

OPTIMAL TIME OF INSEMINATION IN DAIRY CATTLE IDENTIFIED IN ESTRUS BY HEATWATCH®

by

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Thesis submitted to the faculty of the

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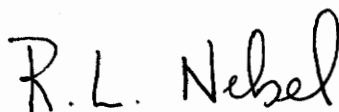
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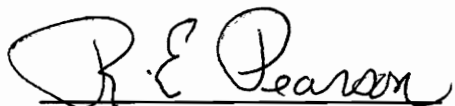
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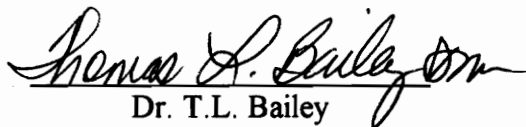
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Dairy Science

(ABSTRACT)

Estrus detection programs practiced on most U.S. dairy farms are not intense enough to provide the information needed to accurately time insemination, thus preventing AI from obtaining its full conception rate potential. Herds ($n = 17$) participated in a trial designed to evaluate percent pregnant relative to various characteristics of estrus. Herds utilized HeatWatch® electronic estrus detection system to detect and record mounting activity for cows in estrus. Inseminations were performed daily during a three hour interval for all cows identified in estrus the previous 24 h. Model characterizing percent pregnant for cows (services = 2661) included effects of interval from first mount to AI ($P < 0.01$), mounts per estrus ($P < 0.01$), DIM at insemination ($P < 0.01$), herd ($P < 0.05$), and season of AI ($P < 0.05$). As mounts per estrus and days in milk increased, percent diagnosed pregnant increased. Interval affected probability of pregnancy with highest odds ratios for percent pregnant occurring >4 to 16 h following onset of estrus. Model

for heifers ($n = 306$) included linear effects of interval ($P < 0.01$), season ($P < 0.05$), and herd ($P < 0.01$). In dairy heifers, as interval from first mount to AI increased, percent pregnant decreased. Timing of insemination in dairy cows can now be performed relative to first mount of estrus, with highest probability of pregnancy occurring between >4 to 16 h after onset. If onset of estrus is not known, inseminations should be performed at the next most convenient time within 3 h.

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Introduction

Reproductive efficiency is an important component of overall herd profitability in today's dairy industry. Accurate detection of estrus and optimum timing of AI remain the major problems limiting reproductive efficiency in many dairy herds (Larson and Ball, 1992; Senger, 1996). Poor reproductive efficiency results in lower profits due to increased days open, leading to lower lifetime milk yields and higher breeding and health costs per cow per year. Studies are available reporting the cost of a day open beyond a "reasonable" amount to range from about \$1.00 to \$2.50 per cow per day open for herds in the U.S. (Olds et al., 1979; Schmidt, 1989).

It is believed that the single most limiting factor to efficient reproductive performance in the national dairy herd is poor estrus detection, with an estimated loss of over \$300 million per year (Senger, 1995). Many farmers simply have insufficient manpower and time available to observe for signs of estrus on a continuing basis. A relatively new system for the identification of mounting activity associated with the expression of estrus known as HeatWatch® (DDx Incorporated, Boulder, CO), has been developed to alleviate the need for visual observation and provide accurate information about the onset of mounting activity (Walker, et al. 1996). The HeatWatch® system consists of pressure sensors that

transmit mounting activity (cow identification, time, and duration of mount) to a remote antenna, coupled with a buffer which stores mount information prior to integration with computer software. One study performed at the VPI Dairy Center showed the accuracy of detection was similar for HeatWatch® when compared with visual observation (96 versus 94%). However, use of the HeatWatch® system increased efficiency of detection (56 to 96 %) during a 4-month period (Walker et al., 1995).

HeatWatch® was the first commercially available system to accurately record the first mount of “standing” estrus, as well as subsequent mounts and duration of mounting activity for each estrus period. Programs practiced on most U.S. dairy farms for the detection of estrus are not sufficiently intense to provide the information needed to accurately time AI, thus preventing AI from attaining its full conception rate potential. The HeatWatch® system provides an accurate method of documenting characteristics of estrus to further examine the optimum timing of AI.

A previous study performed at Virginia Tech examined ovulation in dairy cattle relative to first mount (onset of estrus) as recorded by HeatWatch® (Walker et al., 1996). In this study, Holstein cows ($n = 51$) were either allowed to cycle naturally ($n = 33$) or were induced into estrus with $\text{PGF}_{2\alpha}$ ($n = 86$). Following the onset of estrus, ultrasound examination of ovaries was conducted at 12, 20, and 24 h after

initial mounts and then every 2 h until 40 h to observe the presence and rupture of a dominant follicle. If rupture of the dominant follicle had not occurred by 40 h, ultrasound examination ceased and the cow was designated as anovulatory. Mean ovulation time relative to first mount was 27.6 ± 5.4 h, with no significant difference between spontaneous and PGF_{2α} induced estruses (27.3 versus 28.1 h).

It has been reported that transport of viable sperm in the bovine female reproductive tract takes at least 6 h and possibly more than 12 h for sperm transport to the site of fertilization (Wilmot and Hunter, 1984; Hawk, 1987). Assuming that normal sperm remain viable in the female reproductive tract for 24 h (Hawk, 1987), combined with ova viability of approximately 8 h and a mean ovulation time of 27.6 h (Walker et al., 1996), the optimal timing for AI should be between 6 and 18 h following the first mount recorded by HeatWatch®. This hypothesis provides the basis for research reported in this thesis.

The objective of the research reported herein was to evaluate optimal timing of AI in dairy cattle. Utilizing the HeatWatch® system for precise recording of the first mount of “standing estrus”, which by definition is the onset of estrus in cattle, the specific objectives were 1) to observe effect of timing of AI relative to first mount recorded by HeatWatch® on pregnancy percentages in commercial dairy herds (n = 17) from a three state area; 2) to examine in detail the relationship between characteristics of estrus such as duration and total number of mounts per

estrus as determined by HeatWatch® and pregnancy percentages in dairy cattle; 3) to evaluate effect of milk yield and possible negative energy effects on reproductive efficiency and characteristics of estrus; and 4) to determine additional factors that affect estrus characteristics such as housing, season, and parity.

Results of this study may provide valuable information for improving timing of AI in cattle, as well as add to the basic knowledge of the relationship between characteristics of estrus and milk yield with efficiency of reproduction. This study was conducted in herds from Virginia (n = 12), West Virginia (n = 1), and North Carolina (n = 4), with varying management styles for feeding and reproduction.

Literature Review

The Bovine Reproductive Cycle and Characteristics of Estrus

The estrous cycle of the bovine female exhibits rhythmical changes of both the reproductive system and circulating hormone levels (Salisbury and VanDemark, 1978). Estradiol, combined with the “relative” absence of progesterone, acts on the hypothalamus to induce behavioral estrus (Allrich, 1994). It has been reported that the effects of estradiol may be “all or none”, that is, when a certain level of estradiol is achieved, estrus is induced regardless of subsequent levels of the hormone in circulation. The highlight of the estrous cycle is the period of estrus when the female is receptive to insemination by the male. Most studies have shown the length of the estrous cycle to be between 18 and 24 d (Moeller and VanDemark, 1951; Trimberger and Fincher, 1956; Salisbury and VanDemark, 1978).

Behavioral changes signal the onset of estrus in cattle. Cows in estrus normally stand to be mounted by herdmates in addition to other physical manifestations, such as swelling and redness of reproductive tissues due to increased blood flow and tissue hydration, frequent bawling, and mucous discharge (Foote, 1974). It is believed that as intensity of estrus increases, occurrence of conception also

increases (Stevenson et al., 1983). Nebel et al., (1996) found at the VPI Dairy Center that as number of mounts increased, pregnancy rates or percent pregnant increased.

Hurnik et al., (1975) reported behavior of estrus for Holstein cows (n = 36), each observed continually using two low light video recorders. The distribution of mounts showed the highest frequency for onset of estrus occurred between 1800 to 2400 h; while the highest frequency of the end of mounting activity occurred between 0600 and 1200 h. Nebel et al., (1992) reported that lactating cows (n = 45) exhibited 48% of initial mounts between 0001 and 0800 h. Although the number of cows showing mounting behavior on HeatWatch® reached a peak around 0800 h, the number of mounts per time period peaked near 2000 h. Stevenson et al., (1995) reported that for crossbred heifers (n = 50) equipped with HeatWatch®, 35% of estrus onsets occurred between 0001 and 0600 h as compared with 12.5% occurring between 0601 and 1200 h. Other studies concur that more estrus periods occur in late evening or early morning (Esslemont and Bryant, 1976, Foote, 1978), although Trimberger in 1948 showed an approximately even distribution of cows coming into estrus during both a.m. and p.m. hours. In still another study, the majority of estrus onsets for beef cows (n = 74) occurred between 0601 and 1800 h (Borger et al., 1996).

Duration of estrus was reported by Trimberger and Davis in 1943 to average 17.8 h in lactating cows and 15.3 h in heifers, although Hurnik and King (1987) and Borger et al., (1996) reported the average duration of mounting activity (standing activity) in beef cows to be 4.4 h and 10.0 h, respectively. Gwazdauskas et al., (1983) reported only 17.7% of animals initially observed in estrus were still seen standing to be mounted by herdmates 12 h later. Allrich (1994) reported the mean duration of estrus to be 12 to 16 h in dairy cows, with a range of 3 to 28 h, and Baker and Seidel (1985) reported standing estrus in most European breeds of cattle to last 6 to 18 h.

Number of animals in estrus at any given time may affect expression of estrus; as more cows come into estrus at any given time, the frequency of mounting increases along with duration of estrus (Esslemont et al., 1980). Helmer and Britt (1985) investigated mounting behavior in Holstein heifers ($n = 9$) synchronized with PGF_{2 α} injections 11 d apart. The heifers were then observed four times daily for 1 h periods to observe and quantitate mounting behavior. For each observed estrus ($n = 118$), as the number of heifers in estrus simultaneously increased from 1 to 5, the average attempted mounts per heifer increased from 10.1 to 63.5.

Whittier et al., (1996) described behavioral estrus in beef cows ($n = 110$) either synchronized or not synchronized for estrus. The average estrus consisted of 21.7 ± 19.6 mounts with a duration (h) of 7.9 ± 6.4 for non-synchronized cattle,

and 48.2 ± 42.3 mounts with a duration (h) of 12.4 ± 5.6 for cattle synchronized into estrus. Time of onset (first mount) was documented as well for 6 h periods in both synchronized and non-synchronized cows, with 13.8% of cows beginning mounting behavior at 0000 to 0600 , 34.5% at 0600 to 1200, 24.1% at 1200 to 1800, and 27.6% from 1800 to 2400 in the non-synchronized estrus periods. Estrus synchronization programs may increase intensity of estrus because of the increased numbers of animals in standing estrus at one time.

Stevenson et al., (1996) reported a duration of standing estrus in beef heifers ($n = 39$) as recorded with HeatWatch® to range from 2.6 to 26.2 h with an average of 14 ± 8 h . The mean number of mounts was 50.1 ± 6.4 . Nebel et al., (1992) observed lactating Jersey ($n = 20$) and Holstein ($n = 25$) cows utilizing an early version of the HeatWatch® radiotelemetric estrus detection system for various characteristics of estrus. The average estrus duration was reported to be 12.1 h, with the mean number of mounts per estrus of 14.1.

In one of the most recent studies observing estrus activity, Walker et al., (1996) reported mean estrus duration as recorded by HeatWatch® to be 9.5 ± 6.9 h. In this particular study, total mounts per estrus period was 10.2. The differences in mounting activity between results from Walker et al., (1996) and results reported

here may be explained by age and lactational stress as well as footing conditions (confinement housing versus pasture or drylot).

Britt et al., (1986) reported that duration of estrus as well as number of mounts was greater for cows housed on dirt than those confined to concrete lots. Thirteen lactating cows were ovariectomized 4 to 6 weeks postpartum and induced into estrus six times during the postpartum period. Cows were observed at 8 h intervals during each estrus cycle for 30 min on dirt and 30 min on concrete. When cows were observed on dirt lots, estrus duration was 4.4 h longer than cows observed on concrete lots (13.8 versus 9.4 h). Moreover, average number of observed mounts was greater in the cows observed on dirt lots (7.0 versus 3.2). Britt et al., (1986) reported that standing and mounting activity was 40 to 119% greater on when cows were located on dirt rather than on concrete lots.

Reimers et al., (1985) observed the relationship between signs of behavioral estrus at insemination and percent pregnant for 2696 Holstein cows. Highest rates of pregnancy occurred for cows observed in “standing” estrus (51.3%) when compared to those not standing but showing other physical manifestations of estrus such as: unusual activity, restlessness (49.6%); rough tail head (48.8%); presence of mucus discharge (44.2%); blood on vulva (33%).

Interestingly, Stevenson et al., (1983) examined results from 732 inseminations in Holstein cows and heifers. Females were submitted for AI based on various criteria of estrus; either 1) “standing estrus”, 2) some mounting activity, or 3) no mounting activity but other signs of estrus including excessive vocalization or presence of clear mucus. Higher conception rates occurred with mounting activity or standing estrus, suggesting that mounting activity may promote higher conception rates. Nebel et al., (1994) reported similar results for cows (n = 7240) inseminated in a large field trial, where cows observed in “standing estrus” (n = 4622) showed higher nonreturn (NR) rates (63.4 %) when compared with cows exhibiting only secondary indicators of estrus (n = 1057) such as mounting activity, clear mucus discharge, excessive vocalization (59.5 %). Cows receiving PGF_{2α} injections for synchronization of estrus (n = 126) without observed estrus showed a significantly lower NR rate (35.8 %), and cows submitted for AI on the basis of veterinary recommendation alone (n = 73) had a NR rate of 35.6 %.

Unlike most other species, ovulation in the bovine does not occur until after behavioral estrus. Ovulation is believed to be an inflammatory event, possibly due to the peak of LH that coincides with the onset of estrus behavior (Espey, 1980; Allrich, 1994). Although timing of ovulation relative to stage of estrus has been examined for many years, only with more recent advances in ultrasonography have

researchers been able to verify more exactly the timing of ovulation. As early as 1948, Trimberger reported that cows ($n = 86$) average 10.7 h to ovulation from the end of standing estrus. This information, when combined with the reported mean estrus duration of 17.8 h, would provide for an expected mean ovulation time of approximately 28 h after estrus onset. Allrich (1994) stated that the onset of estrus precedes ovulation by 24 to 30 h. As discussed in the introduction, Walker et al., (1996) used ultrasound scanning of ovaries to observe the presence of follicular rupture using 119 estrus periods expressed by cows located at the Virginia Tech Dairy Center. Mean ovulation time relative to first mount recorded by HeatWatch® was found to be 27.6 ± 5.4 h. Reported ovulation time was not affected by $\text{PGF}_{2\alpha}$ administration for inducing estrus.

It should not be surprising that the two findings 48 years apart are similar since timing of ovulation should not have changed over the years. Researchers have shown that the events leading to ovulation are controlled hormonally and physiologically in the cow, and probably would not be strongly influenced by management changes and increases in milk yield that have occurred in the US dairy industry during the past 48 years.

Sperm transport has been studied extensively in the bovine by Hunter and Wilmut (1983, 1984). Their earlier experiment, using cross-bred Holstein or Charolais heifers, used ligation and removal of oviducts, together with examination of the

ova to establish how quickly after natural service (either 6 , 8 , and 12 h) sperm become available to fertilize the egg. After 6 h, there were no fertilized ova present in the oviducts, however at 8 and 12 h an increasing trend in the number of fertilized ova and accessory sperm was documented.

In 1984, cross-bred heifers were observed “frequently” between 06:30 and 21:00 to detect the onset of estrus. Animals were inseminated via natural service within 8 h after onset of estrous, and ligations of the oviduct were performed at 6, 8, 10 and 12 h post-insemination. Results of the subsequent collection and examination of the oviducts, with analysis of the eggs recovered suggested that the presence of a population of viable sperm was established over a period of time not less than 6 h and probably greater than 12 h (Wilmut and Hunter, 1984). Dobrowolski and Hafez (1970) also suggested viable sperm are present 8 to 12 h after mating in cattle. Utilizing the HeatWatch® system, Walker et al., (1996) reported that ovulation occurs about 28 h after first mounting activity, and when combined with an approximate sperm transport of 12 h, the sperm are apparently stored in the oviduct for 12 to 16 h or greater, possibly allowing for capacitation prior to fertilization. Hawk (1987) stated that fertilization failure is usually a result of failure of sperm to contact the oocyte rather than unfertilizability of ova. This body of research would suggest that timing of AI is vital for high conception rates in cattle.

Estrus Detection Aids

A major limitation for increasing dairy cattle fertility and reduction of calving interval and days open is failure of the detection of estrus (Williams et al., (1981). Foote (1974) stated that accurate detection of estrus is vital for high milk yields and reproductive efficiency. Senger (1994) proposed the following requirements for an “ideal” estrus detection device; 1) continuous (24 h) surveillance; 2) accurate and automatic identification of individual animals in estrus; 3) operational for the life of the cow; 4) minimal or no labor requirements to the manager; and 5) 95% or greater in accuracy of detecting all heats on the farm (“identifying appropriate physiological or behavioral events that correlate highly with ovulation”).

Behavioral signs discussed earlier provide the basis for most methods of estrus detection. Probably the simplest and most common method of detection is visual observation of mounting activity, pacing and restlessness, and discharge and/or swelling of the vulva. (Foote, 1974; Williams et al., 1981). The most frequently stated recommendation for visual observation of estrus is for twice daily observation periods, although it is generally agreed that some estrus periods may be missed without additional use of devices for detection. Tail paint or chalk is also used by many herds to observe signs of activity, where the chalk is placed

regularly on the tailhead of the animal and observed daily for signs of mounting activity.

Chin ball markers placed on sterile bulls or androgenized females are used in some areas for detection of mounting activity, as well as video cameras in herds primarily in the upper Midwest region of the United States.

The KaMar™ (KaMar Marketing Group, Portland, ME) heat mount device was described in detail by Foote (1974) as a pressure sensitive device which could potentially provide continuous monitoring for mounting activity associated with estrus. The KaMar™ device consists of an ink-filled plastic sleeve mounted on the rump of the animal to be observed; when activity takes place, certain amounts of force will rupture the sleeve and turn the indicator red, signaling mounting activity for that animal. The advantages of such a device would include easy application and continuous surveillance for mounting activity. Results were reviewed showing the KaMar™ to be a more efficient device for detection than visual observation. One study observed 107 dairy cows continuously 24 h per day for 21 days, in order to compare visual observation with the KaMar™ devices for efficiency of observation. The KaMar™ devices were activated for 98% of the cycling cows compared with 89% detection for the 24 h visual observation by herdspersons (Williamson et al., 1972).

A contrasting study by Gwazdauskas et al., (1990) evaluated effectiveness of rump mounted devices (KaMar® and Hot Flash®) when either alone or combined with androgenized females equipped with a “chin-ball” marker. The control animals (no estrus detection device) were visually observed twice daily and time of estrus recorded. For the control group, 92.6% were observed in estrus, as compared with nearly 80% observed with the KaMar® devices. There was an increase in percentages of estruses detected (6.2%) when the KaMar® devices were combined with the androgenized females. A high rate of ineffectiveness was revealed for rump-mounted devices when used as the sole method of estrus detection.

Williams et al., (1981) reported a 29% accuracy of estrus detection with the rump mounted devices when compared with a visual detection accuracy of 95%. As noted previously, Nebel et al., (1994) also showed a lower NR rate for cows inseminated on the basis of a positive KaMar® (50.8 %) as compared to animals observed in “standing estrus” (63.4 %).

As might be expected, the disadvantages of the pressure sensitive devices are mainly related to high incidence of false positives from cows rubbing against free stalls, alleyways, branches, and other structures. In addition, the loss rate has been reported as high as 40% (Gwazdauskas et al., 1990) for the rump mounted devices as used on the farm.

Electrical resistance (impedance) of secretions and tissues of the female reproductive tract has been studied in relation to onset of estrus in cattle (Heckman et al., 1979, Kitwood et al., 1993, Foote et al., 1979; Lewis and Newman, 1984). Smith et al., (1988) using an implantable bipolar electronic probe, reported that substantially higher tissue conductivity values are obtained during estrus. It would appear that the changes in conductivity are due to tissue hydration and increased blood flow in the reproductive tract during the period of standing estrus (Lewis and Newman, 1984). Interestingly, Smith et al., (1988) raises the question as to whether the changes in conductivity are more highly correlated with onset of estrus or time of ovulation in the animal. Foote et al., (1979) found that a vaginal “probe” measuring impedance was at least as effective as visual observation of estrus. Kitwood et al., (1993) reported on two experiments designed to investigate the efficacy of a vaginal mucus impedance meter for detection of estrus. Dairy cows (n = 191) were inseminated either after visual observation of standing estrus or with combination of visual observation plus testing for vaginal mucus impedance. It was found that there were no significant differences in reproductive performance (conception rate or detection rate) between the groups. An implanted sensor is not currently available in the industry; however, hand-held probes which measure changes in impedance and claim to identify characteristics associated with estrus are available for use. Limitations of the hand-held probes are readily

apparent; these include high labor requirements for daily probing of animals, proper sanitation of the probe to prevent the transfer of infection, and the necessity of a careful evaluation and recording system to identify false positive readings.

Another detection device currently available is the pedometer, which measures physical activity or number of steps taken during a given period of time. Cows with pedometers have shown significantly higher walking activity during estrus than during late diestrus and proestrus or during metestrus (Farris, 1954). Pedometers are mechanically activated monitors mounted on a leg of the cow to be monitored (Senger, 1994), and normally read when entering the milking parlor. Studies have shown that pedometer readings increase by 200 to 400% during standing estrus (Kiddy, 1977; Lewis and Newman, 1984). Senger reports in his review (1994) that the efficiency of pedometer use versus visual observation can vary greatly and may range from 60 to 100% efficiency. It was estimated by Lewis and Newman (1984) that pedometers can be used to detect a minimum of 37%, 70%, and 74% of the cows in estrus for the first, second, and third ovulation postpartum, respectively .

Another study by Williams et al., (1981) utilized Holstein heifers (n = 12) fitted with both pedometers and KaMar® devices for detection of estrus. Heifers were observed for signs of standing estrus twice daily for 30 minutes. Efficiency of observation reported was 68% for visual detection and pedometer activity peaks at

two times the standard deviation, and 74% for pedometer activity peaks at plus one standard deviation. Not surprisingly, combined techniques for detection yielded higher detection rates than use of a single device for detection. Visual observations plus use of pedometers yielded a 93% detection rate of known estrus periods.

Redden et al., (1993) evaluated pedometer use as a method of estrus detection, utilizing multiparous Holstein cows ($n = 10$). Activity at estrus ($n = 25$) was compared with mean activity since previous estrus. Mean daily activity was increased 2.3 fold during estrus ($P < .0001$), with 80% of estrus periods detected accurately with only four false positives.

A study reported by Liu and Spahr (1993) utilized pedometers (Heat Seeker®, Bou-matic Inc., Madison, WI) attached to cows ($n = 24$) approximately 30 d postpartum. Estrus periods ($n = 66$) were observed when “2 h activity counts” were used. The Heat Seeker® system detected 74% of the predicted periods of estrus compared with 58% observed visually. There are several problems with pedometers, including false readings as a result of increased movement of animals, whether by agitation or management. Williams et al., (1981) showed that the accuracy of detection was lower (83 versus 95%) for pedometers as compared with visual detection. In addition, the use of pedometers in stanchion or total confinement systems may be limited.

HeatWatch® is the newest system available for detection of estrus. Pressure sensitive transmitters are placed in flexible patches and mounted on the tailhead region of the cow or heifer to be observed. When the animal is mounted, the transmitter sends a radio signal to a receiver (antenna) centrally located on the farm. The receiver, hard-wired to a buffer, gathers information for the buffer and computer software. HeatWatch® software collects individual data for each estrus, including cow ID, time of onset, time of individual mount events, duration of mounts, and other breeding and pregnancy information. Originally developed by DDX, Inc. (Boulder, CO), HeatWatch® has been shown to have over 95% accuracy of detecting estrus (Walker et al., 1995). In one study comparing HeatWatch® with visual observation, visual observation identified only 61.3% of estrus periods documented by HeatWatch®, whereas only 2.7% of the estrus periods observed visually were not caught by the HeatWatch® system (Nebel et al., 1995). Stevenson et al., (1995) compared the effectiveness of visual observation for characteristics of estrus with HeatWatch®. The HeatWatch® transmitters were applied to yearling beef heifers (n = 50) and estrus was synchronized with melengestrol acetate (MGA) combined with PGF_{2α} injections 17 d after last dose of MGA. Heifers were visually observed for 45 min twice daily. The HeatWatch® system identified a greater proportion (100 versus 73%) of animals in standing estrus ($P < 0.05$) than twice daily observation.

Borger et al., (1996) compared HeatWatch® with visual observation (twice daily for 30 min) for multiparous beef cows ($n = 74$) over a 22 d period. Estrus detection rate was higher ($P < 0.01$) with HeatWatch® than visual observation (87.8 versus 63.5%), and visual observation detected only 69.2% of the estrus periods detected by HeatWatch®.

The advantages of HeatWatch® include continuous 24 h surveillance for mounting activity, which should increase the incidence of detected estrus periods, and theoretically decrease days open. In a trial at the Virginia Tech Dairy Center, HeatWatch® was utilized for the detection of estrus on 160 cows (Jersey and Holstein) to determine pregnancy frequency and optimum time of AI when initial mount of “standing estrus” was known (Nebel et al., 1996). Pregnancy frequency (defined as estrus detection rate x conception rate) increased from 31% without HeatWatch® to 54% following implementation of the system. Following three estrous cycles with HeatWatch®, 90% of the cows were pregnant as compared with 67% prior to use of the system (Nebel et al., 1996).

The most limiting factor for HeatWatch® on the farm is patch maintenance, although labor involved with HeatWatch® has been shown to be 50% lower when compared to the previous detection programs utilized at the Virginia Tech Dairy Cattle Center (Nebel et al., 1994).

A subcutaneous implantable device (SQID) is currently in early development which may provide many advantages of either a pressure-sensitive transmitter or pedometer activity sensor (McConaha et al., 1996). This device would be inserted under the skin on a proposed “permanent” basis (Parish et al., 1994). Interrogation of mounting or other estrus activity would probably be similar in nature to pedometer technology, where the implant would be “read” when the cow entered or exited the milking parlor (Senger, 1994). One advantage of this system could be a potential decrease in labor requirements for detection of estrus.

Disadvantages of the SQID device may include difficulty of insertion and the inability of mount information to be transmitted to a computer on a real time basis, thus preventing the possibility of accurate timing of insemination.

Timing of Insemination

Optimal timing of insemination relative to stage or onset of estrus has been under investigation for nearly 70 yr. As early as 1918, Gowen reported that ideal timing of insemination occurred 10 to 24 h after onset of estrus. Herman (1939) reported the results of time of breeding based on four time classifications. The time classifications were as follows; 1) 4 to 12 h after first signs of estrus, 2) 12 to 24 h

after first signs of estrus, 3) 24 to 48 h after first signs of estrus, and 4) 48 to 60 h after first signs of estrus. The lowest number of inseminations per pregnancy was 1.71 for the early insemination group. In a review by Trimberger and Davis (1943), it was concluded that fewer services per conception were required when animals were showing active “estrus” activity.

Barrett and Casida (1946) tabulated results for inseminations in herds using AI technician service, where the owner reported the time when cows were observed in estrus (n = 3841). They observed very little effect on conception rates of animals bred at varying intervals from 3 to 25 h after first sign of “standing” estrus.

In a similar trial, Foote (1978) evaluated non-return rates for 44,707 cows bred by technicians affiliated with a major U.S. AI organization. As in the previous study, timing of insemination was examined in relation to when the animals were first “called in” for service by the technician. When NR rates for various intervals to AI were compared, there was no significant change in percent NR for cows inseminated before 1200 h the same morning of observation, when compared with animals inseminated between 1200 and 1800 h, or after 1800 h the same day. Insemination the following a.m. (+24 h) was too late, however, for optimum NR rates.

Roche (1974) looked at optimal timing of AI in heifers. Eighty-four Hereford heifers were randomly assigned to various treatment groups and compared with 24 control heifers inseminated after onset. Progesterone implants were utilized for estrus synchronization. Highest pregnancy rates occurred in heifers inseminated at estrus and either 6 or 12 h after GnRH administration, as compared with heifers inseminated later in the cycle with treatments of estradiol benzoate or HCG.

Nebel et al., (1994) evaluated NR rates relative to either once daily or a.m.-p.m. AI for 7240 first service Holsteins to observe optimal timing of AI. NR rates for once-daily and a.m.-p.m. insemination were 64.6 and 65.6% for 60-d, 60.1 and 60.6% for 75-d, and 58.4 and 57.8% for 90-d NR cycles, with no significant difference in the once-daily and a.m.-p.m. methods used. This suggests not only a large optimal window for AI, but also that early inseminations may not be significantly lower in pregnancy rates than inseminations performed later during estrus. The highest reported NR rates were observed in animals inseminated between 0800 and 1100 h. Gwazdauskas et al., (1981) evaluated first and second service inseminations (n = 1004), comparing cows bred immediately upon observation of estrus with cows bred 12 h after first observation. With twice daily observations, timing of insemination had no effect on fertility, although cows still standing 12 h after first observation of estrus had higher conception rates than

cows not standing 12 h after observed in estrus. This suggests that duration and intensity of estrus may have a noticeable effect on pregnancy results.

In contrast, what has become the “industry standard” for timing of insemination is the early work by Trimberger and Davis (1943), who evaluated conception rates in dairy cattle at various periods during estrus. Although the records are primarily based on Jersey, Guernsey, Ayrshire, and Holstein cattle, this study has remained the most often quoted in the industry, and this paper will be reviewed below in detail.

Cows and heifers (n=295) were observed three times per day for signs of estrus (“complacent when standing to be mounted”), and individuals found to be in estrus were observed every 2 h, in order to more accurately determine end of estrus. Inseminations were with fresh semen, placed cervically, and performed at various stages both during and after estrus. The overall observed pregnancy percentages for the study were 63.4%. The percent pregnant by stage of estrus were reported as follows; start of estrus (defined as animals inseminated the first 6 h of estrus [n = 25]), 44%; middle of estrus (in estrus at least 6 h both before and after insemination [n = 40]), 82.5%; end of estrus (in heat at least 12 h before insemination [n = 40]), 75%; 6 h post-estrus,[n = 25] 62.5%; 12 h post-estrus, [n = 25] 32%; and 18 h post-estrus [n = 25] of 28%. Number of inseminations at each time interval ranged from 25 inseminations performed for cows during the

first 6 h of estrus to 40 inseminations for groups bred in middle to end of estrus. Trimberger and Davis concluded that best rates of conception are obtained when insemination occurred during the middle to end of “standing estrus”. In 1948, Trimberger would go on to implement what is known in the industry as the “a.m.-p.m. guideline”, where cows first observed in estrus in the a.m. should be inseminated the afternoon of the same day, and cows first observed in the evening should be inseminated before 1200 h the following day. This rule is widely accepted by the industry, and is still implemented today (Nebel et al., 1994).

Broadway et al., (1975) looked at time of insemination related to season and embryonic loss in a herd of Holsteins at Texas A&M University. Although embryonic loss was greatly attributed to heat stress and warm season effects, it was concluded that inseminating cows according to the “a.m.-p.m. rule” or 12 h after observation of estrus was not optimal, especially during warm seasons of the year.

Rankin et al., (1992) used data from 372 virgin heifers to study the effect of timing of insemination relative to onset of estrus. Heifers were observed several times daily, with most estruses observed between 0700 and 1600 h daily. Inseminations (n = 676) were performed 0 to 33 h after first observed estrus, and the percentages of pregnant heifers were calculated for three time groups; 0 to 7 h, 7.1 to 13.5 h, and >13.6 h. For first AI, least squares analysis showed the highest pregnancy

percentages (55%) occurred in heifers inseminated within 7 h of first observation. Heifers inseminated > 13.5 h post observation had a 19% decrease in conception rate when compared with the early group. The group inseminated during the middle of the cycle was not significantly different for percent pregnant from the early AI group, at 51%.

An interesting trial looking at time of AI in Boran (*Bos indicus*) cattle looked at pregnancy percentages following AI at varying times after PGF_{2α} induced estrus (Mukasa-Mugerwa et al., 1989). Both cows and heifers (n = 56) were inseminated at either 6, 12, and 18 h following observation of estrus. Time of AI affected pregnancy percentages ($P < 0.05$), with the highest pregnancy percentages occurring in cattle inseminated 6 h after observation of estrus.

Although optimal time of AI has been investigated for many decades, recent advances in estrus detection technology have allowed for a larger scale study on timing of AI relative to pregnancy percentages. Larger studies with greater numbers of animals, combined with a variety of management scenarios, will allow for more accurate estimation of ideal timing of AI. In addition, more information on virgin heifers relative to timing of AI should be evaluated. The purpose of this research is to determine what effects time of insemination relative to stage of estrus have on increasing rates of pregnancy in dairy cattle. The HeatWatch® system provides a method of continuous, accurate observation of mounting

characteristics and allows for more careful documentation of timing of AI for research and in the field.

Materials and Methods

Seventeen commercial herds participated in a study designed to evaluate effect of timing of insemination on resulting pregnancy in dairy cattle. The herds were chosen to represent a wide variety of herd sizes (65 to 629) and management styles. Three of the herds were classified as total confinement (no specified observation period for estrus off concrete housing surface), two herds utilized grazing programs, and the remainder were classified as “free stall to pasture or dry lot” herds, with possible extended pasture periods during any 24 h period. Three of the seventeen herds employed three times per day milking, and herds on DHI testing programs had rolling herd averages ranging from 7330 to 10847 kg per year. Three herds were not on any DHI testing program. All herds consisted of Holstein cattle, with the exception of the Virginia Tech dairy herd, which also had Jersey cattle.

All herds utilized the HeatWatch® system to detect onset of estrus and record mount activity. Mounts of greater than one second were recorded in the program software, and the program software classified a “standing” estrus as three mounts in any 4 h period; fewer mounts than this standard were noted and animals observed for secondary signs of estrus such as restlessness or mucus discharge by the herds person. The ultimate determination of estrus and decision to AI was

made by the inseminator. Inseminations were performed daily during a three hour period chosen by the herdowners for all animals identified in estrus by HeatWatch® the previous 24 h. The once-daily inseminations provided for data collection for all intervals 0 to 26 h following first mount recorded by HeatWatch®. Five of the herds utilized HeatWatch® on heifers in addition to lactating cows.

Time of AI was recorded by inseminator in a daily logbook provided by researchers. Number of inseminators per herd varied (1 to 4); herd effects for percent pregnant were included in the overall model for percent pregnant. Return to estrus information, individual cow mount data, and monthly breeding records were collected on a monthly or bi-monthly basis from program software. Timing of AI information was collected at this time, along with other relevant information (service sire, lactation number, calving date). Data from each herd were sent via computer to DDx, Inc.,(Boulder, CO) for verification and translation into individual records which included cow number, date, time (month, day, hour, and minute), and duration of each mount.

For herds on DHI, production information including test day milk closest to insemination, summit milk, within herd rating, and 305-d projected mature equivalent (ME) milk was gathered using Direct Access to Records by Telephone (DART) in order to further examine effect of production on reproductive

efficiency, mounting activity, and estrus duration. Summit milk consisted of the average of the two highest milk weights during the first three test periods. The 305-d ME records were calculated by adjusting projected milk for age and season of calving. Within herd rating categorized cows in five production classes based on their current lactations. Using a 3.5% fat corrected ME, ratings were as follows: A - cows more than 110% of herd average fat corrected ME; B - 100 to 110% of herd average; C - 90 to 100% of herd average; D - 80 to 90% of herd average, and E - less than 80% of herd average for ME. Pregnancy status was determined monthly or bi-monthly with uterine palpation via rectum by the herd practitioner.

Preliminary models characterizing percent pregnant included effects of herd, insemination interval relative to onset of estrus, parity, number of mounts per estrus, duration of estrus (h), days in milk at insemination, season effects, and production variables. Insemination intervals were divided into seven categories based on 4 h increments, in order to investigate timing of AI relative to periods following onset of estrus. Estrus duration was defined as the time interval (h) from first to last mount as documented by HeatWatch® system software. Mount classification (number of mounts) was categorized into three groups (< 3 mounts, 3 to 15 mounts, and >15 mounts), based on HeatWatch® program software, which classifies a standing estrus as 3 or more mounts within 4 h.

Production variables in the preliminary model included effects of test day milk closest to AI, summit milk, 305-d milk ME, and DHI production rating within herd. Test day milk was evaluated in order to observe the effect of milk production at the time of insemination. Production categories were grouped into quartiles within herd according to distribution of that production variable.

Season effects were evaluated according to climactic data for mean temperature from records provided by the National Oceanic and Atmospheric Administration. Only a small number ($n = 160$) of observations were performed during the summer months. Seasons were broken down as follows: Season 1 = November, December, January, and February; Season 2 = March, April, May; Season 3 = June, July, August; Season 4 = September and October.

Simple linear and quadratic regression equations were calculated to observe the effect of interval from first mount to AI on resulting pregnancy in lactating cows and heifers in the study.

Data were analyzed according to logistic binomial regression analysis procedures from SAS (SAS® Institute; Cary, NC). Logistic procedures fit linear logistic regression models for binary response variables such as pregnancy by utilizing maximum likelihood estimates.

Preliminary analyses were conducted to evaluate relationships and associations between variables and percent pregnant for cows and heifers. The preliminary

model for lactating cows was analyzed using the logistic method from SAS, and variable selection was utilized both to find the factors in the model most strongly associated with higher pregnancy percentages, as well as to provide information about possible confounded variables. In addition, herds 7, 11, and 13 were not on DHIA testing programs, so data from these herds were not used in the preliminary model analysis. Final model for cows (services = 2661) included variables that were significant in the preliminary model and deleted non-significant factors. Significance of a variable in the model was calculated by comparing the -2 log likelihood chi-square statistic between the model and a reduced model where the variable to be evaluated was deleted. Resulting differences in the likelihoods provided a chi-squared statistic for determining significance of that variable relative to pregnancy in the model. Confidence intervals (CI, 95%) were calculated in order to observe differences between categories for variables affecting percent pregnant. If the range of the CI contained 1.0, the category was not significantly different from the baseline category for that variable. Calculated odds ratios provided probabilities of resulting percent pregnant for various categories of each variable. Odds ratios (probabilities) for resulting percent pregnant were calculated for seven classes of AI following the onset of estrus; 1) 0 to 4 h; 2) >4 to 8 h; 3) >8 to 12 h; 4) >12 to 16 h; 5) >16 to 20 h; 6) >20 to 24 h; and 7) > 24 h. An odds ratio is defined as the odds of animals becoming

pregnant for each category within a variable when compared with a “baseline” category within the same variable, where baseline odds ratio equals one.

The final statistical model for characterizing percent diagnosed pregnant in lactating cows was as follows:

$$Y_{ijklm} = \mu + H_i + I_j + M_k + D_l + S_m + e_{ijklm}$$

where

Y_{ijklm} = percent diagnosed pregnant 35 to 50 days post AI

μ = overall mean

H_i = the effect of the i^{th} herd ($i = 1, 2, 3, \dots, 17$)

I_j = the effect of the j^{th} interval ($j = 0-4, 5-8, \dots, >24$)

M_k = the effect of the k^{th} mount class ($k = < 3, 3 \text{ to } 15, > 15$)

D_l = the effect of the l^{th} days in milk group ($l = < 75, 75 - 100, > 100$)

S_m = the effect of the m^{th} season ($m = 1, 2, 3, \text{ or } 4$)

e_{ijklm} = random error

Odds ratios were calculated for all reduced model components, including interval from first mount to AI, herd, mount class, (< 3 mounts, $3 \text{ to } 15$ mounts, and > 15

mounts as recorded on program software), DIM at insemination (≤ 75 d, 76 to 100 d, and > 100 DIM), and season at AI.

Logistic analysis was also performed utilizing heifer estrus data (services = 306) from 6 herds. Factors evaluated in the heifer models included herd, interval from first mount to AI, estrus duration (h), mounts per estrus, and season.

The statistical model for characterizing percent diagnosed pregnant in heifers was as follows:

$$Y_{ijklm} = \mu + H_i + I_j + M_k + D_l + S_m + e_{ijklm}$$

where

Y_{ijklm} = percent diagnosed pregnant 35 to 50 days post AI

μ = overall mean

H_i = the effect of the i^{th} herd ($i = 1, 2, \dots, 5$)

I_j = the effect of the j^{th} interval ($j = 0-4, 5-8, \dots > 24$)

M_k = the effect of the k^{th} mount class ($k = < 3, 3 - 15, > 15$)

D_l = the effect of the l^{th} estrus duration class ($l = 0-6, 7-12, \dots$
18-4)

S_m = the effect of the m^{th} season ($m = 1, 2, 3, \text{or } 4$)

e_{ijklm} = random error

Table 1. Profile of herds that participated in research trial (services = 2967)

Herd	Services ^a	Percent Pregnant	Milking Frequency ^b	DHI rolling herd average
	—(no.)—			—(kg)—
1	520	56.7	2x	9785
2	403	41.9	3x	10227
3	214	47.2	2x	9324
4	143	44.0	2x	7579
5	468	47.4	3x	10847
6	132	49.2	2x	7331
7 ^c	73	53.4	2x	-
8	209	47.4	2x	8399
9	186	37.1	3x	10862
10	68	54.4	2x	9356
11 ^c	78	44.9	2x	-
12	45	57.8	2x	9655
13 ^c	89	44.9	2x	-
14	67	41.8	2x	8675
15	34	38.2	2x	7769
16	22	54.6	2x	9039
17	216	43.5	2x	7330

^a Cows and heifers included in total number of services per farm.

^b Frequency of milking was either twice daily (2x) or three times daily (3x).

^c Herd did not participate in DHI programs.

Results and Discussion

Herd and Estrus Characteristics

The overall data set consisted of 2967 inseminations from seventeen herds. Profiles of estrus characteristics for lactating cows (services = 2055) and heifers (services = 292) across herds are presented in Tables 2 and 3. As noted in these tables, herds 3, 4, and 7 removed patches voluntarily prior to the end of standing estrus, and these herds were removed from the final analysis of estrus characteristics.

Using Tukey's studentized range tests, lower pregnancy percentages ($P < 0.05$) were documented for inseminations ($n = 260$) following one mount recorded by HeatWatch® when compared with inseminations ($n = 2401$) for cows which exhibited 2 or more mounts (36 versus 46 %). Because of these differences, cows with only one mount were removed from the data set prior to calculation of means for estrus characteristics to account for possible inseminations of cows not in estrus. Cows with one mount were included in the logistic analysis.

For lactating cows (Table 2), mean mounts per estrus were 8.5 ± 6.6 , ranging from a low of 6.2 (herd 15) to a high of 12.8 (herd 11). For nulligravid heifers (Table 3), mean mounts per estrus were 15.0 ± 12.4 , with a low of 12.0 (herd 16) and a high of 17.3 (herd 1).

Table 2. Profile of estrus characteristics for lactating cows across herds (n = 17) for estrus periods identified by HeatWatch® electronic estrus detection system.

Herd	No.	Characteristics of estrus			
		Mounts ^a		Estrus duration ^b	
		(no.)		(h)	
		\bar{X}	SD	\bar{X}	SD
1	362	7.7	7.2	7.3	5.8
2	351	8.2	6.4	6.9	4.9
3 ^c	176	6.0	5.0	5.8	5.6
4 ^c	115	5.5	4.0	5.2	4.6
5	307	7.4	6.1	6.6	4.8
6	128	8.2	5.8	8.1	5.6
7 ^c	55	3.8	2.4	4.5	5.9
8	202	8.7	7.0	6.4	4.8
9	125	6.4	4.4	6.5	6.6
10	68	9.4	6.1	6.9	4.2
11	76	12.8	9.9	7.8	5.2
12	36	12.0	10.6	8.0	6.0
13	80	7.1	5.1	5.5	5.0
14	63	8.7	6.9	7.9	6.5
15	32	6.2	5.0	5.0	3.8
16	20	8.4	4.0	10.6	6.8
17	205	8.2	8.0	6.3	5.7
Total ^d	2055	8.5	6.6	7.1	5.4

^a Estrus periods with one mount removed from herd and total means.

^b Estrus duration defined as time interval from first to last mount as recorded by HeatWatch®.

^c Patches removed voluntarily after cows were identified in estrus by HeatWatch®.

^d Herds which removed patches prior to end of estrus removed from totals.

Table 3. Profile of estrus characteristics for dairy heifers across herds (n = 6) for estrus periods identified by HeatWatch® electronic estrus detection system.

Herd	Characteristics of estrus				
	No.	Mounts ^a		Estrus duration ^b	
		(no.)		(h)	
		\bar{X}	SD	\bar{X}	SD
1	116	17.3	13.2	10.6	5.6
3 ^c	12	10.0	6.8	8.6	3.8
5	117	15.2	10.0	9.5	5.0
9	51	16.2	9.9	9.6	5.6
12	7	14.4	16.5	10.5	3.9
16	1	12.0	-	9.4	-
Total ^d	292	15.0	12.4	9.9	5.0

^a Estrus periods with one mount were removed from herd and total means.

^b Estrus duration defined as time interval from first to last mount as recorded by HeatWatch®.

^c Patches removed voluntarily after cows were identified in estrus by HeatWatch®.

^d Herds which removed patches prior to end of estrus removed from totals.

Estrus duration (time interval from first to last mount recorded by HeatWatch®) was 7.1 ± 5.4 h for lactating cows. Mean estrus duration (h) in cows ranged from a low of 5.0 (herd 15) to a high of 10.6 (herd 16). In heifers, mean estrus duration (h) was 9.9 ± 5.0 , with a range of 9.4 (herd 16) to 10.6 (herd 1).

This is the first study to quantitate estrus characteristics on a large scale across many herds. The results reported here for mean mounts per estrus in lactating cows are similar to previous reports from studies utilizing HeatWatch® for detection of mounting behavior (Walker et al., 1996). Britt et al., (1986) reported that housing conditions may affect expression of estrus, with cows observed on dirt lots having both a longer duration of estrus and more total mounts than cows observed on concrete. In addition, number of cows in estrus at one time may have a positive relationship with mounting activity. In a 1975 trial, Hurnik et al. reported that the number of mounts per estrus increased from 11.2 with one cow in estrus to 52.6 mounts per estrus when three cows were in estrus at the same time. Synchronization programs have also shown more mounting activity as compared to naturally occurring estrus periods, perhaps due to increased numbers of cows in estrus at one time (Whittier et al., 1996).

As might be expected from the early work by Trimberger in 1948, heifers exhibited both higher number of mounts per estrus and a longer duration of estrus than lactating cows. Lower activity in lactating cows was most likely due to the

stress of lactation and possible effects of negative energy balance, as well as housing that may not have provided the optimal footing conditions necessary for higher mounting activity. Heifers may be similar to beef cows in both energy balance requirements and environment, and consequently show more activity during the estrus period.

Gwazdauskas et al., (1983) reported that mounting activity increased as maximum daily temperature increased to 25° C. Declines in mounts per hour were observed for temperature increases beyond 25° C, suggesting that extreme cold or warm temperatures beyond the cow's comfort zone may inhibit the expression of estrus. Results reported for mean mounts in heifers are lower than results from Stevenson et al., (1995) who reported a mean number of mounts of 50.1, with a duration of 14 h in beef heifers. The system used in this 1995 trial was an earlier version of HeatWatch® which recorded mounts less than one second, possibly including a large number of false mounts. This may explain the higher mounts per estrus as compared with the dairy heifers in the research reported here. Stevenson also employed the feeding of melengestrol acetate (MGA) and injecting PGF_{2α} for the synchronization of estrus in this study, possibly increasing the numbers of heifers in estrus at one time and increasing total mounts.

Distributions of the time of day of onset and termination for estrus periods documented by HeatWatch® in both cows and heifers are shown in Table 4.

In lactating cows, 28.4% of estrus onsets occurred from 0601 to 1200 h, with 19.8% of onsets occurring from 1201 to 1800 h. There were no differences for distribution of onset and end of estrus in lactating cows (services = 2401) among the 6 h periods. Previous studies reported more estrus activity during the late evening and early morning hours (Hurnik et al., 1975; Nebel et al., 1994; Gwazdauskas et al., 1983). In heifers (services = 304), the lowest number of onsets (19.1%) occurred from 0001 to 0600 h, and 34.9% of the estrus periods were terminated between 0601 to 1200 h. This contrasts markedly with the findings of Stevenson et al., (1996) who reported more onsets occurring from 0001 to 0600 h (35%) in beef heifers. The differences in the two studies may be due to different breeds of cattle and timing of synchronization for the beef heifers in Stevenson's study.

Preliminary analysis on a combined data set (heifers and cows) revealed effects ($P < 0.01$) of parity on percent diagnosed pregnant (Figure 1.). Mean pregnancy percent was 65% for heifers (services = 306) as compared with 45% for lactating cows (services = 2661). It was decided to separate heifers and cows for the final analysis, and logistic binomial regression was performed separately for heifers and cows. Odds ratios were calculated for significant effects in the preliminary and final models.

Table 4. Percentage distribution of the time of onset and termination of standing estrus for a 24 h period for lactating cows (services = 2401) and heifers (services = 304) identified in estrus by HeatWatch® electronic estrus detection system. ^{a,b}

Time	Onset of estrus ^c		Termination of standing estrus ^d	
	Cows	Heifers	Cows	Heifers
—(h)—	—(%)—	—(%)—	—(%)—	—(%)—
0001 to 0600	24.5	19.1	24.8	21.4
0601 to 1200	28.4	28.3	27.8	34.9
1201 to 1800	19.8	27.6	23.4	18.7
1801 to 2400	27.3	25.0	24.0	25.0

^a Mounting activity identified by HeatWatch® electronic estrus detection system.

^b Estrus periods with one mount were removed from means for onset and termination of estrus.

^c Occurrence of first mount

^d Occurrence of last mount

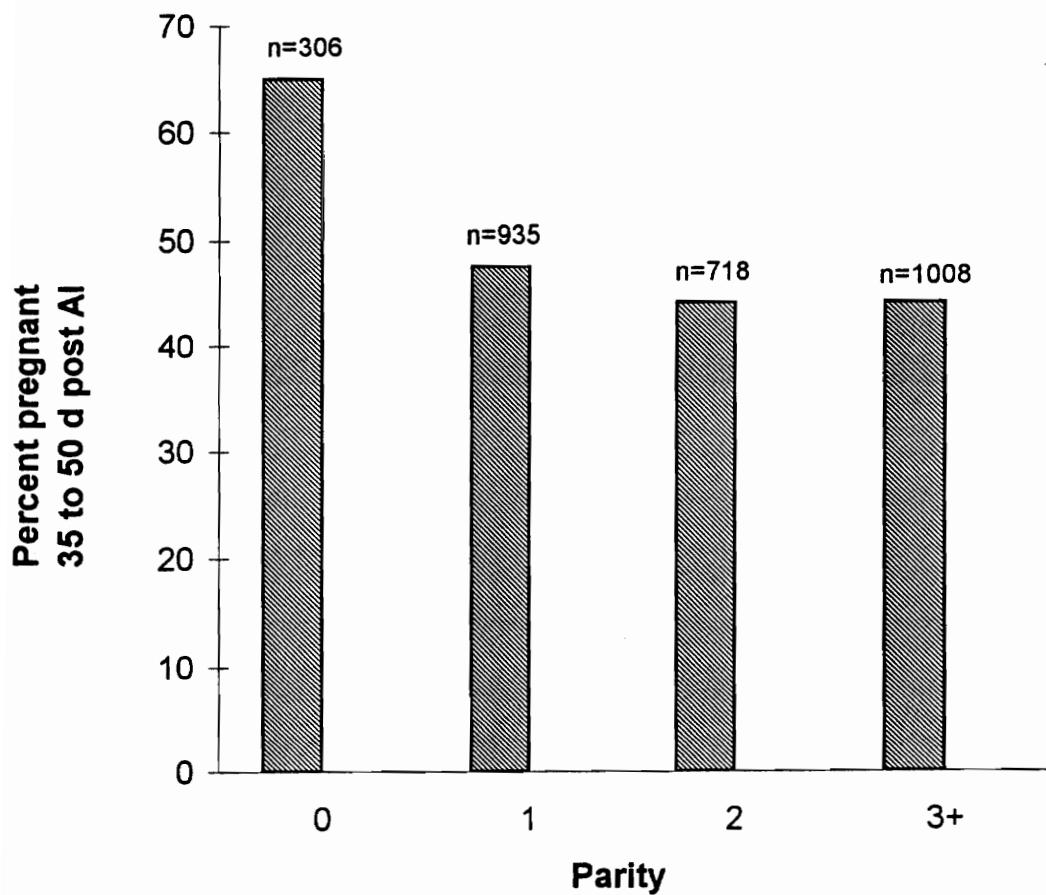


Figure 1. Percentage diagnosed pregnant 35 to 50 d post AI relative to parity ($P < 0.01$) for dairy cows (services = 2967) identified in estrus by HeatWatch.

Factors Associated with Probability of Pregnancy in Lactating Cows

For lactating cows (services = 2661), logistic analysis was performed on a preliminary model including herd, interval, mounts per estrus, parity, season, estrus duration (h), and all production variables. Results from the model analysis and odds ratios for interval in the preliminary model are presented in Tables 11 and 12 in the appendix.

In the preliminary model, cows inseminated 5 to 12 h following first mount had a higher ($P < 0.05$) expected probability of pregnancy when compared to the baseline group of cows inseminated between 0 and 4 h after first mount (Table 12, appendix). In addition, there was a lower probability ($P < 0.05$) of pregnancy for cows inseminated 17 to 20 h following first mount when compared to cows inseminated 0 to 4 h following first mount.

Yield variables in the preliminary model were found to have no effect on resulting percent diagnosed pregnant for lactating cows. This was surprising, as previous studies have reported negative effects of increased summit milk (Lean et al., 1989), test day milk (Faust et al., 1988), and 305-d fat corrected milk (Ray et al., 1992) on reproductive performance. The results reported here may differ due to differences in season of calving, days in milk at insemination, and herd effects on pregnancy percentages.

In order to increase number of observations used to calculate odds ratios for various classes, production variables in the model were deleted so the final model would include observations from herds not on DHIA. Additional non-significant factors of estrus duration and parity were also removed from the preliminary model prior to analysis.

In the final model, factors associated with resulting percent diagnosed pregnant included: interval from onset of estrus to AI, herd, mounts per estrus, season, and days in milk. Effects of these factors are shown in Table 13, appendix.

The final model revealed effects of interval ($P < 0.01$) on percent diagnosed pregnant 35 to 50 days post AI for lactating cows. As the interval increased beyond 16 h, probability of pregnancy decreased.

Table 5 characterizes the logistic binomial regression results for the effects of interval from first mount to AI (services = 2661) on pregnancy percentages. There were lower ($P < 0.05$) odds of pregnancy for animals inseminated later than 17 to 24 h after onset when compared with animals inseminated earlier during estrus. Odds ratios ranged from .18 for inseminations > 24 h following onset, to 1.35 for inseminations occurring from 5 to 8 h following onset of estrus. Highest expected probability of pregnancy occurred for inseminations performed 5 to 12 h following first mount recorded by HeatWatch®, with odds ratios of 1.35 (5 to 8 h) and 1.33 (9 to 12 h).

These figures follow closely the early work by Trimberger and Davis (1943), where the highest pregnancy results were for inseminations occurring during the middle to end of estrus. Differences in the two studies result from a longer duration of estrus reported by Trimberger and Davis (17.8 h) as compared with the mean estrus duration reported for lactating cows in this study (7.1 h). In addition, Trimberger and Davis were not able to pinpoint exact timing of the onset of estrus. For both studies, the recommended time of AI would coincide with the middle to end of standing estrus in dairy cattle. Trimberger utilized 295 cows and heifers in the earlier study, as compared with 2967 overall observations in the research reported here.

Table 5. Logistic-binomial regression results for the effects of interval from first mount to AI (services = 2661) on pregnancy percentages for dairy cows identified in estrus by HeatWatch®^a.

Category	Estimate	SE	OR ^b	95% CI ^c
Intercept	-.61	.21	-	-
—(h)—				
0 to 4	0	-	1	-
5 to 8	.30	.14	1.35	(1.02,1.78)
9 to 12	.28	.14	1.33	(1.00,1.74)
13 to 16	.11	.15	1.12	(0.85,1.50)
17 to 20	-.68	.17	.51	(0.36,0.71) ^d
21 to 24	-.57	.22	.57	(0.37,0.87) ^d
> 24	-1.70	1.09	.18	(0.02,1.55)

^a ($P < 0.01$).

^b OR = “odds ratio”. The OR is the estimated odds of a dairy cow getting pregnant in any one interval when compared to the baseline interval for cows inseminated 0 to 4 h following first mount where baseline interval OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes interval different ($P < 0.05$) from baseline interval.

In the 1943 study, only 25 cows were inseminated prior to the middle of the estrus period compared to 966 services before 8 h in the present study. In addition, the classic study reported by Trimberger and Davis was performed in one herd (University of Nebraska Experiment Station), with four breeds of cattle including Jersey, Guernsey, Ayrshire, and Holstein. The results of the present study include data from 17 herds with varying management styles and housing systems, and primarily Holstein cattle were used in the analyses.

The results from this study are similar to those reported by Rankin et al. (1992) and Mukasa-Mugerwa et al. (1989), where earlier insemination during the estrus period revealed higher rates of pregnancy.

Effect of herd ($P < 0.05$) on pregnancy percentages are shown in Table 6. As expected, herd affected percent diagnosed pregnant in the final model. Herds varied widely in percent pregnant, from 37.1 to 57.8 % (Table 1). This might be expected from differences in reproductive management and herd fertility between herds. In addition, the effect of different technicians may have contributed to these herd differences.

Table 6. Logistic-binomial regression results for the effects of herd on pregnancy percentages for dairy cows identified in estrus (services =2661) by HeatWatch®.^a

Herd Category	Estimate	SE	OR ^b	95% CI ^c
Intercept	-.61	.21	-	-
—(herd)—				
1	0	-	1	-
2	-.31	.15	.73	(0.55,0.98) ^d
3	-.25	.18	.78	(0.55,1.11)
4	-.40	.20	.70	(0.45,0.99) ^d
5	-.45	.16	.64	(0.47,0.87) ^d
6	-.14	.21	.87	(0.57,1.31)
7	-.06	.26	.94	(0.56,1.56)
8	-.28	.18	.76	(0.53,1.07)
9	-.85	.22	.43	(0.28,0.66) ^d
10	.01	.27	1.01	(0.59,1.71)
11	-.48	.26	.62	(0.37,1.03)
12	.06	.35	1.06	(0.53,2.10)
13	-.41	.24	.66	(0.41,1.06)
14	-.48	.29	.62	(0.35,1.09)
15	-.64	.38	.53	(0.25,1.11)
16	-.02	.46	.98	(0.39,2.40)
17	-.49	.18	.62	(0.43,0.87) ^d

^a ($P < 0.05$)

^b OR = “odds ratio”. The OR is the estimated odds of getting a dairy cow pregnant in a particular herd when compared to the baseline herd 1, where baseline herd OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes herd different ($P < 0.05$) from baseline herd 1.

Effects of other factors associated with percent pregnant in the final model are shown in Table 7. Stevenson et al., (1983) reported the best temperature for conception ranged from 0 to 30° C for 732 inseminations performed on Holstein cows and heifers. Ingraham et al., (1974) reported average daily temperature and humidity accounted for 80% of the variation in conception by months. For effects of season of AI, the baseline group consisted of cows inseminated in September and October. Season affected pregnancy, with a higher expected pregnancy percent ($P < 0.05$) occurring for inseminations between March and May. Odds ratio for inseminations occurring between March and May was 1.45, with a 95% CI of (1.12,1.86). Odds for percent diagnosed pregnant in cows inseminated during the summer months of June to August were 12% lower than for inseminations performed from November to February, although this difference was not significant. This expected drop in conception is not surprising considering the mean temperatures for the region during those summer months and the additional stress the cows in the study experienced during the warm months. This agrees with Broadway et al., (1975), who evaluated time of insemination related to season in Holsteins, and reported higher rates of embryonic loss during periods of heat stress.

Table 7. Logistic-binomial regression results for the effects of season, days in milk, and mounts per estrus (services =2661) on pregnancy percentages for dairy cows identified in estrus by Heatwatch®.

Category	Estimate	SE	OR ^a	95% CI ^b
Intercept	-.61	.21	-	-
—(Season) ^d —				
Nov.-Feb.	.13	.12	1.14	(0.90,1.47)
Mar. - May	.37	.13	1.45	(1.12,1.86) ^e
June - Aug.	-.13	.22	.88	(0.57,1.35)
Sept. - Oct.	0	-	1	-
—(Days in milk) ^e —				
<= 75	0	-	1	-
76 to 100	.12	.12	1.12	(0.89,1.42)
> 100	.38	.10	1.46	(1.20,1.78) ^e
—(Mounts per estrus) ^f —				
<= 2	0	-	1	-
3 to 15	.35	.10	1.41	(1.16,1.73) ^e
> 15	.34	.16	1.41	(1.02,1.92) ^e

^a OR = “odds ratio”. OR is the estimated odds of dairy cows getting pregnant in one category within a variable when compared to the baseline category within that variable, where baseline category OR = 1.

^b CI = confidence interval

^c CI not containing 1.0 denotes category different ($P < 0.05$) from baseline category of that variable.

^d ($P < 0.05$)

^e ($P < 0.01$)

^f ($P < 0.01$)

Mean percent diagnosed pregnant relative to season of year for lactating cows in the seventeen herds is shown in Figure 2. As suggested by the logistic analysis, highest pregnancy percentages occurred for inseminations performed between March and May. The three other seasons did not differ in percent pregnant. Gwazdauskas in 1985 reported that extreme cold or warm temperatures adversely affected expression of estrus, and it appears that either extreme affected percent diagnosed pregnant in this study.

Reimers et al., (1985) reported higher rates of pregnancy for cows inseminated greater than 120 DIM (60%) when compared with cows inseminated before 70 DIM (49%). Relationship between DIM at insemination and percent diagnosed pregnant for the seventeen herds in the study is shown in Figure 3. As DIM increased at AI, percent diagnosed pregnant increased accordingly. Assuming that DIM and service number would follow a similar relationship, these results also follow those of Stevenson et al., (1983), who reported that conception rates for third services were higher (58%) than those for first and second service cows (40%).

In the logistic analysis (Table 7), DIM affected percent pregnant ($P < 0.01$), with the probability of pregnancy increasing as DIM at insemination increased. This could be explained due to lower production levels and less stress during later lactation.

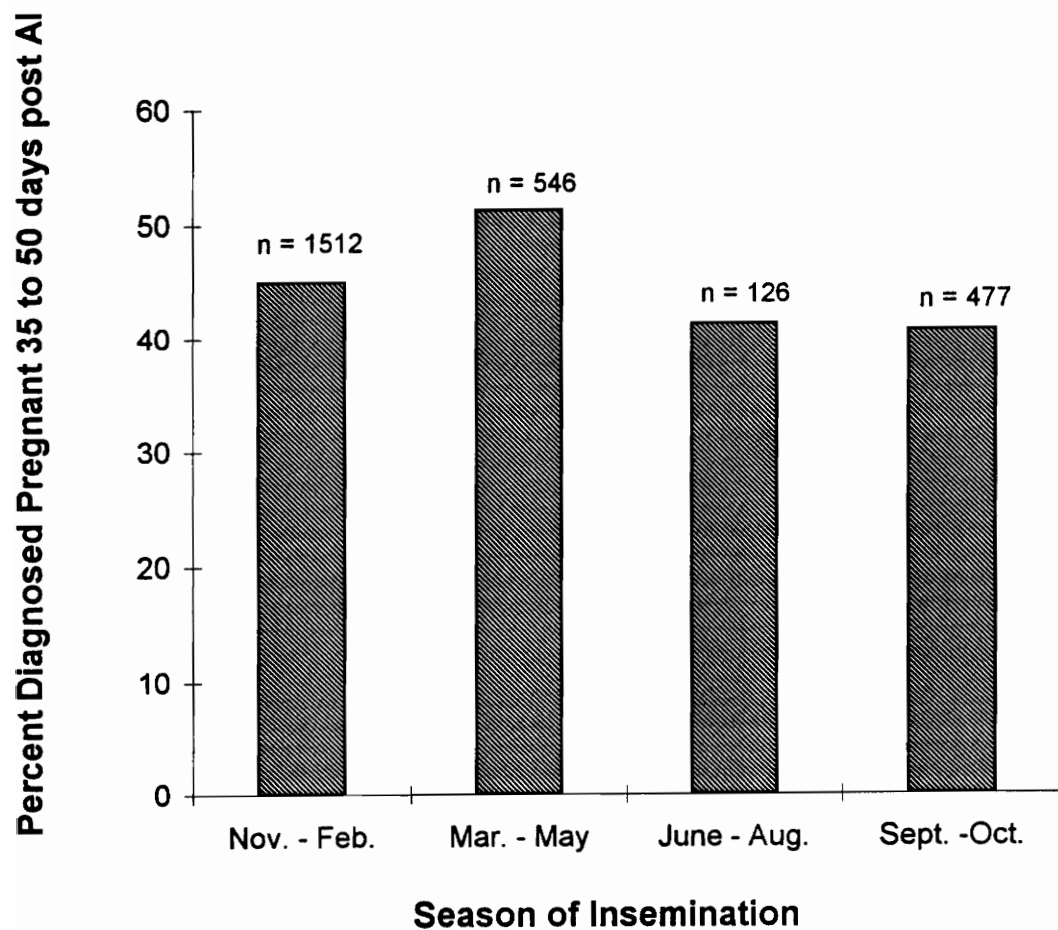


Figure 2. Percentage diagnosed pregnant relative to season of insemination for lactating cows (services = 2661) identified in estrus by HeatWatch

Percent diagnosed pregnant 35 to 50 days post AI

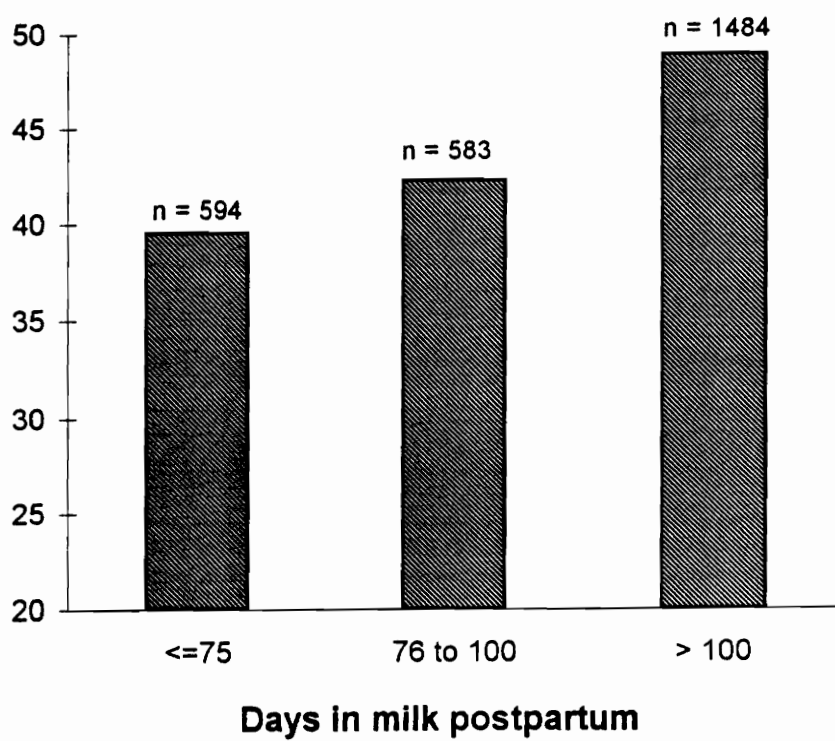


Figure 3. Percent diagnosed pregnant relative to DIM for lactating cows (services = 2661) identified in estrus by HeatWatch

Baseline group for DIM consisted of inseminations occurring less than 75 d postpartum. Inseminations occurring greater than 100 d postpartum had a higher probability ($P < 0.05$) of resulting in pregnancy than inseminations earlier in the lactation, with an odds ratio of 1.46 compared to a baseline of 1.00 for inseminations occurring before 75 d postpartum.

Interestingly, mounts per estrus also affected ($P < 0.01$) the probability of pregnancy in the final model. The baseline group with less than 3 mounts prior to insemination (corresponding to the “suspect” list in HeatWatch®) had a lower ($P < 0.05$) odds ratio for pregnancy than cows inseminated following ≥ 3 mounts. Cows exhibiting ≥ 3 mounts had odds ratios for probability of pregnancy of 1.41. It was also hypothesized that some cows exhibiting less than three mounts with HeatWatch® may have not been in estrus at insemination, contributing to a lower percent pregnant for those inseminations.

Figure 4 shows the means for percent pregnant relative to mounts per estrus in the seventeen herds. As evidenced in the logistic analysis, cows with less than three mounts were significantly lower ($P < 0.05$) for resulting pregnancy than other groups.

These findings corroborate those of Nebel et al., 1994 and Stevenson et al., 1983, which suggest that increasing mount activity may be associated with higher pregnancy results. It is not known, however, whether the estrus periods with

lower activity are actually less fertile, or whether many animals with fewer mounts were not in estrus at time of AI.

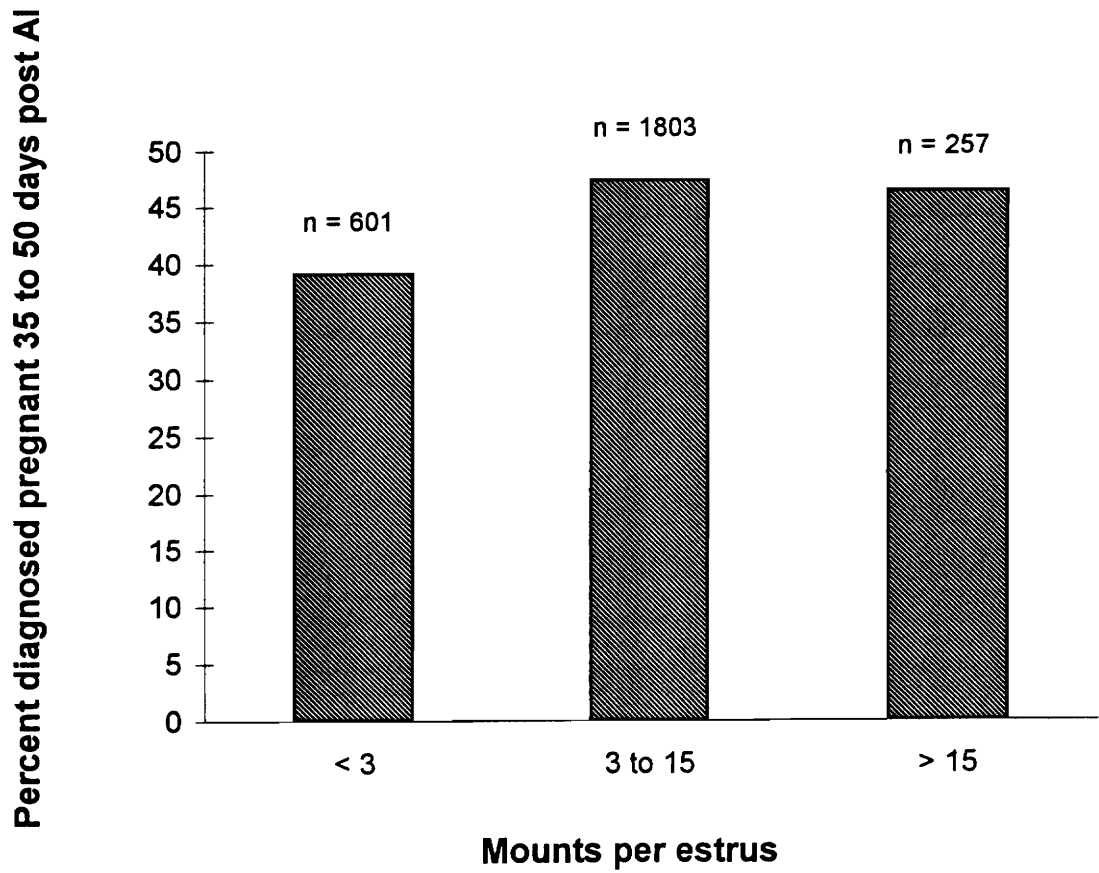


Figure 4. Percent diagnosed pregnant relative to mounts per estrus for lactating cows (services = 2661) identified in estrus by HeatWatch

Factors Associated with Probability of Pregnancy in Heifers

Heifers (services = 306) were analyzed following the same procedure used for cows in the study. Factors associated with pregnancy in heifers that were investigated included interval, herd, estrus duration, mounts per estrus, and season of AI.

In the overall model for heifers, herd ($P < 0.05$), interval ($P < 0.01$), and season ($P < 0.01$) affected probability of pregnancy. Non-significant factors in the model included mounts per estrus and estrus duration (appendix, Table 15.). Heifers in this study exhibited more mounts per estrus than lactating cows (15 versus 8.5).

Logistic binomial regression results for effect of interval ($P < 0.01$) from first mount to AI on pregnancy percentages for dairy heifers (services = 306) are shown in Table 8. The number of heifer inseminations are very comparable to the 295 services in the 1943 study by Trimberger and Davis which served as the basis for the a.m. - p.m. guidelines used by herds even today.

The heifers follow a more linear relationship between interval from onset of estrus to AI and resulting pregnancy when compared to lactating animals. Highest odds ratio for percent pregnant occurred for heifers inseminated immediately (0 to 4 h) following onset of estrus.

Table 8. Logistic-binomial regression results for the effects of interval from first mount to AI (services = 306) on pregnancy percentages for dairy heifers identified in estrus by HeatWatch®^a.

Interval	Estimate	SE	OR ^b	95% CI ^c
Intercept	3.02	1.31	-	-
—(h)—				
0 to 4	0	-	1	-
5 to 8	-0.06	0.58	0.93	(0.30,2.93)
9 to 12	-0.27	0.60	0.76	(0.24,2.47)
13 to 16	-0.16	0.62	0.85	(0.25,2.87)
17 to 20	-1.26	0.59	0.28	(0.09,0.90) ^d
21 to 24	-0.86	0.64	0.42	(0.12,1.48)
> 24	-1.19	1.09	0.30	(0.03,2.57)

^a ($P < .01$).

^b OR = “odds ratio”. The OR is the estimated odds of getting a heifer pregnant in any one interval when compared to the baseline interval for heifers inseminated 0 to 4 h following first mount where baseline interval OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes interval different ($P < 0.05$) from baseline interval.

The lower number of heifers increased the standard error to where the only group significantly different ($P < 0.05$) from baseline (inseminations occurring 0 to 4 h following first mount) consisted of those heifers inseminated 17 to 20 h following onset of estrus. These heifers inseminated between 17 and 20 h following onset showed a lower expected odds of pregnancy of .28 as compared to heifers inseminated 0 to 4 h following onset.

Herd also affected pregnancy ($P < 0.05$) in the heifer model (Table 9). Housing conditions varied for heifers in the study, with one herd utilizing a “counterslope” heifer facility (total confinement) and other herds using pasture to house heifers for observation of estrus.

Effects of season ($P < 0.05$) are shown in Table 10. Heifers inseminated during the baseline period of September to October had a significantly higher probability of pregnancy when compared with heifers inseminated the rest of the year.

This may be an artifact of low sample numbers, as there were a fewer heifers ($n = 30$) inseminated during the months of September and October, with a higher percent diagnosed pregnant (87 versus 63 %).

Table 9. Logistic-binomial regression results for the effects of herd on pregnancy percentages for dairy heifers identified in estrus (services = 306) by HeatWatch®.^a

Herd	Estimate	SE	OR ^b	95% CI ^c
Intercept	3.02	1.32	-	-
1	0	-	1	-
3	.05	.69	1.10	(0.27, 4.06)
5	-.20	.38	.81	(0.39, 1.72)
9	-1.50	.45	.22	(0.09, 0.54) ^d
12	.18	.91	1.20	(0.20, 7.12)

^a ($P < 0.01$)

^b OR = "odds ratio". The OR is the estimated odds of getting a heifer pregnant in a particular herd when compared to baseline herd 1, where herd 1 OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes herd different ($P < 0.05$) from baseline herd.

Table 10. Logistic-binomial regression results for the effects of season (services = 306) of insemination on pregnancy percentages for dairy heifers identified in estrus by HeatWatch®.^a

Season ^c	Estimate	SE	OR ^b	95% CI ^c
Intercept	3.02	1.32	-	-
Nov.-Feb.	-1.97	0.66	0.14	(0.04, 0.51) ^d
Mar. - May	-1.95	0.67	0.14	(0.04, 0.53) ^d
June - Aug.	-2.14	0.77	0.12	(0.03, 0.53) ^d
Sept. - Oct.	0	-	1	-

^a ($P < 0.05$)

^b OR = "odds ratio". OR is the estimated odds of heifer pregnancy resulting from inseminations performed during one season when compared to the baseline season of inseminations occurring in September and October, where baseline season OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes seasons different ($P < 0.05$) from baseline season.

Pregnancy percentages reported here were higher ($P < 0.05$) for heifers (65%) than cows (45%). This agrees with Stevenson et al., (1983), who reported higher rates of pregnancy for nulliparous heifers (58%) as compared to lactating cows (40%). Gwazdauskas et al., in 1981 reported results from a field trial with 1004 inseminations where parity significantly affected overall percent pregnant. In this previous study, heifer inseminations ($n = 275$) yielded a 59.5 % pregnancy percent, and percent pregnant declined with each subsequent increase in lactation number, ranging from 50.8% in first lactation cows to a low of 38.2% in fourth and greater lactation cows.

Means for percent diagnosed pregnant relative to season for dairy heifers (services = 306) are shown in Figure 5. The highest rates, as evidenced by the odds ratios, occurred for heifers inseminated in September and October. There were no differences in means for heifers inseminated from November to August.

Percent Diagnosed Pregnant 35 to 50 days post AI

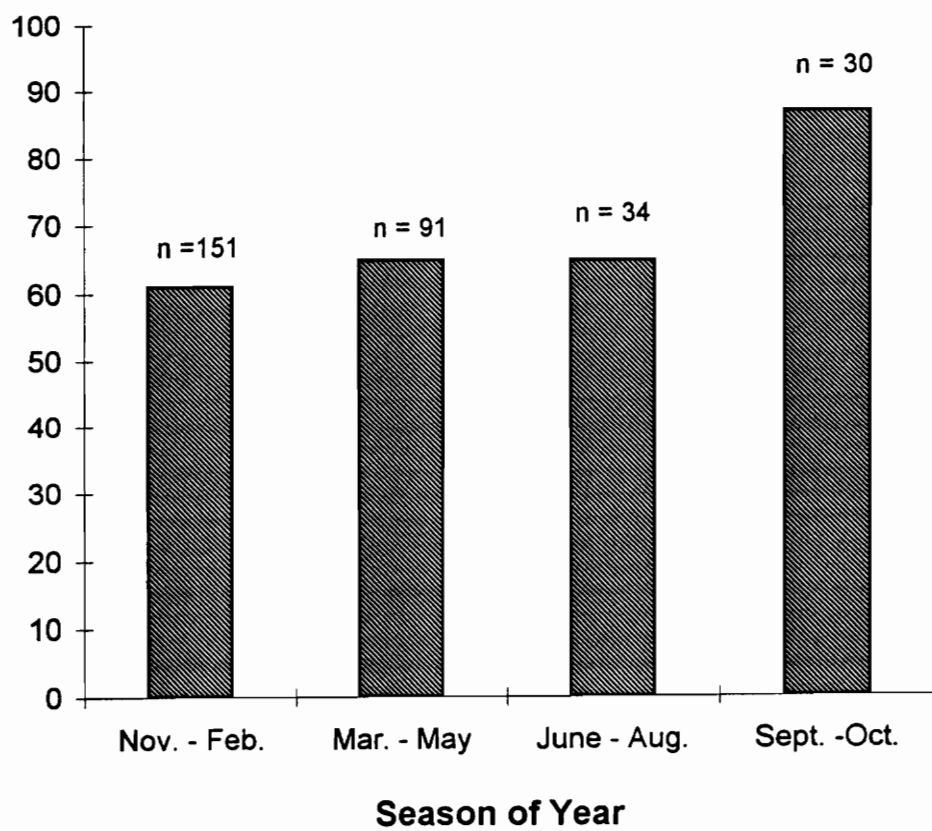


Figure 5. Percent diagnosed pregnant relative to season of insemination for dairy heifers (services = 306) identified in estrus by HeatWatch

Optimal Time of Insemination in Dairy Cows and Heifers

In lactating cows, a simple regression was calculated for interval from first mount to AI relative to percent diagnosed pregnant. The regression curve characterizing effects of interval on percent pregnant for cows is shown in Figure 6. The curve represents the quadratic equation: percent diagnosed pregnant = $.42985 + (.019 \times \text{interval}) - (.0013 \times \text{interval}^2)$; ($P < 0.01$). The odds ratios (Table 5) revealed no differences ($P > 0.05$) for percent pregnant in cows inseminated less than 16 h following onset of estrus, although the odds ratios suggest a 35% lower probability of pregnancy for inseminations performed 0 to 4 h after onset when compared to the 5 to 8 h interval group (Table 4). This is also shown in the regression curve (Figure 8), where there is an observable decrease in percent diagnosed pregnant for cows bred during the first 3 h of “standing estrus”.

Additionally, Figure 7 graphically represents mean percent diagnosed pregnant for lactating cows (services = 2661) relative to interval from first mount to AI by four hour increments. The bar graph shows a curvilinear relationship between interval and pregnancy, with highest rates for cows inseminated >8 to 12 h following first mount identified by HeatWatch®. The results from both analyses suggest that the optimal time of AI in cows would be between >4 and 16 h following onset of estrus as determined with HeatWatch®.

For heifers, the simple linear regression of interval from first mount to AI on percent pregnant is shown in Figure 8. The line represents the linear equation: percent diagnosed pregnant = $.871 - (.017 \times \text{interval})$; ($P < 0.01$).

As noted earlier, the relationship is linear, in contrast to the relationship between interval and pregnancy for cows which showed a quadratic response. There was an overall decrease in percent diagnosed pregnant from heifers inseminated earlier in the estrus period to those inseminated later during estrus. These differences between heifers and lactating cows may be physiological, or could be explained by possible herd within season interactions. The numbers of heifers in this study are comparable to the numbers from Trimmerger and Davis' 1943 study. Another study should be performed relative to timing of AI using heifers equipped with HeatWatch® to provide mount and onset of estrus information.

Effects of interval from first mount to AI on percent pregnant for heifers (services = 306) are graphically represented in Figure 9. The highest rate of pregnancy occurred for heifers inseminated 0 to 4 h following onset of estrus, with an additional peak for heifers inseminated >12 to 16 h following onset.

Pregnancy rates declined from a high of 84.2% (for inseminations performed 0 to 4 h following onset) to a low of 45.7 % for inseminations performed > 20 h after onset of estrus (Figure 9).

The guidelines for timing of insemination set forth by Trimberger (1948) suggest to wait approximately 12 h after observation of standing estrus for optimal pregnancy results. Results reported here would suggest that timing of insemination should be performed earlier after observation of estrus. Using Trimberger's "a.m. - p.m." guideline would effectively lower the probability of resulting pregnancy, as many cows observed in the morning may have been in estrus for several hours previous to observation.

Previous studies have reported that when onset of estrus is not known, once daily insemination for cows observed in "standing" estrus can be used as effectively as the "a.m. - p.m." method with no differences in resulting pregnancy (Nebel et al., 1994; Foote, 1978). Our results would suggest that if onset is not known, AI should be performed at the next most convenient time within 3 h of observation of estrus for optimum results. Results from lactating cows in this study fit closely with our hypothesis of timing presented in the introduction; where the physiology of sperm transport, life of sperm and ova, and timing of ovulation would predict optimal timing for AI to be between 6 and 18 h following the first mount recorded by HeatWatch®.

The heifer data differs from this hypothesis, with the earliest inseminations yielding optimal pregnancy results. If the heifer data are representative of the population, it would suggest either an earlier ovulation time or prolonged sperm

viability in the female reproductive tract of heifers as compared to cows. Interesting studies on timing of ovulation in heifers could now be performed using HeatWatch® for identification of onset of estrus.

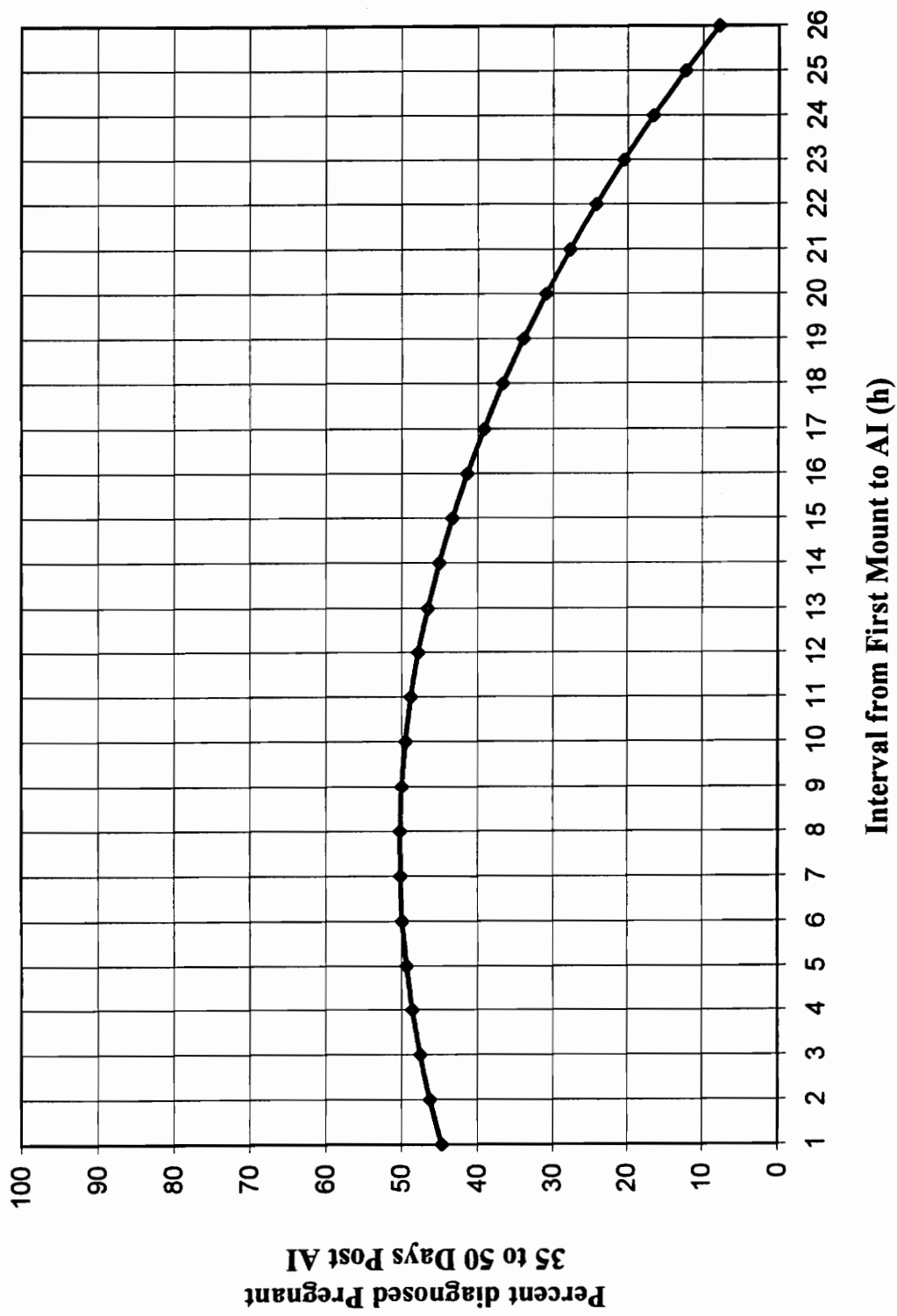


Figure 6. Optimal time of insemination for lactating cows from 17 herds (services = 2661) identified in estrus with the HeatWatch electronic estrus detection system.

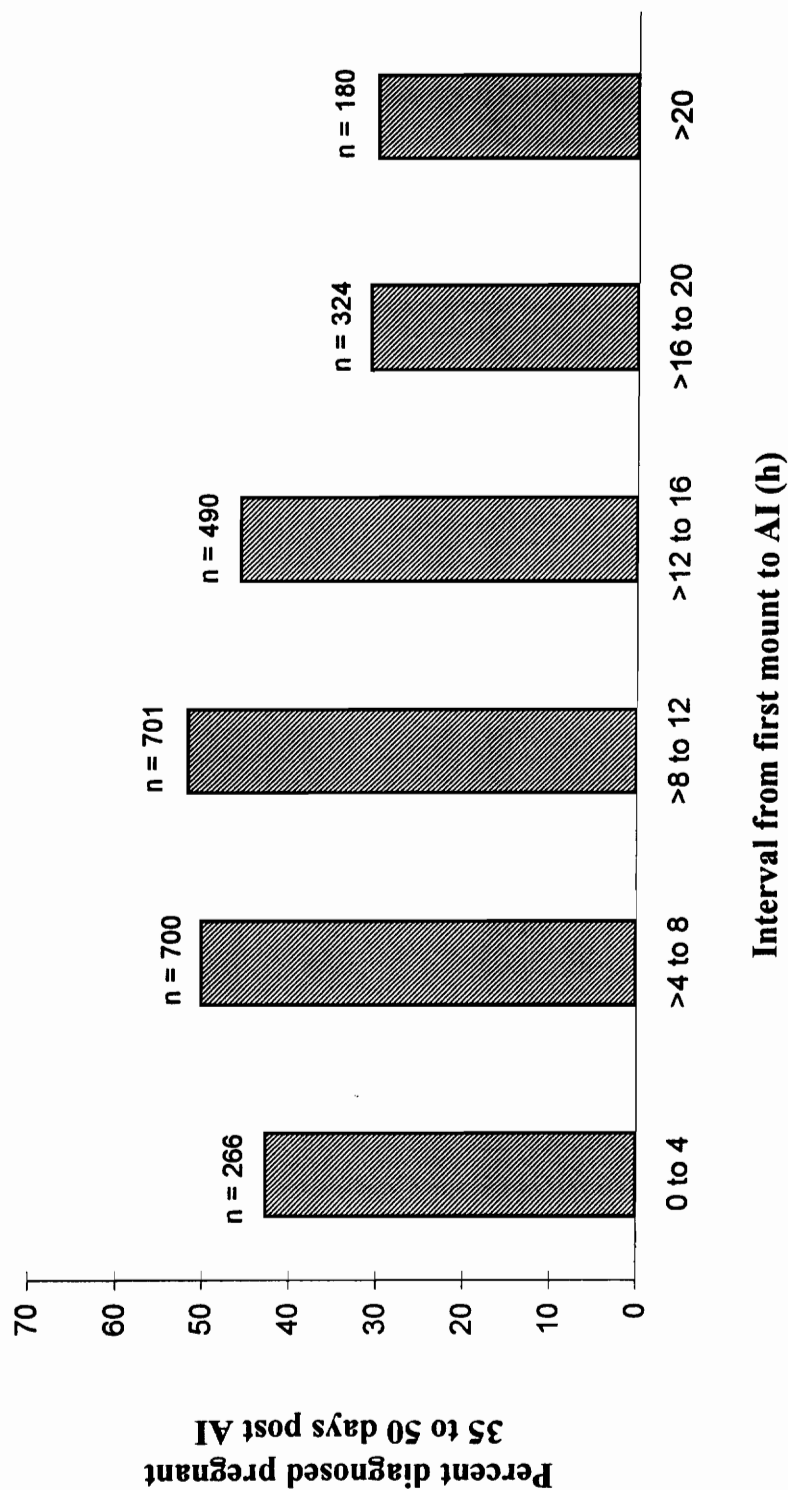


Figure 7. Percent lactating cows diagnosed pregnant relative to interval from first mount to AI (h) in 17 herds for estrus periods (n = 2661) identified by HeatWatch electronic estrus detection system

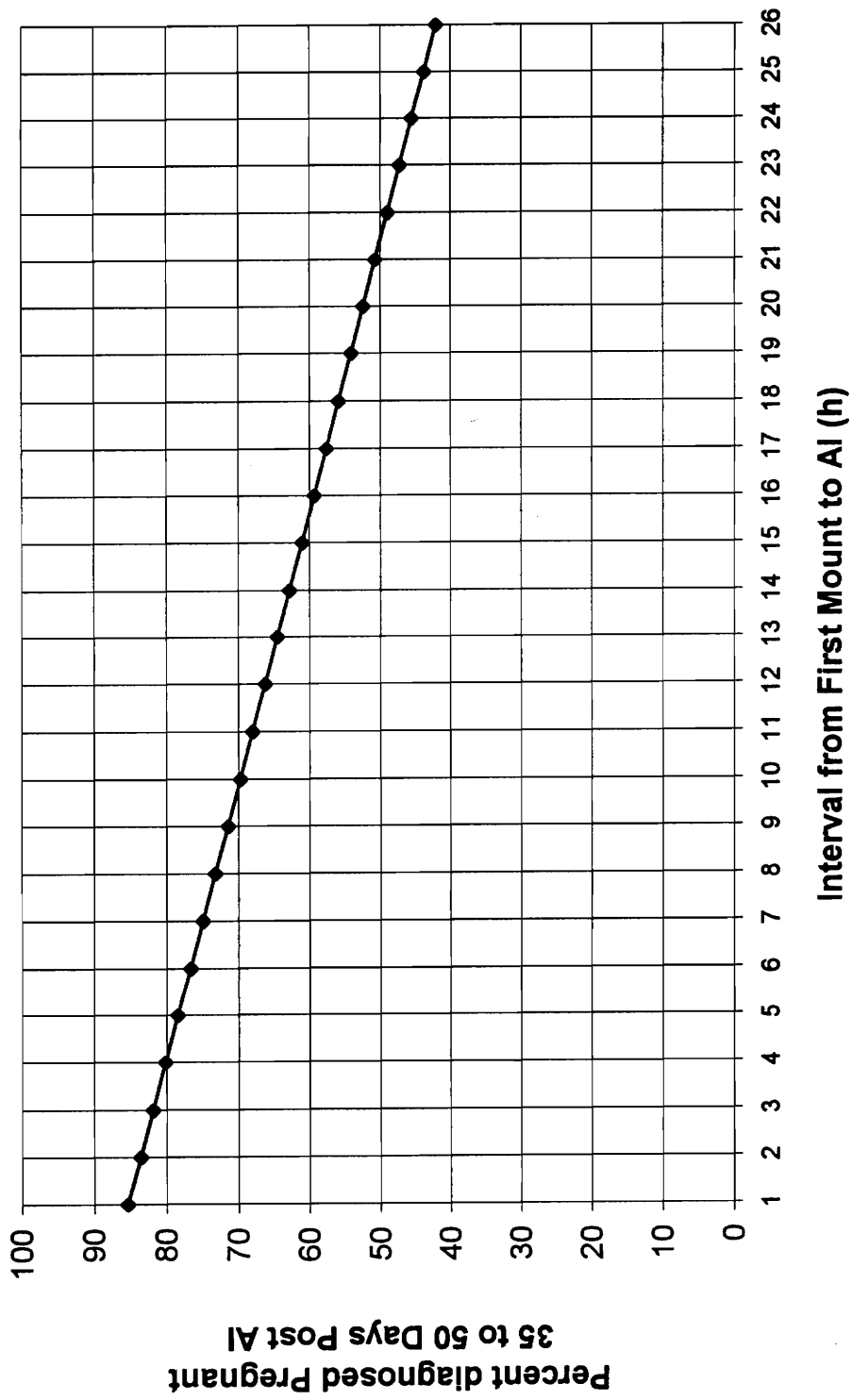


Figure 8. Optimal time of insemination for dairy heifers from 6 herds (services = 306) identified in estrus with the HeatWatch electronic estrus detection system.

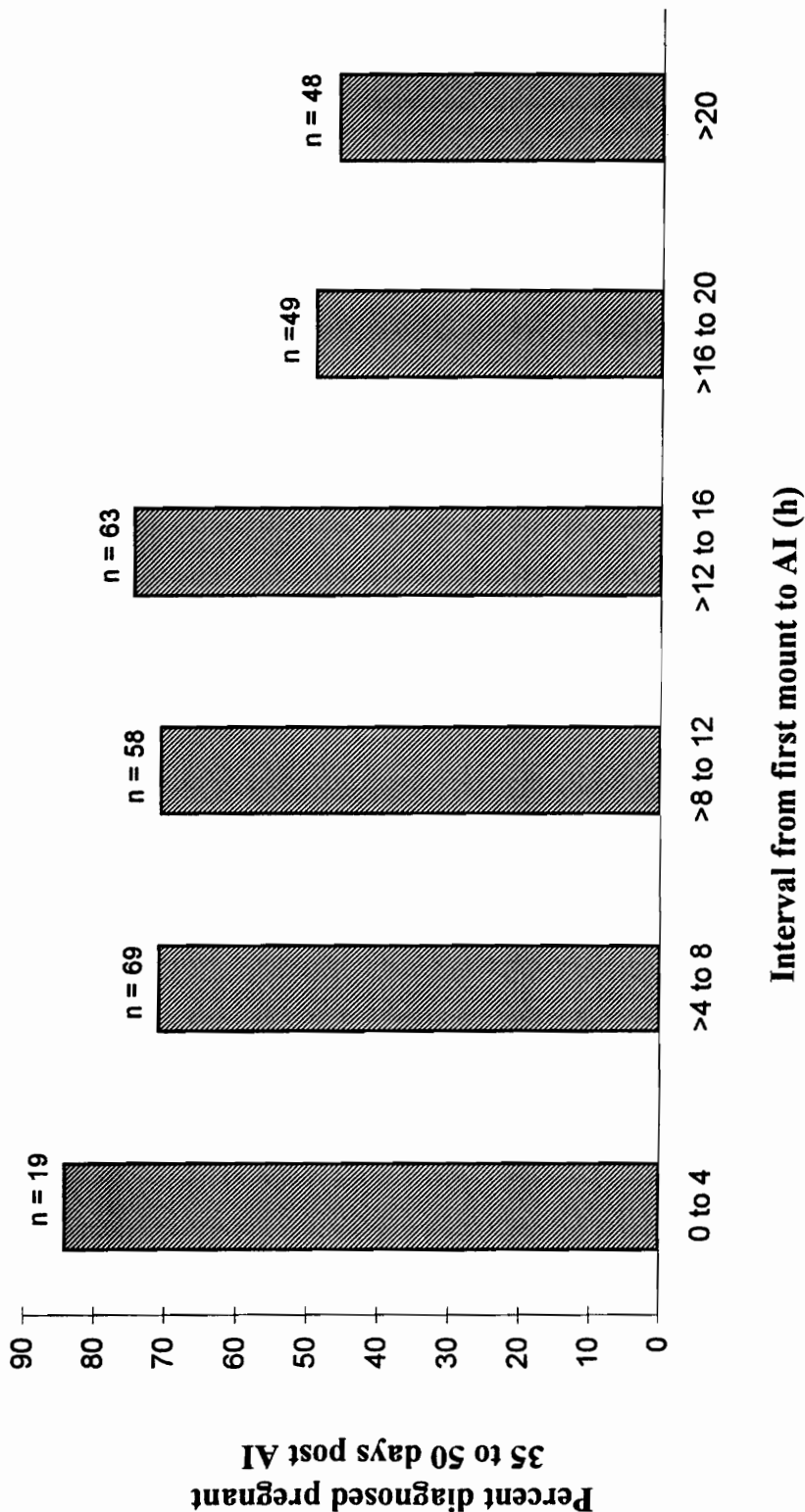


Figure 9. Percent heifers diagnosed pregnant relative to interval from first mount to AI in 6 herds for estrus periods (n = 306) identified by HeatWatch electronic estrus detection system

Conclusions

Results of this experiment have shown the optimal time of AI in lactating cows to be between >4 and 16 h following first mount detected by HeatWatch®. In heifers, inseminations earlier in the estrus period resulted in higher percentages diagnosed pregnant.

Many dairy herds will never employ HeatWatch® in their reproductive programs; however, results reported here can be utilized for optimal pregnancy rates on the farm. In herds using methods other than HeatWatch® for the detection of estrus, cows should be inseminated earlier during the estrus period than the industry standard “a.m. - p.m.” guideline recommends. As discussed earlier, herds employing the “a.m. - p.m.” guideline are observing cows in the morning, but inseminating in the afternoon or evening. Our results suggest that cows observed in estrus in the morning should be inseminated at the next most convenient time within 3 h, and cows observed to be showing signs of estrus in the afternoon should be inseminated that evening, rather than waiting until the following morning.

Additional information from this study will be useful in explaining factors affecting pregnancy in most dairy herds. Season affected percent diagnosed pregnant in both heifers and cows for the trial, suggesting that temperature may

have a strong relationship with reproductive efficiency in dairy cattle, as shown by previous studies (Gwazdauskas et al., 1979, Broadway et al., 1975).

This study was conducted in the Southeast, where extreme ambient temperatures and humidity combined with longer growing seasons are characteristic of the region in the summer. It would be interesting to observe more closely the effects of cold temperatures on reproduction in cattle from cooler regions.

Although little can be done to change season effects on reproductive performance, cooling and misting systems should be implemented during warm months. It would appear from the data that there is no significant change in heifer pregnancy percentages during warm seasons, as previously reported by Gwazdauskas et al., (1981).

Mounds per estrus also had a positive relationship with pregnancy. As mounds increased, pregnancy percentages also increased. For herds using HeatWatch®, animals on the “suspect list” (< 3 mounds) should be evaluated closely for other signs of estrus before insemination. This would also apply to herds utilizing other methods of detection, where animals not observed in “standing estrus” would be either palpated for signs of estrus or observed for discharge or other secondary signs before determination to AI is made. This practice could increase probability of pregnancy by as much as 41% (Table 7).

Cows inseminated later than 100 d postpartum had a 46 % higher predicted odds of pregnancy than those inseminated before 75 d. DIM affected pregnancy percentages, with increased probability of pregnancy as DIM at insemination increased. Herds utilizing HeatWatch® and results reported here for ideal timing of insemination should have lower services per conception and higher rates of pregnancy, allowing for a longer voluntary waiting period (VWP).

This study may be the “tip of the iceberg” for learning more about AI in dairy cows and estrus characteristics in cattle of all types. HeatWatch® should continue to provide more new information for researchers regarding estrus, timing of ovulation, and reproductive efficiency in cattle. Future studies could be conducted to investigate the association between timing of AI and synchronization programs, timing of AI in beef cattle, and the interrelationships between timing and physiological events during estrus. Studies with larger numbers may provide additional information about timing of AI in heifers.

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Appendix

Table 11. Preliminary model logistic analysis of factors affecting pregnancy percentages in dairy cows (services = 2244)

Variable	<i>P</i> <
Herd	0.001
Interval ^a	0.01
Mounts per Estrus	0.05
Parity	<i>NS</i>
Days in Milk ^b	0.01
Season ^c	<i>NS</i>
Estrus Duration (h)	<i>NS</i>
TDM ^d	<i>NS</i>
Rating ^e	<i>NS</i>

^a Interval from first mount to AI (h)

^b Days from calving to AI

^c Season of AI

^d Test day milk closest to AI

^e Rating within herd

Table 12. Preliminary model logistic-binomial regression results for the effects of interval from first mount to AI (services = 2244) on pregnancy percentages for dairy cows identified in estrus by HeatWatch®^a.

Interval	Estimate	SE	OR ^b	95% CI ^c
Intercept	-0.78	.26	-	-
—(h)—				
0 to 4	0	-	1	-
5 to 8	0.32	.15	1.38	(1.1,1.9) ^d
9 to 12	0.31	.16	1.37	(1.1,1.9) ^d
13 to 16	0.24	.17	1.28	(1.0,1.9)
17 to 20	-0.70	.19	.50	(0.4,0.7) ^d
21 to 24	-0.38	.24	.68	(0.4,1.1)
> 24	-1.64	1.10	.19	(0.3,1.6)

^a ($P < .01$).

^b OR = “odds ratio”. The OR is the estimated odds of getting pregnant in any one interval when compared to the baseline interval for animals inseminated 0 to 4 h following first mount where baseline interval OR = 1.

^c CI = confidence interval

^d CI not containing 1.0 denotes interval different ($P < 0.05$) from baseline interval.

Table 13. Final model logistic analysis of factors affecting pregnancy percentages in dairy cows (services = 2661)

Variable	<i>P</i> <
Herd	0.05
Interval ^a	0.01
Mounts per Estrus	0.01
Days in Milk ^b	0.01
Season ^c	0.05

^a Interval from first mount to AI (h)

^b Days from calving to AI

^c Season of AI

Table 14. Logistic analysis of factors affecting pregnancy percentages in dairy heifers (services n = 306)

Variable	<i>P</i> <
Herd	0.01
Interval ^a	0.01
Mounts per Estrus	<i>NS</i>
Estrus Duration (h)	<i>NS</i>
Season ^b	0.05

^a Interval from first mount to AI (h)

^b Season of AI

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