 60% and a conception rate of 50%. Enhancement of reproductive performance on a day-to-day basis and increases in profit are then most likely to occur from improvements in herd management (Britt, 1985). To achieve such performance, older cows must reproduce regularly and fertility must be maintained.

Many measures are used to assess reproductive performance of the dairy herd; the average herd calving interval is one of the most commonly used parameter (Plaizier et al., 1997). For optimal milk production and reproductive efficiency, dairy cattle should calve at 12 to 13.5-mo interval (Nebel et al., 2000). Inefficient detection of estrus has been found to be the leading cause of extended calving intervals (Rounsaville et al., 1979). Increasing the detection of estrus reduces days open and increases profitability with a higher impact at lower estrus detection rates (Pecsok et al; 1994). After calving, cows should conceive within 130-d to attain this interval. Frequently, conception rates (number of cows diagnosed pregnant by palpation or ultrasonography per rectum divided by the total number of cows inseminated) less than 50% make it difficult to obtain this goal (Nebel et al., 2000).

Comparisons between projected calving interval (projected minimum average days open for cows plus a standard 280-d gestation, divided by 30.4 d) and net revenue showed an inverse relationship between these two variables (Plaizier et al., 1997). Negative regressions were found between net revenue and adjusted calving interval (average interval from calving to pregnancy plus a gestation length of 280-d divided by the fraction of the cows not culled for failure to conceive and the average duration of 1-mo (30.5-d). The reduced income was described as

primarily due to reduced milk revenue and increased replacement expense (French and Nebel, 2003).

Several researchers have calculated costs for additional days open that were attributable to production losses and increases in breeding, veterinary, and replacement costs, implying marginal benefits for decreased days open. These costs ranged from about \$0.40 to \$3.6 annually per cow for a change of 1-d open (Pecsok et al., 1994). Many of the causes of poor reproductive performance can be explained by an estimation of loss of profit. An emphasis on profit losses prevention should be an incentive for identification and correction of the underlying causes of poor reproductive management by estimating the economic values that give a high correlation with profitability. To gain a better understanding of methods for estrus detection and factors associated with artificial insemination (AI), and conception rates, the following objectives were investigated.

## Objectives

- 1) Compare efficiency of estrus detection by visual observation (Visual), HeatWatch (HW; DDx Inc., Boulder, CO), ALPRO (ALPRO; DeLaval Inc., Kansas City, MO) systems, and combinations of systems.
- 2) Compare estimates of onset of the estrus determined by the ALPRO and HW systems.
- 3) Compare conception rates for inseminations performed based on visual, HW, ALPRO, and various combinations of the three systems.
- 4) Determine factors that influence conception rates for estrus detected by each system and various combinations of the three systems.
- 5) Determine factors affecting standing activity during the expression of estrus.
- 6) Determine the optimum time for AI with the ALPRO activity system in conjunction with standing activity measured by HW and Visual.

## Literature Review

### Basic endocrinology of estrus

The major release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) occurs at estrus. This surge release of gonadotrophins is triggered by a positive feedback of estradiol-17 $\beta$  from the preovulatory follicle. During the immediate postestrus period, when ovarian steroid concentrations in blood are relatively low, FSH increases in the absence of any increase in LH. This rise in FSH may be due to removal of the negative feedback of inhibin when its source is destroyed by ovulation. The increase in FSH may play a role in recruitment of preantral follicles. At approximately 3 to 4 d postestrus, a large follicle appears on the ovary. Estrogen from this follicle plus progesterone from the newly formed corpus luteum feedback negatively on LH (Allrich, 1994).

During the luteal phase of the estrous cycle, gonadotrophin secretion is under the negative feedback influence of estradiol-17 $\beta$  and progesterone. Following luteal regression, a slight but significant increase in LH occurs, which may be important in inducing follicular maturation and the proestrus rise in estradiol-17 $\beta$  (Hansel and Convey, 1983).

The action of estradiol-17 $\beta$  upon the hypothalamus in the relative absence of progesterone is the factor responsible for the induction of estrus (Allrich, 1994; Lyimo et al., 2000). The induction of estrus by estradiol-17 $\beta$  has been demonstrated in ovariectomized cattle (Lefebvre and Block, 1992). Immunization of cattle against estradiol-17 $\beta$  inhibited the expression of estrus behavior

(Martin et al., 1978). Because the antibodies specifically bind estradiol-17 $\beta$  and no other hormones, this evidence supports the hypothesis that estradiol-17 $\beta$  is indeed the endogenous hormone that induces estrus (Allrich, 1994).

Estradiol-17 $\beta$  induces the preovulatory LH surge as an “all or nothing” event. More specifically, after a certain threshold of estradiol-17 $\beta$  is reached, there will be a LH surge, which will result in ovulation or not. No reports suggest a “low” or “minor” LH surge, which results in a “minor” ovulation. However, with respect to estrus behavior, which is also supposed to be induced by estradiol-17 $\beta$ , a large variety exists (Lymo et al., 2000). The “all or nothing” event may reference also that once the concentration of plasma estradiol-17 $\beta$  is sufficient to induce estrus, additional amounts have no further stimulatory influence on expression of estrus behavior. However, additional amounts may be required for reproductive tract function, such as secretion of specific proteins required to nourish the early embryo (Allrich, 1994).

The “all or nothing” event has also been described for progesterone. Serum concentrations of progesterone are very low during proestrus and estrus, which is necessary prerequisite to avoid the inhibitory properties of progesterone over the expression of estrus behavior (Allrich, 1994). The cause of inhibitory effect of progesterone on estrus behavior may be related to a decrease in estradiol-17 $\beta$  receptors in the brain, thereby blocking the effect of estradiol-17 $\beta$  (Davidge et al., 1987). Once progesterone concentrations increase to a threshold level, estrus is inhibited even when estrus-inducing concentrations of estradiol-17 $\beta$  exist. Administration of progesterone near the onset of estrus has also been shown to prevent the preovulatory surge of LH (Lee et al., 1988). The prior exposure or absence of progesterone prior to estradiol-17 $\beta$  which induces estrus

behavior is controversial (Alrich, 1994). The studies that support previous progesterone actions, indicate that progesterone may act at the hypothalamic level to remove a refractory condition caused by high estradiol-17 $\beta$  levels during late pregnancy; alternatively progesterone may act at the ovarian level to modify the response of the preovulatory follicle to gonadotrophin secretion to stimulate the synthesis and secretion of estradiol-17 $\beta$  (Davidge et al., 1987).

Other hormones like testosterone can induce estrus behavior in cattle; however, large amounts are required (100 to 400 mg), and the response is never as pronounced as when using estradiol-17 $\beta$  (Allrich, 1994). Allrich et al. (1989), testing the effects of corticoids in estrus behavior, found that dexamethasone (a synthetic glucocorticoid) reduced the percentage of heifers in estrus. In another study, Ashimine et al. (1991) showed the infusion of cortisol for 90 h to proestrus heifers completely blocked the LH surge and estrus behavior without altering plasma estradiol concentrations. Lyimo et al. (2000) reported that cortisol levels were elevated during the periods of estrus because estrus itself apparently causes some stress. Stoebel and Moeberg (1982) showed that repeated stress, which elevated total corticosteroid concentrations during the follicular phase, prevented the surge release of LH in some heifers but did not interfere with the expression of estrus.

Nonpubertal estrus is a condition, defined by Allrich (1994), characterized as behavioral estrus not followed by ovulation and subsequent formation of the corpus luteum. Fortunately, nonpubertal estrus usually involves the first behavioral estrus noted in heifers near puberty and not heifers of breeding age. Ovulation without estrus is described as a “silent” ovulation. High concentrations of estradiol-17 $\beta$  at the end of pregnancy are postulated to induce a state of

refractoriness (at the hypothalamus level) to the estrus-inducing concentrations of estradiol-17 $\beta$  present at the first postpartum ovulation (Kyle et al., 1992). Another explanation to this phenomenon includes a decrease in estradiol production at the first postpartum ovulation compared with subsequent ovulations (Allrich, 1994).

### **Signs of estrus**

Williamson et al. (1972) performed a study in a large commercial dairy herd to describe the signs of estrus. They found presence of signs of estrus from highest to lowest were:

- 1) Standing immobile to be mounted. This was the most important single sign and was observed in 79% of cows in estrus. Standing behavior was observed in 4.5% of cows not in estrus and 7.3% of pregnant cows.
- 2) Mounting other cows repeatedly; exhibiting homosexual activity.
- 3) Ruffled, scraped, or rubbed rump and tailhead. These signs were misleading as they persisted for 4 to 6-d.
- 4) Group activity of sexually active cows was important especially at night, including cows in estrus or proestrus and nymphomaniacs. These cows often stand close together within a few feet of each other.
- 5) Restlessness. If on pasture the interruption of grazing and roaming with increase in activity.
- 6) Vulva swollen, edematous, and relaxed: This was seen in only a few cows and was unreliable.

- 7) Stringy mucus suspended from vulva especially after mounting or lying down. This was useful but infrequent.
- 8) Sniffing and licking of vulva, flehmen reaction, also chin resting and rubbing cow on rump.
- 9) Tail raising and switching, frequent urination, and bellowing were also seen in cows in diestrus.

The standing behavior event has been found in other studies to be the most important sign of estrus behavior. Hurnik et al. (1975) used for their definition of estrus: “the interval during which the cow makes no effort to escape when mounted by others (standing estrus)”. Thatcher and Wilcox (1973) using 577 cows under visual observation found that the percentages of cows exhibiting standing estrus prior to 60-d postpartum were: 25.9%, 38.7%, 28.0%, 7.2%, and 0.3% for 0, 1, 2, 3, and 4 estrus periods, respectively. LeBlanc et al. (1998) using 348 synchronized cows found that the standing behavior was present in 53% of the cows.

Reimers et al. (1985) showed the relationship between signs of estrus at AI and error rates of estrus detection based on milk progesterone assays. Their data indicated that “standing” and “riding other cows” were the most accurate signs of estrus. When “standing” was used in combination with other secondary signs of estrus such as: rough tail head, riding other cows, unusually active, mucus on vulva, bawling, triggered heat-mount detector, and no milk let-down, the accuracy of these secondary signs was improved greatly. Comparing the relationship between various signs of estrus and conception rates at the first service, they reported that cows to be “standing” (n=2696) had the highest conception rate of 51.3%. This was different ( $P < 0.05$ ) from the conception rate for cows not reported to be “standing” but exhibiting other signs of

estrus (47.7%; n=1174). Gwasdauskas et al. (1986) reported that inseminations without observed standing estrus resulted in approximately 20% lower conception rate than when standing behavior was detected.

In other studies, standing behavior to define the period of estrus has been less important. Van Vliet and Van Eerdenburg (1996) reported that standing behavior was observed in only 37% of the periods of estrus. They postulated that other estrus signs, such as mounting other cows, restlessness, mucous discharge, being mounted but not standing, cajoling, sniffing vagina of other cows, and resting with chin on other cows are probably less reliable, but may be more than an indication when observed often in one observation period or in successive periods. Lyimo et al. (2000) estimated a low correlation between standing behavior and plasma estradiol-17 $\beta$  concentrations associated with behavioral estrus. Vliet and Van Eerdenburg (1996) postulated that a possible cause for a low number of standing events reported in some studies may be related to the small size of the herds utilized, i.e., 45 to 50 cows.

Hurnik et al. (1975) found that the duration of estrus based on video recording varied with the number of cows in estrus simultaneously, increasing from 7.5 to 10.1 h for 1 or 3 cows in estrus at one time, respectively. Helmer and Britt (1985) using synchronized heifers studied the mounting activity during the different stages of the estrous cycle. They found that 66% of all attempted mounts were by estrual animals, and over 85% were by estrual and prestrual stages of the estrous cycle. With a higher number of simultaneously sexually active animals, the mounting and standing activities were higher.

The number of standing events has been recorded in several studies. Britt et al. (1986) using visual observation estimated that the number of mounts averaged ( $\pm$  SE)  $3.7 \pm 0.3$  when cows were located on dirt and  $2.5 \pm 0.2$  when cows were on concrete; standing events were  $3.8 \pm 0.3$  (dirt) and  $2.7 \pm 0.2$  (concrete). Walker et al. (1996) using HW system found that the mean number ( $\pm$  SD) of standing events per estrus was  $10.1 \pm 9.9$  with  $6 \pm 7.2$  mounts  $\geq 2$  s of duration and a mounting duration of  $24.1 \pm 29.4$  s. Dransfield et al. (1998) using the same estrus detection device registered an overall mean ( $\pm$  SD) number of standing events per estrus of  $8.5 \pm 6.6$  (n=2,661).

Physical activity measured by steps has been also recorded as a sign of estrus in some studies. Pennington et al. (1986) observed sexual activities in 26 Holstein cows in estrus continuously for 5 d. In their study, mounting with pelvic thrusts, mounting with standing only, attempted mounting, orientating to mount, chin resting, sniffing genitalia, licking back, rubbing, butting, cajoling, cycling, and following were all correlated with pedometer readings and periodic 30-min visual observations; correlations ranged from 0.40 to 0.75. Van Vliet and Van Eerdenburg (1996) using 100 dairy cows, reported correlations between signs of estrus “mounting other cows” and “resting with chin on other cow” and pedometer readings of 0.5 and 0.39, respectively. In their study, with 12 observation periods per day, they registered each of the sexual activities according to the Van Vliet and Van Eerdenburg (1996) score and considered a cow to be in estrus when the animal obtained maximum points available (Table 1).

Other signs of estrus may be detected by the development of new sensor systems. Cycling cows release an attractant pheromone 2 to 3 d before standing estrus. Analysis of the vapor phase of

body fluids showed that acetaldehyde, one of the volatile compounds, seems to be related to standing estrus and elevated blood levels of estradiol and progesterone. Analysis of 164 blood samples from 18 estrous cycles indicated that acetaldehyde concentration rose suddenly and then fell just prior to or at standing estrus (Klemm et al., 1994).

Redden et al. (1993) using 13 lactating cows fitted with radio transmitter thermometers inserted in the vagina found that temperature increased by  $0.6 \pm 0.3$  °C at estrus and remained elevated by at least  $0.3$ °C for  $6.8 \pm 4.6$  h. These techniques of estrus detection have had variable results, and further research is required to determine accuracy and efficiency of such devices.

Table 1. Scoring scale for observed symptoms of estrus behavior<sup>1</sup>.

Estrus symptoms	Scoring scale
<i>Other symptoms</i>	
Mucous vaginal discharge	3
Cajoling	3
Restlessness	5
Sniffing of the vagina of other cow	10
Resting with chin on other cow	15
<i>Mounting symptoms</i>	
Mounted by other cow but notstanding	10
Mounting (or attempting) other cows	35
Mounting headside of other cow	45
Standing heat	100

<sup>1</sup>Reprinted from Theriogenology, 53, Lyimo, Z. C., M. Nielen, W. Ouweltjes, T. A. M. Kruij, and F. J. C. M. Van eerdenburg, Relationship among estradiol, Cortisol and Intensity of estrous behavior in dairy cattle, 1783-1795, Copyright (2000), with permission from Elsevier.

## **Factors that affect detection of estrus**

There are diverse factors that influence the detection of estrus. Gwazdauskas et al. (1983) performed a study in which 1000 estrus cycles were characterized to find possible influencing factors. Mounting activity was defined as the number of times the cow stood to be mounted during 30 min of group detection and was doubled to standing events per hour. In this study, the researchers found that mounting activity at first detection of estrus was affected by sire within genetic group, suggesting possible genetic influences on estrus activity. This hypothesis was first established by Rottersen and Touchberry in 1957 by indicating significant differences on degree of expression of estrus between progeny groups. Hackett et al. (1984) also found differences among genetic lines for standing and mounting activity in cows with conception at fourth or subsequent service and for overall conception. Differences in time for onset of estrus between genetic lines were also found by Hackett and McAllister, 1984.

Another variable discussed in the study by Gwazdauskas et al. (1983) was the influence of circadian rhythm. Sexual activity at the initial observation of the estrus was significantly greater during the evening ( $7.7 \pm 0.3$  standing events per hour, average  $\pm$  SE;  $n=391$ ) than during the morning ( $6.0 \pm 0.2$  standing events per hour;  $n=609$ ). A total of 61% of the cows were first observed in estrus during the 0801 to 1000 h observation period, supporting the study of Hurnick et al. (1975), who reported a greater frequency of estrus onset at 0800 h and a nadir of activity at 1900 h. DeSilva et al. (1981) found that during the morning visual observation, 51% ( $n=101$ ) of the animals first were detected in estrus, whereas 49% ( $n=98$ ) were observed in estrus at the evening visual observation. Animals in estrus in the morning (a.m.) displayed more activity (11.4

$\pm 5.6$  standing events per hour,  $P < 0.01$ ) than those first seen in the evening (p.m.) ( $7.6 \pm 5.6$  standing events per hour; average  $\pm$  SD).

Hacket and McAllister (1984) using chin-ball markers for detection of estrus in 56 cows found about 65% of the cows were marked during night hours (1800 to 0600 h). Foote (1979) using data from 44,707 cows and heifers with visual observation for detection of estrus reported that the majority of cows were first observed in estrus in the morning, and presumably most came into estrus during the night. Hurnik et al. (1975) using continuously time-lapse video recording revealed the true onset of estrus was near mid-night, and most cows displayed first signs of estrus between 2100 and 0400 h. They monitored behavior continuously for 80 d and found that 70% of the standing activity occurred between 1900 and 0700 h. This observation suggested that cows were more likely to exhibit standing activity when not distracted by other activities, such as feeding, milking, and barn cleaning. Dransfield et al. (1998) using HW system with 24 h surveillance found no differences in the distribution of onset and end of estrus among four periods of 6 h each. Van Vliet and Van Eerdenburg (1996) using pedometers activity found no significant differences in time of onset of estrus between four periods of 6 h.

The effect of primary housing area on the intensity of estrus at the first observation has been also reported (Gwazdauskas et al., 1983; DaSilva et al., 1981). In these studies, cows confined in barn except for visual observation and milking exhibited more standing events per hour than cattle housed in free stalls or on pasture. These differences may be due to the amount of confinement and association with cattle that are not in estrus but willing to mount females in estrus (Gwazdauskas et al., 1983). Britt et al. (1986) reported that footing surfaces where cows were

observed was an important factor influencing duration of estrus and both standing and mounting activity. When cows were observed on dirt lots, they were in estrus longer and showed more total mounts and stands than when observed in concrete alley. Walker et al. (1996) and Pennington et al. (1986) found similar results with higher percentages of standing activity and total activity in the locations with best footing and least crowding.

Another factor that influences estrus behavior is parity. Gwasdauskas et al. (1983) found that estrus activity at first observation of estrus was lowest in heifers and increased for cows in their fourth lactation or older. Walker et al. (1996) described that duration of estrus was nearly 50% shorter for primiparous cows ( $7.4 \pm 1.4$  h) than for multiparous cows ( $13.6 \pm 2.0$  h). In this study, cow within parity was a major source of variation for total standing events, standing events  $\geq 2$  s of duration, and standing duration. These data agree with other studies (DeSilva et al., 1981; Hackett et al., 1984; Britt et al., 1986) and suggest that increased activity occurs with advancing lactation number and may be subject to conditioning or sexual experience.

Maximum environmental temperature on day of estrus had a significant curvilinear relationship with estrus activity at the first observation of estrus (Gwasdauskas et al., 1983). As maximum daily temperature increased to approximately 25°C, standing activity detected by visual observation also increased. Above 30°C, a decline in standing events per hour occurred. These data suggest that cold temperatures inhibit expression of estrus activity, and as ambient temperature increases, activity can be maximized within the temperature comfort zone.

Seasonal factors have been also related with environmental temperatures. DeSilva et al. (1981) using visual observation described that estrus behavior during the winter months (November through May with average maximum and minimum temperatures of 12.8 °C and 0 °C, respectively) was higher ( $P<0.05$ ) ( $11.2 \pm 8.9$  standing events per hour) than during summer months (June through October with average maximum and minimum temperatures of 25.7 °C and 13.6 °C, respectively) when the number of standing events per hour was low  $4.7 \pm 4.4$ . At-Taras and Spahr (2001) reported that the duration of standing activity was significantly affected by weather; hot weather (June through August) resulted in a shorter duration (2.97 versus 6.76 h) of standing activity than cool weather (September through May). However, other studies have not found significant effects of environmental temperatures on duration of estrus (Britt et al., 1986; Walker et al., 1996), concluding that willingness to mount or to exhibit estrus might contribute to secondary behavioral characteristics, and environmental temperature might not have been high enough to affect duration of estrus.

The relation between expression of estrus and production traits has been estimated in several studies. Pennington et al. (1986) found a positive independent regression coefficient between days postpartum and body condition scores and sexual variables defined as estrus activities received and initiated, mounts received, time in standing estrus and increase in physical activity recorded by a pedometer. Conversely, sexual variables decreased as milk production per unit metabolic body weight and weight changes from the time of calving increased. In the same study, a stepwise regression indicated that body weight change since calving most affected all sexual variables.

Recently, Van Eerdenburg et al. (2002) reported that the level of a 305-d milk yield was not correlated with a score that estimated the estrous behavior. Previously, similar results were found by Britt et al. (1986) with a daily milk yield not affecting the standing or mounting activity. Butler et al. (1981) found a significant negative correlation between average energy balance and number of days to ovulation.

### **Efficiency and accuracy of estrus detection**

Estimation of the efficiency and accuracy of estrus detection are useful tools to evaluate systems for the detection of this physiological activity. Efficiency of detection of estrus is usually expressed as the percentage of possible estruses that were detected over a given period. The principal factor related with failure in AI success is inefficient detection of estrus. The mean efficiency of detection of estrus for Virginia and North Carolina with 799 herds processed at the Raleigh DRPC for the year 2002 was  $41.7\% \pm 17.4$  (Dairy Metrics, DRMS, Raleigh, NC).

Accuracy of detection of estrus is defined as the percentage of estrus periods observed that are true estrus periods. The ability of dairy farm managers to interpret the signs of estrus correctly and, therefore, to detect cows in estrus accurately is highly variable and significantly affects conception rate (Elmore, 1989; Nebel, 1988; Nebel et al., 1987; Reimers et al., 1985). Reimers et al. (1985) reported that 5.1% of the cows presented for insemination were not in estrus with an error rate varied from 0 to 60% among herds, and 10% or more of the cows inseminated were not in estrus in 30% of the herds. Nebel et al. (1987) reported that 25% of the cows determined to be

in estrus by visual observation had high progesterone levels measured by an on-farm ELISA test. Elmore (1989) found high progesterone levels in 8% of the cows presented for insemination in three herds with a range of 1 to 12%. Senger et al. (1988) using milk progesterone assays indicated that between 7.6 and 25.3% of all inseminations occurred in cows that were not in estrus.

Low conception rates are not always related to inaccurate detection because many factors influence conception rate. However, accuracy of detection of estrus should be estimated as part of the evaluation of herds with a low conception rate. Insemination of cows that have been observed in standing estrus and have low progesterone levels should yield the highest accuracy possible (Heersche and Nebel, 1994). However, Ruiz et al. (1989) questioned the economic value of on-farm tests for progesterone. They found a decreased ( $P < 0.05$ ) in net return per cow per year so the costs associated with the test were not balanced by the benefits. They concluded that the economic profitability of the test depends on incidence on rate of errors in detection of estrus and semen costs.

Walker et al. (1995) using 120 Holstein and 40 Jerseys cows compared accuracy and efficiency of the electronic pressure sensing system HW and visual observation. They found that accuracy of detection was similar for both methods (HW 96%; visual 94%) and HW increased efficiency of detection by 40% (91 versus 51%). Nebel et al. (1995) estimated that visual observation identified 61.3% of estrus detected by HW, whereas only 2.7% of the estruses detected visually were not identified by HW. Rorie et al. (2002) suggested that cows detected by visual

observation but not by HW could tear loose their transmitters by mounting activity early in estrus and/or failing to record enough mounts in the time period specified by the system parameters.

Stevenson et al. (1996) using 50 beef heifers fitted with the HW system found that the efficiency of twice daily visual observation (detection of all periods of estrus; 73%) was less ( $P < 0.01$ ) than that achieved by pressure-sensitive radiotelemetric devices (100%). The accuracy of detected estrus was 100% for both visual observation and HW system. Borger et al. (1996) also compared HW and the visual observation of estrus detection in 74 mature beef cows. They reported that HW improved the efficiency of estrus detection over visual observation (91.1% vs. 65.8%). The accuracy of estrus detection for the HW and visual observation was 87.5 and 91.5%, respectively, and conception rates were 56.8 and 51.4%, respectively.

The literature describing the effectiveness of pedometry-aided detection of estrus (when compared with visual observation) is quite variable and ranges from 60 to 100%, depending on the study (Lehrer et al., 1992). Pennington (1986) reported an efficiency for visual observation of 45% and for pedometers of 78 to 96% (with 100 and 200% increase in activity, respectively). In the same study, accuracy of estrus detection based on milk progesterone samples was of 88 to 100% for pedometers. Williams et al. (1981) reported for twice daily visual observation and pedometer readings efficiencies of 68 and 74%, respectively. Accuracy reached a peak of 95 and 83% for visual and pedometer, respectively. These two methods combined increased the efficiency to 93% but the accuracy was 80%.

## **Requirements for an estrus detection system**

Senger (1994) postulated that in order to eliminate the need of visual observation and marginally effective facilitators of estrus detection, new technology should provide highly effective automated methods for identifying cows in estrus. Following this observation, this author recommended five requirements on which to base the “ideal” system for the detection of estrus:

- 1) Continuous surveillance of the cow: the system should monitor continually for a repeatable, measurable change in behavior or physiology with the requirements of adequate power to monitor continually for the appropriate event and record its occurrence, appropriate sensitivities to recognize that the event has taken place, and stable attachment of the device to the cow for a sustained period.
- 2) Accurate and automatic identification of the cow in estrus: permanent identification and coding of the cow with the information of the ovulation-related event.
- 3) Operation for the productive lifetime of the cow: an approach would enable the detection of estrus over the lifetime of the animal. Surgical implantable technology can be readily applied to dairy cattle providing a regulatory and food safety criteria.
- 4) Minimal labor: significantly reducing or even eliminating labor must accompany any technology if it is to be cost effective. Elimination of labor would enable funds that are now spent on detection of estrus to be transferred to investments in new technology hardware and software.
- 5) Accuracy of identification of the appropriate physiologic or behavioral events: identification with a high degree of certainty (>95%), one or more behavioral or physiology events of the

animal (standing to be mounted, changes in ionic characteristics of the reproductive tract, or changes in hormone level) that are highly correlated with the time of ovulation so that AI can be timed appropriately.

### **Development of the HW system**

The HW system has been developed to alleviate the need for estrus detection by visual observation and to provide an accurate determination of the initiation of estrus. An initial study was performed to evaluate this system (Nebel et al. 1992) using 20 Jersey and 25 Holstein lactating cows. The pressure sensor was mounted over the backbone allowing the mounting cow to contact the sensor with the radiotransmitter lying in the concave area between hip and pin bones. A fixed remote receiver connected to a computer, collected and temporarily stored each signal consisting of transmitter number, date and time of pressure, and duration of each pressure event.

The estrus periods recorded were composed of an average of 14.1 standing events (4.9 of which were  $\geq 2$  s) over 12.1 h for the duration of the estrus period. These results demonstrated no differences between Jersey and Holstein cattle. Of initial standing events, 48% occurred between 0000 and 0800, whereas 27% occurred between 0900 and 1600, and 25% between 1700 and 0000. The results were similar to those previously reported by Hurnick et al. (1975).

Nebel et al. (1995) reported on the integration of the HW system at the Virginia Tech University dairy. They used 120 Holstein and 40 Jersey cows and compared the HW system with visual

observation. They developed a system for transmitter attachment, daily monitoring and maintenance of transmitters and patches, and systematic evaluation of mounting activity. The components of the HW system included: 1) battery-powered reusable pressure sensing radio frequency transmitters, secured within disposable burlap patches; 2) signal receiver (0.4 km range); buffer which receives and stores mounting activity data until downloaded to HW software; and 3) HW software that sorts information by cow and generates management lists (standing estrus, suspect estrus, etc.). The patches containing transmitters were glued to the base of the tail and secured by 24-gauge wire to a 5-mm long electrical tie-strap that was subcutaneously implanted.

They calculated that the labor requirement for this system, which included daily maintenance and installation of the transmitters and patches and review of computer generated management lists to determine cows to inseminate, was approximately 25 min per day. They concluded that HW was a viable alternative to visual detection and required half of the labor the previous visual detection program employed required.

An additional study was performed that same year (Walker et al., 1996) for the characterization of the estrus activity using HW. The system was installed in 51 Holstein cows with 86 induced ( $\text{PGF}_{2\alpha}$ ) and 33 spontaneous estrous cycles. They reported that the mean ovulation time relative to first standing event was  $27.6 \pm 5.4$  h and was not different between spontaneous and induced cycles. The overall means  $\pm$  SEM for estrus parameters were  $7.7 \pm 0.9$  total standing events,  $4.6 \pm 0.6$  standing events  $\geq 2$  s,  $7.1 \pm 0.7$  h for estrus duration, and  $18.3 \pm 2.5$  s for time spent

mounting. They concluded that the HW system was a viable alternative to visual observation for detection of estrus.

Stevenson et al. (1996) conducted a study to compare the effectiveness between twice daily visual observation and radiotelemetric, pressure-sensitive, rump-mounted devices (an earlier generation of the HW) in 50 peripubertal, crossbred yearling beef heifers. They feed melengestrol acetate for 14 d then injected PGF<sub>2α</sub>. Heifers identified in estrus average ( $\pm$  SEM)  $50.1 \pm 6.4$  standing events over a period of  $14 \pm 0.8$  h. They concluded that heifers with fewer standing events (19.3 vs. 60.5;  $P < 0.001$ ) of shorter duration (8.4 vs. 15.6 h;  $P < 0.001$ ) were not identified by visual observation, and concluded that the HW was a useful tool in identifying a greater proportion of heifers in estrus with accuracy similar to visual observation.

In 2001, At-Taras and Spahr characterized estrus in 71 lactating dairy cows comparing HW system, pedometer (recording increase in physical activity ratio algorithm and count algorithm), and visual observation. They performed two trials; a first trial without synchronization and a second trial with cows synchronized using PGF<sub>2α</sub>. Increase in physical activity was defined by means of an individually calculated threshold value for increased activity count. Differences for estrus duration using different systems were significant ( $P < 0.0001$ ) between pedometers (increase in activity count), and HW ( $5.0 \pm 0.9$ ). Efficiencies for estrus detection were 86.8 and 71.7% (trial 1 and 2) for HW, 82.6 and 90.6% for pedometer (increase in activity count) and 54.4 and 54.7% for visual observation. The conclusions of the study were that HW and pedometers resulted in improved detection of estrus over visual observation.

Cavalieri et al. (2003) synchronized 20 lactating cows with intravaginal progesterone releasing devices (CIDR-B) and estradiol benzoate. Between 24 to 60 h after the removal of the CIDR-B, estrus characteristics were compared using continuous visual observation and HW. Significant differences ( $P < 0.05$ ) were obtained between the mean number of standing events (42.7 vs. 17.8), duration of estrus (14.4 vs. 10.9 h), total duration of standing event (223.9 vs. 33.1 s), mean duration per standing event (5.6 s vs. 1.9 s), the number of standing events per hour (3.5 vs. 2.0), and the interval to first recorded standing event associated with estrus (41.2 vs. 42.8 h) between values determined by visual observation compared with HW. Results demonstrated a low agreement between these systems for estrus detection, which could be related to the fact that the estrus periods were synchronized, underestimating the magnitude of estrus detected by HW compared with visual observation. Mounting activity occurred simultaneously, whereas HW system can only record a single event every 45 s.

### **Development of the pedometry**

Increase in physical activity during estrus has been a phenomenon described in mammals for approximately 80 yr. Wang (1923) described that the activity cycle of the female white rat was highly correlated to the estrous cycle with the peak of activity coinciding with the height of estrus using vaginal smears and mating tests. Another study showed an increase in running activity (recorded with an automatic recording system) during female receptivity to coitus in the Wistar rat (Farris, 1941). In a subsequent study, Farris (1944) demonstrated that women also exhibit variation in the walking activity due to the estrus cycle. He described three activity peaks

during the menstrual cycle: a) during the menses, b) during the period of ovulation, and c) a late peak on about d 24 of the cycle.

The first study that described increased physical activity of dairy cows during estrus was performed in 1954 by Farris. He installed mechanical pedometers on 13 Guernsey cows and obtained records of activity of 5 d before, during, and 5 d after estrus identified by visual observation. He reported a gradual increase in activity in all cows for 48 h with clinical signs of estrus observed on the second day of increased activity. He also reported a reduction in activity evident the day prior to, and following, the increase in estrus activity. This was the first presentation of a method to record activity in cattle for detection of estrus.

Twentythree yr later, Kiddy (1977) described the first potentially useful field application of a pedometer system. He used 40 and 28 cows in free and comfort stalls, respectively. Mechanical pedometers were enclosed in plastic cages and attached to the lower rear legs with a plastic ankle strap. Readings of the pedometers were taken twice daily when the cows were milked. Visual observation occurred at least twice daily. Activity at estrus was greater than the nonestrus period by two standard deviations above the mean activity in 98 and 93% of the estrus periods of cows located in free and comfort stalls, respectively. The average increase in activity at the time of estrus was of 393% for cows in free stalls and 276% for cows in comfort stalls. Comparing the efficiency of the two systems of estrus detection, 21% more estrus periods were detected for cows located in free stalls but only 6% more estrus periods were detected for cows located in comfort stalls. The main conclusions of this study were that development of a better carrier for the pedometers was necessary, false positive diagnosis was not a problem but questions did arise

occasionally, and physical activity must be monitored continuously for pedometry to be more effective (Kiddy, 1977).

In 1996, Van Vliet and Van Eerdenburg conducted a study in approximately 100 lactating cows to compare sexual activities measured by an estrus score and electronic pedometer readings (NEDAP, The Netherlands). Pedometer readings were correlated with sexual activities such as mounting other cows (0.5), resting the chin on other cow (0.39), and total estrus score (0.5). They concluded that pedometer registers only part of the sexual activities, so a combination of visual observation with pedometry was necessary for optimal detection of estrus.

### **Timing of artificial inseminations**

Many researchers agree that the major challenge to improving reproductive and economic efficiencies of many dairy farms, involves an efficient and accurate detection of estrus and resulting timing of AI (Foote, 1974; Gwazdauskas et al., 1986; Heersche and Nebel, 1994; Senger, 1994). Many methods have been developed to determine time for ovulation. Some researchers have used frequent visual observation (DeSilva et al., 1981; Larsson, 1987), frequent exposure to teaser animals or other cows (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Mattoni et al., 1988), or a combination of frequent visual observation with estrus detection aids (Rajamahendran et al., 1989). Palpation of the ovaries per rectum at frequent intervals (Brewster and Cole, 1941; Nalbandov and Casida, 1942; Trimberger, 1948; Mattoni et al., 1988) or frequent ultrasonography (Larsson, 1987; Rajamahendran et al., 1989) have been techniques applied in most of the studies. Ovulation has been timed from the cessation of estrus (Brewster

and Cole; Nalbandov and Casida, 1942; Trimberger, 1948) and from onset of estrus (Larsson, 1987; Mattoni et al., 1988; Rajamahendran et al., 1989; Dransfield et al., 1998).

In 1948, Trimberger developed the a.m. – p.m. guideline for AI using visual observation. This system recommended that cows observed in estrus in the a.m. should be submitted for AI the following p.m., and cows observed in estrus in the p.m. will be inseminated the next a.m. He utilized 46 heifers and 86 cows that were observed for estrus three times daily; at 0700, 1200, and 1800. The classification for time of the first observed estrus was described as a.m., forenoon, or p.m. The time of the ovulation was determined by rectal palpation of the ovaries at several times during estrus and at 2-h intervals after the onset of estrus. Relative to the time estrus was first observed the following divisions were used: a.m. (estrus first observed before 0900), forenoon (estrus first observed between 0900 and 1200), and p.m. (estrus first observed 1230 or thereafter during the afternoon). To determine the end of estrus, females were tested every 2 h, and thus it was possible to determine the end of estrus within a 1-h interval. Cows were bred at various time intervals before and after ovulation. The results showed that the average duration of the estrus (defined as time between first and last standing event) was 15.3 h for heifers and 17.8 h for lactating cows. The 132 females ovulated on average, 10.5 h after end of estrus. The higher conception rates were found in females bred more than 6 h but less than 24 h before ovulation. An important finding in this study was that highest rates of conception were obtained from services in the middle and toward the end of the estrus period. Finally, this study concluded that proper timing of AI was very important for obtaining high conception rates.

A description of the time of ovulation using the HW system and ultrasound ovarian scanning in dairy cattle was reported by Walker et al. (1996). In this study, 51 lactating Holstein cows were used to describe estrus occurring spontaneously (n = 33) and induced by PGF<sub>2α</sub> (n = 86). Onset of estrus was determined by HW, and ovulation was determined by ultrasound examinations at 12, 20, and 24 h after the initial standing event and then every 2 h until 40 h. Blood samples were taken for serum progesterone detection and estrus confirmation. A progesterone concentration of ≤ 1 ng/ml was used to declare a cow in estrus. The results showed that the mean time of ovulation was 27.6 ± 5.4 h relative to first standing event with a significant positive relationship between duration of estrus and time of ovulation. Seventyeight percent of the cows ovulated within 40 h of onset of estrus. Of the 22% not ovulating by 40 h, a large proportion (73%) was from those cycles induced by PGF<sub>2α</sub>. They found that the time from administration of PGF<sub>2α</sub> to estrus and to ovulation was 73.1 and 103.6 h, respectively. Warmer (>13°C) and cooler (<13°C) temperatures increased and decreased, respectively time from administration of PGF<sub>2α</sub> to first recorded standing event. The importance of this study was the time of ovulation in reference to the first standing event of estrus accurately detected by HW, allows for an accurate timing of AI relative to visual observation of estrus.

The effect of timing of AI using the HW system was determined by Dransfield et al. (1998), using 17 commercial dairy herds and 2661 inseminations. The percentage of cows pregnant by hour relative to timing of AI from onset of standing estrus was determined. Conceptions rates were higher for cows receiving AI after ≥ 2 versus only one standing event (46 vs. 36%). For cows that had estrus periods of low intensity (< 1.5 standing events per hour) and long duration (≥ 7 h from first to last standing event) conception rates were 45.5 and 49.8%, respectively for

cows with estrus period classified as high intensity ( $\geq 1.5$  standing events per hour) and long duration ( $\geq 7$  h). Using logistic regression analysis, the interval from the first standing event to AI affected the probability of pregnancy; the highest conception rates was found for cows inseminated between 4 to 12 h after the onset of estrus compared with a baseline interval of 0 to 4 h after the onset. The probability of pregnancy was also higher for cows  $> 100$  DIM and inseminated during March, April, or May. Results reported here suggested that timing of AI should be performed earlier following observation of estrus than previously reported by Trimberger (1948), whose a.m. - p.m. system would lower the probability of resulting pregnancy. With the a.m. - p.m. guideline, most cows that were observed most likely had been in estrus for several hours previous to observation. Final conclusion suggests that if onset of estrus is unknown, AI should be performed within 4 to 12 h of observation of estrus.

Description of the conception rates in relation to the time from onset of estrus until AI, according to pedometers readings, was the objective of Maatje et al. (1997). In a previous study, pedometer activity readings coincided closely with actual cow activity regarding the onset and cessation of standing estrus (Varner et al., 1994). In the study of Maatje (1997), pedometers (Boumatic Heat-seeker-TX<sup>®</sup>; Dairy Equipment Co., Madison, WI) were attached to the inside of the right hind leg just above the distal expansion of the tarsal bone of each cow. Activity data was stored for each 2-h period for a maximum of 10 d. If the mean activity of the last six periods was more than double the mean activity of the last six corresponding periods for the previous 2 d, an alarm signal (flashing light) was produced. The period in which the signal started was recorded, so retrospectively, the exact time of the onset of estrus and the number of hours from the beginning of increased activity to the time of AI could be determined from the computer program. Cows

were randomly assigned to one of the three groups: early (< 10 h), average (10 to 20 h), or late (>20 h) after the onset of increased activity.

Pedometer activity indicated the onset of estrus with a >100% increase in physical activity. The significant reduction in conception rates (approximately 15%) were useful for determining the disadvantages of later (>24 h) AI in comparison with previous detection of estrus. The statistical analysis showed that AI at 11.8 h after estrus had the higher probabilities of conception and therefore was the optimal time to breed cows. This value coincided with the midpoint of the interval of 6 to 18 h based on the first mount detected by HW (Dransfield et al., 1998). With these results, pedometers were described as an important tool to determine the optimal time for AI during the estrus period.

### **Estrus characteristics under heat stress conditions**

Yousef (1984) defined heat stress as the sum of forces external to a homeothermic animal that acts to displace body temperature from the resting state. Such stress can disrupt the physiology and productive performance of an animal. Physiological adaptations that homeotherms undergo during heat stress can compromise other physiologically important systems. An example is the redistribution of blood flow from the viscera to the periphery during heat stress.

Heat stress reduces the length (Abilay et al., 1975) and intensity (Gwasdauskas, 1983) of estrus. In Virginia, Nebel et al. (1997) reported that Holsteins in estrus during summer had 4.5 standing events per estrus vs. 8.6 for those in winter. Changes in estrus activity caused by heat stress

reduce the likelihood that estrus will be detected by dairy personnel. The percentage of undetected estrus periods on a commercial dairy in Florida was estimated at 76 to 82% during June through September vs. 44 to 65% during October through May (Thatcher and Collier, 1986). Some effects of heat stress may involve adrenocorticotrophine hormone (ACTH). Heat stress can cause increased cortisol secretion (Roman-Ponce et al., 1981; Wise et al., 1988a; Elvinger et al., 1992), and ACTH has been reported to block estradiol-induced sexual behavior (Hein and Allrich, 1992). In certain studies, though, heat-stress induced elevations in circulating cortisol concentrations were transitory (Christison and Johnson, 1972; Miller and Alliston, 1974; Elvinger et al., 1992). In others, there was no increase in cortisol concentrations associated with heat stress (Wise et al., 1988b; West et al., 1991) or heat stress depressed cortisol concentrations (Abalay et al., 1975). Accordingly, effects of heat stress on estrus behavior likely include actions independent of the pituitary-adrenal axis. Some reports indicate that heat stress causes a reduction in peripheral concentrations of estradiol-17 $\beta$  at estrus (Gwasdauskas et al., 1981; Wilson et al., 1988), although this effect has not always been observed (Rosenberg et al., 1982). It is possible that the major reason that heat stress reduces the expression of estrus is because of the physical lethargy produced by heat stress. Reduced physical activity is itself probably an adaptive response that limits heat production.

The mechanism by which heat stress causes decreased fertility is probably multifactorial and may vary depending on the magnitude of heat stress. When rectal temperatures in the hot season were low (39.0 °C) because of environmental cooling, most early embryonic mortality associated with the hot season occurred between d 6 and 14 of pregnancy (Ryan et al., 1993). In contrast, Putney et al. (1988) observed that experimental application of a heat stress that caused rectal

temperatures to rise to 41.1 °C resulted in a large reduction in embryonic development at an earlier time (d 7 after estrus). Wolfenson et al. (2000) reported that heat stress affected fertility by suppressing dominant follicles and steroidogenic capacity of theca and granulosa cells. Progesterone secretion by luteal cells was lowered during summer, and in cows subjected to chronic heat stress, this was also reflected in lower plasma progesterone concentration. Heat stress impaired oocyte quality and embryo development, and increases embryo mortality. They found that high temperatures compromised endometrial function and alter its secretory activity, which may lead to termination of pregnancy. Since the process by which heat stress leads to embryonic loss may be different following mild vs. severe heat stress, it follows that the optimal strategy for improving fertility during heat stress may depend on climatic conditions. Elucidation of the physiological and biochemical pathways through which heat stress causes disruption of embryonic survival are therefore important for development of techniques to intercept those pathways and to improve fertility.

## **Materials and Methods**

### **Location**

This study was performed on a large commercial dairy farm (Rocky Creek Dairy) located in Olin, NC during the summer of 2002 between the months of May and August. For the month of June, the average production for approximately 1,000 cows housed in free stall barns was 32.1 kg per cow per milking with a rolling 365-d herd average of 11,118 kg. The reproduction variables averaged 139 average days open, 3.1 services per pregnancy, and 80 days to first service.

### **Systems for the detection of estrus**

Two hundred and sixty six lactating Holstein cows at 37 to 45 DIM were fitted with HW and ALPRO transmitters and weekly enrolled to the study from May to July. The visual method for the detection of estrus was performed by one person observing cows for 30 min periods, at approximately 0730 and 1300 with another individual observing at 0100. The primary characteristic of estrus that declared a cow in estrus was “standing to be mounted”, and the time of first standing event observed was recorded as the onset of estrus.

The HW pressure-sensing system for detection of mounting activity include a battery-powered, reusable pressure-sensing transmitters, a signal receiver with a 0.4-km range, a buffer that received and stored mounting activity data, and computer software that sorted information by

cow, date, and time and generated management lists using a personal computer (Gateway, Inc. N. Sioux City, SD). The HW transmitters were enclosed in a Whirlpack™ bag (Nasco, Fort Atkinson, WI) and contained in a durable, tightly woven, nylon envelope that formed a pouch on a 25 x 20-cm disposable nylon patch. This sensor-transmitter patch was glued to the sacrum region with contact-type adhesive (The W. J. Ruscoe Company, Akron, OH). The remote receiver was placed approximately 4 m above the ground and within 50 m of the free-stall barn that housed the cows. All areas of cow traffic were within detection range of the transmitter signal. The HW software was reviewed a minimum of every 8 h to determine time of first standing event for commencement of estrus. Data received by HW software included cow identification, transmitter number, time and duration of standing event, and signal strength. Standing events of greater than one second in duration were recorded in the program software, and the program software classified a “standing” estrus as three standing events in any 4-h period.

The ALPRO herd management system included a milk yield meter, an activity antenna, a parlor gate sort system, a panel processor, and a battery-powered reusable activity-sensing transmitter. The activity-sensing transmitters were securely attached to the neck of the cows with nylon bands. These transmitters detect the physical activity of the cows and sent a signal to two antennas placed approximately 3 m above the ground within 25 m of the herds, one at each end of the barn. Activity signals were received by the antennas on real time basis and transmitted to a processor that stored hourly activity. Data received by this processor included cow identification, transmitter number, and time of increase in physical activity. The processor classified the increase in physical activity with 1, 2, or 3 plus (weak, medium, or strong threshold), which were

based on constants achieved from maximum and minimum historical values of specific hours of activity for this cows. This pattern is determined in bases of 4 to 5 h of baseline or “normal” activity. For the calculation and prediction of the movements of the cow, the ALPRO system used a Kalman filter. This filter can correct errors made in the time update data, improving the prediction for the next movement of the cow.

With this comparison between each individual hour, the system determined the start of estrus by the increase in physical activity. The ALPRO system also included software (Windows 2000, Microsoft Corporation) that recorded the time for increase of physical activity (onset of estrus), milk yield, feeding rations, and breeding calendars.

### **Timing of artificial inseminations**

A total of 506 inseminations were performed by one of five AI technicians. Timing of AI was previously determined for each system (HW: Dransfield et al., 1998; ALPRO: Maatje et al., 1997; Visual: Trimberger, 1948). For visual observation and HW, AI was performed 4 to 14 h after the estimated onset of the estrus. With the ALPRO system, cows were inseminated 6 to 16 h after the time for increased physical activity. A protocol for insemination was designed for cows when more than one system detected estrus. The priority for the timing of AI was given first to HW, then ALPRO, and finally to visual observation.

Pregnancy status was determined at 26 d post AI by ultrasonography and confirmed later at 35 to 49 d by palpation of the uterus via rectum.

### **Data analysis**

Records obtained included cow number, install date, calving date, AI date, DIM at AI, parity, AI time, inseminator, estrus detection system, time of visually observed estrus, activity level by the ALPRO system, onset of estrus by HW and ALPRO systems, and total and last standing event of the HW system. Parity was grouped as follows: 1, 2, and  $\geq 3$ . A total of 5 inseminators were used. Each detected estrus was classified by system (s) that identified the cow for each of the three individual systems, two systems in combination, and if all three systems identified the cow in estrus. Time of visual observation was classified as: AM, PM, and night. Number of standing events was classified as: 1 or 2; 3 to 9, and  $\geq 10$ . Activity level by the ALPRO system was classified as 2-fold increase in physical activity over the baseline for 1, 2 or 3 h consecutively.

Conception rates were determined for all cows detected by a single or combination of the multiple systems. Also the conception rate by each system and all combinations of the three systems were compared by month of AI. Estrus detection efficiency was also calculated using estrus periods detected by all and each of the individual systems, divided by the total theoretical number of estrus periods detected (subtracting days from transmitter install from days open and dividing by 21 for all cows).

The optimal time for insemination was determined by comparing the conception percentages for 4 intervals (0001 to 0600, 0601 to 1200, 1201 to 1800, and 1801 to 2400 h) between estimated onset of estrus and AI for visual, ALPRO, and HW systems.

Other variables analyzed were conception rate by activity level for ALPRO, number of standing events detected by HW, service, DIM at AI, parity, and inseminator. For the average mounting and physical activity the effect of parity, DIM, and month of AI was calculated.

Environmental temperature and humidity were compared with conception rates and standing activity. Maximum dry bulb temperatures in degrees Celsius and percentage of relative humidity were recorded daily from the National Weather Service at Hickory, NC located approximately 64 km from the dairy. A temperature-humidity index (THI) was calculated for the day of estrus for each cow as described by Tarazon-Herrera et al. (1999).

Briefly, THI was calculated as follows:

$$THI = 0.45 T + 0.55 TH - 31.9 H + 31.9$$

where

T = dry bulb temperature expressed in ° C and H= percentage of relative humidity.

A THI of < 72 was indicative of no heat stress. A THI range of 72 to 76 was considered mild heat stress. Moderate heat stress was defined as a THI of > 76.

## Statistical analysis

Statistical analysis was performed using procedures from SAS (SAS<sup>®</sup> Institute; Cary, NC). Difference between conception rates by systems of detection of estrus was estimated using linear contrast analysis from the GLM procedure. Conception rates for the four time intervals following onset of estrus were compared using logistic regression. Characterization of the conception rates for cows diagnosed pregnant by each of the variables present in the model was calculated by analysis of variance using the GLM procedure. The final statistical model for conception rate for estrus periods detected by HW system was as follows:

$$Y_{ijklmn} = \mu + P_i + D_j + M_k + IN_l + I_m + S_n + e_{ijklmn}$$

where

$Y_{ijklmn}$  = percentage diagnosed pregnant at 35 to 49 d post AI.

$\mu$  = overall mean

$P_i$  = the effect of the  $i^{\text{th}}$  parity group (1, 2, 3...>3)

$D_j$  = the effect of the  $j^{\text{th}}$  DIM (<60, 60 to 79, 80 to 99, 100 to 139, >140)

$M_k$  = the effect of the  $k^{\text{th}}$  month (May, June, July and August)

$IN_l$  = the effect of the  $l^{\text{th}}$  inseminator (1, 2, 3, 4 or 5) <sup>1</sup>

$I_m$  = the effect of the  $i^{\text{th}}$  interval (0001 to 0600, 0601 to 1200, 1201 to 1800, and 1801 to 2400)

$S_n$  = the effect of the  $m^{\text{th}}$  standing event (1 to 2; 3 to 9, and >10)

$e_{ijklmn}$  = random error

The final statistical model characterizing conception rate for estrus detected visually and by the ALPRO system was as follows:

$$Y_{ijklmn} = \mu + P_i + D_j + M_k + T_l + I_m + e_{ijklmn}$$

where

$Y_{ijklmn}$  = percentage diagnosed pregnant at 35 to 49 d post AI.

$\mu$  = overall mean

$P_i$  = the effect of the  $i^{\text{th}}$  parity group (1, 2, 3...>3)

$D_j$  = the effect of the  $j^{\text{th}}$  DIM (<60, 60 to 79, 80 to 99, 100 to 139, >140)

$M_k$  = the effect of the  $k^{\text{th}}$  month (May, June, July and August)

$IN_l$  = the effect of the  $l^{\text{th}}$  inseminator (1, 2, 3, 4 or 5)<sup>1</sup>

$I_m$  = the effect of the  $i^{\text{th}}$  interval (0001 to 0600, 0601 to 1200, 1201 to 1800, and 1801 to 2400)

$e_{ijklmn}$  = random error

<sup>1</sup> Inseminations performed by technicians 6, 7, and 8 were combined with technician 5.

## Results and Discussion

### Efficiency of detection of estrus

The efficiency of detection of estrus for each system (Visual, ALPRO, and HW) and combinations of the three systems are presented in Figure 1. A theoretical number of 707 estrus periods was calculated by subtracting days from transmitter install from days open and dividing by 21 for all cows. The efficiency of detection of estrus is based on the number of estrus periods detected by ALPRO, HW, visually, and the three systems combined (235, 285, 324, and 506, respectively) over the theoretical number of 707 possible estrus periods. Visual observation detected the highest number (45.8 %) of estrus periods in contrast with ALPRO (33.2 %) and HW (40.3 %). The total percentage of estrus periods detected by the three systems was 71.6 % thus the three systems complemented each other to detect a greater number of estrus periods than each system individually. Visual observation detected 128 estrus periods not identified by either the HW or ALPRO systems. However, the conception rate for cows inseminated when visual observation was the only system detecting estrus was the lowest ( $P < 0.05$ ) of all systems and combinations of systems (Figures 5 and 6). Consequently, either cows detected visually were not correctly identified as being in estrus or the timing of insemination for these cows was grossly out of the range in relation to ovulation, to obtaining expected conception rates.

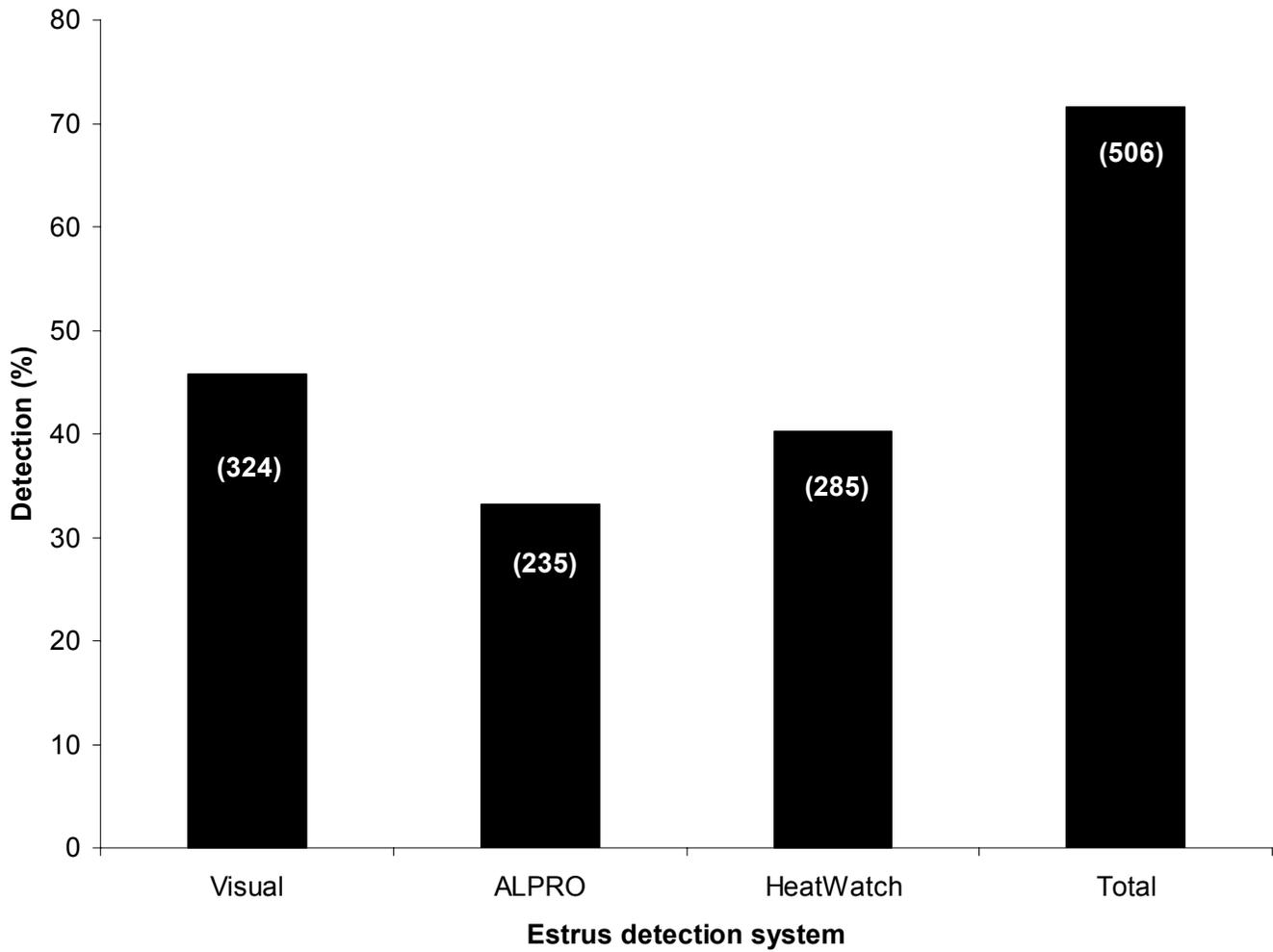


Figure 1. Frequency of detection of estrus for each system, Visual, ALPRO, and HW and combination of the three systems. Numbers in parentheses indicate periods of estrus detected.

The efficiencies of detection of estrus for each system alone (Visual, HW, and ALPRO) and in combination (Visual + ALPRO, Visual + HW, ALPRO + HW, and Visual + ALPRO + HW) are presented in Figure 2. The higher frequency of efficiency was obtained by visual observation (18.1%) as in Figure 1. For the other systems and combinations, efficiencies of estrus detection ranged from 6.1% (Visual + ALPRO) to 11.0% (Visual + ALPRO + HW). Fifty cows were solely detected in estrus by the ALPRO system, thus physical activity increased above the calculated baseline without detection of standing activity; however this occurred in only 7.1% of the expected estrus periods. The combination of visual observation and ALPRO systems identified 43 estrus periods not detected by the HW system. In contrast, 64 cows were identified in estrus by both the ALPRO and HW systems that were not detected by visual observation. The HW system identified 68 cows not detected by either visual observation or the ALPRO system.

Walker et al. (1996) using 120 Holstein and 40 Jerseys cows, compared efficiency of HW systems versus twice daily visual observation. The HW system increased efficiency of detection of estrus by 40 percentage points (91 vs. 51%). Nebel et al. (1995) reported that visual observations identified 61.3% of the estrus periods detected by HW whereas only 2.7% of the estrus periods detected visually were not identified by HW. Stevenson et al. (1996) using 50 beef heifers reported the efficiency in detection of estrus by HW (100%) was higher ( $P < 0.01$ ) than twice daily visual observation (73%). Borger et al. (1996) using 74 beef cows reported efficiencies of 91.1% and 65.8% for HW and visual observation, respectively.

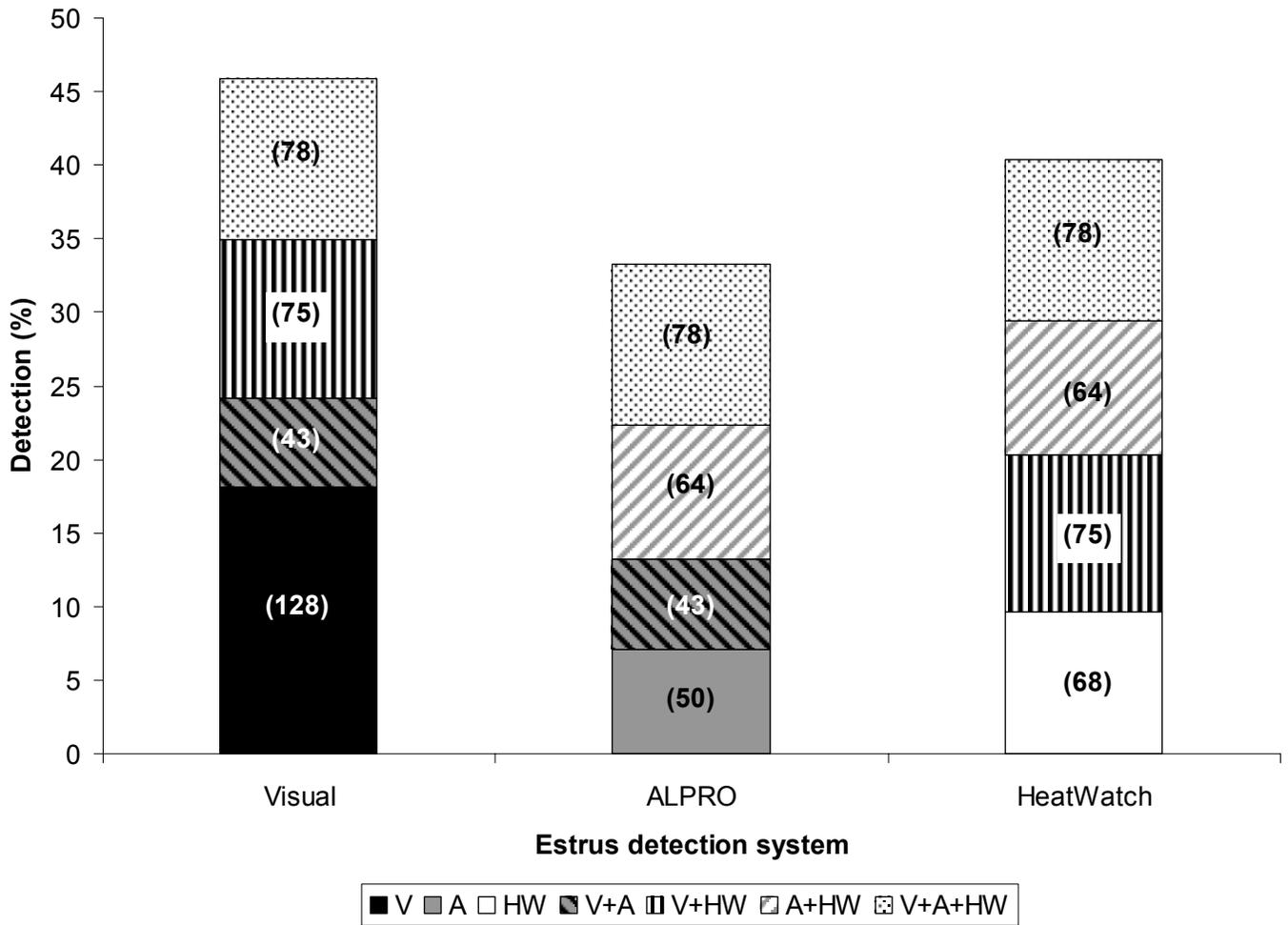


Figure 2. Frequency of detection of estrus for each system alone and in combination with the other two systems (V = visual, A = ALPRO, and HW = HeatWatch). Numbers in parentheses indicate periods of estrus detected.

To the knowledge of the author, no previous studies evaluate the efficiency or application of the ALPRO system. However there are studies that report efficiencies for other pedometer systems. Pennington (1986), using 23 cows reported efficiency for visual observation of 45% and 78 to 96% for pedometers (with a 100 and 200% increase in activity, respectively). He also estimated efficiencies for estrus detection systems combined, obtaining 83 and 78% for mount detector, chalk plus 100 and 200% increases in activity detected by pedometer, respectively. Combination of estrus detection systems obtained high percentages of efficiencies in detection of estrus periods and also decreased the number of false positives compared by singles estrus detection aids. Williams et al., (1981) using 12 mature Holstein heifers reported single estrus detection systems had efficiencies of 68, 70, 74, and 68% for twice daily visual observation, heat mount detector, and pedometer readings at 1 SD and 2 SD, respectively. Combinations of systems increased the percentage of estrus detected from 84% for visual observation plus heat mount detectors to 93% for visual observation plus pedometer activity peaks at 1 SD. They concluded that the efficiencies of detection of estrus did not differ greatly between techniques, and two or more techniques showed additive effect on efficiency.

All of these studies for visual observation, HW, and pedometers systems were performed during standard environmental conditions without reference to temperature or relative humidity. No previous studies have evaluated the HW system or pedometry under heat stress conditions. There are very little data as to whether these systems can improve estrus detection during heat stress. In this study, the detection of estrus due to heat stress conditions decreased approximately 40 percentage points in relation to previous studies. Efficiency in detection of estrus declined during heat stress conditions in large part because of a reduction in the intensity and duration of estrus.

Gwazdauskas et al. (1983) using 1,000 periods of estrus visually observed twice daily reported maximum environmental temperature on day of estrus had a significant curvilinear relationship with estrus activity at the first observation. As maximum daily temperature increased from  $-20^{\circ}\text{C}$  to approximately  $25^{\circ}\text{C}$ , mounting activity also increased from 2 to 9 standing events per hour, but above  $30^{\circ}\text{C}$ , a decline to 8 standing events per hour occurred. Using the HW system, Nebel et al. (1997) reported that standing events per estrus were approximately doubled for winter (8.6) versus summer (4.5) months. Estrus duration has also been reported to decline from 17.0 h to 12.5 h when Guernsey heifers were maintained at  $18.2$  vs.  $33.5^{\circ}\text{C}$ , respectively (Abilay et al., 1975).

The circadian frequency for onset of estrus detected by ALPRO ( $n = 235$ ) and HW ( $n = 285$ ) are presented in Figure 3. Estrus periods detected by ALPRO had a higher ( $P < 0.05$ ) frequency of onset of estrus between 0001 to 0600 h (43.0%) compared with the others time intervals, indicating a higher increase in physical activity as sign of onset of estrus during night. Hackett and McAllister (1984) using chin-ball markers for detection of estrus in 56 lactating cows found about 65% of the cows were marked during night hours (1801 to 0600 h). Hurnik et al. (1975) used continuously time-lapse video recording on 36 Holstein cows for 80 d. They found that 56% of cows displayed first signs of estrus between 1801 and 0600 h and concluded that cows were more likely to exhibit standing activity when not distracted by other activities, such as feeding, milking, and barn cleaning.

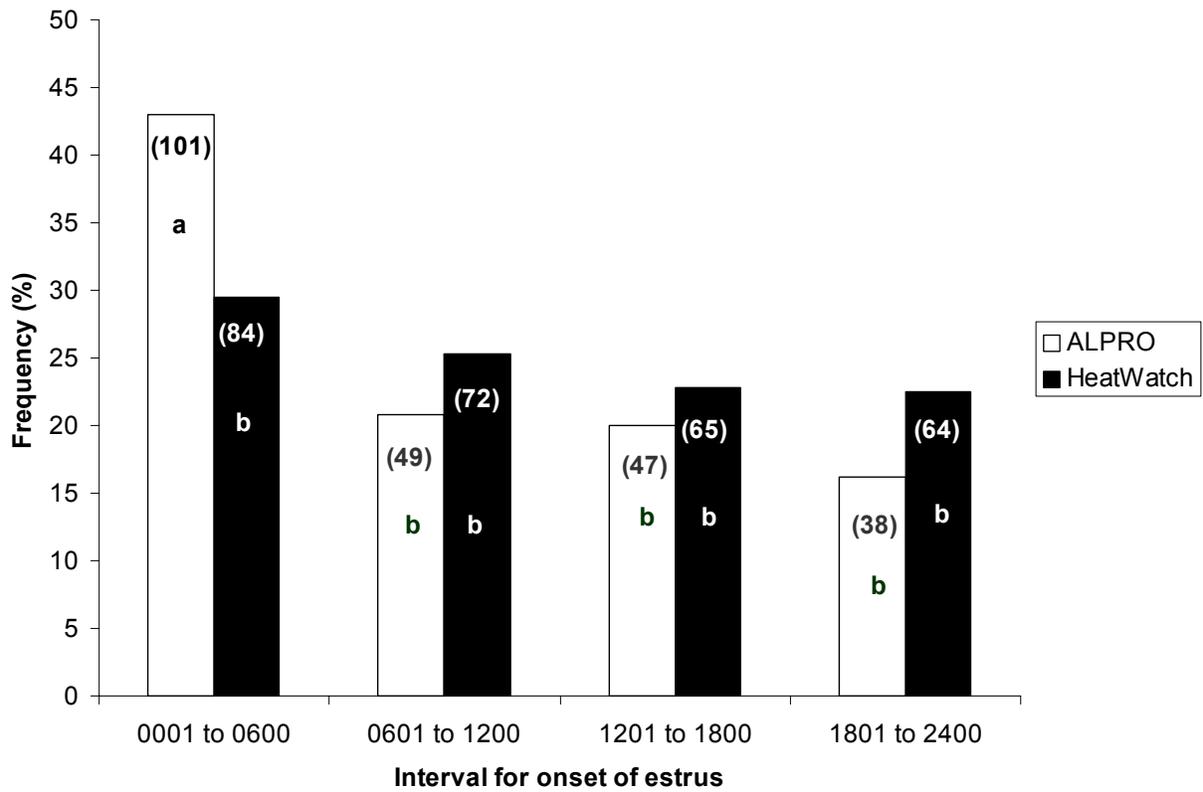


Figure 3. Circadian distribution for onset of estrus detected by ALPRO (n=235) and HW (n=285). Numbers in parentheses indicate periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

In our study the higher frequency of onset of estrus during night hours can also be related with a decrease in disturbing activities such as inseminations, veterinary treatments, and barn cleaning; however, the night milking session was performed at 0200 with three times daily milking and cows should have had minimal distractions.

There was also a difference ( $P < 0.05$ ) between ALPRO and HW systems for the interval between 0001 to 0600 h (43.0 vs. 29.5%), indicating higher expression of estrus by increasing physical activity but not in standing activity. Estrus periods detected by HW did not present differences ( $P < 0.05$ ) between intervals (29.5, 25.3, 22.8, and 22.5% for each interval, respectively). Dransfield et al. (1998) reported similar results with no differences in distribution of onset of estrus among the same 6-h time intervals (24.5, 28.4, 19.8, and 27.3%, respectively).

In Figure 4 the circadian frequency for estrus periods detected visually (n=324) are presented. During 0730 observation period a 62.7% of the onset of periods of estrus was observed and was higher ( $P < 0.05$ ) compared with the 21.6% and 15.7% of 1300 and 0100 time periods, respectively. This higher frequency of onset of estrus observed during the morning could be related to increase in physical activity that started at night, as showed in Figure 3 by the ALPRO system, and continued during the a.m. period. In 44,707 cows and heifers using visual observation, Foote (1979) reported that the majority of the cows were first observed in estrus in the morning (72.6 and 73.2 % for cows and heifers, respectively) and presumably most came in estrus during the night.

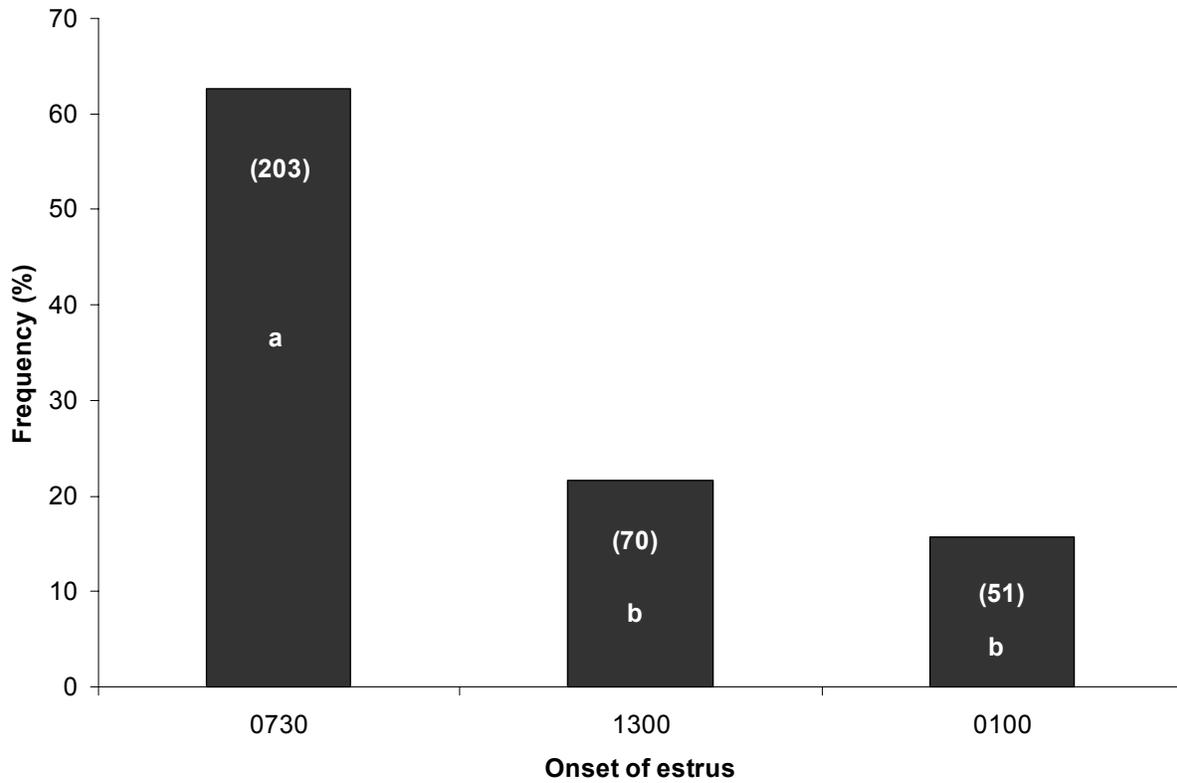


Figure 4. Circadian distribution for onset of estrus detected by visual observation (n=324). Number in parenthesis indicates periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

## Conception rates for estrus detection systems

Conception rates for estrus periods detected by each system (Visual, ALPRO, and HW) either alone or in combination are presented in Figure 5. A higher ( $P < 0.05$ ) conception rate occurred for cows detected in estrus by the HW system (24.6 %) in comparison to cows detected visually (18.2 %). The conception rate for cows detected by ALPRO (21.7 %) was not different ( $P < 0.05$ ) from the other two systems. The number of cows that were found pregnant by inseminations performed for estrus period detected by each system were 59, 51, and 70 for Visual, ALPRO, and HW, respectively. Thus, if the ALPRO system would have been the only system of detection of estrus, it would have accounted for approximately half the number of pregnant cows by the end of August. Whereas, 60.2 and 71.4% would have been pregnant if only visual observation and HW system were used. The total number of cows that were found pregnant in this study was 98, with a combined conception rate for all systems and combinations of 19.4%. Of the 266 cows used in this study, 36.8 % were pregnant at the end of the trial.

Least square means of conception rates for estrus periods identified by each system either individually or in combination with the other systems are presented in Figure 6. The highest efficiency of detection occurred with visual observation (Figure 1); however, cows detected visually without detection by the other two systems had the lowest conception rate (7.1%). Although the conception rate was lowest for cows detected only by visual observation, it was not different than the conception rates for cows inseminated when identified in estrus solely by HW system or with the combination of visual observation and the ALPRO system.

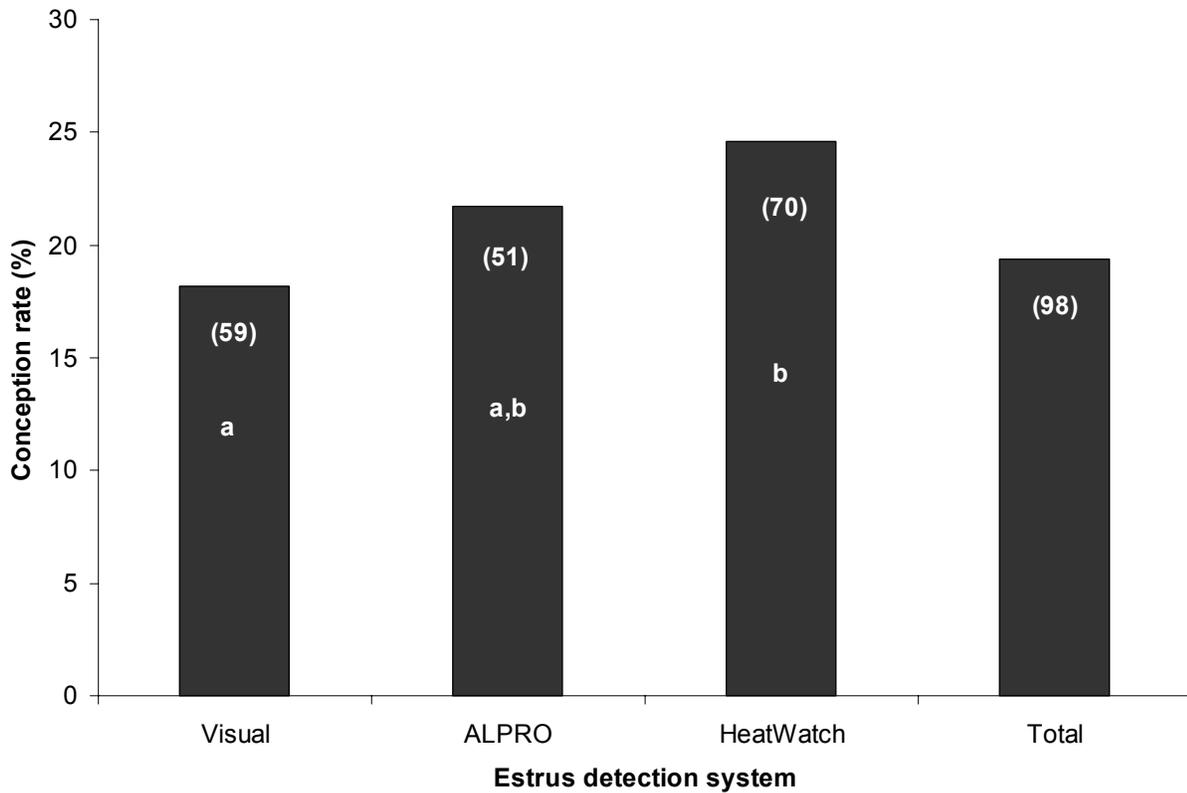


Figure 5. Conception rates for estrus periods detected by each system (Visual, ALPRO, and HeatWatch) either alone or in combination. Numbers in parentheses indicate cows diagnosed pregnant. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

Cows detected by both the HW system and visually had the highest conception rate (27.6%) of any system either alone or in combination and was higher ( $P < 0.05$ ) in almost 20 percentage points over estrus detected only visually (Figure 6). Appropriate timing for AI by the HW system could be the cause for this difference in conception rate; additionally, signs of estrus other than standing activity may be missed by the person who checked estrus, decreasing the conception rate for visual observation. Van Vliet and Van Eerdenburg (1996) using approximately 100 lactating cows scored signs of estrus and correlated them with pedometer readings. They concluded that observation of other signs of estrus other than increase in physical activity and standing to be mounted was important for estrus detection efficiency. They reported that estrus detection aids recorded only part of sexual activities of high producing cows housed in freestall with cubicles and a concrete slatted floor. Therefore, visual observation would be necessary for detection of other signs of estrus.

It is important to consider the heat stress conditions for this study. Cavestany et al. (1985) performed a study in Florida using 1,145 Holstein cows to determine conception rates under high environmental temperatures. They used twice daily visual observation in which the average maximum daily temperature for the months of May, June, July, and August was 32.9 °C with an average conception rate of 11.3%. This study showed some similarities in the environmental conditions, with an average maximum daily temperature for the same months of 39.2 °C but with a higher conception rate of 19.4%.

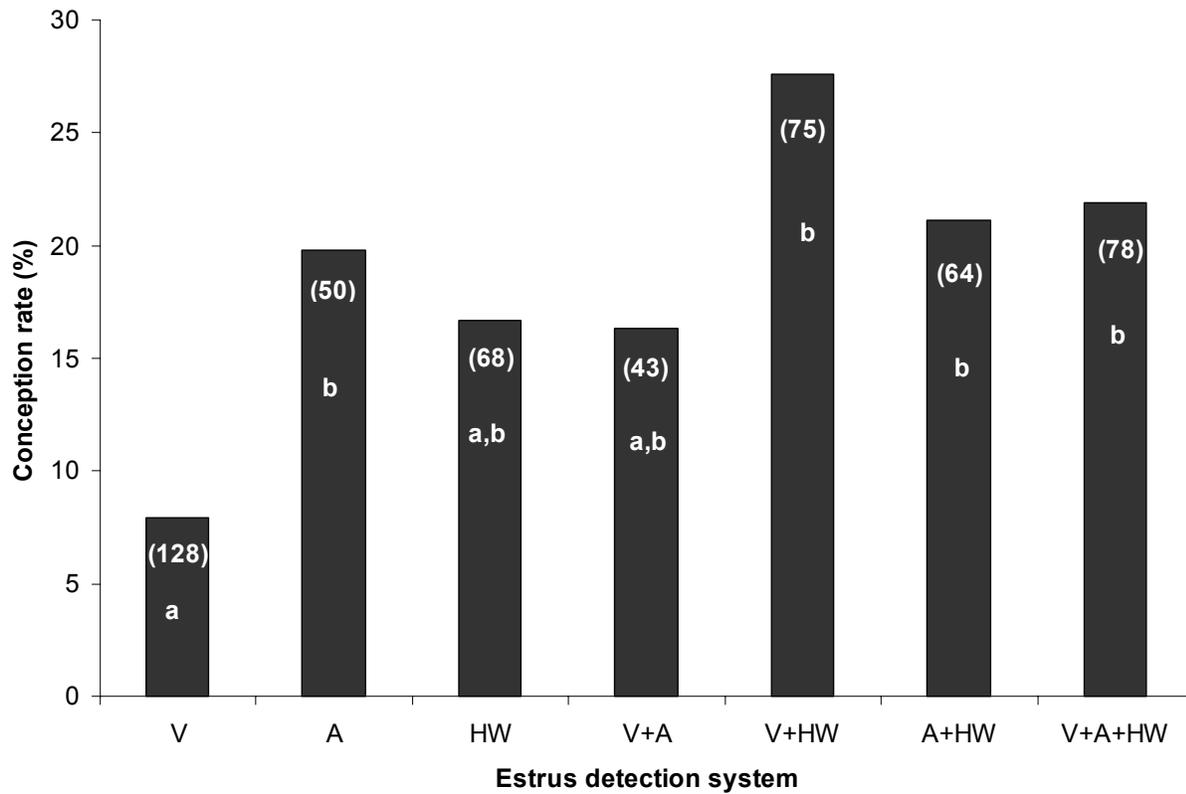


Figure 6. Conception rates (LSM) for estrus periods identified by V=Visual, A=ALPRO, HW=HeatWatch, and all combinations of two and three systems used for detection of estrus (n=506). Numbers in parentheses indicate periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

Conception rates for cows in estrus periods detected visually by ALPRO and HW system relative to service are presented in Figure 7. For first service, a difference was found between systems, with the highest conception rate for estrus detected by HW (50.0%) followed by ALPRO (44.4%) and visual observation (37.3%). The decrease in conception rate from first to second service could be a consequence of more fertile cows becoming pregnant at first service. In second service, conception rates were similar between systems, and ranged from 17.2% for estrus detected visually to 19.6% for HW; the same occurred for the fourth service. A decrease in conception rate was found for estrus detected visually at third service (7.5 %) probably associated with more “problem” cows expressing less estrus behavior.

Conception rates for estrus periods detected by either the ALPRO system or visually and classified as a “suspect estrus” by HW are shown graphically compared with cows classified as in “standing estrus” by the HW system (Figure 8). Cows classified by HW as having a suspect estrus either had 1 or 2 standing events recorded within a 4-h period. The conception rate for estrus periods of cows classified as suspect by the HW system was not different from cows classified as standing by the HW system. Similar results were obtained for cows classified as low or medium increase in physical activity versus strong increase in physical activity above baseline for estrus periods detected by the ALPRO system (Figure 8).

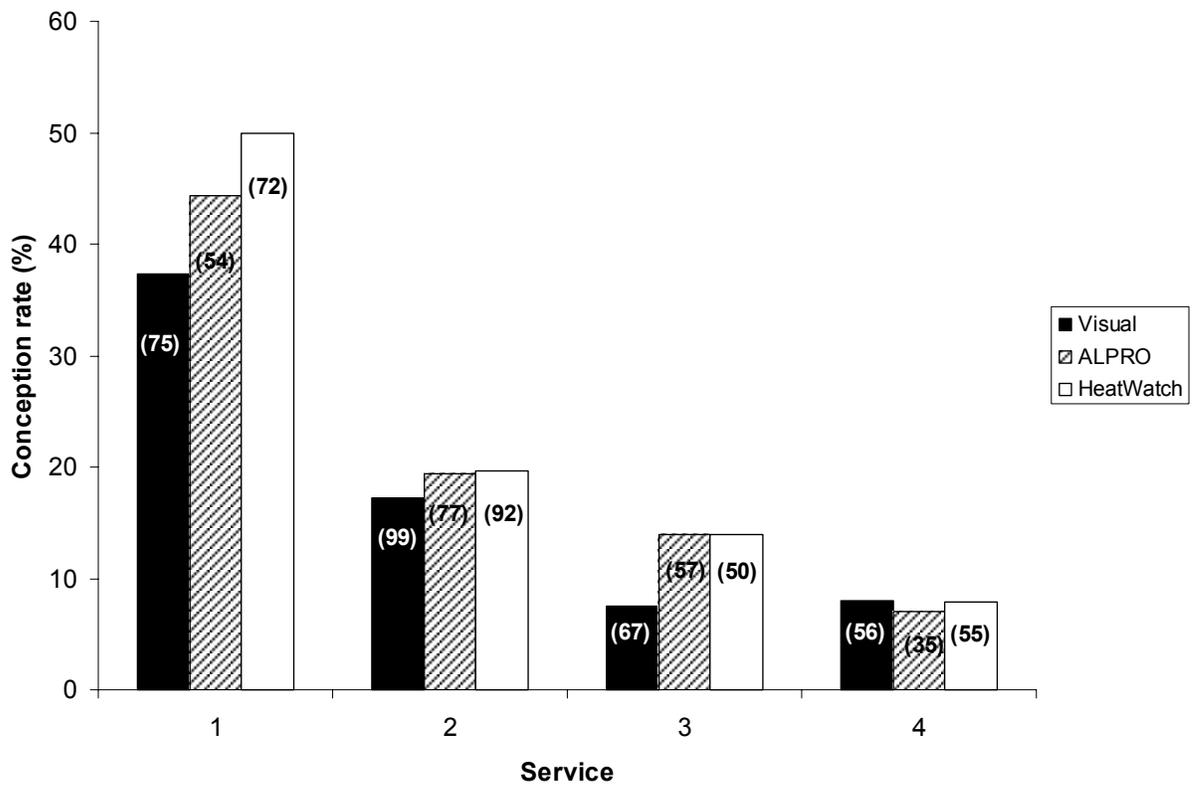


Figure 7. Conception rate for cows in estrus period detected visually by ALPRO and HW system relative to service number. Numbers in parentheses indicate periods of estrus.

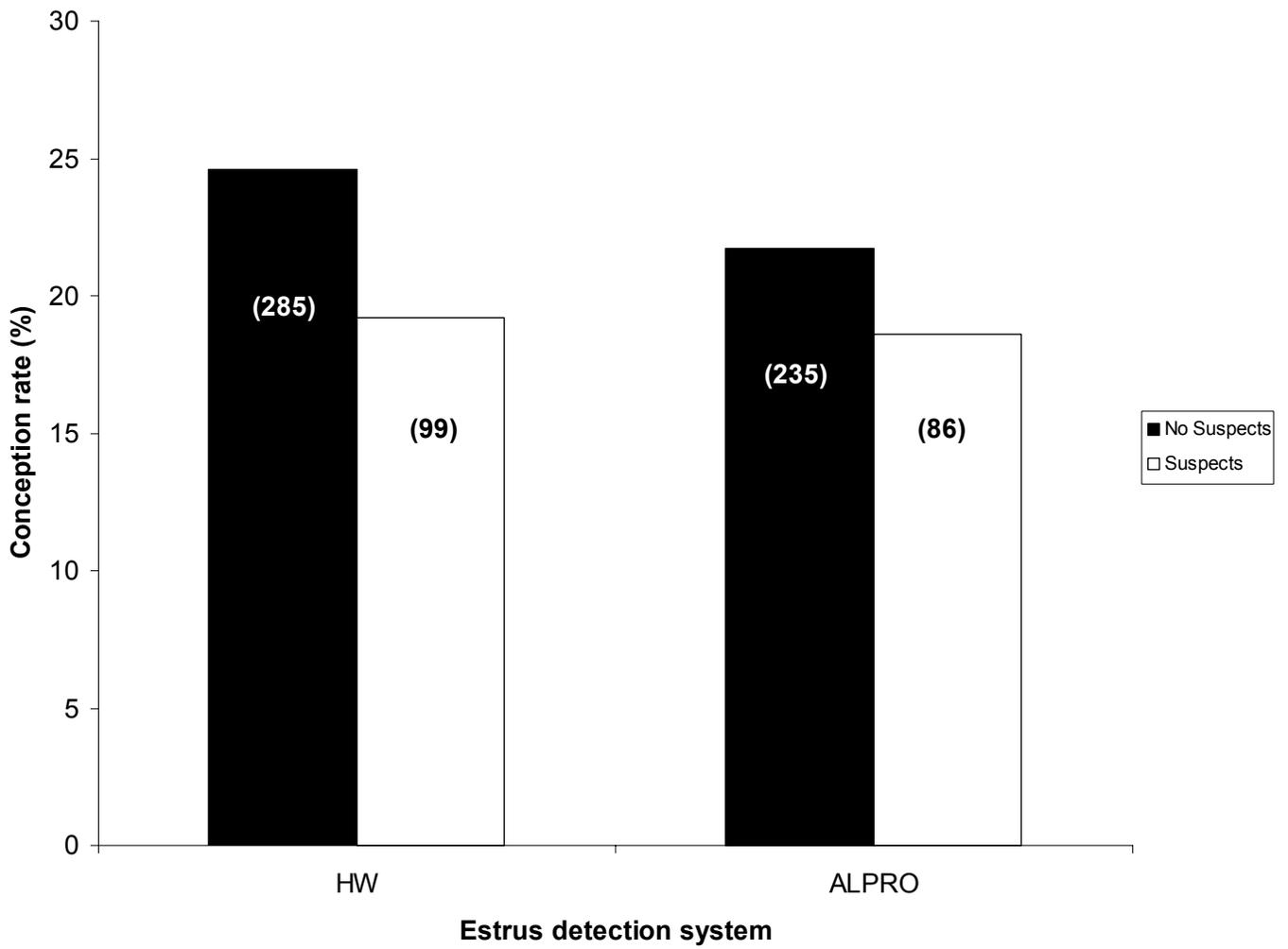


Figure 8. Conception rates considering suspects and no suspect estrus periods identified by HW and ALPRO systems. Numbers in parentheses indicate periods of estrus.

## Factors affecting conception rate

Factors significantly affecting conception rate during this study were system of estrus detection and parity. Least squares means of conception rates (LSM) for estrus periods (n=506) by parity are presented in Figure 9. A higher ( $P < 0.05$ ) conception rate for first lactation (26.2%) compared with second (18.0%) and third or more lactations (12.0%) may be related to an interaction of heat stress and production level. Barton et al. (1996) reported differences ( $P < 0.05$ ) in fertility between primiparous and multiparous cows. They found that older cows had 17.5 more days open, required 0.42 more services per conception, and had a 20% conception rate at first service versus 61.7% for first lactation cows. In a previous study, Carrol et al. (1988) reported a difference in plasma urea nitrogen concentrations between primiparous and multiparous cows (16.6 vs. 17.9 mg/dl;  $P < 0.05$ ). Several studies have proposed a possible interaction between elevated concentrations of ammonia, urea, or other toxic by-products of nitrogen metabolism that may negatively impact fertility in older dairy cows (Carrol et al., 1988; Ferguson et al., 1993). Heat stress has also been related to have a higher negative effect on older cows. Badinga et al. (1985) reported that nulliparous heifers had higher conception rates (50%) than lactating cows (34%) under heat stress conditions. They concluded that this reduction in fertility in older animals may be related to lactational stress associated to increased body temperature in high milk producing cows. They concluded that environmental conditions may contribute to aggravate the stress status of lactating dairy cows.

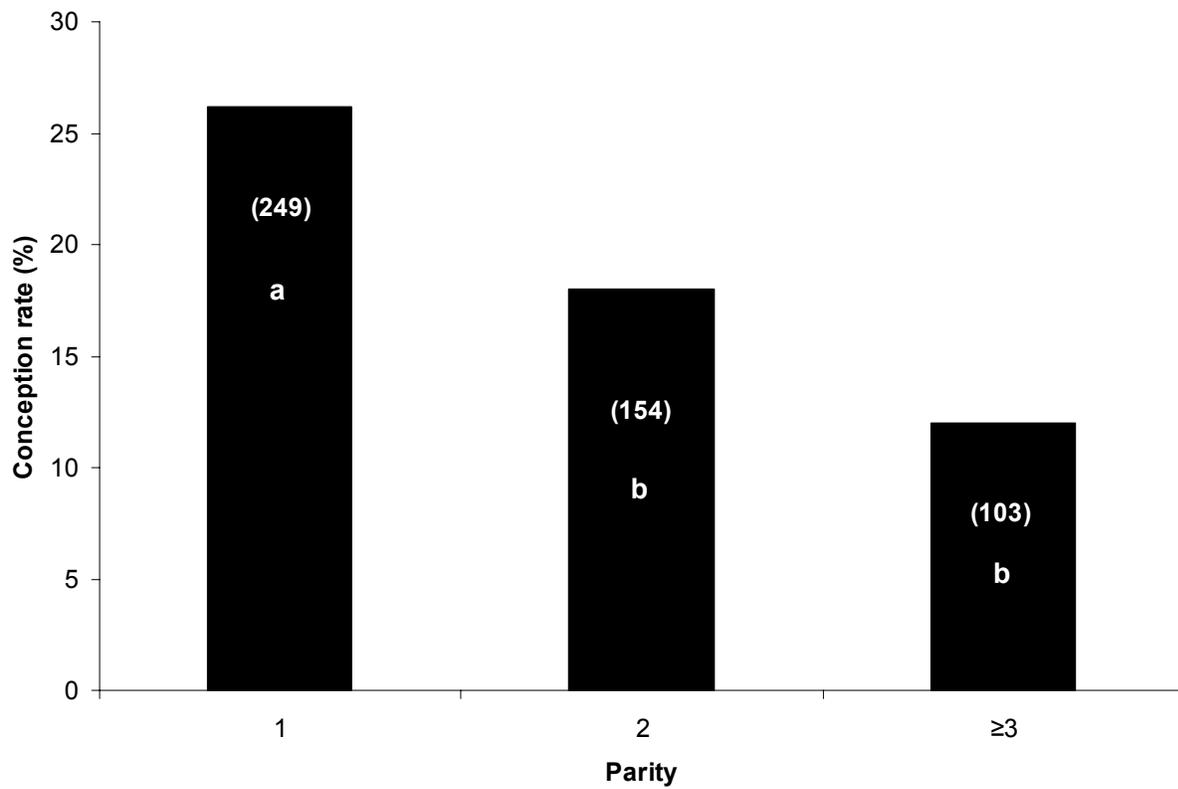


Figure 9. Conception rate (LSM) for estrus periods relative to parity (n=506). Number in parenthesis indicates periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ )

The relation of month of AI, considering that this study was performed under heat stress conditions was considered. Conception rates for cows visually detected in estrus and with the ALPRO or HW systems in relation to month are presented in Table 3. Visually detected cows had the highest conception rates during June (19.3%) and July (23.1%); whereas, July and August had higher conception rates (23.5 and 24.1%, respectively) for cows detected with ALPRO. Conception rates for HW system were similar across months (May 33.3%, June 24.2%, July 24.2%, and August 24.2%).

Conception rates for cows detected by each system either alone or in combination of two or three systems by month are presented in Table 4. During May half of the estrus periods were detected solely by visual observation (23 of 47). This may be due to the fact that the person responsible of the HW and ALPRO systems operation was not currently living at the farm, as well as to the reluctance of farm personnel to AI cows not detected visually. During the month of June, the combination of HW and visual observation had the highest conception rate of 37.5% across all months and systems. During August, conception rates ranged from 7.4% for 54 cows detected visually to 40.0% for 10 cows detected in estrus by the combination of ALPRO system and visual observation. For the total percentages, conception rates ranged from 8.6% for 128 cows detected visually to 29.3% for 75 cows detected by the combination of HW system and visual observation.

The THI did not influence conception rates and may be explained by its lack of variation during the 4 mo studied. The index classified according to the stress level of the cow was: <72-no heat stress, 72 to 76-mild heat stress, and >76-moderate heat stress. Table 5 shows

that all monthly values for THI were classified as moderate heat stress. However, the conception rates for each month (11.0, 19.6, 20.0, 20.4% for May, June, July, and August, respectively) and the overall conception rate (19.4%) could be considered being suppressed by heat stress (Table 4). The daily maximum temperatures during this study ranged from 35.7°C for the month of May to 41.4°C for the month of July. These values were for environmental temperatures without considering the effect of the cooling system (fans) available at the dairy. Experimental exposure of cattle to heat stress has been shown to reduce conception rates (Dunlap et al., 1971) and embryonic survival (Ealy et al., 1993). Dransfield et al. (1998) recorded using 2661 AI in 17 Virginia herds reported that the odds of pregnancy for cows inseminated during summer months were 12 percentage points lower than for AI performed during November to March. Badinga et al. (1985) performing 12,038 inseminations during 2 yr reported a 20% depression in conception rates during the summer months in Florida. Conception rate decreased from 52 to 32% as maximum air temperature increased from 23.9°C in March to 32.2°C in July. Udomprasert and Williamson (1987) using 5845 inseminations in 23 herds of Holstein cows in Minnesota, compared conception rates for each month of the year during 3 yr. Conception rates ranged from 43.0% for the month of May to 26.4% for August, with daily maximum temperatures of 19.7 and 28.9 °C, respectively. They reported that conception rates decreased from approximately 48% for the month of March to 30% during the month of August.

Table 2. Monthly conception rates for cows detected in estrus visually with the ALPRO or HW systems <sup>1</sup>.

System/Month	May		June		July		August		Total	
	Total	%	Total	%	Total	%	Total	%	Total	%
V	41	12.2	57	19.3	91	23.1	133	16.5	334	18.2
A	23	17.4	48	16.7	85	23.5	79	24.1	235	21.7
HW	12	33.3	62	24.2	91	24.2	120	24.2	285	24.6

<sup>1</sup>V = Visual observation, A = ALPRO, and HW = HeatWatch.

Table 3. Monthly conception rates for cows detected solely by each system of estrus detection and in combinations of two or three systems (n=506)<sup>1</sup>.

System/Month	May		June		July		August		Total	
	Total	%	Total	%	Total	%	Total	%	Total	%
V	23	4.3	18	16.7	31	9.7	54	7.4	128	8.6
A	0	0	10	10.0	23	22.0	17	24.0	50	20.0
HW	1	0	20	15.0	17	5.9	30	33.3	68	2.6
V+A	12	0	11	9.1	10	2	10	40.0	43	16.3
V+HW	0	0	16	37.5	22	36.4	38	21.1	75	29.3
A+HW	5	0	14	36.0	24	21.0	21	24.0	64	23.4
V+A+HW	6	66.7	13	7.7	28	28.6	31	19.4	78	24.4
Total	47	11.0	102	19.6	155	20.0	203	20.4	506	19.4

<sup>1</sup>V = Visual observation, A = ALPRO, and HW = HeatWatch.

Table 4. THI, daily maximum temperatures, and relative humidity by month.

Month	May	June	July	August	Total
THI <sup>1</sup>	78.9	84.5	86.0	82.3	83.6
MXT °C <sup>2</sup>	35.7	39.6	41.4	39.9	39.2
RH <sup>3</sup>	92.3	90.3	90.2	88.8	90.4

<sup>1</sup>THI= Temperature-humidity index =  $0.45 T + 0.55 TH - 31.9 H + 31.9$ .

<sup>2</sup>MXT °C= Daily Maximum temperature.

<sup>3</sup>RH= Relative humidity.

Conception rates relative to interval from estimated onset of estrus to AI for estrus periods detected by ALPRO and HW are presented in Figure 10. Using logistic regression, no differences were found between conception rates of the four intervals for either HW and ALPRO system. However, for cows detected in estrus by ALPRO system the conception rate for interval between 1201 to 1800 h (31.7%) was different compared with the interval 1801 to 2400 h (16.7%) at the  $P < 0.08$  level.(Figure 10 and Table 6).

Maatje et al. (1997) using pedometers and visual observation performed 186 breedings in 121 cows and reported that AI performed after 24 h following onset of estrus resulted in conception rates  $< 20\%$ . The probability of conception for AI performed between 6 to 17 h after onset of estrus was optimum with conception rates of approximately 80%. The precise optimal time for AI from onset of estrus was 11.8 h, coinciding with the midpoint of the optimal interval detected by Walker et al. (1996) of 6 to 18 h after onset of estrus using the HW system. Dransfield et al. (1998) using the HW system performed a similar study to determine the optimal timing for AI since first mount of estrus. They reported that intervals from onset of estrus to AI higher than 16 h were negatively related to the probability of conception. A curvilinear relationship was found between intervals for AI from onset and percentage of cows pregnant. Conception rates were highest for cows that were inseminated from 4 to 14 h following the first standing event of estrus. Inseminations performed between 4 and 12 h following the onset of estrus achieved a conception rate of approximately 50%. For AI performed after 16 h, conception rate was only 30%. However, the vast majority of their data was not collected during the summer months of higher temperatures.

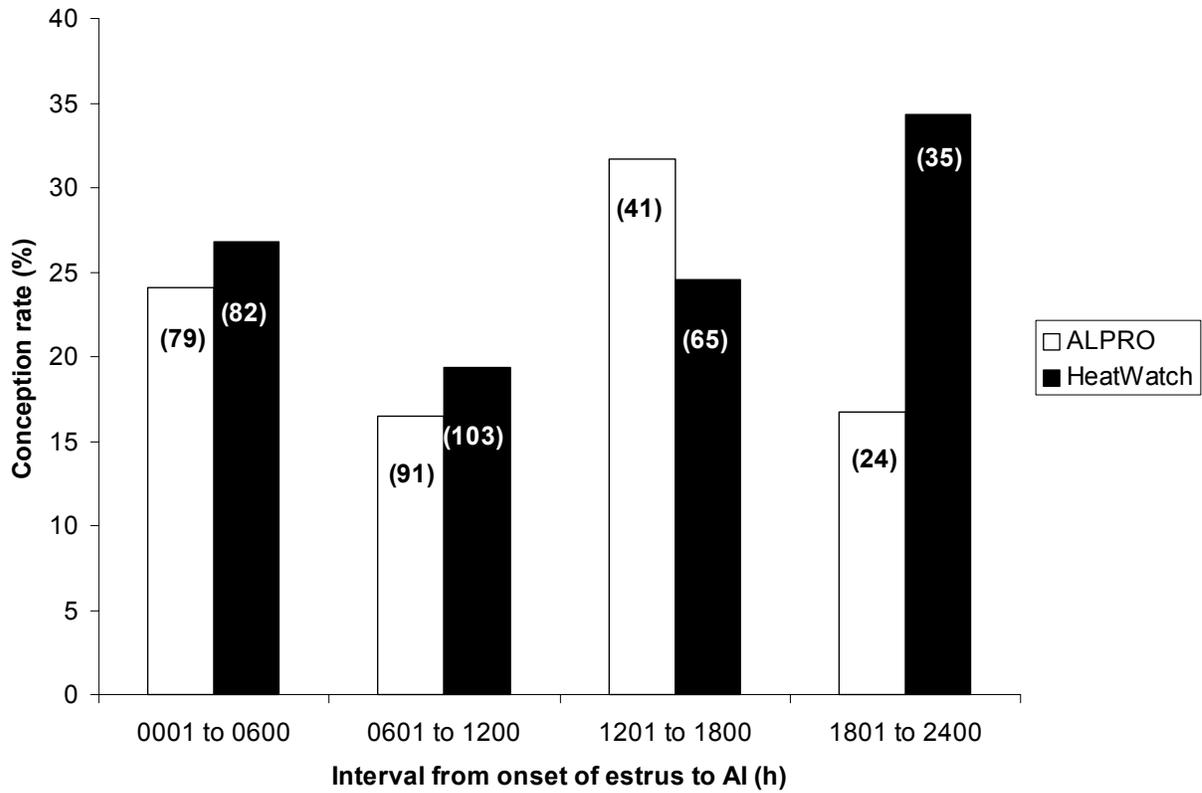


Figure 10. Conception rates relative to interval from first standing event to insemination for estrus periods detected by ALPRO (n=235) and HW (n=285) systems. Numbers in parentheses indicate periods of estrus.

Table 5. Logistic-binomial regression results for the effects of interval from first mount to AI (services=235) on conception rates for lactating cows identified in estrus by ALPRO.

Interval	Estimate	SE	OR <sup>1</sup>	P > ChiSq	95% CI <sup>2</sup>
Intercept	1.29	0.20	-	<0.0001	-
0001 to 0600	-0.14	0.26	0.63	0.6	(0.19,2.08)
0601 to 1200	0.34	0.27	1.01	0.2	(0.30,3.40)
1201 to 1800	-0.52	0.30	0.43	0.08	(0.12,1.52)
1801 to 2400	0	-	1	-	-

<sup>1</sup>OR = "odds ratio". The OR is the estimated odds of a dairy cow getting pregnant in any one interval when compared to the interval between 19 to 24 h following first mount where baseline interval OR = 1.

<sup>2</sup>CI = confidence interval CI not containing 1.0 denotes interval different ( $P < 0.05$ ) from baseline interval.

For the HW system, the highest conception rate of 34.3% was found for AI performed > 1800 h after onset of estrus. This result is not consistent with previous studies but may be related to the heat stress. Inseminations closer to the time of ovulation may be beneficial during periods of heat stress. This may be related to a reduced life of sperm or ova during periods of elevated temperature.

### **Factors affecting standing estrus**

Factors that significantly affected standing estrus (number of standing events measured by HW) during this study were month, parity, and DIM. Standing events and THI relative to month of AI for cows detected in estrus by HW are presented in Figure 11. An increase ( $P<0.05$ ) in standing events was found for the month of July (8.9) in relation to the month of June (6.8). A slight increase in the value of the THI was found between these 2 mo (86.0 vs. 84.5). A decrease in standing activity for the month of June could be influenced by the increase in environmental temperature and humidity from the previous month. The THI had increased from May (78.9) to June (84.5), which could be the cause of the significant differences in standing events between these 2 mo (9.7 vs. 6.8). Nebel et al. (1997) using the HW system in Holstein cows reported an approximate doubling in standing events for the winter season (8.6) versus summer (4.5). Nebel et al. (2002) monitored estrus characteristics during a 7 yr period. They found for heifers an average of 24 standing events and were similar during spring and fall whereas, winter and summer were also similar with 18 standing events. Holstein cows had  $5.7 \pm 0.8$  standing events during the summer; however, season had no effect on standing events on cows probably due to the ability of the cooling systems to

decrease the heat stress during summer. Gwasdauskas et al. (1983) reported that maximum environmental temperature on day of estrus had a significant curvilinear relationship with estrus activity at the first observation of heat. As maximum daily temperature increased to approximately 25°C, standing activity also increased. With temperatures higher than 30°C, a decline in standing events per hour occurred. With 35°C they found approximately 9 standing events per hour. This study showed similarities in the results; maximum daily temperatures ranged from the 35.7°C for the month of May to 41.4°C for the month of July with 9.7 and 8.9 standing events for each month. Considering all the estrus periods detected by HW, the average standing events was of  $7.9 \pm 10.6$  and the average duration of estrus was  $7.9 \pm 5.6$  (average  $\pm$  SD) (Table 7) similar to previous studies (Dransfield et al., 1998; Nebel et al., 2002).

Duration of estrus and THI for month of AI is presented in Figure 12 and Table 7. As THI increased from June to July, the duration of estrus had a slight decrease (8.2 h vs. 7.2 h). The dramatic increase in duration of estrus for the month of June (8.2 h) relative to May (5.9 h) is coincident and may be due to the finding that only 12 cows were detected by HW in May. However, heat stress conditions did not affect duration or number of standing events per estrus. Nebel et al. (2002) reported similar results during summer conditions with duration of estrus of  $7.3 \pm 0.2$  h and  $10.6 \pm 0.3$  for Holstein cows and heifers, respectively.

Abilay et al. (1975) artificially exposed six nulliparous synchronized Guernsey cows to environmental temperatures of 18.2 and 33.5 °C. They found that the duration of estrus

decreased from 17.0 to 12.5 h for 18.2 and 33.5°C. The average duration of estrus was 7.0 h (Table 7), which was similar to that reported by Dransfield et al. (1998) using the HW system.

Physiological effect of heat stress on the expression of estrus is variable. Some effects of heat stress may involve changes in adrenocorticotrophin hormone (ACTH). Heat stress can cause increased cortisol secretion (Roman-Ponce et al., 1981; Wise et al., 1988b; Elvinger et al., 1992), and ACTH has been reported to block estradiol-induced sexual behavior (Hein and Allrich, 1992). Heat-stress induced elevations in circulating cortisol concentrations may be transitory (Christison and Johnson, 1972; Miller and Alliston, 1974; Elvinger et al., 1992). Accordingly, effects of heat stress on estrus behavior likely include actions independent of the pituitary-adrenal axis. Some reports indicate that heat stress causes a reduction in peripheral concentrations of estradiol-17  $\beta$  at estrus (Gwazdauskas et al., 1981; Wilson et al., 1988). It is possible that the major reason that heat stress reduces the expression of estrus is the physical lethargy. Reduced physical activity is itself probably an adaptive response that limits heat production (Hansen and Arechiga, 1999).

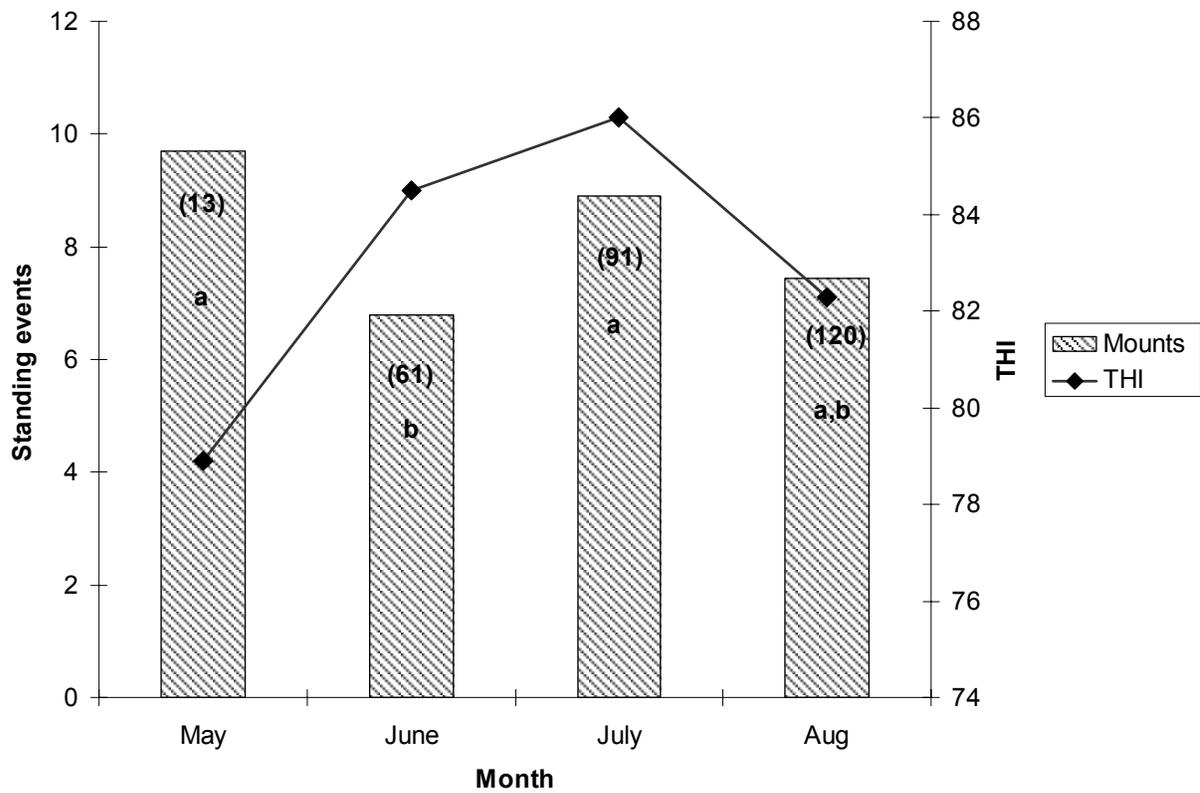


Figure 11. Standing events and the THI relative to month for cows detected in estrus by HW (n=285) system. Numbers in parentheses indicate periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

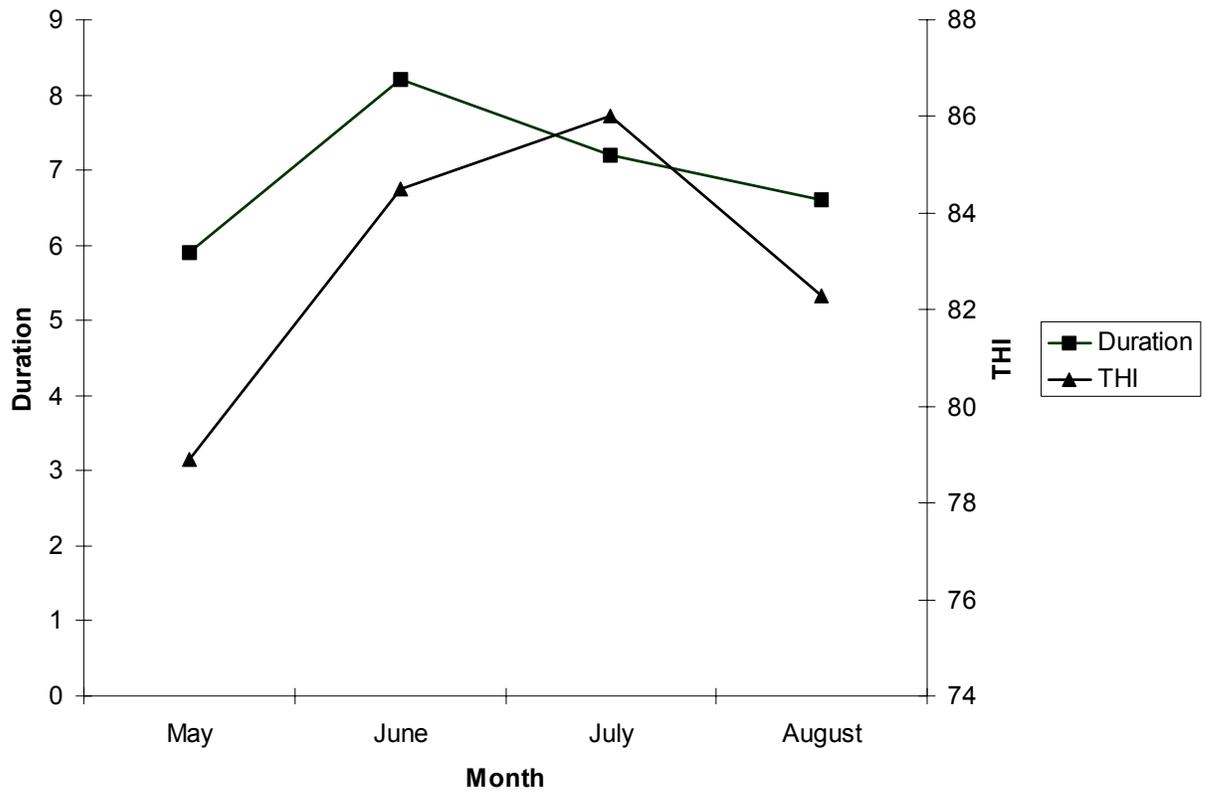


Figure 12. Duration of estrus and THI relative to month of AI for cows (n=285) detected in estrus by HW system.

Table 6. Duration and Standing events for periods of estrus detected by the HW system (n=285).

Month	May	June	July	August	Total
Duration, h <sup>1</sup>	5.9 ± 10.4	8.2 ± 11.1	7.2 ± 10.7	6.6 ± 10.4	7.1 ± 10.6
Standing event <sup>1</sup>	9.6 ± 6.1	6.8 ± 5.5	8.9 ± 6.6	7.4 ± 4.7	7.9 ± 5.6

<sup>1</sup>Arithmetic mean ± SD.

Standing events relative to parity for cows detected in estrus (n=286) by HW are presented in Figure 13. There was a significantly higher ( $P<0.05$ ) number of standing events (9.3) for first lactation cows compared with second (6.3) and third (5.7) lactation cows. Feet conformation and condition may be limiting factors in older cows for standing behavior. Lameness has been associated with reduced mounting activity and fertility in dairy cattle. Enevoldsen and Grohn (1991) reported an increase in number of cows affected by heel erosion, interdigital dermatitis, and hyperplasia for second to ninth lactation in relation to first lactation. Lucey et al. (1986) reported that cows that were lame because of sole or white line lesions between 36 and 70 d of lactation had a calving to conception interval which was longer, on average, 30 d. They concluded that in herds with good estrus detection, lameness may be an important limiting factor in maintaining optimal levels of fertility.

However, studies have found different results from this report. De Silva et al. (1981) and Gwasdauskas et al. (1983) analyzed the relationship between estrus behavior and lactation number. They found that older cows exhibited significantly more mounting activity than younger animals. They concluded that older animals may be more dominant and more experienced in exhibiting estrus behavior than younger subordinates. These animals may attract others by their dominance and thus may have a greater opportunity to interact. Van Vliet and Van eerdenburg (1996) using a scoring scale for observed symptoms of estrus behavior found similar results. Primiparous cows scored less ( $P<0.05$ ) points ( $361 \pm 82$ ) for standing activity than multiparous cows ( $578 \pm 331$ ).

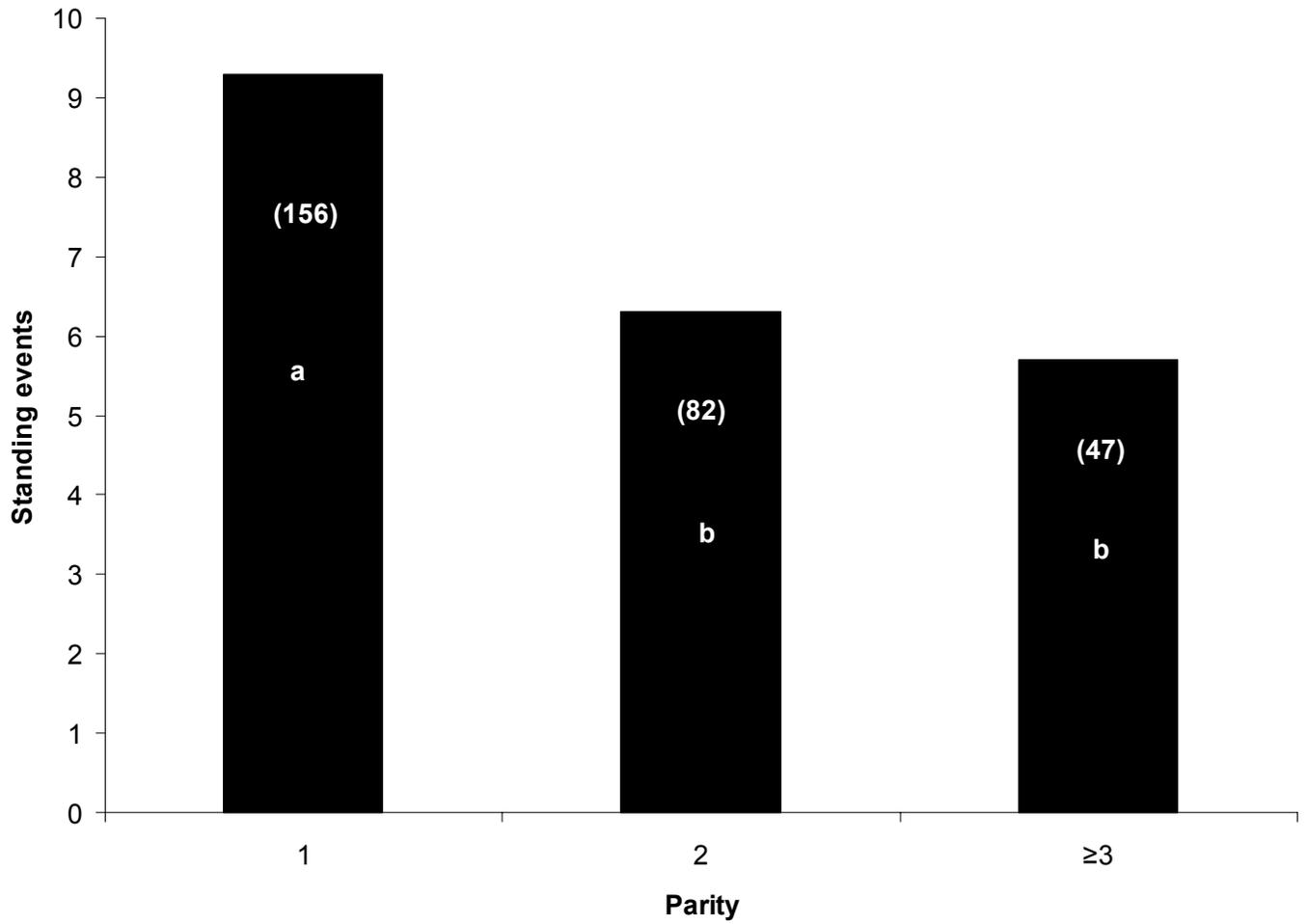


Figure 13. Standing events relative to parity for cows detected in estrus (n=285) by HW system. Numbers in parentheses indicate periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

Standing events relative to DIM at AI for cows detected in estrus by HW (n=285) are presented in Figure 14. Cows detected in estrus during the interval between <60 and 60 to 79 DIM had significantly higher ( $P<0.05$ ) average standing events (9.1 and 9, respectively) than cows detected in estrus between 80 to 99 (6.8), 100 to 139 (7.2), and >140 DIM (7.5). A review of the literature on postpartum ovarian activity and uterine involution indicated the interval from parturition to first observed estrus varied from 30 to 76 d in dairy cows. The time required for uterine involution based on rectal palpation and clinical observations and measurements varied (26 to 52 d). In general, the interval from calving to first estrus was greater in cows with higher production and in cows milked four times a day (Roberts, 1984). Silent estrus occurred in 77% of the first postpartum ovulation, 54% of the second, and 36 % of the third ovulations and was more common in high-producing cows (Morrow et al., 1969).

Significant ( $P<0.05$ ) differences between standing events of cows detected in estrus between <60 to 79 d and 80 to >140 d were probably a consequence of our voluntary waiting period of 50 d for first service. The decrease in standing events after 79 d postpartum could be related to a higher number of cows that conceived > 79 d in relation to <79 d postpartum. Thus, a higher proportion of problem cows exist after 80 DIM.

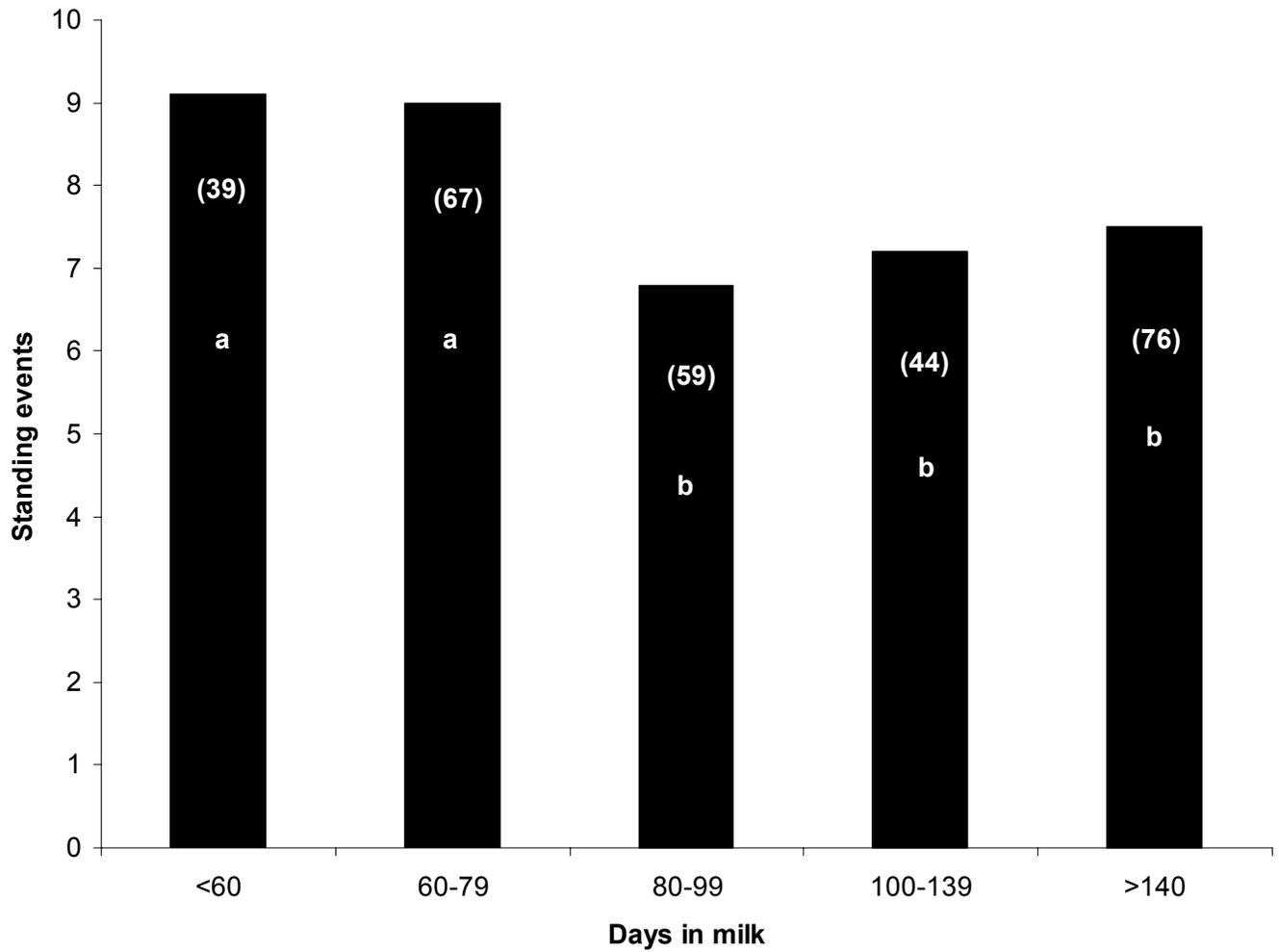


Figure 14. Standing events relative to days in milk at insemination for cows detected in estrus by HW (n=285) system. Numbers in parenthesis indicates periods of estrus. Bars with no superscripts (a, b) in common differ ( $P < 0.05$ ).

## Summary and conclusions

Efficiency of a system for the detection of estrus should not be sole criteria of evaluation without considering the resulting conception rate. A high number of cows detected in estrus visually that were inseminated did not result in conception probably because of erroneous observation of estrus or timing for AI. Among systems for detection of estrus, cows detected in estrus by HW obtained a higher conception rate compared with cows detected visually or with the ALPRO system. Consequently, a higher number of pregnant cows resulted after for estrus periods were detected by the HW system (70) in comparison to ALPRO (51) and visual observation (59). The combination of systems for estrus detection, such as visual observations and HW, resulted in a superior strategy to obtain higher conception rates and efficiency than the use of a single system, thus a greater number of pregnant cows.

Parity as well as estrus detection system influenced conception rates. First lactation cows obtained higher conception rates compared with older cows, and this was probably a result of the interaction of body size and heat stress. Interval of time from the onset of estrus to AI did not significant influence conception rates. However, similar to previous studies, AI performed after 18 h from the onset of estrus had lower conception rates.

Month of AI significantly affected standing activity as measured by the HW system. Environmental factors associated with heat stress probably were the cause of this influence but duration and intensity of estrus were not correlated with the calculated THI. Parity was a variable that directly influenced standing behavior. First lactation cows had a higher average standing

activity compared with older cows. Standing behavior monitored by the HW system was decreased as DIM advanced past the period associated with peak milk or approximately 80 d. This depression in estrus expression may be explained by fewer normal cows not pregnant by this time as first service yielded approximately a 45% conception rate across all three systems of detection. Environmental characteristics and parity were variables that also affected the efficiency of estrus detection.

Efficiencies in estrus detection and timing for insemination are important factors to be considered for investment in an estrus detection system. If the number of pregnant cows resulting from AI is the prime criterion for evaluating the three systems studied, HW resulted in 11 more pregnancies with 39 fewer inseminations than visual observations, and 19 more pregnancies with 50 more inseminations compared with the ALPRO system. Visual observations resulted in 8 more pregnancies compared with cows inseminated based on recommendations from the ALPRO system; however, 89 more inseminations were required. Dairy producers must be able to operate with lower marginal costs because of increasing expenses and low milk prices. Consequently, economical costs and benefits for the application of any new reproductive technology should be considered in future research

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## **Professional Experience**

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### **Meetings**

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Latin American Environmental Meeting, 1998, Santiago, Chile.

National Veterinary Medicine Meeting, 2000, Santiago, Chile.

ADSA-ASAS Meeting, 2002, Quebec City, Canada.

Graduate Student Assembly Research Meeting, Virginia Tech, 2003, Blacksburg,  
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### **Publications**

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