VAGINAL TEMPERATURE, EAR SKIN TEMPERATURE AND ACTIVITY IN RELATION TO ESTRUS IN DAIRY COWS

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Kimberly D. Redden

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ABSTRACT

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Methods to improve detection of estrus in dairy cows have been studied extensively without wide acceptance of any one method. In the present study, twice daily activity levels of 13 lactating cows were monitored using pedometers. Concurrent vaginal and ear skin temperature measurements were recorded continuously using radiotelemetry. Activity increased ($p<.0001$) at estrus for the 8 h daytime period (0630-1430 h), during which cows were given a 5 h turnout period; but not for the 16 h overnight period (1430-0630 h), throughout which cows were confined to tie stalls. Individual increases in activity resulted in 80% estrus detection and 4 false positives. Vaginal temperature increased .6 C $\pm$ .3 C ($p<.0001$) at estrus and remained elevated for a total of 6.8 $\pm$ 4.6 h. Individual increases in temperature resulted in 81% estrus detection and 3 false positives. Estrus detection was similar between the two methods studied and greatly exceeded the detection rate currently being achieved by the dairy industry.

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From east to west, I thank Laurie, Merridy and Tracy for their support and friendship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

Figure | Page

INTRODUCTION

One of the more prevalent reproductive problems facing dairy producers is that of detecting when a cow is in heat and determining the correct time to inseminate. The extent of the problem is evident from longer than desired calving intervals (Gilson, 1987) arising primarily from inadequate heat detection. According to Barr (1975), less than 60% of all heats are detected. To date, there is no widely accepted heat detection aid in use by dairy producers that consistently improves accuracy of heat detection over casual observations, with a minimal input of labor.

Monitoring activity and changes in body temperature are among the wide variety of heat indicators that have been investigated in recent years. Kiddy (1977) was first to explore the use of pedometers to monitor estrus-related changes in cow activity. While results of this and subsequent studies have indicated a high accuracy of heat detection (78% to 93%), there is currently no "cow pedometer" available in Canada for producer use. Several researchers (Ball et al., 1978; Maatje and Rossing, 1976 and Zartman, 1983) have demonstrated an estrual rise in body and milk temperature for cows but the effectiveness of correctly identifying an individual cow in estrus is generally poor.

A major limitation of these earlier studies may be related to technique and/or duration/frequency of temperature measurements.

The current study was designed to examine the potential of continuous vaginal and ear skin temperature monitoring and to reevaluate pedometers as aids for estrus detection.

LITERATURE REVIEW

Importance of Estrus Detection

Maximizing productivity in any animal husbandry system requires a high level of reproductive efficiency. The dairy industry is no exception as the rebreeding of cows is imperative for maintaining lactation. While a calving interval of 365 days is considered optimal, Morris et al. (1976) calculated an average value of 123.8 days open and a calving interval of 404 days for all cows recorded on the Canadian Dairy Record of Performance Programme. Pelissier (1972) found a calving interval of approximately 410 days to be consistent with the United States Dairy Herd Improvement Association data. More recently, Gilson (1987) reported calving intervals of 420 days (140 days open) to be rather typical of dairy cows. Barr (1975) examined data from Ohio dairy herds and found that the number of days open for fertile cows were much more variable than could be attributed to variation in services per conception. It was estimated that 14.7 days were lost due to failure to conceive whereas 40.3 days were lost due to missed heat periods. From this, approximately 53% of heats were considered missed.

All missed heats are due to failure of the cow to behaviorally express estrus or failure of the dairyman to observe the expressed behavior. Since non-detected heats are not observed it is difficult to distinguish between missed estrus and silent estrus. Although ovulation without associated behavior is known to occur in cattle at some rate, O'Farrell (1984) claims it is erroneous to assume that ovulations which are unaccompanied by overt signs of heat are "silent". Rather, it is more a reflection of estrus detection efficiency. In a study by King et al. (1976), two groups of postpartum cows were monitored for onset of ovarian function and occurrence of estrus. One group was continuously observed for estrus with a time lapse videorecorder and the second group was casually observed by the herdsmen as the cows passed through the milking parlor and during the periods the cows were exercising. Plasma progesterone levels revealed no differences in the time to the initiation of ovarian function and the occurrence of regular ovarian cycles between the two groups of cows. Mean time to first observed estrus was 34.5 ± 12.8 days in the continuously observed and 56.6 ± 26.5 days in the casually observed group ($p<.01$). The percentage of cows in which estrus was detected at the first, second and third ovulation were 20%, 44%, and 64% for the casually observed group and 50%, 94%, and 100% for the continuously observed group ($p<.01$). These findings demonstrate the more common cause of poor breeding performance to be the inability to detect estrus.

In a study of estrus and related behavior in postpartum cows, Hurnik et. al (1975) found both the intensity of expression and length of time that characteristic behavioral symptoms of estrus were evident varied considerably between individuals and was also influenced by the degree of estrus synchronization and social factors. True estrus, as defined by the interval during which the cow made no effort to escape when mounted by others, varied from 7.5 ($\pm$ 2.37) h when only one cow was in estrus to 10.1 ($\pm$ 2.36) h when three cows were in estrus at the same time. As reported by Boyd (1984), estrus expression is also intermittent and may be supressed by numerous factors. O'Farrell (1984) found that the highest proportion of heats were recorded in the 12 h period between 1800 and 0600 h.

Considering the nature and pattern of estrus behavior, good estrus detection requires frequent observations by skilled persons at appropriate times. Donaldson (1968) reported that 3 observations for one hour at 0700, 1500 and 2300 hours detected 90% of heats. O'Farrell (1984) found half hour observations at dawn, midday and dusk generally gave over a 70% detection rate. While rate of estrus detection may increase with observation time, this is becoming more and more difficult with the current trends in dairying. Economic conditions have resulted in a move towards larger herd sizes and an overall decrease in labor invested per cow. Consequently, it is more difficult to correctly identify which cow is in estrus and there is a reduction in man hours available for this task.

Due to the difficulties in detecting estrus, many forms of estrus detection aids have been investigated for their potential in improving efficiency at a reasonable expense. Methods to aid in the detection of estrus rely on some form of behavioral or physiological expression of estrus.

Estrus Detection Methods

Behavioral Indices

Heat Mount Detectors. Heat mount detectors glued to the rump of a cow, are a means of monitoring standing behavior. When an estrus cow is mounted by another cow, pressure on the device causes dye to be released from a small tube within the detector. Pennington and Callahan (1986) found a higher detection rate with heat mount detectors (93.9% heats detected) than with 3 separate 30 minute observation periods (60.6% heats detected). However, they also reported a 28% rate of false detections with the heat mount detectors. Considering their overall accuracy of 49.4% (detection rate x no. detected/(no.detected + no.false positives)), heat mount detectors were not superior to human observations. Stevenson and Britt (1977) and Williams et al. (1981) found heat mount detectors had only 34% and 29% accuracy in two studies. High rate of false positives were attributed to mounts to nonestrus cows when escape was not possible. Tail paint is another form of heat mount detector. Paint applied just in front of the tailhead is rubbed off when the cow stands to be ridden. Subject to the same tendencies for false positives as other mount detectors, Sawyer et al. (1986) reported lower accuracy with tail paint than with visual observations.

Detector Animals. A second approach for monitoring standing behavior is to use detector animals which are fitted with a marking device. Detector animals may be surgically altered bulls, or steers and cows treated with testosterone. In addition to requiring general maintenance, surgically altered bulls may lose or have lowered libido and hormone treated cows or steers must be given hormone therapy every 10 to 14 days (Fulkerson et al., 1983). Fulkerson et al. (1983) reported the ratio of detector animals to cows should be 1 in 50. The detection rate in this study, using hormone treated steers and marking devices which were updated twice per day, was 79%. As pointed out by Gilson (1987), the placing and amount of markings must be considered carefully for correctly distinguishing between a cow marked due to standing behavior and cows marked due to chin resting or other reasons. The difficulties in this were illustrated by Stevenson and Britt (1977) who reported an overall heat detection accuracy of only 41%.

In general, estrus detection rates are improved through use of detector animals or mount detectors when estrus detection is poor to begin with or when used in combination with regular observations.

Pedometers. Hurnik et al. (1975) monitored the activity of cows using a videorecorder and found a large increase in the amount of time spent walking around the time of estrus. The use of pedometers to quantify this increase in activity at estrus has been investigated by several researchers (Doherty et al., 1987; Farris, 1954; Kiddy, 1977; Peter and Bosu, 1986 and Wiliams et al., 1981;). Pedometers are instruments, primarily for human use, designed to indicate distance travelled. The pedometer responds to up/down movement as a result of a weighted arm attached to a spring.

Kiddy (1977) modified pedometers for placement on a cow's rear ankle. Pedometers were worn by cows in free stall and tie stall housing. From 4 weeks after calving until estrous cycles ceased, the pedometers were read twice daily while cows were in the milking parlor. Cows housed in tie stalls spent about one hour in a holding lot at each milking. Data was collected on 87 estrous periods of 40 cows in free stall and on 39 estrous periods of 28 cows in tie stall. Free stall cows showed an average increase in activity of 393% at the time of estrus. Activity of individual cows at estrus exceeded each cow's nonestrus mean by 2 or 3 standard deviations in 98% or 93% of the cases, respectively. Changes in activity at the time of estrus were less distinct for cows in tie stall housing, possibly due to the restriction of cow movement. Average increase in activity at the time of estrus was 276% for tie stall cows. Activity of individual cows at estrus exceeded each cow's nonestrus mean by 2 or 3 standard deviations in 93% or 72% of the cases, respectively. Rate of false diagnosis of estrus from activity data was not reported by Kiddy (1977) but was not considered a problem since false positives were eliminated by noting the number of days since the cow's last estrus and the current behavior of the cow.

Williams et al., (1981) examined the use of pedometers for estrus detection of 12 mature, loose housed heifers. Pedometers of the same model and modification as used by Kiddy (1977) were strapped to the lower foreleg and read twice daily, approximately 12 hours apart. While Kiddy (1977) did not report loss of pedometers to be a problem, Williams et al. (1981) indicated that replacement was required, on average, every 18.7 days. This may have been due to the location of the pedometer. Placing the pedometer on the front leg (Williams et. al, 1981) may also have resulted in a low detection rate. Activity of individual cows at estrus exceeded each cow's nonestrus mean by 2 standard deviations in only 68% of the cases. Smaller sample size or use of heifers could also have yielded the lower detection rate observed by Williams et al. (1981) as compared to Kiddy (1977).

Estrus related changes in the motor activity of female dairy goats have been investigated by Doherty et al. (1987). Pedometers worn around the neck of free stall dairy goats and read every 12 hours showed estrus activity averaged 2.4, 2.6, and 2.7 times greater than mean levels of activity during diestrus for day ($p<.005$), night ($p<.001$) and 24 h periods ($p<.001$), respectively. A two-fold or greater increase in activity during estrus was noted for 40% (8 of 20) of the does during daytime hours, 71% (15 of 21) at night, and 61% (11 of 18) of the does combining both day and night activity. There were 5 instances of decreased activity of does in estrus which was attributed to heavy rainfall and/or close proximity to a neighboring buck pen. Does in estrus were frequently seen standing, relatively inactive, close to the fenceline bordering the buck pen. In addition, the authors felt diestrus activity may have been elevated, in some cases, by involvement with estrus females in the same enclosure.

Peter and Bosu (1986) found the use of pedometers for estrus detection was superior to twice daily observations for signs of estrus. Activity Monitors, developed by the Dairy Equipment Company, were worn on the lower front leg of 47 Holsteins from day of calving to 60 days postpartum. When the current activity level reached 2, 3, or 4 times greater than the average activity level, one of three possible light emitting diodes on the monitor was activated, indicating by how much activity had increased. Cows were maintained on pasture, observed for 30 minutes twice a day, and had their pedometers examined twice daily at milking. Occurrence of ovulation was determined by rectal palpation of the ovaries and serum progesterone concentrations. Fifty seven percent (24/42) of all first postpartum ovulations were associated with increased activity as indicated by the pedometer, while only 19% (8/42) were associated with observed behavioral signs of estrus. Similarly, 32 of 35 (91%) second postpartum ovulations and 13 of 14 (93%) third postpartum ovulations were accurately predicted by pedometer readings. Only 37% and 79% of these second and third postpartum ovulations were associated with observed behavioral signs of estrus. Overall, of the total of 91 ovulations recorded, 76% (69/91) were detected by pedometer and 35% (32/91) were detected by visual observation. Of the 22 ovulations not predicted by pedometer readings, 18 were first postpartum ovulations. All cows visually observed in estrus (32/91) were also detected by the pedometer with the mean interval from pedometer indication of estrus to observed estrus being 9 ± 4.46 hours. All pedometer detected estruses were followed by subsequent ovulation, indicating 0% incidence of false positives.

Results of investigations with pedometers indicate their potential use in detecting estrus cows, particularly when free movement is permitted. In contrast to other methods relying on behavioral expression, accuracy is high and overall detection efficiency can be improved in the absence of visual observations.

Physiological Changes

Progesterone. Monitoring physiological changes associated with estrus has the potential advantage of permitting detection of even silent heats. The advent of rapid on farm progesterone test kits means that there is access to a quick and economical assay of progesterone (Rajamahendran et al., 1990). Use of progesterone assays to detect estrus is sound to the extent that in the cycling cow, prior to ovulation, progesterone production from the corpus luteum falls at a known rate and time (Boyd 1984). However, even in cycling cows, a fall in progesterone is not always followed by estrus and ovulation. There are also documented cases of cows with an unusually long period from fall in progesterone level to ovulation (Boyd 1984). For these reasons, progesterone tests can only accurately identify cows not in estrus. In addition, in order to achieve a high detection rate and adequate timing of insemination, daily progesterone measurement would be required. For example, Foulkes et al. (1982) found a 98% estrus detection rate and a 61% conception rate when insemination time was based on daily progesterone levels. When estrus detection and insemination time were based on visual observations of estrus behavior, fewer cows (71%) were detected but a slightly better conception rate (67%) was obtained.

As a consequence of high estrogen in the absence of progesterone, electrical resistance of the vagina is high at estrus (Lewis et al., 1989). Most literature (Boyd 1984; Gartland et al., 1976 and Heckman et al., 1979) suggests a change in electrical resistance of the vagina at estrus. This change however, is an unreliable detector of estrus because considerable variation arises due to positioning of the probe in the vagina (Heckman et al., 1979). Gartland et al.(1984) found measuring electrical resistance every second day was unreliable in distinguishing physiological states. Daily intravaginal examination is labor intensive and inflammation often arises and produces variable readings (Heckman et al., 1979). More current research (Lewis et al., 1989 and Smith et al., 1989) has involved developing a telemetric system for measuring vaginal changes in electrical resistance which would reduce variability in measurements due to positioning and inflammation, and reduce labor input as well.

Kiddy et al.(1978) reported on the ability of trained dogs to detect odour-related changes of cows in estrus. Dogs with previous experience in olfactory detection were trained to detect and respond to vaginal swabs from estrus cows. After several weeks of preliminary discrimination training, dogs were exposed to diestrus and estrus pairs of samples from different cows in a farmyard setting. Scores for individual dogs ranged from 71.1% to 93.3% correct. Overall level of correct detection of estrus samples was 79.5%.

<u>Core and Milk Temperatures</u>. Studies relating milk or body temperature to estrus have also been conducted. The etiology of estrual rises in body temperature remains inconclusive but hormonal changes characteristic of estrus and ovulation are thought to have a large influence (Abrams et al., 1973). In addition, increased activity of cows in estrus would increase heat production and possibly body temperature. While estrus-related peaks in temperature may preceed (Maatje and Rossing, 1976) or not be accompanied by any obvious behavioral display (Fordham et al., 1988), Walton and King (1986) reported that the estrus cows with the largest increases in rectal temperature were also visually observed in estrus during the exercise period that immediately preceeded the temperature measurement. Some evidence exists (Maatje and Rossing, 1976 and Fordham et al., 1988) for a slight decrease (.05 to .08 C) in body temperature on the day before and the day after estrus but these changes were not statistically significant. There has been some recent efforts (Ball et al. 1978; Fordham et al. 1988; Hurnik et al.,1985; Maatje and Rossing, 1976; Zartman and DeAlba, 1982 and Zartman, 1983) to determine if estrus changes in temperature are measureable and unique enough to

estrus to be a reliable predictor.

Early attempts to monitor temperature changes in milk (Lira et al., 1975) at estrus were unsuccessful as temperatures were influenced by ambient temperature and speed of milking. Improvements in milk temperature sensing equipment as described by Maatje and Rossing (1976) resulted in better correlations between milk temperature and rectal temperature. With these improvements, 16 of 19 (84%) cases of heat were accompanied by a morning (or evening) milk temperature rise of at least .3 C over the average temperature for that cow on the preceeding 6 milkings at the same time of day. Similar to Maatje and Rossing (1976), Ball et al. (1978) measured milk temperature twice a day and found 13 of 16 (81%) estrus periods were detected using the same criteria. Whereas Maatje and Rossing (1976) did not report on the incidence of false positives, Ball et al.(1978) calculated the rate of falsely detecting a cow in estrus was 12% based on milk temperature.

Other efforts to improve accuracy (Ball et al., 1978) involved use of two separate criteria: (1) a morning or afternoon temperature that was at least X C higher than the mean corresponding temperature over the previous *n* days, and (2) a morning or afternoon temperature that was at least X C higher than the corresponding temperature on any of the previous *n* days. Each of these techniques was applied using a range of values for X and n. Criteria to increase detection of estrus also increased the incidence of false positives. Conversely, minimizing false positives increased the likelihood of missing estrus. The best criterion was determined to be either a morning or afternoon milk temperature that was at least .1 C higher than the corresponding temperature on any of the previous 15 days. Application of this criterion to the data resulted in detection of 12 of 14 heats, a detection rate of 85.7%. The rate of false positives using this milk temperature criteria was 2.6%, or 6 incidences of false detection of estrus out of a possible 236 nonestrus days.

Rectal temperatures measured once daily (Ball et al., 1978) gave very high rates of false positives and false negatives. Similarly, Walton and King (1986) measured rectal temperature with a mercury in glass thermometer every morning and found the recorded temperatures to be very unreliable in detecting estrus. While once daily measurement could be considered too infrequent to detect short duration temperature rises at estrus, the temperature measurement procedure may have contributed to variation resulting in poor accuracy as well. Zartman and DeAlba (1982) measured body temperature once daily at 0630 hours over a 5 minute period using transmitters with thermistors leading into the peritoneal cavity. All but one cow was reported to show a temperature spike at estrus. Aside from stating that tests for outliers agreed well with emperical conclusions, there was no further evidence given by these authors concerning incidence of false positives, actual number of estruses monitored, or statistical manipulations.

Hurnik et al. (1985) used thermal infrared imagery to monitor 27 Holsteins daily for 90 days, starting at 14 days postpartum. The thermal infrared imagery focused on the gluteal region which included the anal and vulval areas, the posterior zone of the udder attachment, and the two posterior lobes of the udder. Overall results indicated that skin surface temperature increased at estrus and the magnitude of the increase rose with each successive postpartum estrus over the 100 day period after calving. In contrast, Fordham et al. (1988) found little evidence that a rise in milk temperature became more pronounced as the number of estruses since parturition increased. Ninety three percent of cows were correctly detected in estrus at least once when passing through their postpartum estrus sequence (Hurnik et al.,1985). However, of the 18 cows that experienced a third estrus, only 78% were detected and there was a false positive rate of 33%. Infrared scanning measurements showed strong dependence on dry bulb air temperature and this was considered to have contributed to the high frequency of false positives.

Fordham et al.(1988) reported an overall significant rise ($p<.001$) in both milk and body temperature by approximately .3 C (range of 0 to 1.0 C) on the day of estrus for 15 cows in early lactation. Approximately 60% of the 33 estrus periods were considered to be associated with a limited rise at estrus, 27% associated with a pronounced rise and 13% associated with no temperature rise. There was only a 47% detection rate by Fordham et al. (1988) using the criteria of a minimal rise of .3 C over the corresponding 6 day mean (which gave Maatje and Rossing, 1976 an 84% detection rate). Using the same criteria as Ball et al. (1978), where estrus was defined as a rise of at least .1 C above the corresponding temperature on any of the preceeding 15 days, only 33% of all heats were detected. The criteria resulting in the optimum compromise for a high rate of correct diagnosis of estrus and low false positive detections was based on a rise of .2 C over the mean of the preceeding 3 days (Fordham et al., 1988). This resulted in detection rates of 73.3% for milk temperature measured at the top of each of 4 short milk tubes, 70% for milk temperature measured in the claw piece, and 80% for vaginal temperature measured at each milking. Corresponding false positives were 10.8%, 11.2% and 10%, respectively.

Vaginal Temperature. Measurements of vaginal temperature in the study by Fordham et al. (1988) gave slightly better detection and accuracy levels than milk temperature. Although differences arising from temperature measurement site may not have been significant, there is evidence to suggest that vaginal temperature may have greater potential as a predictor of estrus. As described by Abrams and Stolwijk (1972), estrogens released endogenously are known to lower the resistance to blood flow in the uterus of the ewe and it can be presumed that a similar reduction occurs in the blood vessels of the vaginal wall. Abrams et al. (1973) constructed a probe to measure the effects of injections of estradiol-17B on the thermal conductance of the vagina in diestrus heifers. With a 40 to 60 minute lag following estradiol-17B injection, vaginal thermal conductance increased ($p<.01$) markedly in a curvilinear fashion. It was assumed that the main increase was due to an increase in vaginal blood flow. The well known effects of estrogen on metabolism of uterine tissue *in vitro* and possibly vaginal tissue may have contributed to the response as well (Abrams et al.,1973).

Temperature-transmitting devices were inserted into the vagina of 9 cows approximately 50 days postpartum and remained in place for the duration of the study of Zartman et al., (1983). Vaginal temperatures were received from the transmitters once daily at 0600 h. Generally, cows were considered in estrus when temperature on a given day was at least .4 C greater than the mean of the previous 5 days. Cows were inseminated on the basis of a temperature spike and the number of services to conception and number of days open were compared to 9 control cows bred on the basis of visually observed signs of estrus. Services to conception averaged 2.4 and number of days open averaged 148.4 for the temperature monitored group. At the time of publication, only 7 of the 9 control cows had been confirmed pregnant. Provided the 2 remaining cows conceived from their last service, the average number of days open would be 164 and services to conception would be 3.2 for the control group.

Zartman et al. (1983) also examined the safety and feasability of intravaginal temperature transmittance using radiotelemetry. Radiotransmitters were implanted non-surgically into the vagina of cycling heifers, positioned with a plastic anchor, and remained in place for 107 days. Estradiol-17B and progesterone comparisons were made between fitted and control heifers to assess the impact of the vaginal object on steroid hormones related to reproduction. The presence of a radiotransmitter in the vagina did not appear to pose any threat to fertility or physical health. The radiotransmitter did not attenuate ($p>.10$) either estradiol-17B or progesterone levels during the first 15 days of the cycle. During the 4 days prior to the second estrus, radio-fitted heifers had higher ($p<.03$) progesterone concentrations than did controls. Progesterone for the radio-fitted heifers declined from 6.1 to .5 ng/ml during the 5 days preceeding estrus, while the control values declined from 3.5 to .3 ng/ml. These findings suggest that the intravaginal device may have slightly delayed the time of luteal regression. Zartman et al. (1983), however, concluded this to be of little consequence since both groups had similar progesterone values by the day of standing heat and the number of services to conception did not differ.

Considerable variation exists among studies concerning the temperature criterion which best define estrus and the levels of accuracies these criteria yield. These differences may be due to the equipment used, site of measurement, and/or the time of measurement. The time(s) of temperature measurement tended to be consistent within a particular experiment since diurnal fluctuations affecting variability in body temperature of lactating cows are well documented (Araki et al., 1987; Bitman et al., 1984; and Wrenn et al., 1961). However, timing of temperature measurements were different among studies and it is possible that time of day affects estrus detection accuracy. Zartman and DeAlba (1982) measured temperature at 0630 h because it was believed that the animals' lowest point in diurnal temperature occurred between 0500 and 0700 h. Temperature increases due to estrus were thus believed to be more easily detected at that time of day. Araki et al. (1987) monitored ambient temperatures and vaginal temperatures of lactating Holsteins every 15 minutes for 17 days and found fluctuations in body temperatures at night were not as dependent on ambient temperature as fluctuations that occurred during the day. In addition to absolute time of day; time of day relative to other activities such as feeding, milking, and turnout periods could also influence the accuracy of temperature monitoring for estrus detection. Continuous monitoring of body temperature and factors that may influence body temperature (ie. ambient temperature) would likely reduce the number of false positives and negatives encountered using body temperature to predict estrus. Considering the variable duration of the temperature rise, continuous monitoring should also detect a greater proportion of estruses. Mosher et al. (1990) found vaginal temperature spikes of estrus heifers to range from 4 to 21 h (11.00 ± 5.91 h). Clapper (1990) reported the average time temperature was elevated during estrus in 7 postpartum cows to be 8.14 ± 3.48 h.

Should continuous temperature monitoring prove to be reliable in predicting estrus, an automated system could be developed for improving estrus detection and possibly conception rate as well. Investigations concerning the temporal relationship between rise in vaginal temperature and ovulation (Clapper et al., 1990; Mosher et al., 1990 and Rajamahendran et al., 1989) indicate that temperature rise and surge in luteinizing hormone is more consistent than drop in progesterone, increase in estrogen, or behavioral expression and luteinizing hormone. Given this, the time of temperature rise may be the best indicator of when to inseminate.

With the recent research efforts of Kennedy et al.

(1989), continuous radiotelemetric monitoring of body temperature is feasable under most production conditions. The objectives of the current study were to continuously monitor vaginal temperature of lactating cows as an indicator of estrus, and ear skin temperature as an indicator of cow thermoregulatory status. In addition to temperature measurements, activity as indicated by twice daily pedometer readings, was to be evaluated for its potential use in estrus detection.

MATERIALS AND METHOD

Animals and Routine

Activity and body temperatures of 10 multiparous and 3 first parity Holstein cows in early lactation were monitored while under regular herd management practices and routine. Cows were turned out to an exercise paddock (60 m x 40 m) from 0830 h to 1330 h but were otherwise housed in tie stalls. Milking was done by pipeline twice daily between 0630-0800 h and 1430-1600 h. Cows were fed concentrate, in amounts dependent on stage of lactation and production, at 0600 h and upon return to stalls from the exercise area. Hay was available *ad libitum* while in the exercise area and after the afternoon milking. Grass silage was fed once daily at 1330 h in combination with the concentrate. Visual observations for estrus were conducted by the barn staff and usually occurred at least once daily during the turnout period. Artificial insemination was performed on cows which were at least 60 days postpartum and visually observed in estrus. Cow 11 was superovulated for embryo donor purposes. Treatment began 111 days postpartum with twice daily intramuscular injections of Folltropin (Vetrepharm Inc., London, Ont.) for 4 consecutive days. On the fourth day, Lutalyse (Tuco Products Comp., Orangeville, Ont.) was also administered intramuscularly.

All cows to be monitored calved within a 2 month period between July 22, 1990 and September 19, 1990. All data collection took place over a 4 month period between August 14 and December 5, 1990. Average barn temperature (as measured by a temperature transmitter at cow chest level) was 12 C and ranged from 2 to 26 C during the experimental period. Mean daily minimum and maximum barn temperatures were 9 $\pm$ 4 C and 14 $\pm$ 4 C, respectively, with an average daily range of 5.5 $\pm$ 2 C.

Activity Monitoring

Battery operated, digital pedometers (DIGI-WALKER MINI, EM-201, Yamax Corp., Yokohama) manufactured for human use with a display of steps taken (up to 100,000), were modified to be worn by cows. Modification involved encasing the pedometer in plexiglass and attaching two elastic straps as illustrated in Figure 1.

The case protecting the pedometer was made by cutting 5 mm thick plexiglass into 68 x 63 mm squares and removing 10 x 10 mm from each corner. The thickness of the remaining 10 mm wide flaps were ground to 3 mm. After shaping, plexiglass was heated in a 300 C oven for 20 minutes and then molded to the form of the pedometer. Straps were made from elastic fabric cut to 45 cm lengths with 10 cm each of hook and loop tape sewn to the ends. Parts were assembled by bolting each of two straps to the face of the case

Figure 1. Digital pedometer (DIGI-WALKER MINI, EM-201, Yamax Corp., Yokohama) modified for cow use by encasing it in plexiglass and attaching straps.

through holes drilled in the plexiglass. After removal of the belt clip attached to the pedometer, the latter was placed inside the case and secured with duct tape.

Modified pedometers were strapped just above the hock, beginning 20 days postpartum. Readings from the pedometers were recorded twice daily at each milking.

Temperature Monitoring

Body temperature monitoring began within 32 - 51 days postpartum and continued through to 77 - 125 days postpartum. Each cow was monitored continuously for a period of 42 - 83 days with an average ($\pm$ S.E.) of 66.5 $\pm$ 13.8 days per cow.

Transmitters

Ear skin temperature was monitored using radiotransmitters and an implantation technique described by Kennedy et al. (1989). Each transmitter, weighing 50 g, contained a high quality precision quartz crystal and lithium battery. The external thermistor consisted of a probe sheathed in a polyolefin shrink tube with an inner melt core. For attachment to the ear, the body of the transmitter was wrapped in duct tape and riveted to the ear with two commerial ear tags. The probe was held to the skin surface by suturing protective foam pads to the dorsal and ventral ear surfaces. The probe, which lay in a groove in the ventral foam pad, was held close to the ventral skin surface when sutures were tied.

Similar radiotransmitters were modified for internal use (Wildlife Materials Inc., Carbondale, Ill.). Changes included mounting the thermistor and antennae internally, and encapsulating the transmitter body in a noninvasive paraffin based coating (Figure 2). A vaginal anchoring device was designed to encase the transmitters and permit placement into the vagina for an indefinite period of time. The overall characteristics of the anchor were a hollow body which fit tightly over the transmitter. Eight finger-like projections, 30 - 34 mm long and rounded at the ends, protruded from the body in a spherical pattern (Figure 3).

In making the hollow body, a metal mold was welded to the size and shape of the transmitter. The mold was heated over a bunsen burner and then dipped into Plastisol (F.H. and Sons Manufacturing Ltd., Rexdale, Ont.) for 30 seconds. The mold and surrounding Plastisol were baked for 30 minutes at 125 C. The resulting sleeve, which was open at one end and round at the other, was trimmed to 80 cm at the open end. Anchor projections were made by inserting test tubes into a rack covered with aluminum foil, so that the open tops were just below the surface of the rack. Plastisol was injected into the tubes and allowed to overflow onto the foil covered rack. Once filled, test tubes and rack were placed in a 125 C oven for 90 minutes. The resulting solid finger projections, with bases trimmed to 15 mm, were then

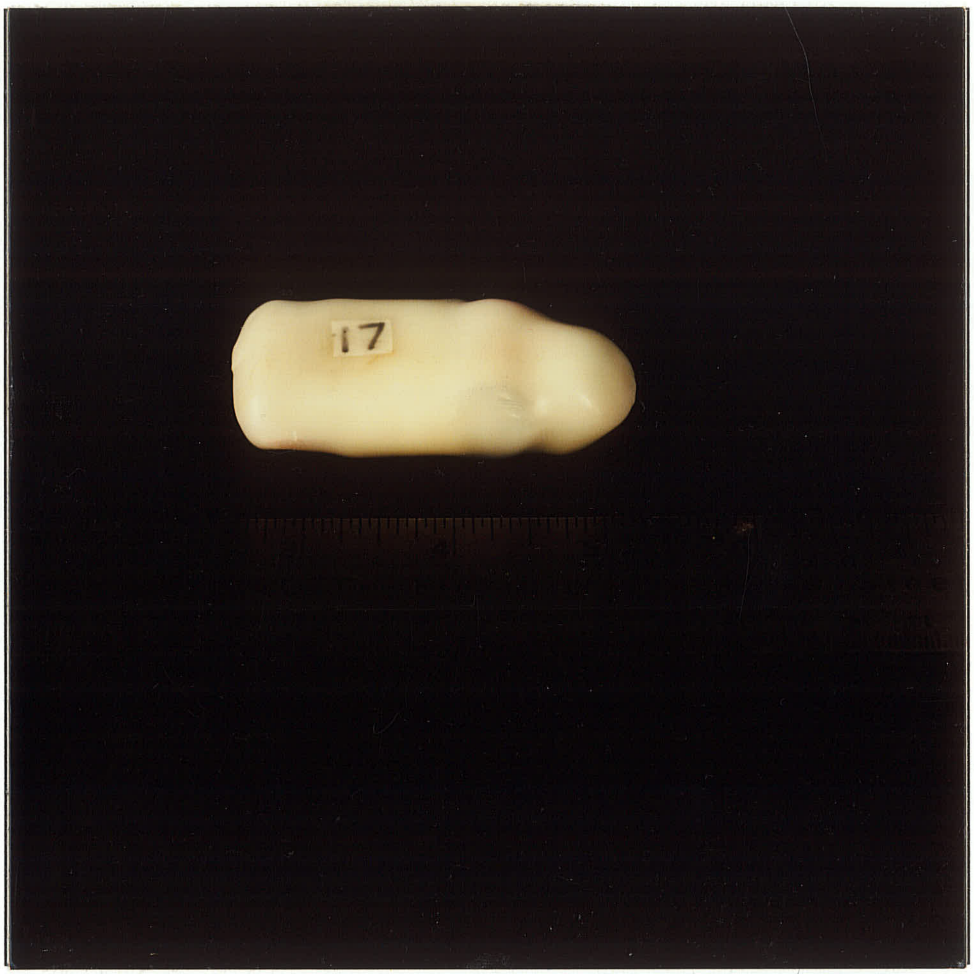

Figure 2. Internal vaginal transmitter with internal thermistor and antennae.

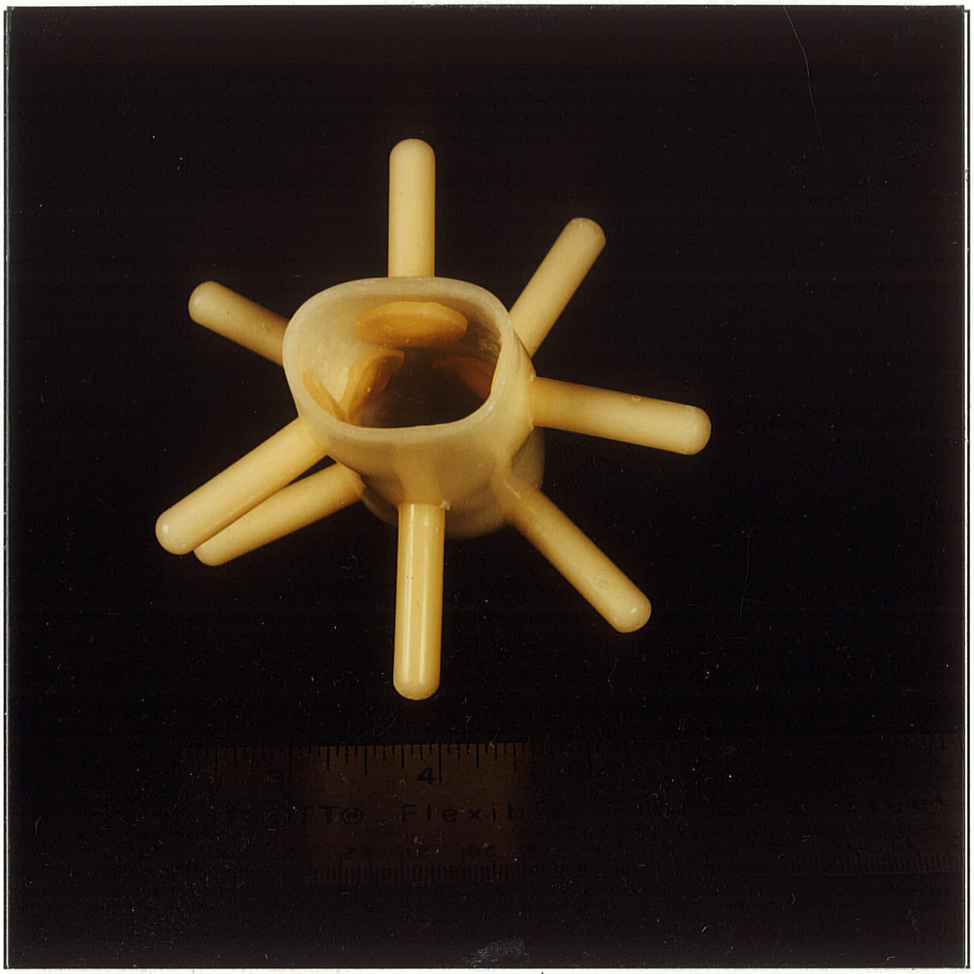

Figure 3. Vaginal anchoring device designed to encase vaginal transmitters and prevent expulsion.

inserted into holes made in the hollow body with a hole punch.

Before placement in the vagina, radiotransmitter and anchoring device were soaked in a container of Benzalkonium Chloride solution (1:750) for a minimum of 30 minutes. The container was then immersed in hot water, allowing the anchoring device to reach approximately 37 C; a temperature at which the anchor projections were more flexible. To facilitate insertion, the anchor/transmitter unit was held in hand (using a sterile, lubricated glove) with the protruding fingers of the anchor flattened against the body. The anchor/transmitter unit was lubricated and then inserted into the vagina to a depth of 20 cm. Transmitters remained in the vagina for the duration of the test period. Cows observed in estrus were artificially inseminated without removal of the device.

Eight of the cows (Cows 1,2,3,5,7,8,9 and 11) monitored were involved in earlier efforts to design a suitable transmitter anchoring device. Initial devices were unsuccessful in preventing expulsion and were sometimes associated with excessive vaginal discharge. A minimum of 10 days was allowed to pass between removal (or expulsion) of earlier devices and placement of the final prototype described.

Radiotransmitters were tested in a water bath with a mercury thermometer before and after the data collection period. Transmitters were found to have varying accuracies (Appendix 1, Table 1) but were precise to >.1 C $\pm$ .01 C. Calibration drift over the experimental period was negligible (Appendix 1, Table 2).

Telemetry Monitoring System

The telemetry equipment used was similar to that described by Kennedy et al. (1989). In collaboration with Wildlife Materials Inc., modifications were made to accomodate simultaneous use of ear and vaginal transmitters and to make the system compatable for use on IBM computers.

Each transmitter sends a unique identification signal at a common frequency of 150.25 MHz. Time between signals is dependent on temperature and transmitter type: approximately every minute for ear transmitters at 30 C and every 4 minutes for internal transmitters at 39 C. Time between successive signals was linearly related to temperature for each transmitter type. A 4 element yagi antenna (Cushcraft Inc., Manhester, N.H.) attached upright to the roof of the barn was used to aid in signal reception while cows were outside. The antenna was 5.2 m above ground level and 80 m from the furthest edge of the exercise paddock. A second antenna was suspended lengthwise along the ceiling inside the barn. Maximum distance between the indoor antenna and indoor-housed cows was 6 m.

Two receivers, specific for receiving signals from either ear transmitters or internal transmitters, were connected to a 386-SX, 60 Mb computer through seperate serial communication ports. The receivers converted individual transmitter signals into 4 byte packets. A privately written data logging program operating on the computer received the packet which contains as the first byte, the transmitter I.D.; the remaining 3 bytes make up the receiver's internal counter value at the time the signal was recieved. When another signal was received from the same transmitter, the elapsed time between signals was calculated and the corresponding temperature computed by reference to a time interval: temperature table.

The output of the data logging program consisted of a screen displaying transmitter I.D.s, time of current signal reception and current calculated temperature. Also displayed were current time and most recent signal I.D. A data file for each transmitter containing time of day and elapsed time between signals was generated on the hard drive. All data files automatically closed (and new files opened) every two hours to minimize data loss in the event of a power failure.

Data Processing

Activity Data

Activity data, as indicated by the difference between 2 sucessive pedometer readings (daytime = 8 hr: 0630-1430 h and nighttime = 16 h: 1430-0630 h), were entered directly into the mainframe at the end of the study. Daytime readings for cow(s) kept inside during the regular turnout period were regarded as missing observations to avoid artificially lowering the mean and increasing the variation of the cow's activity level for that time of day.

Temperature Data

Temperature data files for vaginal radiotransmitters were manipulated at least twice weekly and often daily during a 1 - 4 h period between 0800 h and 1200 h. Incoming signals were missed during this time. Manipulation of the data involved using a privately written program to convert two hour files (containing time of day and elapsed time between signals) to one continuous data file (containing time of day and temperature) for each vaginal transmitter. Continuous data files were modified to 15 minute mean values using a program written within a Quattro-Pro (Borland International Inc., Scotts Valley, Ca.) spreadsheet. Mean fifteen minute values were plotted for viewing and saved in ASCII text files for future uploading (Procomm Communication Software, Datastorm Technologies Inc., Columbia, MO.) to the University of Manitoba Amdahl Mainframe computer. Upon termination of the study, ear temperature data was similarly manipulated into 15 minute mean values and saved into ASCII text files.

For the purpose of determining estrus detection ability, a variety of criteria (as described in the results) were applied to hourly means of vaginal (Tv) and ear skin (Tes) temperature.

Confirmation of Estrus

Commencing 20 days postpartum, milk samples were collected daily during the morning milking and frozen for future progesterone assay. Milk progesterone was measured by radioimmunoassay (Tekpetey et al., 1987) for milk collected 5 days before and 5 days after each observed and/or suspected estrus. In cases of uncertainty, milk samples for the cow's entire test period were assayed. Estrus was assumed to have occurred between 2 and 3 days after the initial drop in progesterone to less than 1 ng/ml in cases when behavioral observations were not available to determine time of estrus.

Statistics

Statistics describing means, ranges and standard deviations of each individual cow, for the three parameters measured, are included in Appendix 2.

Activity data was analysed using a paired t-test to test for differences between activity at estrus and mean activity for all previous, nonestrous days. To test for effect of individual cow and estrous cycle number on activity at estrus, a general linear model including cycle number as the independent variable was used.

Main effects of individual cow and reproductive status (pregnant following estrus or open following estrus) on the increase in Tv at estrus were tested using a general linear model with status X cow specified as the error term. Period (54 - 90 h before, 18 - 54 h before, during, 18 - 54 h after, and 54 - 90 h after estrus) comparisons were made on least square means using a general linear model specifying period X cow as the error term. Correlations between Tv and Tes were determined for each cow for all days, each individual day of estrus and each individual day of false positives. All statistics were performed on SAS version 5.16 (1986).

RESULTS

Confirmation of Estrus

Progesterone values confirmed a total of 26 estrous cycles among 10 cows (Cows 1 - 10), with cows having a range of 1 - 4 cycles during the experiment. Cows 1 and 7 exhibited prolonged luteal phases (greater than 30 day estrous cycles) prior to their first estrus of the monitoring period. In contrast, Cow 5 exhibited prolonged low milk progesterone for 35 days prior to estrus at 62 days postpartum. Speculations on the influence that the early vaginal anchoring devices may have had are provided in the discussion. Progesterone profiles for the remaining 3 cows (Cows 11 - 13) revealed irregular ovarian activity which could not be characterized conclusively in the absence of profiles for other reproductive hormones.

Data for the 10 cycling cows only were included in statistical manipulations and calculations of estrus detection accuracy. Data for the remaining cows were considered individually with speculation on the cow's reproductive status.

Activity

Modification of the pedometers was effective but the strapping material sometimes stretched beyond its length adjustment towards the end of the study. As a result, activity data were not collected on one of the 26 estrus days. Standard deviations in activity throughout the experimental period, for each cow, are provided in Appendix 2. Activity at estrus (n=25) was compared to the mean activity of preceeding nonestrus days with results illustrated in Figure 4. Mean total daily activity (0630-0630 h) was 2.30 times greater at estrus ($p<.0001$) than on the preceeding nonestrus days. The 8 h daytime period (0630-1430 h; including the 5 h turnout period) comprised the majority of total daily activity and increased 2.8 times ($p<.0001$) at estrus. Overnight activity (1430-0630 h) did not increase ($p<.5$) at estrus. Figure 5 shows changes in daytime activity throughout early lactation for a typical cow (Cow 1). While there appeared to be a tendency for estrus activity to be less at the initial estrus than for subsequent estruses, estrous cycle number did not have a significant effect ($p<.9$) on activity at estrus. There was also no cow effect ($p<.2$) on increase in activity at estrus.

To determine the effectiveness by which pedometers could identify estrus cows, criteria were examined which might yield a high detection rate with a low incidence of false positives. The best detection rate, while maintaining a minimal number of false positives, was achieved by adhering to a two-step detection criterion. First,

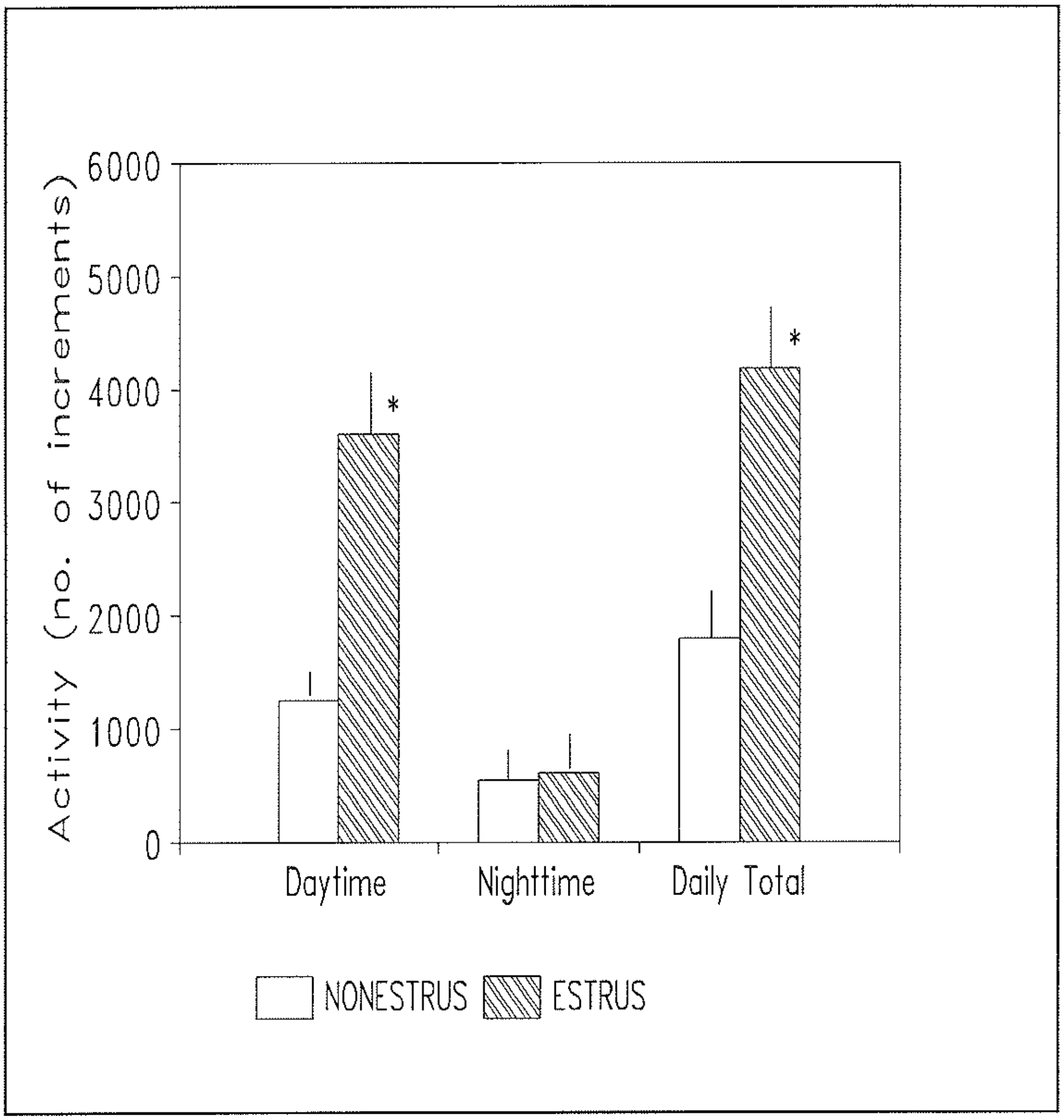

Figure 4. Estrus versus preceeding nonestrus activity (mean ± s.e.) during daytime (0630-1430 h), nighttime (1430-0630 h), and daily total (0630-0630 h).
* estrus > nonestrus; $p < .0001$

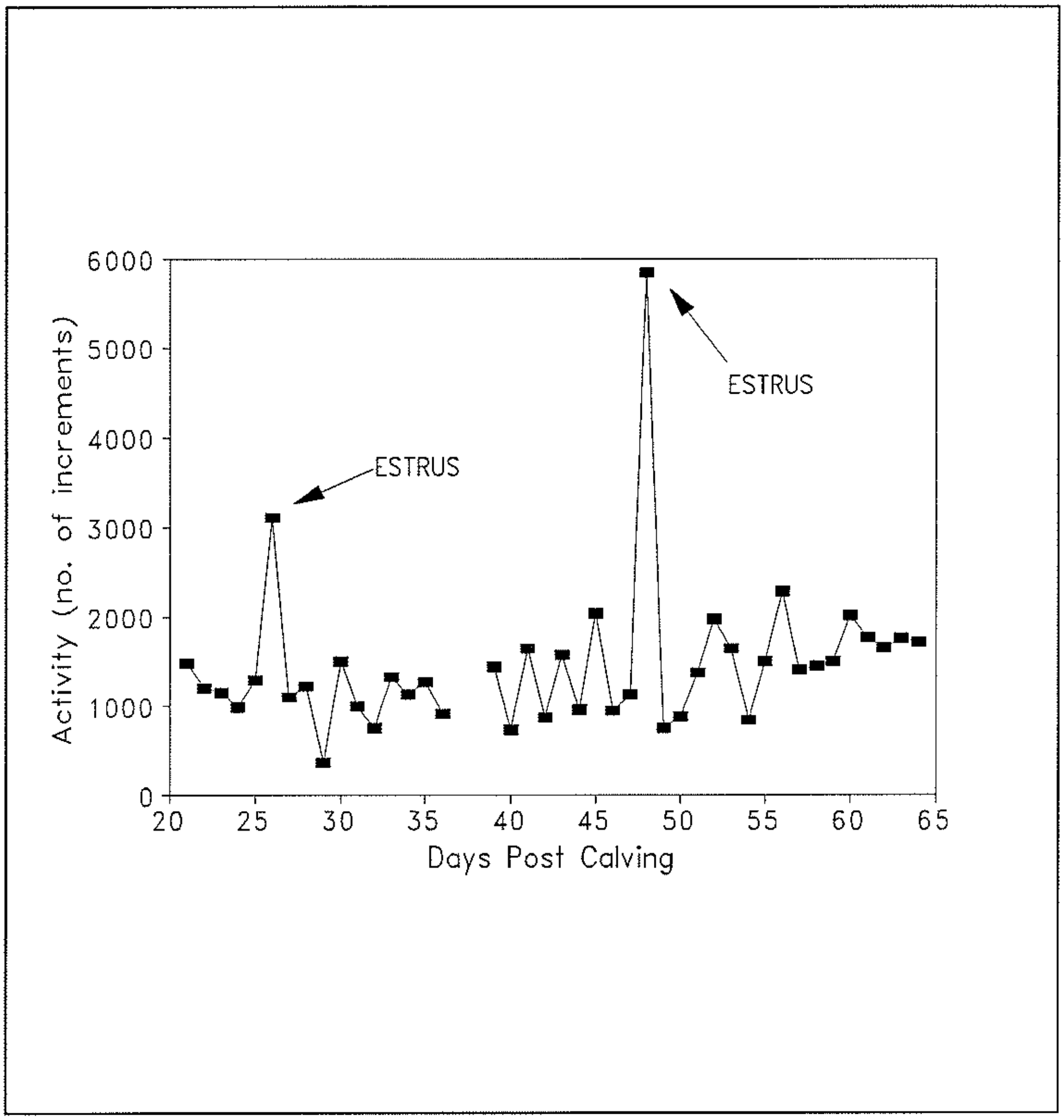

Figure 5. Daytime activity (0630-1430 h) of a typical cow (Cow 1) throughout early lactation.

potential heats were indicated by an increase in daytime activity exceeding the current nonestrus mean by at least 50%, a minimum of 5 days being necessary to establish the nonestrus activity mean. Secondly, to minimize the incidence of false positives, if the increase in activity occurred less than 15 days after an increase of greater magnitude, the second increase was not considered to be estrus-related. If estrus was indicated less than 15 days after an increase of lesser magnitude, the previous increase was considered a false positive indication of estrus and the second increase was assumed to be estrus-related.

Application of this criterion to the data (Cows 1-10) resulted in detection of 20 of the 25 (80%) estrus days and 4 false positives. Cow 10 was particularly variable in her day to day activity (Figure 6). Removal of this cow's 3 ovulations from the data set improved estrus detection to 86% and reduced the number of false positives to 2. Milk progesterone and daytime activity for the 3 irregular cows are illustrated in Figures 7-9. In general, none of the cows showed increased activity when progesterone was above 1 ng/ml (as with normal luteal function). Cow 11 (Figure 7) showed no increases in activity except on the day of induced heat, 116 days postpartum. Ovulations may have occurred prior the increases in progesterone beginning on days 28 and 69 postpartum. If so, lack of increase in activity prior to these days should be regarded as false negatives.

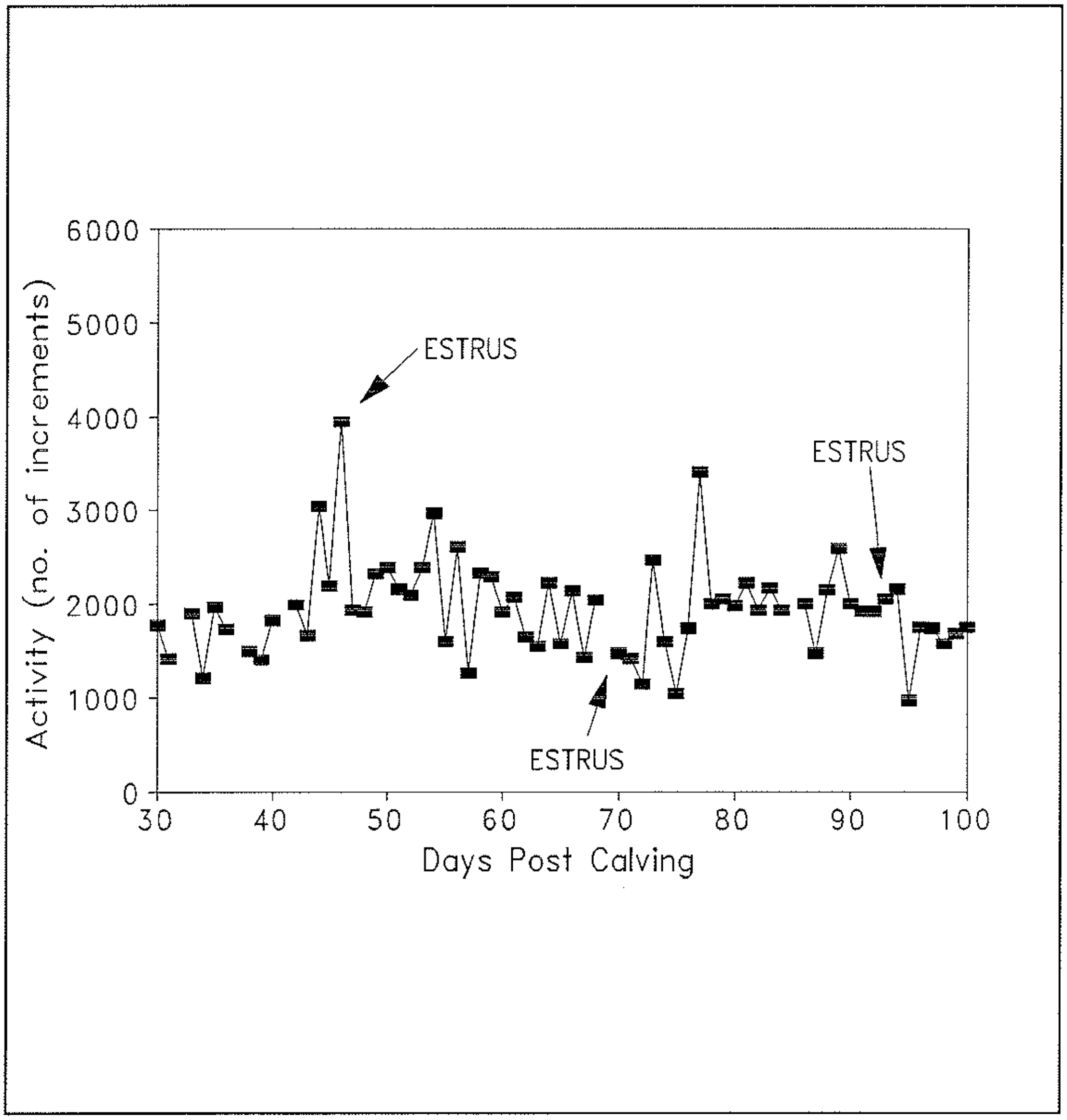

Figure 6. Daytime activity (0630-1430 h) throughout early lactation for Cow 10.

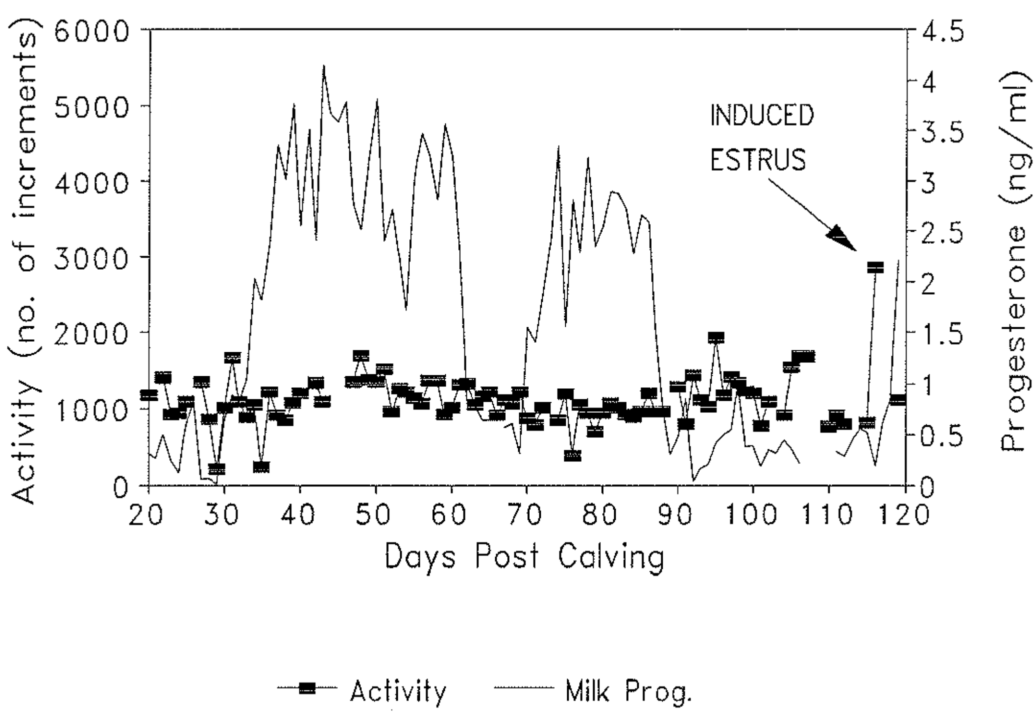

Figure 7. Daytime activity (0630-1430 h) and milk progesterone of Cow 11. No increases in activity were detected except on day of induced heat (day 116).

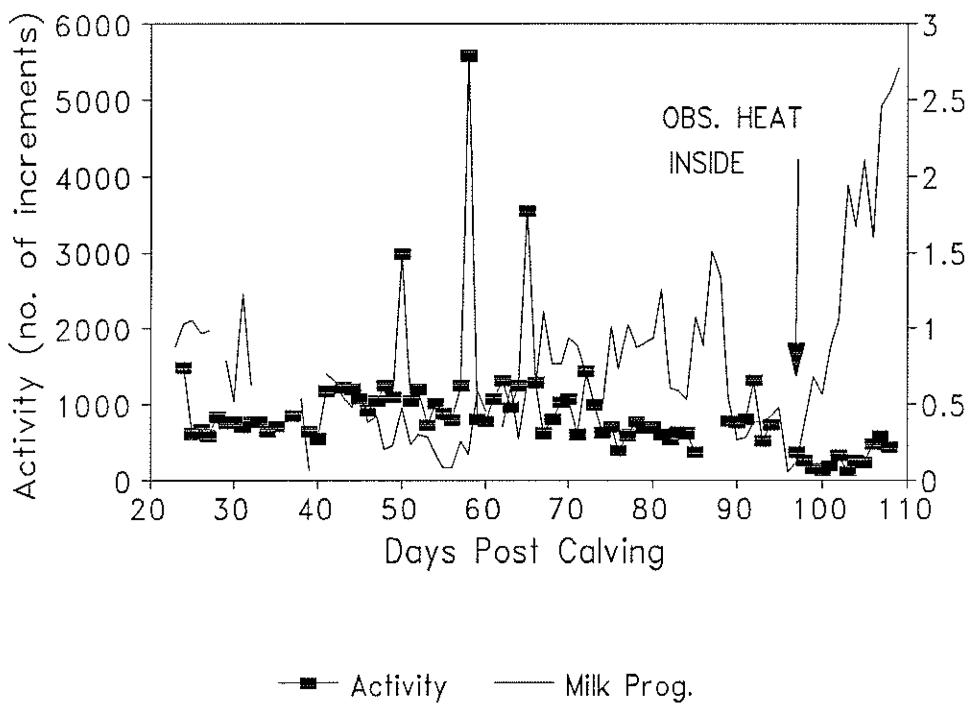

Figure 8. Daytime activity (0630-1430 h) and milk progesterone of Cow 12. Arrow indicates observed heat while cow remained inside during the regular turnout period.

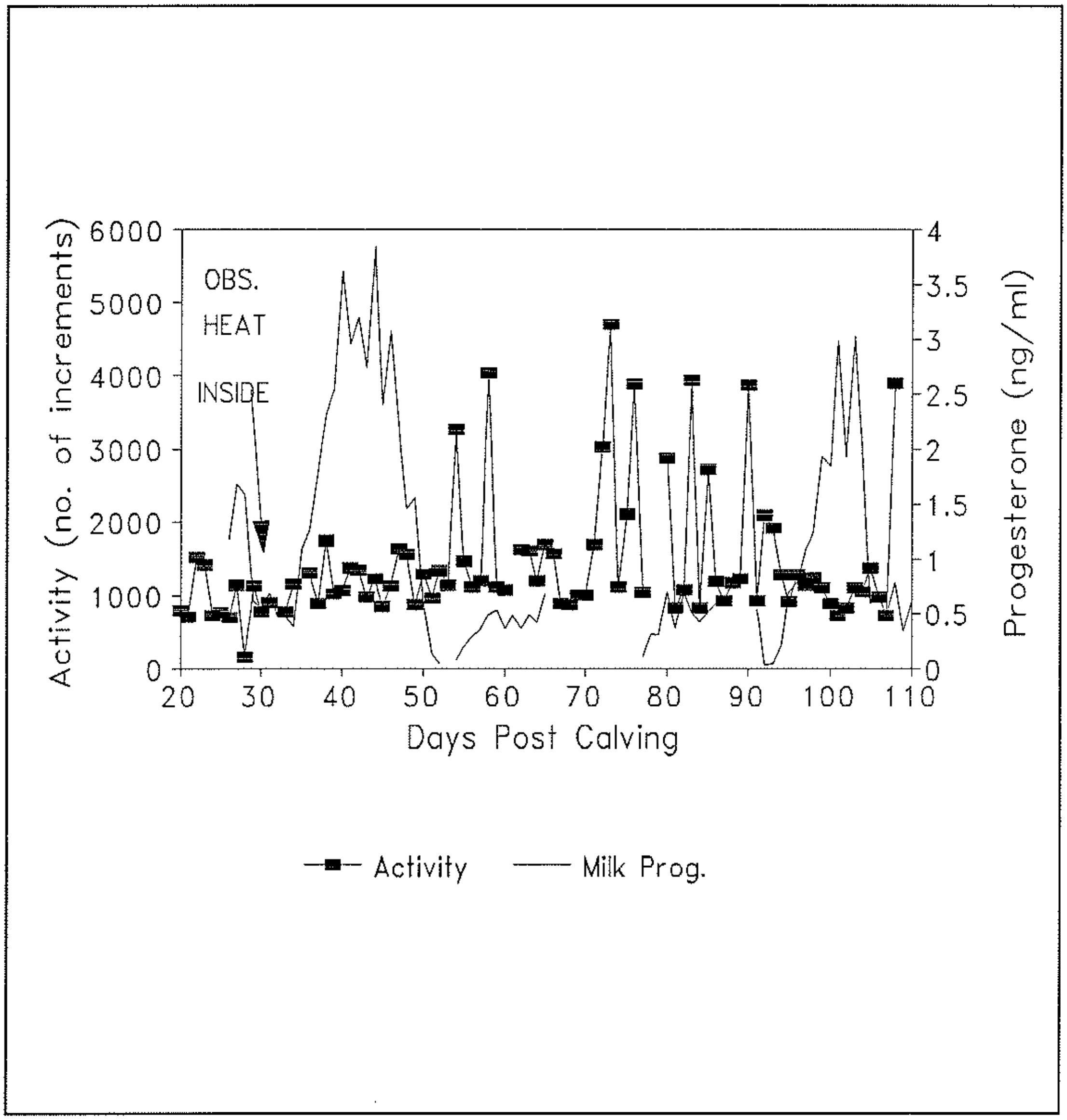

Figure 9. Daytime activity (0630-1430 hr) and milk progesterone of Cow 13. Arrow indicates observed heat while cow remained inside during the regular turnout period.

Considering the irregular time intervals for changes in progesterone (in particular being high for 30 days, low for 8 days and low for 25 days) and the high milk production of this cow (Appendix 3), it is also possible that no estrus occurred prior to 116 days postpartum.

None of the increases in activity of Cow 12 (Figure 8) could be associated with ovulation but each increase was accompanied by visually observed standing/mounting behavior; speculated to be due to follicular cyst(s). Progesterone profile and behavioral observations indicated Cow 13 (Figure 9) was in estrus 32 days postpartum. No increase in activity was indicated by the pedometer measurements on this day but it should be noted that this cow remained inside during the regular turnout period. Following day 50, progesterone indicated Cow 13 to be acylic for at least 40 days. Frequent increases in activity during this time may have been in response to changing estrogen levels in the absence of progesterone. Standing/mounting behavior was observed with the activity increases on day 83 and 108. Increased activity on day 90 may have been associated with ovulation and the subsequent rise in progesterone (return to normal cycling).

Temperature

Computer/receiver system malfunction resulted in only 35 h of missed data collection during the 85 day period of continuous temperature monitoring.

There was only one incident of transmitter expulsion. Anchoring devices remained in the vagina until removal 42 - 83 days after insertion. Vaginal temperatures were collected for 21 estrous cycles in the 10 regularly cycling cows.

Ear transmitters required occasional maintenance to ensure proper placement of the thermistor. Some ear transmitters were unreliable in either signal transmission or signal reception by the receiver. As a result, ear skin temperature data was collected from only 6 cows during a total of 12 estrous cycles.

Vaginal Temperature

Development of Criteria. Mean vaginal temperature (Tv) for every hour of data collection was calculated for each cow. A variety of criteria were investigated to determine whether a rise in Tv occurred at estrus and whether or not it was unique to estrus. Initially, it was necessary to establish what amount of increase would be considered to exceed normal variation in Tv and the measuring devices. In agreement with other literature, Fordham et al (1988) reported a mean estrual rise in body temperature of approximately 0.3 with a range of 0 to 1.0 C. Upper limits of temperature increases were also considered to avoid fever-related increases in body temperature meeting the criteria of estrus. For these reasons, a range of values between .1 and 1.0 C were considered in defining the first element of the criteria: the required increase in Tv.

It was also necessary to define a baseline temperature against which increases would be compared. In establishing a baseline temperature, previous researchers have examined the usefulness of using a value representing the mean or maximum temperature recorded over a number of previous days. In the current study with continuously monitored temperatures, the calculated mean temperature for every cow for every hour was available. Therefore, each hour could be compared to the mean of previous hours of the same day, the number of previous hours included being a changing element of the criteria. Preliminary examination revealed this form of baseline temperature resulted in a high number of false positives, likely due to diurnal variation in body temperature. Also, calculation of daily means and establishing baselines which varied in the number of previous days included was found to result in poor detection of estrual rises in Tv. This was possibly due to a short term increase in Tv at estrus having little impact on the total daily mean. Using means obtained from previous days as the baseline temperature improved detection when each day was divided into 4 or 6 equal time periods and each period was compared to the corresponding period mean of previous days. This increased the impact that an estrus-related increase in temperature would have on the current mean.

Further improvements in estrus detection were expected to be made by comparing each hour of the 24 hour day to its corresponding hour over previous days. Thus, each hour would have its own baseline and the number of previous days included (number of days lagged) in calculating the baseline became the second element in establishing the criteria which best defined estrus. While a more accurate nonestrus baseline can be established by increasing the number of days lagged, it is possible that too much previous history may be including information no longer pertinent about the cow and that information pre-estrus would become diluted. Considering this, it was expected that some compromise between the two extremes would be optimal.

While some researchers report using the maximum temperature recorded instead of the mean temperature recorded (over previous n days) as the baseline value, this aspect was not investigated in the current study. However, some criteria presently examined did define the increase required in terms of a range of standard deviations above baseline temperature (baseline + 2, 3 or 4 standard deviations of baseline).

When applying criteria that varied in definition of an increase in temperature and in definition of baseline temperature, it became evident that increases above baseline were somewhat common on nonestrus as well as on estrus days. It was also evident that during nonestrus, the criteria was more likely to be met for only one hour; whereas during estrus, the criteria was more likely to be met for more than one consecutive hour. For this reason, a third element was included in setting the criteria where a defined temperature increase (X C or X s.d.) over a defined baseline (n days) had to be exceeded for a specified minimum duration. Temperature increases above baseline were tested for durations ranging from 2 to 4 consecutive hours and also for a duration defined as a total of 4 hours within a 12 hour period.

Inclusion of all possible combinations of the varied elements resulted in excess of 196 criteria to be examined. These criteria were examined on a 3 cow sample chosen on the basis of appearing to display liberal increases in Tv at estrus. With this initial screening, many of the examined criteria were eliminated due to their obvious contribution to poor estrus detection or numerous false positives. The remaining criteria were applied to all 10 cycling cows for an overall indication of their effectiveness to maximize estrus detection and minimize the incidence of false positives.

Optimum detection of estrus was considered to occur where overall accuracy (detection rate x no. detected/(no. detected + no. false positives)) was greatest. The criterion which maximized overall accuracy defined estrus as a rise in Tv $\geq .3 \leq 1.0$ C above the previous 4 day baseline for at least 3 consecutive hours. Application of this criterion detected 17 of 21 (81%) estruses and yielded 3 false positives which represents an overall accuracy of 69%.

General trends for changing the three elements of the criteria are shown in Figures 10-12. For the purpose of simplification, only shown are the effects for changing one variable where the other two variables are held constant. Figure 10 illustrates the effects of changing the minimum temperature increase required from .35 C to .30 C to .25 C while the number of days lagged and number of consecutive hours required are held constant at 4 and 3, respectively. Initially, the benefit of making the criteria less strict (from .35 C to .30 C) is apparent since a large increase in detection rate is realized at no expense of additional false positives. However, as the Tv increase required decreases from .30 C to .25 C, no increase in detection rate is gained and there is an increase in the number of false posititves.

Figure 11 illustrates the relationship between detection rate and the number of false positives as the number of days lagged decreases from 7 to 2 and the Tv increase and number of consective hours are held constant at .30 C and 3, respectively. When the number of days included exceeds 5, the number of false positives and detection rate are based on 20 possible estruses due to one estrus having data for the previous 5 days only. As the number of previous days included in determining baseline temperature

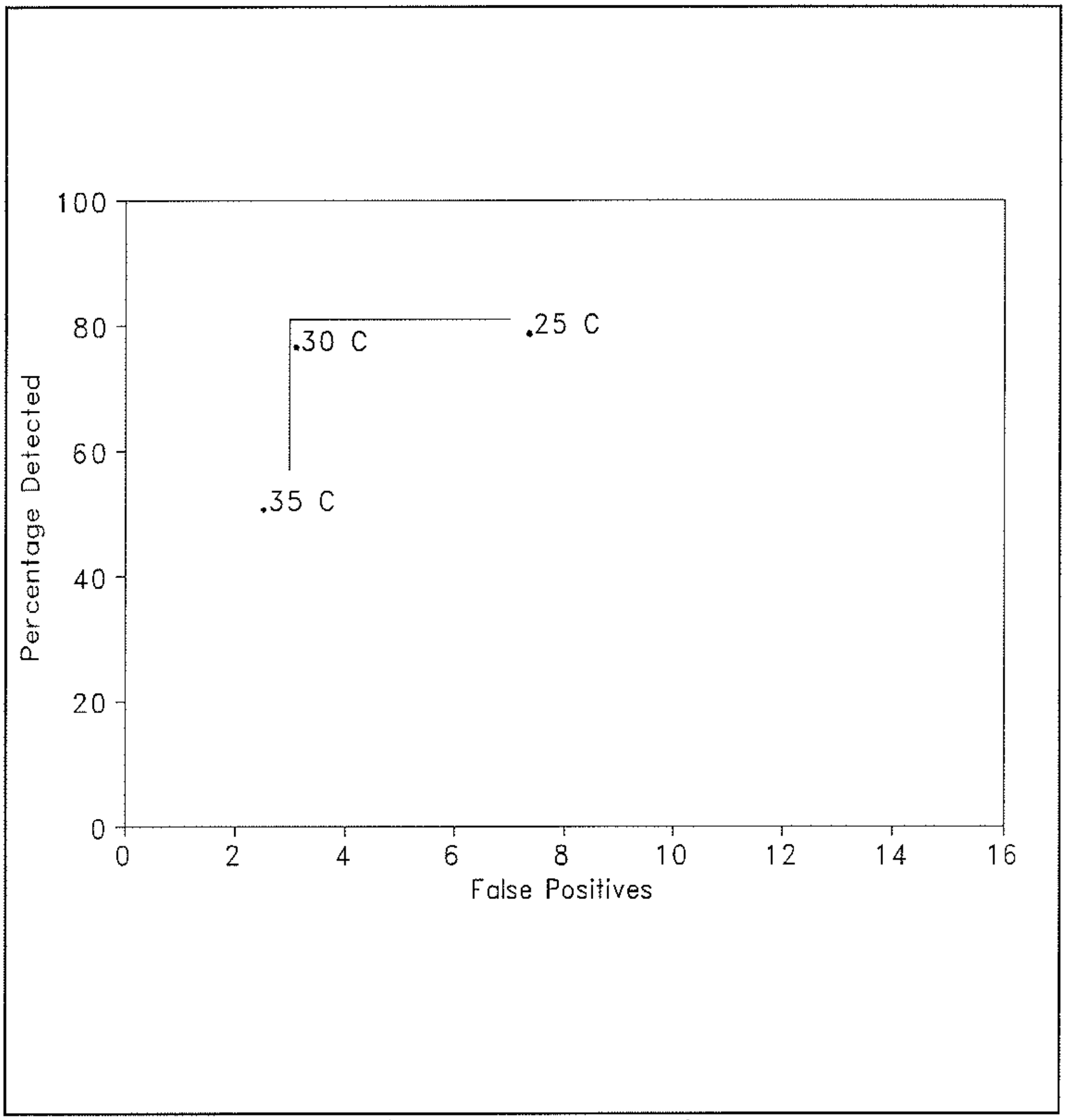

Figure 10. Changes in estrus detection and false positives for changing the temperature increase required. Number of days lagged and number of hours required are held constant at 4 and 3, respectively.

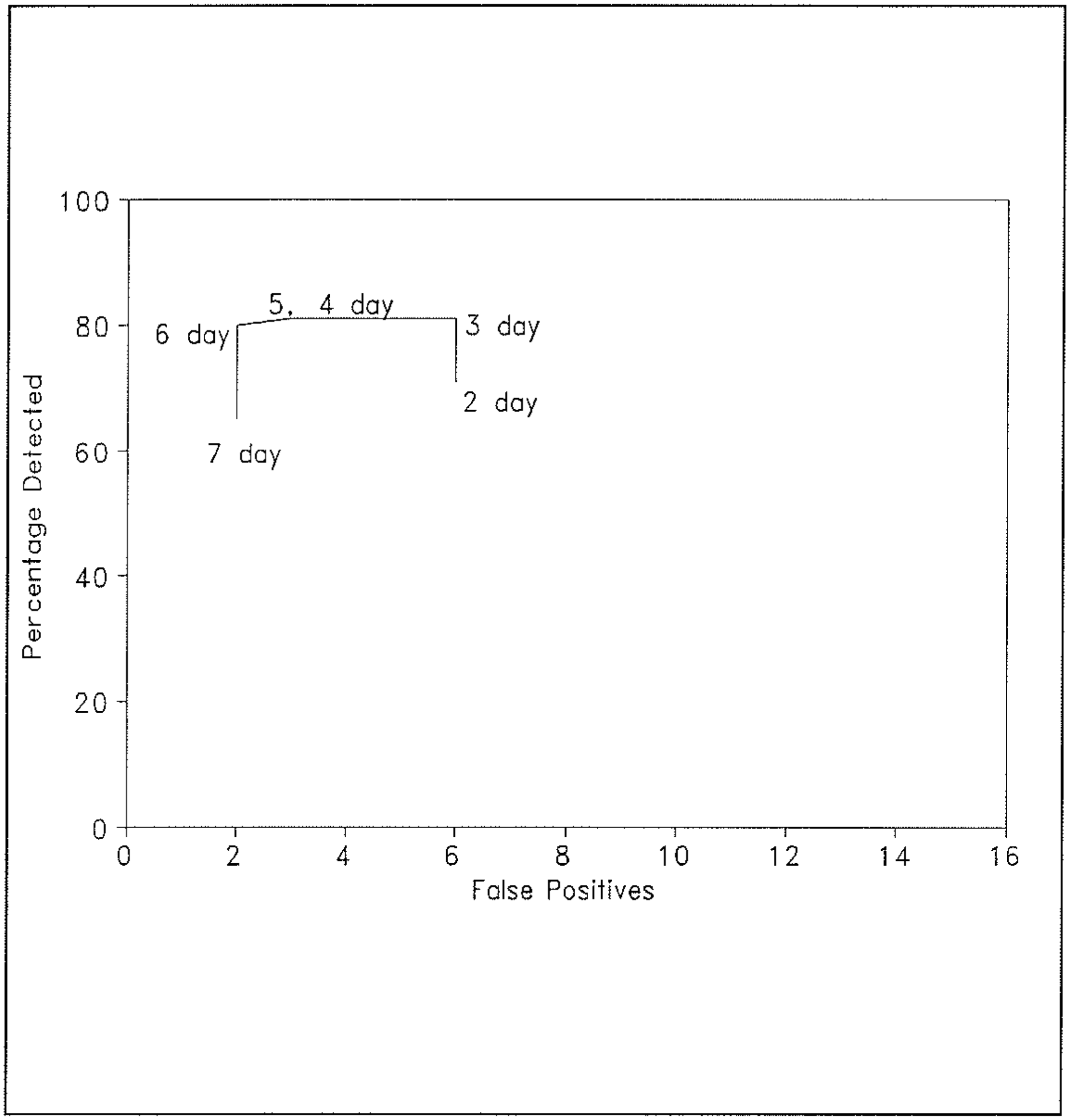

Figure 11. Changes in estrus detection and false positives for changing the number of days lagged. Tv increase and number of hours required are held constant at .3 C and 3, respectively.

decreases to 4, detection rate improves and the number of false positives remains constant. Detection rate is maximized and the number of false positives are relatively minimal for both a 4 and 5 day lag (represented by the same point on the line). Using less than a 4 day lag, the number of false positives increases (3 day lag) and is followed by a reduced detection rate (2 day lag).

Figure 12 illustrates the tradeoff between detection rate and false positives as the number of consecutive hours required changes from 4 to 3 to 2. The temperature increase required is held constant at .30 C and the number of days lagged is held constant at 4. Similar to the effects of changing the magnitude of the increase (Figure 10), there is an initial benefit in making the number of hours required less strict in order to improve detection rate. However, as the number of hours required decreases from 3 to 2, the increase in the number of false positives exceeds any improvement in detection.

Standard deviations in Tv throughout the experimental period, for each cow, are provided in Appendix 2. Increases in Tv for each individual estrus and false positive are illustrated in Appendix 4. Maximum change in Tv from baseline, at each estrus, was .65 $\pm$ .3 C (range = .27 - 1.7 C). Temperature remained elevated by at least .3 C for an average total of 6.8 $\pm$ 4.6 h (range = 0 to 22 h).

Figure 13 shows the mean and standard error Tv change

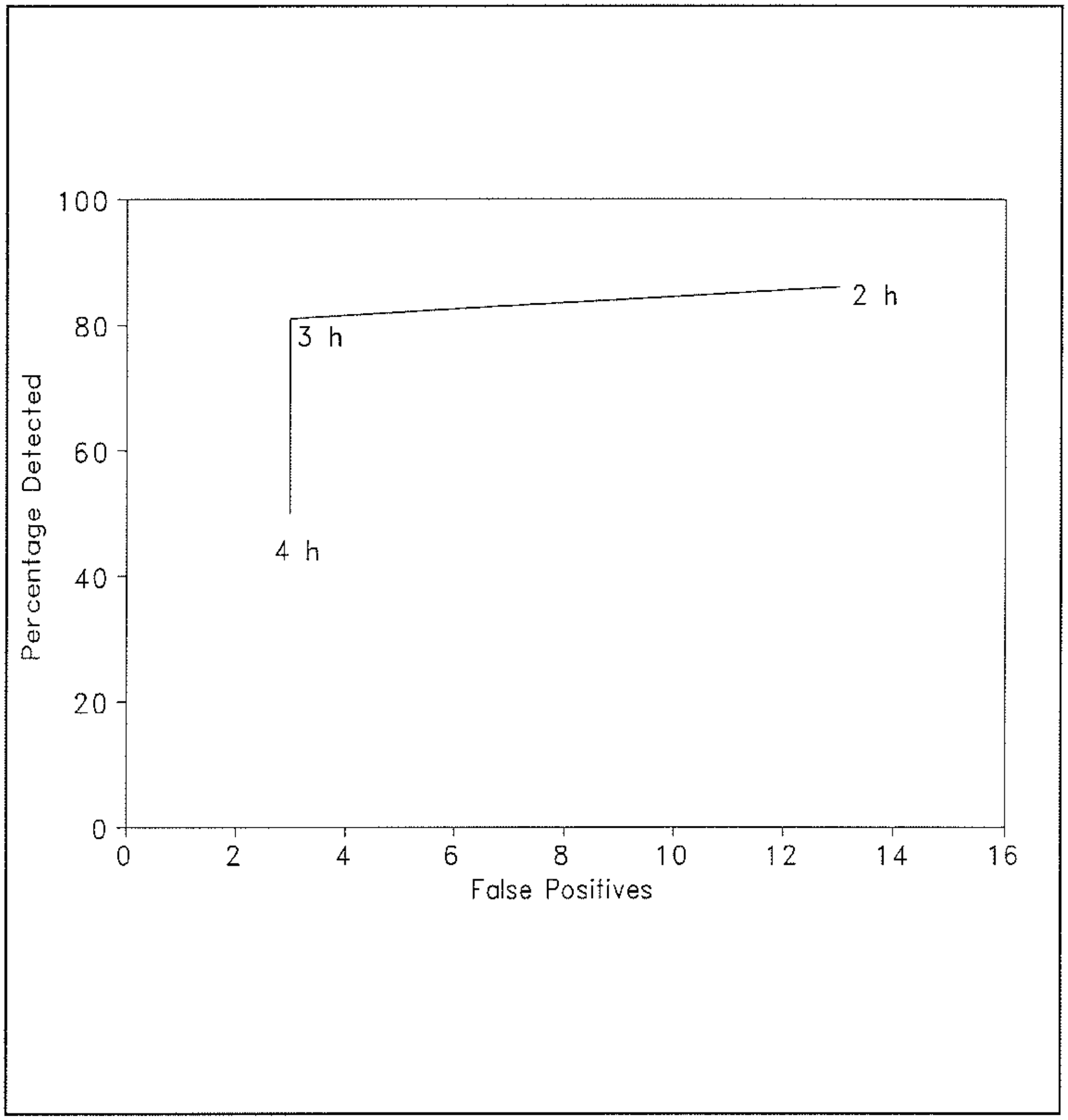

Figure 12. Changes in estrus detection and false positives for changing the number of consecutive hours required. Number of days lagged and increase required are held constant at 4 and .30 C.

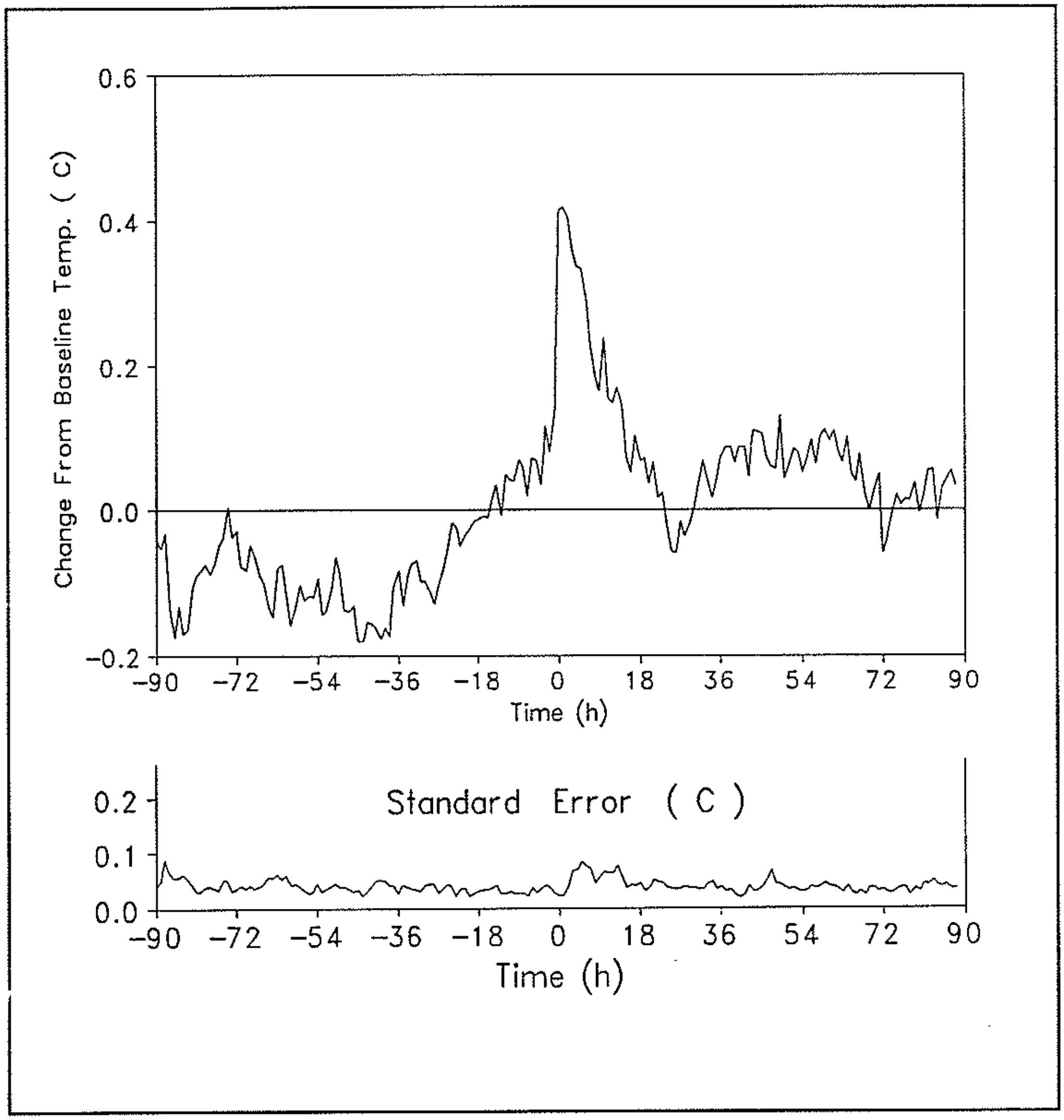

Figure 13. Mean and s.e. for change in vaginal temperature from baseline (4 day lag) for all estruses (n=21). Temperatures arranged around time of intial rise of .3 C (time 0).

from baseline for all estruses (n=21) when temperatures for each estrus were arranged around the time of initial rise to .3 C above baseline (time 0). Mean temperatures for the three false positives were also calculated (Figure 14) and appear to lack the more sytematic pattern as observed for the true estruses.

Time preceeding and following estrus (time 0) was divided into 36 h periods (Table 1). Change in temperature from baseline was significantly higher for the 36 h period surrounding time 0 than for either the preceeding ($p>.0001$) or following ($p>.01$) 36 h periods. Temperatures prior to the 36 h period surrounding estrus were below baseline and significantly less ($p>.0001$) than temperatures following the 36 h period surrounding estrus. The sources of variation and respective degrees of freedom and mean squares resulting from the analysis of variance are provided in Appendix 5. Initial observations suggested a trend for larger differences between temperature and baseline following estrus for cows which were inseminated and subsequently confirmed pregnant than for cows which were not bred or bred and confirmed nonpregnant. However, analysis revealed no significant differences between pregnant and open cows 18 to 54 h or 54 to 90 h after estrus ($p>.6$, $p>.5$, respectively).

Milk progesterone, daytime activity and occassions of Tv exceeding .3C for a minimum of 3 hours for the irregular cows are illustrated in Figures 15-17. Cow 11 (Figure 15)

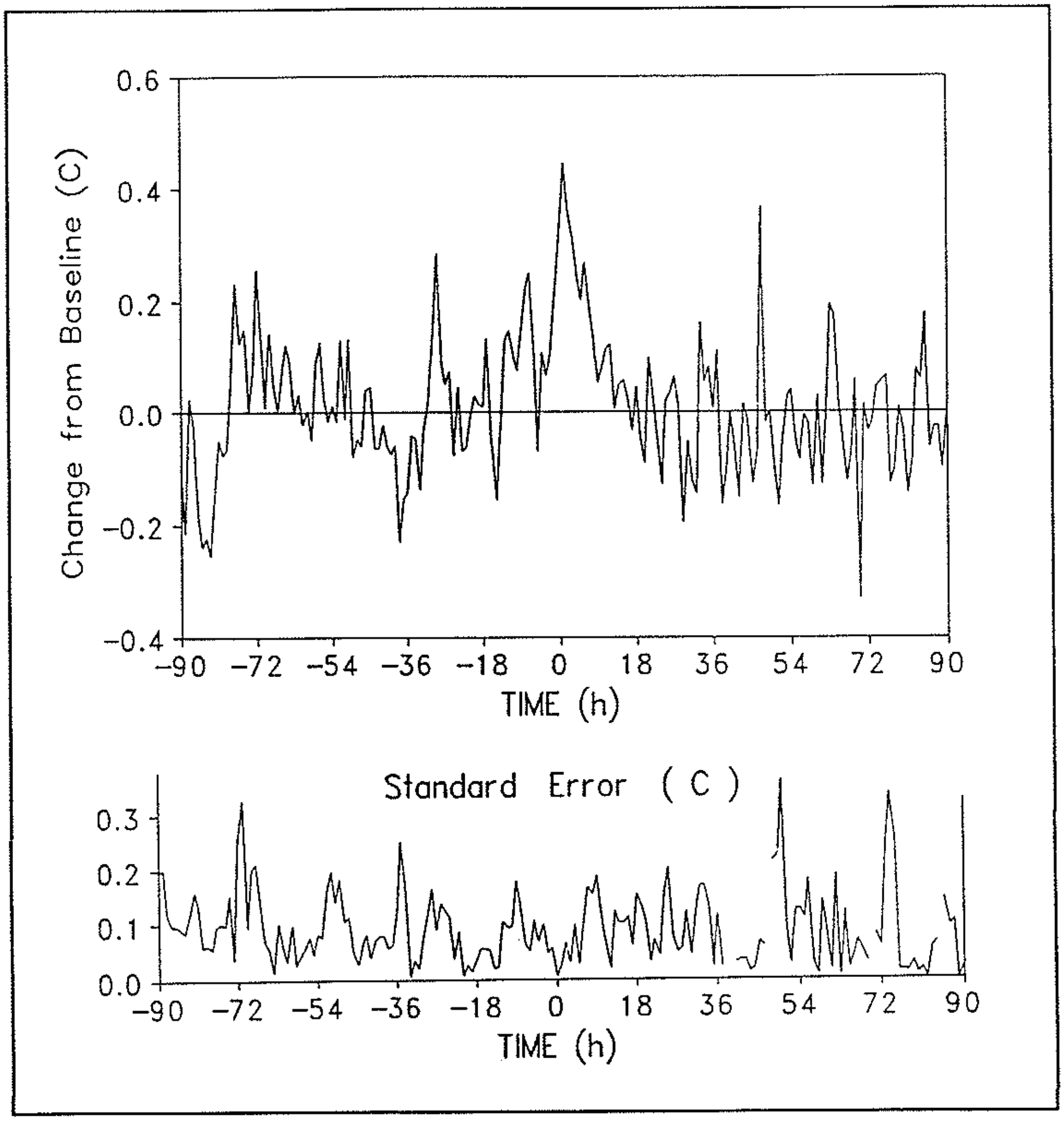

Figure 14. Mean and s.e. for change in vaginal temperature from baseline (4 day lag) for all false positives (n=3). Temperatures arranged around time of initial rise to .3 C (time 0).

Table 1. Mean ($\pm$ s.e.) change from baseline temperature for 36 h time periods surrounding estrus (n=21).

Period (hr)	LSmean (C)	Standard Error (C)	P > .05
1 ($> -90 \leq -54$)	- .07	.02	C
2 ($> -54 \leq -18$)	- .09	.02	C
3 ($> -18 \leq +18$)	+ .13	.02	A
4 ($> +18 \leq +54$)	+ .05	.02	B
5 ($> +54 \leq +90$)	+ .06	.02	B

* Means with the same letter are not significantly different.

* Standard errors and probabilities calculated using the mean square for period X cow as the error term.

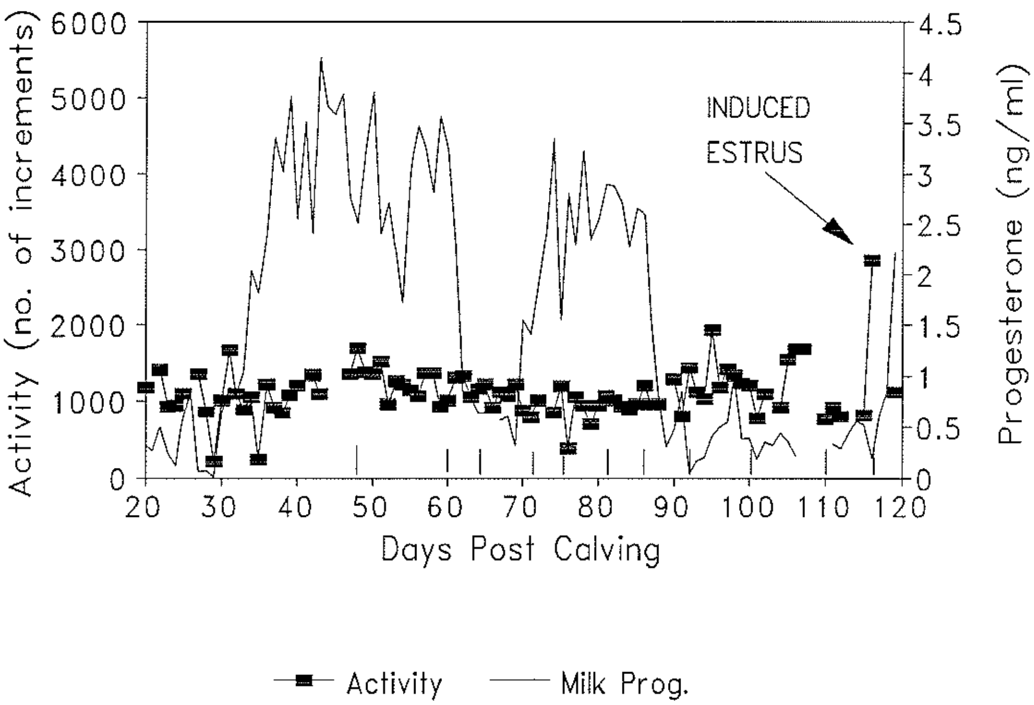

Figure 15. Milk progesterone, activity and occasions of Tv increases of Cow 11. Vertical lines above the x axis indicate Tv increases $\geq$ .3 C for a minimum of 3 consecutive hours.

showed frequent Tv increases of varying magnitude (per incidence maximum .5 to 1.1 C). Cow 2 (Figure 16) showed increases in temperature corresponding to increases in activity and observed standing/mounting behavior. Cow 13 (Figure 17) showed only 2 increases in Tv which occurred on days 100 and 108.

Comparison of detection rate and number of false positives among activity, Tv, and casual observations for each progesterone confirmed estrus are presented in Table 2. Activity and Tv were similar in detection rate (activity 20/25, 4 false positives; Tv 17/21, 3 false positives) and were better than casual observations (14/26, 1 false positive). Two of the false negatives (Cow 10) and none of the false positives overlapped for activity and Tv. Only one estrus not detected by Tv was detected by observations and observations did not detect any heats not detected by activity monitoring.

Ear Skin Temperature

Standard deviations in ear skin temperature (Tes) are given in Appendix 2.

Tes for available cows and estrous cycles were manipulated similar to Tv but no criteria could be found for detecting any of the 12 estruses. Tes were available on two of the three false positives that arose from Tv monitoring. The correlations between Tv and Tes on these 2 days did not appear to differ from their correlations on days of estrus

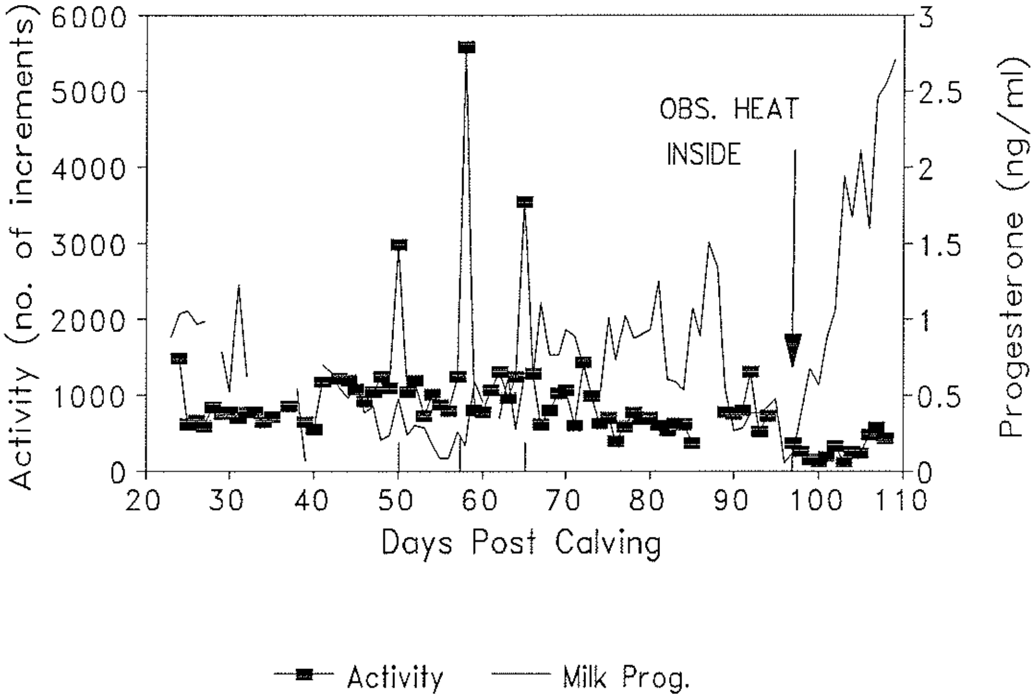

Figure 16. Milk progesterone, activity and occasions of Tv increases of cow 12. Vertical lines above the x axis indicate Tv increases $\geq$ .3 C for a minimum of 3 consecutive hours.

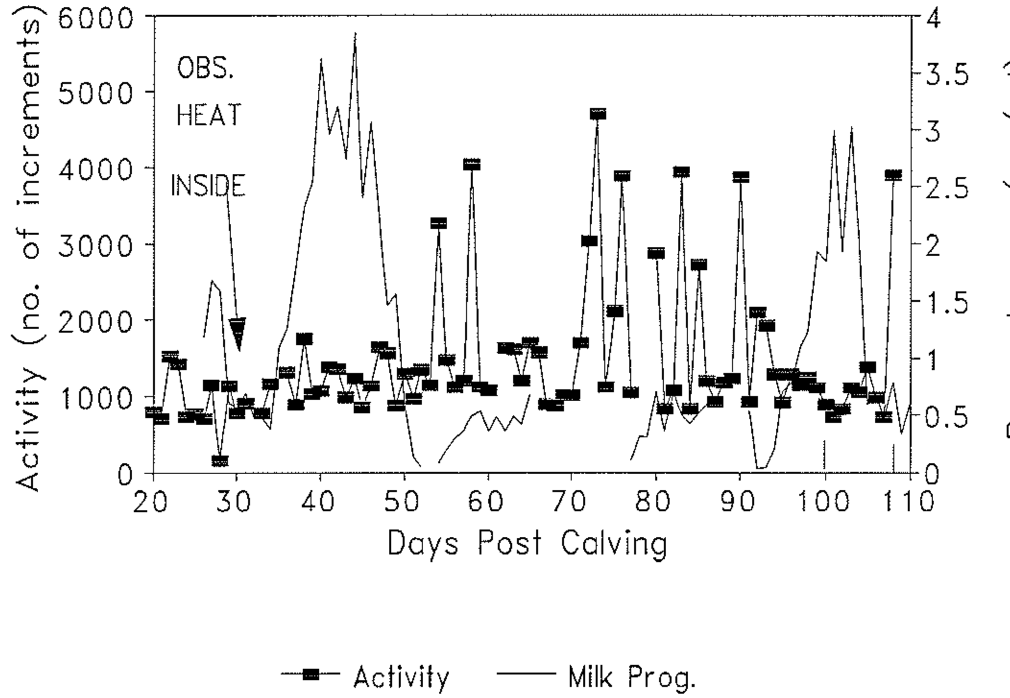

Figure 17. Milk progesterone, activity and occasions of Tv increases of cow 13. Vertical lines above the x axis indicate Tv increases $\geq$ .3 C for a minimum of 3 consecutive hours.

Table 2. Comparison among pedometer, vaginal temperature and casual observations in detecting estrus.

		Pedometer		Temperature		Observations	
Cow	Estrus*	Detected	F.P.	Detected	F.P.	Detected	F.P.
1	1 (61)	yes	0	no	0	no	1
1	2 (83)	yes	0	yes	0	yes	0
1	3 (113)	yes	0	yes	0	yes	0
2	1 (32)	no	0	N/A	N/A	no	0
2	2 (55)	yes	0	yes	0	yes	0
2	3 (78)	yes	0	yes	1	yes	0
3	1 (37)	yes	0	N/A	N/A	no	0
3	2 (58)	yes	0	yes	0	no	0
3	3 (79)	yes	0	yes	0	yes	0
4	1 (26)	yes	0	N/A	N/A	no	0
4	2 (48)	yes	0	yes	0	yes	0
4	3 (72)	N/A	N/A	no	0	yes	0
5	1 (62)	yes	0	yes	0	yes	0
6	1 (35)	yes	0	N/A	N/A	no	0
6	2 (60)	yes	0	yes	0	yes	0
7	1 (67)	yes	0	yes	0	yes	0
7	2 (89)	no	0	yes	0	no	0
7	3 (116)	yes	1	yes	0	yes	0
8	1 (68)	yes	0	yes	0	yes	0
9	1 (50)	no	0	N/A	N/A	no	0
9	2 (72)	yes	1	yes	0	no	0
9	3 (96)	yes	0	yes	0	no	0
9	4 (118)	yes	0	yes	1	yes	0
10	1 (46)	yes	1	yes	0	yes	0
10	2 (70)	no	1	no	0	no	0
10	3 (93)	no	0	no	1	no	0

* estrous cycle number (days postpartum)
N/A data not available
F.P. false positives; indicated are the number of false positives that occurred during the current estrous cycle

(Table 3). However, the incidence of false positives was too low to subject the correlations to statistical comparisons.

Table 3. Correlation between Tv and Tes on all available days, days of true estrus and days of Tv false positives.

Cow	Correlation between Tv and Tes (r) all days	true estrus	false pos.
1	-.49	+.32 (heat 1)	
		+.13 (heat 2)	
2	+.12	+.17 (heat 2)	
		-.17 (heat 3)	+.78
3	+.34	+.31 (heat 2)	
		-.56 (heat 3)	
4	-.18	-.26 (heat 2)	
		+.18 (heat 3)	
5	-.23	+.39 (heat 1)	
9	+.27	-.12 (heat 1)	
		-.49 (heat 2)	
		-.15 (heat 3)	+.32

DISCUSSION

Activity

Activity increased at estrus during the 8 h daytime period (0630-1430 h) when a 5 h turnout period was permitted. Pedometers did not indicate significant increases in activity for the 14 h nighttime period (1430-0630 h). Poor nighttime detection likely relates to the low activity during the night due to the degree of confinement imposed by tie stalls. Estrus activity for each cow did not increase with each passing estrus. If the experimental period had included first ovulations, estrous cycle number might have proven significant. Within cow variation in activity at estrus has been attributed to differences in the degree of estrus synchronization within the herd (Hurnik et al., 1975) and weather (Doherty et al., 1987).

The criteria developed to detect estrus identified 80% with only 4 false positives, a detection rate superior to that of casual observations alone (54% detected, 1 false positive). The criteria used was designed to simulate decisions that would be made emperically from viewing plots of the raw data. Because it was developed post hoc, it does require application to a new, similar data set for validation. The results obtained here agree with results of

Kiddy (1977) who used pedometers on cows primarily housed in tie stalls. With cows spending 1 h twice daily in a holding lot prior to each milking, 72% of all heats were detected.

The false positives in this study could have arisen due to increased diestrus activity in response to another cow in heat as was found for goats wearing pedometers (Doherty et al., 1987). In trying to minimize false positives, every suggested estrus could be confirmed by behavioral observations and/or milk progesterone tests. However, the low incidence of false positives would not warrant the efforts and cost of extensive screening.

Pedometer false negatives can arise due to true silent heats, poor weather and possibly a decrease or no change in ambulatory movement even though standing behavior may be overt. In this study, the majority of pedometer false negatives could have arisen from estrus not coinciding with the turnout period.

While detection of estrus-related activity was very good, a once daily indicator of activity may not be a precise indicator of the ideal time to inseminate. Therefore, under management systems similar to those of this study, improving both estrus detection and number of services to conception may require behavioral observations. In free stall housing or tie stall housing with more than one period of free movement activity, pedometers alone may yield even better estrus detection rates and may also provide information pertaining to the optimal time for artificial insemination as well. In support of this, Kiddy (1977) found larger increases in activity at estrus among free stall cows than tie stall cows.

While the strapping material used to modify the pedometers had deteriorated by the end of the study, the general design functioned very well and placing the pedometer on the rear leg above the hock was effective. The simplicity and effectiveness of pedometers shown in this study suggest that they could become a regular part of dairy herd management. In addition, continuous activity monitoring is feasable (through technology similar to that used to monitor Tv and Tes) and could be used to automate and further improve the accuracy of pedometers.

Temperature

The criteria applied to vaginal temperatures to detect estrus were also developed post hoc but were similar to that of Mosher et al. (1990) who defined a putative temperature spike as the first hour of a period of at least 3 consecutive hourly elevations in temperature $\geq$ .3 C above the average temperature at the same time of day on the previous 3 days. While a tradeoff between estrus detection and incidence of false positives existed, the optimal criteria detected the majority of estruses with a very low rate of false positives. Other studies measuring milk or

Tv twice daily have attained a detection rate equivalent to that of this study only at the expense of a large number of false positives. This, combined with the average duration of estrus-related temperature increases observed in this study (6.8 $\pm$ 4.6 h), illustrate the importance of continuous monitoring.

Twelve of the 17 Tv detected estruses initially increased to $\geq$ .3 C above baseline within the 5 hour turnout period (Appendix 4). This suggests that increased activity may be responsible for the increase in Tv measured at estrus. It may also be that behavioral estrus slightly preceeded the estrual rise in temperature and both contributed to the temperature increase measured.

As expected, Tv increased significantly at estrus. Mean temperatures 18 - 90 hours before the increase were found to be below baseline for true estruses but not for the false positives. It should be noted that the increase from baseline Tv on day of estrus is greater when preceeded by a drop since baseline Tv is defined as the mean of the previous 4 days. A preceeding drop in Tv could explain why a 4 day (96 h) lag gave the best detection rate. A slight drop in temperature prior to estrus has been observed previously (Fordham et al., 1988 and Maatje and Rossing, 1976) and may reflect luteal regression. Future studies to investigate this phenomenon more specifically would be worthwhile.

While differences in Tv from baseline, up to 90 hours after the initial rise, were not significantly different between pregnant and open cows, a larger sample size may have yielded different results. Also, the time periods tested may not have been those that would have illustrated this since it appears that any difference between the two begins about 80 hours after the initial estrual increase. However, in the current study, Tv were available for more than 90 h after breeding for only 2 cows in the pregnant status. Again, future studies to investigate this phenomenon more specifically would be worthwhile.

In addition to the 3 irregularly cycling cows (Cows 11, 12 and 13), Cows 1, 5, and 7 had more than an average number of days between first and second ovulation or between second and third ovulation. In the case of Cow 5, the extended interval between ovulations was characterized by low progesterone. While Zartman et al. (1983) found no influence of a plastic anchor in the vagina on reproductive fitness, the early anchoring devices used in this study were associated with discharge and erratic (frequent increases) Tv profiles. If physical irritation of the vaginal wall resulted from the devices, it could have led to increased corticosteroids capable of blocking ovulation through inhibition of the LH surge (Stoebel and Moberg, 1982).

In the case of Cows 1 and 7, the extended interval batween ovulations was characterized by high progesterone.

While it is known (Peter and Bosu, 1987) that uterine infections can result in levels of prostaglandins sufficient to initiate luteal regression, it is not known by what mechanisms the initial devices could have resulted in prolonged luteal phases.

The safety of the final anchoring device is partially substantiated by the abilty of the cows to resume cyclicity and the observation that 8 cows conceived with the anchor in place. Still, it is considered necessary to further investigate any influences of the final anchoring device, particularly during the early postpartum period.

Three cows (Cows 11, 12 and 13) continued to be either acyclic or irregular throughout the monitoring period. Stress can cause cycle irregularities as Liptrap and McNally (1976) were able to induce follicular cysts (as characterized by Cow 12) experimentally by administration of ACTH. However, Cow 12 never received the initial anchoring device and there is evidence from behavioral observations that she was cystic prior to receiving the final anchor type. Cow 13 also did not receive the early anchoring device. Cow 11 was the only cow, classified as irregular, to receive the initial anchoring device. While the early part of the progesterone profile for this cow was similar to that of Cow 1, Cow 11 did not resume regular cyclicity. Although the cause of the irregularities of Cows 11, 12 and 13 is not known, the incidence at which these irregularities occurred throughout the experiment is not considered unusually high.

Interestingly, Cow 12 and Cow 13 both showed frequent pedometer increases in activity but only increases by Cow 12 were consistently accompanied by increases in Tv. It can be assumed that Cow 12 possessed cysts which were (as described by Cook et al., 1990) dynamic in nature. If a follicular cyst becomes luteinized before being replaced by a second cyst, the resulting changes in progesterone and estrogen would simulate those preceeding ovulation and could therefore cause increases in both activity and vaginal temperature. Cow 13, however, had persistantly low progesterone. Periodic increases in estrogen due to waves of follicular growth may have been sufficient to cause the increases in activity without overt standing behavior, especially if there were other cows in estrus. Increases in estrogen in the absence of decreasing progesterone however, may not be a suitable stimuli for estrus-like increases in Tv. If so, this would eliminate the possibilty of detecting the first postpartum ovulation through monitoring of Tv. However, it is also likely that the increases in estrogen due to normal waves of follicular growth are not of the magnitude of estrogen increases due to a follicular cyst. For both cows, initial pedometer readings would be classified as false positives but due to the high frequency of false positives, reproductive failure would be suspected and the cow in question would be examined for possible ovarian abnormality. Repeated increases in Tv, or continued absence thereof, would also indicate reproductive failure for these cows. Thus, both activity and Tv monitoring could be useful aids in herd health evaluation.

Cow 11 showed frequent increases in temperature of varying magnitude, some in excess of 1 C. Since her radiotransmitter tested reliable before and after the experiment, the simplest explanation for these increases would be that they were fever related. This cow was observed to have a persistent rash on her udder but feed intake and milk production were unaffected. The 305-day lactation for this cow was 10,967 Kg of milk which is above the 7500 Kg U.S. National average for Holsteins (Harrison et al.,1990). While not directly affected by high production (Harrison et al., 1990), reproductive function can be compromised in high producing cows if energy requirements are not met. In view of this and the irregular changes in progesterone, it is not known whether or not the increases in progesterone were preceeded by ovulation.

It was not expected to be able to detect estrual increases in Tes but instead determine if there was a consistent relationship between ear skin temperature and vaginal temperature. It was assumed that, on the day of estrus, this relationship might be disturbed. Failure to establish the presence of this may be a reflection of its complexity more than its existence.

The number of false positives arising from other studies when trying to use body temperatures as a predictor of estrus, suggested than some measure of thermoregulatory status may be worthwhile. Measuring ear skin temperature could have indicated degree of heat liberation for a certain period and from this determine whether increases in vaginal temperature were estrual related. The two occurrences of false positives for which both ear skin temperature and vaginal temperature were available were insufficient to determine any consistencies. In addition, the temperate ambient conditions throughout the experiment may have minimized both the relatonship between ear skin temperature and vagina temperatures during false positives and the incidence of false positives themselves.

The estrus detection rate obtained through vaginal temperature monitoring alone are promising. The technology required is not yet available at a commercial level or at commercial prices but further studies validating the usefulness of continuous monitoring could provide incentive for meeting commercial needs. Computer software could also be improved to include necessary data manipulations and provide a yes/no response to whether a cow is in estrus.

CONCLUSIONS

Modification of pedometers for placement on the cow's rear leg to monitor activity was effective. The use of radiotelemetry was effective for continuously monitoring vaginal temperature whereas technical difficulties were encountered in measuring ear skin temperature. The final anchoring device designed prevented expulsion of the internal (vaginal) transmitters without causing any apparent physical harm. Further studies are required to determine the effects, if any, the anchor may have on reproductive function.

Increases in activity and vaginal temperature were observed at estrus in postpartum cows. Application of post hoc developed criteria to activity data detected 20/25 (80%) estruses and resulted in 4 false positives. Three false positives and an estrus detection rate of 81% (17/21) arose from criteria applied to vaginal temperatures. Both methods of detecting estrus were superior to casual observations (14/26, 1 false positive).

Further investigations are reqiured to validate, and possibly improve, the criteria developed here and to determine their effectiveness in other management systems.

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Appendix 1. Table 1. Precision of individual vagina transmitters (Trans. #) as determined by the difference between water bath temperature and transmitter temperature over a period of 1 hour.

Trans. #	38 (C)	39 (C)	40 (C)	41 (C)	42 (C)
5	+.23 ± .03	+.06 ±.09	+.17 ±.10	+.11 ±.10	-.01 ±.01
6	-1.00 ±.06	-1.02 ±.10	-.91 ±.09	-.99 ±.11	-1.06 ±.04
9	-1.33 ±.04	-1.37 ±.03	-1.42 ±.09	-1.50 ±.09	-1.60 ±.04
10	-1.51 ±.08	-1.65 ±.09	-1.52 ±.03	-1.59 ±.09	-1.66 ±.03
15	-1.68 ±.07	-1.77 ±.09	-1.66 ±.06	-1.75 ±.10	-1.82 ±.02
17	-1.44 ±.04	-1.50 ±.02	-1.51 ±.09	-1.62 ±.10	-1.71 ±.03
22	-1.73 ±.03	-1.76 ±.03	-1.78 ±.08	-1.86 ±.08	-1.93 ±.04
25	-2.34 ±.06	-2.49 ±.08	-2.62 ±.10	-2.77 ±.09	-2.88 ±.01
27	-1.64 ±.05	-1.69 ±.09	-1.60 ±.10	-1.70 ±.09	-1.75 ±.03
33	-1.78 ±.02	-1.84 ±.03	-1.70 ±.08	-1.81 ±.09	-1.88 ±.06
39	-.54 ±.07	-.61 ±.08	-.49 ±.09	-.58 ±.09	-.65 ±.05
47	-.65 ±.08	-.69 ±.10	-.56 ±.08	-.67 ±.09	-.76 ±.05
50	-.82 ±.03	-.86 ±.03	-.82 ±.08	-.94 ±.08	-1.03 ±.05

Appendix 1. Table 2. Change in precision between initial and final transmitter test.

Trans. #	38 (C)	39 (C)	40 (C)	41 (C)	42 (C)
5	+1.1	+1.4	+1.0		
6	-.2	0.0	-.4	-.5	-.4
9	-.5	-.2	-.5	-.6	-.4
10					
15	+.9	+1.2	+.7	+.7	+.8
17	-.1	0.0	-.2	-.3	-.1
22	+.2	-.6	+.2	+.1	+.2
25	+.6	+.9	+.7	+.6	+.6
27					
33	0.0	+.2	-.2	-.2	-.1
39	-.1	-.2	-.2	-.2	-.1
47	-1.9	-1.9	-2.3	-2.4	-2.4
50					

Transmitter numbers 10, 27 and 50 were unavailable for testing at the end of the experimental period.

Transmitter number 5 changed in precision to the extent that time intervals corresponding to temperatures above 40 C exceeded the upper limit of the computer lookup table.

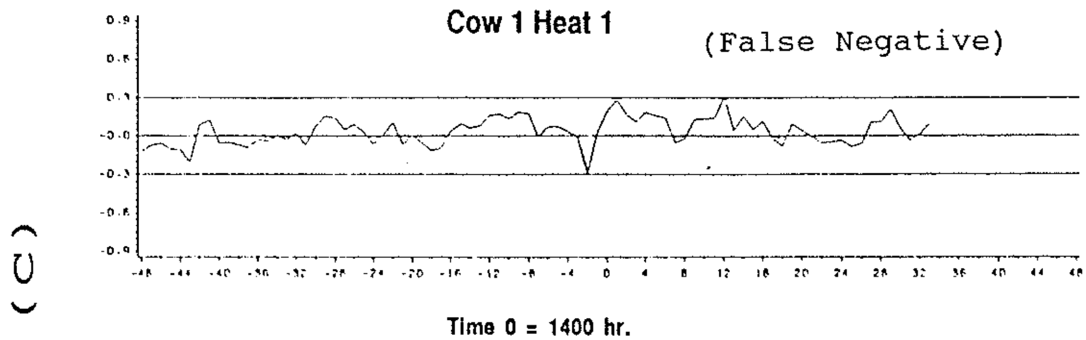
Appendix 2. Standard deviation in activity, Tv and Tes throughout the experimental period, for each cow.

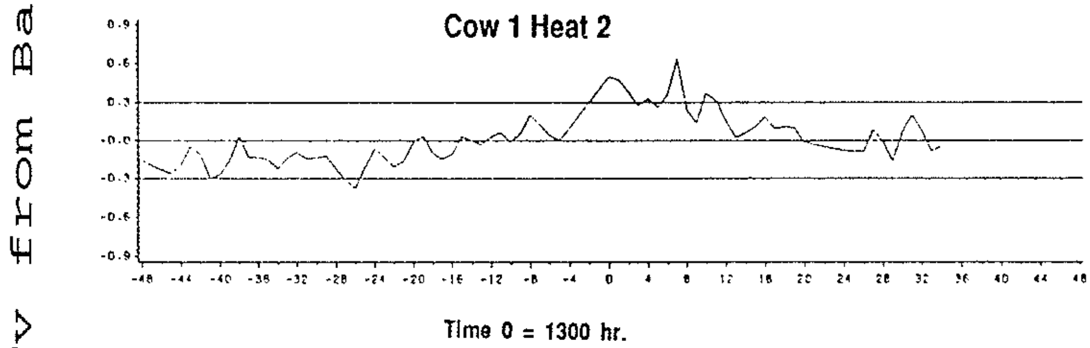
	Standard Deviation		
Cow	Activity (increments)	Tv (C)	Tes (C)
1	553	.3	2.89
2	702	.39	2.24
3	789	.31	2.31
4	1056	.36	1.82
5	1326	.80	1.84
6	727	.36	
7	769	.58	
8	1424	.31	
9	751	.47	2.44
10	568	.29	
11	212	.37	
12	1141	.43	
13	681	.75	

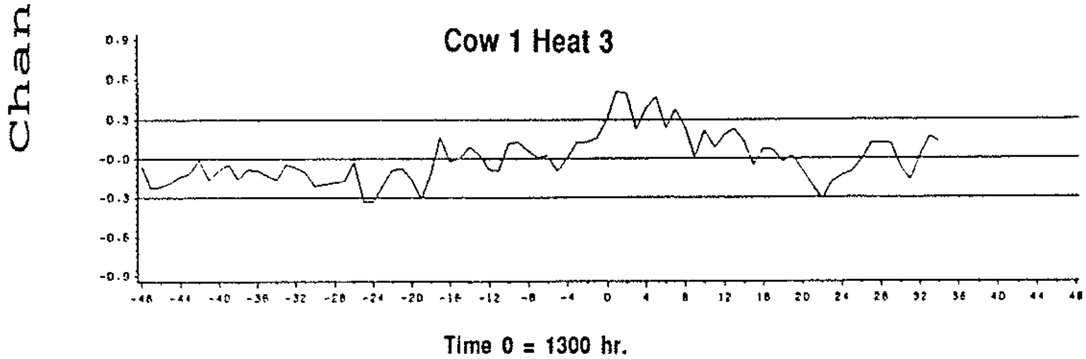
Appendix 3. Milk production and lactation number for all cows monitored.

Cow	305 day milk production	Lactation
1	6338	3
2	6634	3
3	9443	3
4	8929	3
5	7952	3
6	8796	1
7	8401	4
8	9361	1
9	7627	3
10	7030	1
11	10697	5
12	9858	3
13	9102	1

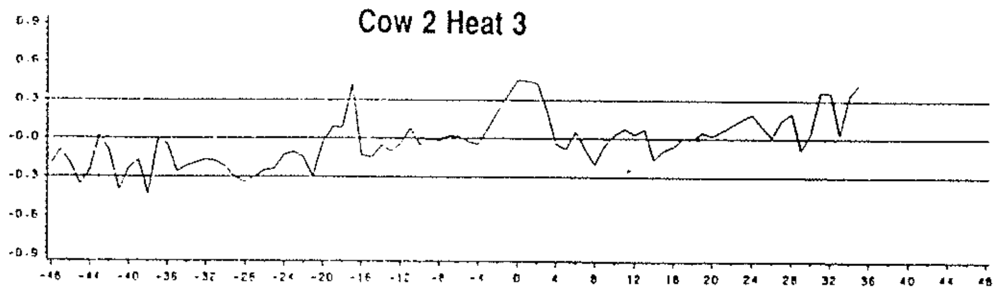
Appendix 4. Individual change in vaginal temperatures from baseline around time of estruses and false positives.



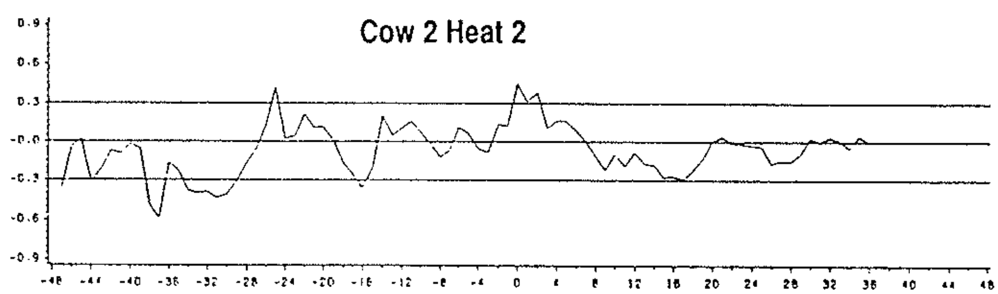




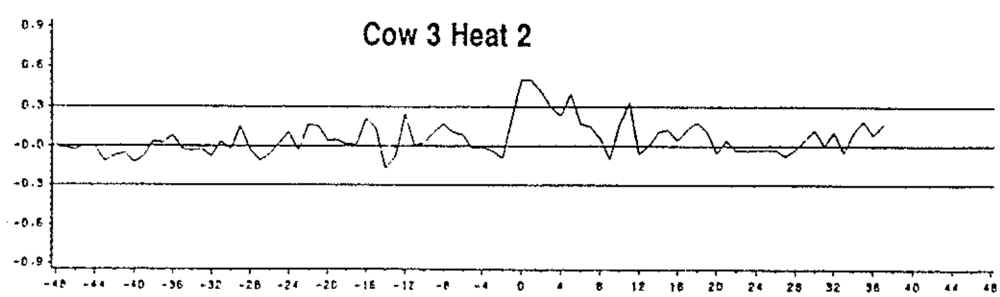
Change in Tv from Baseline (C)



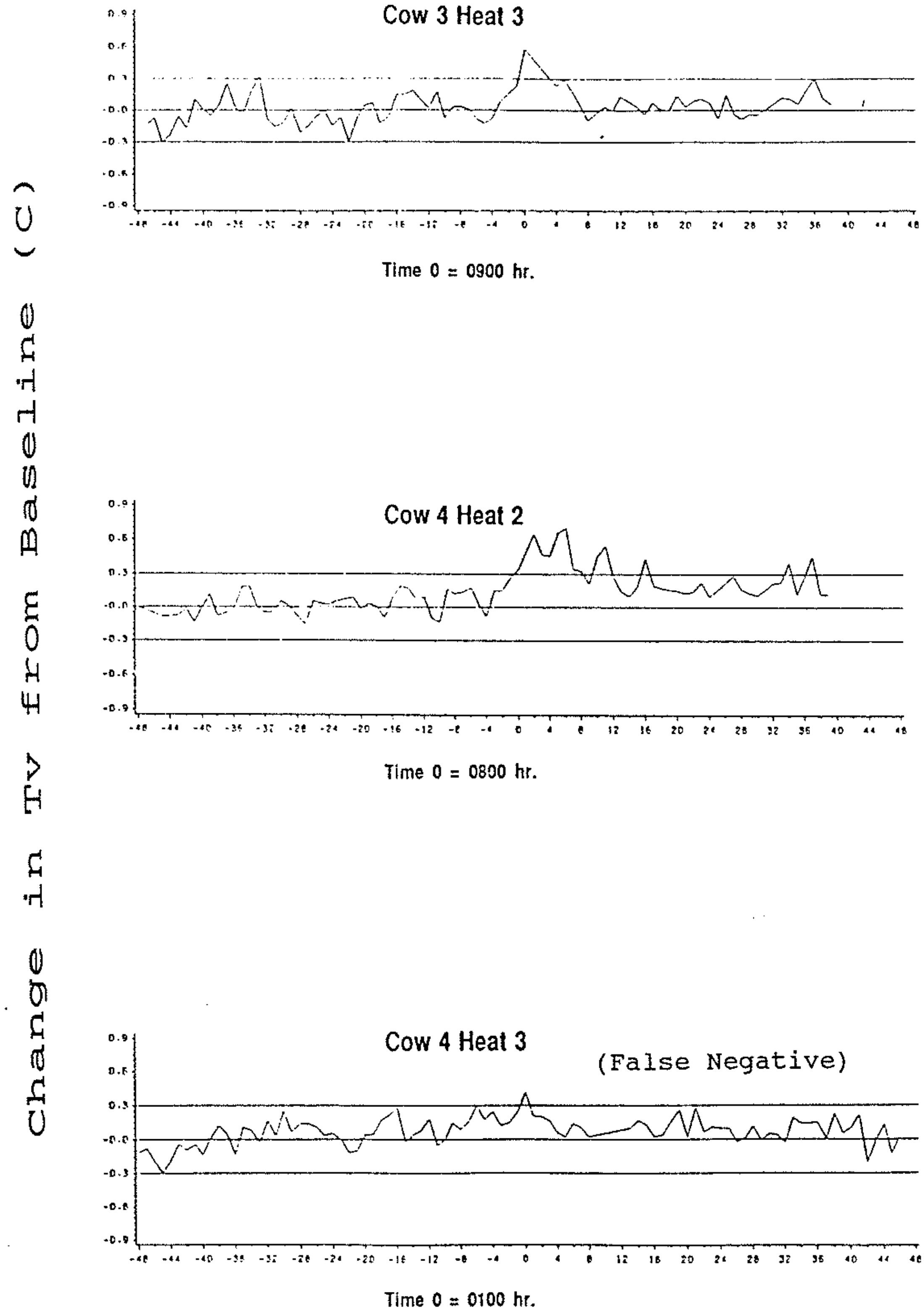
Time 0 = 1200 hr.

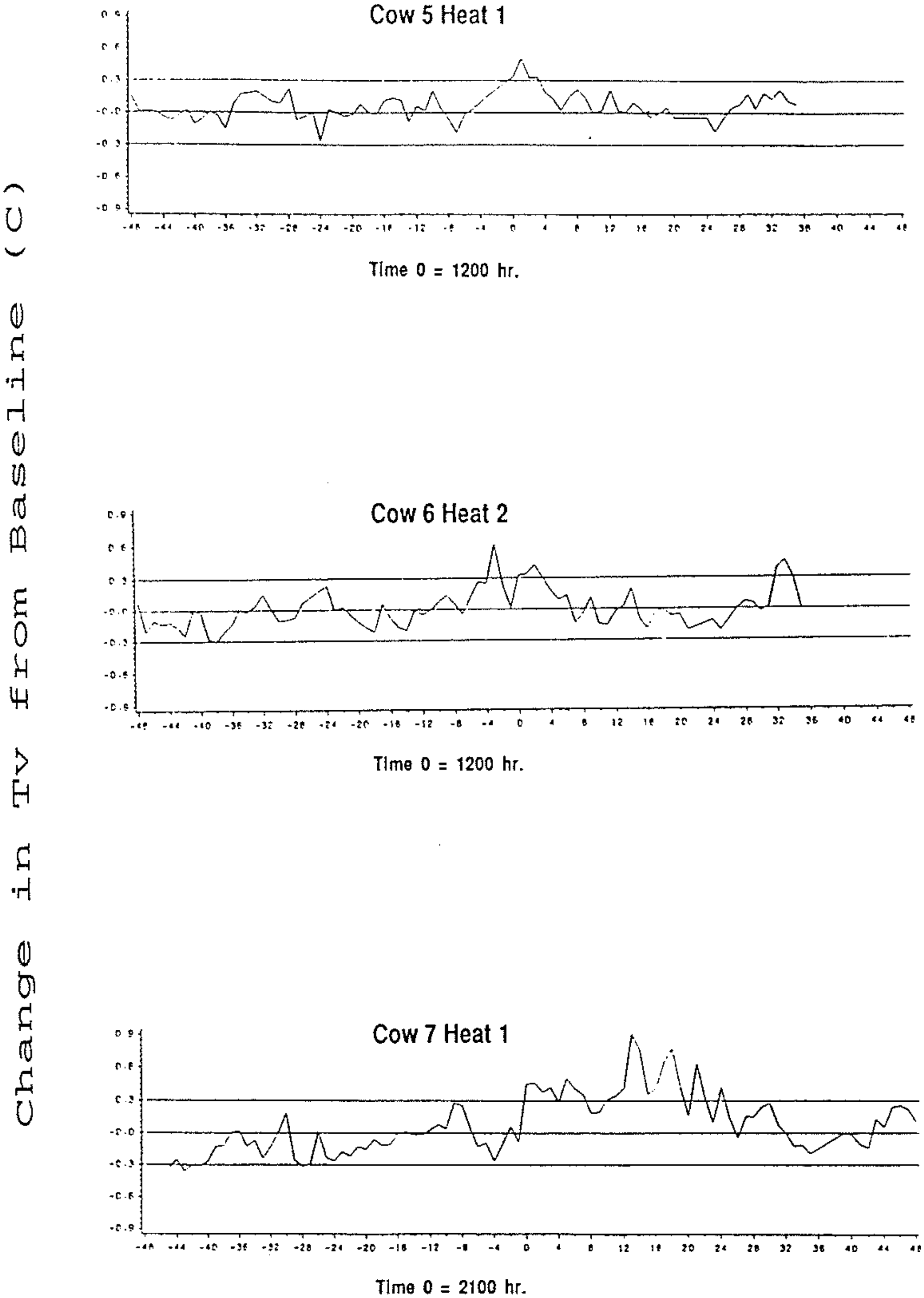


Time 0 = 1100 hr.

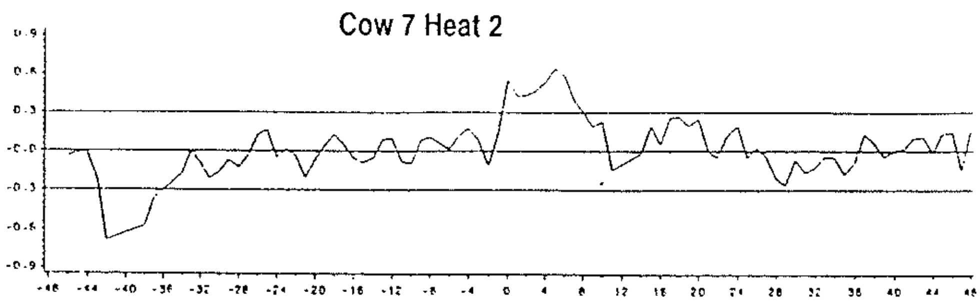


Time 0 = 1000 hr.

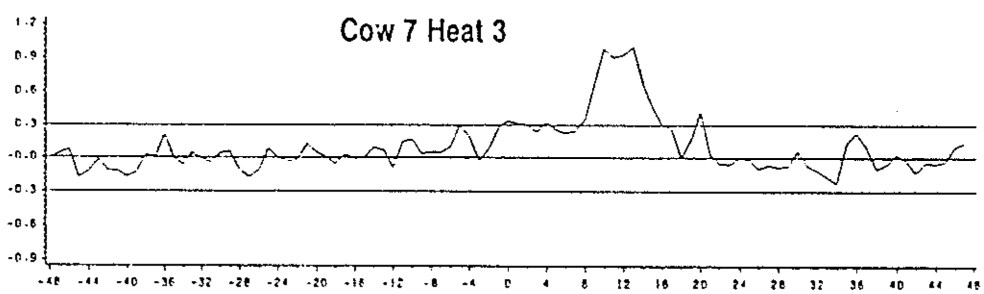

Change in Tv from Baseline (C)
Cow 3 Heat 3
Time 0 = 0900 hr.
Cow 4 Heat 2
Time 0 = 0800 hr.
Cow 4 Heat 3
(False Negative)
Time 0 = 0100 hr.


Change in Tv from Baseline (C)
Cow 5 Heat 1
Time 0 = 1200 hr.
Cow 6 Heat 2
Time 0 = 1200 hr.
Cow 7 Heat 1
Time 0 = 2100 hr.

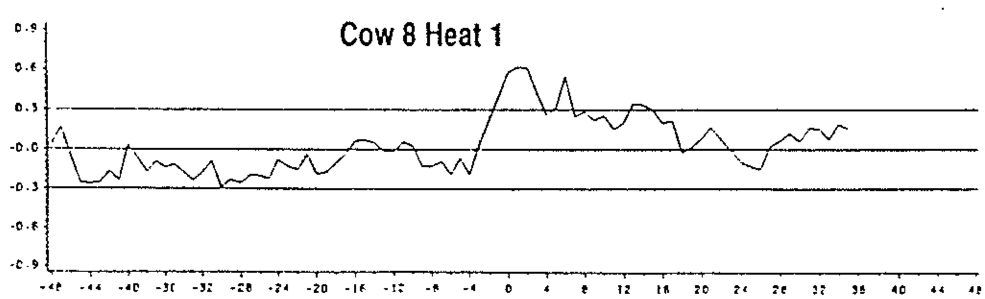
Change in TV from Baseline (C)



Time 0 = 2200 hr.

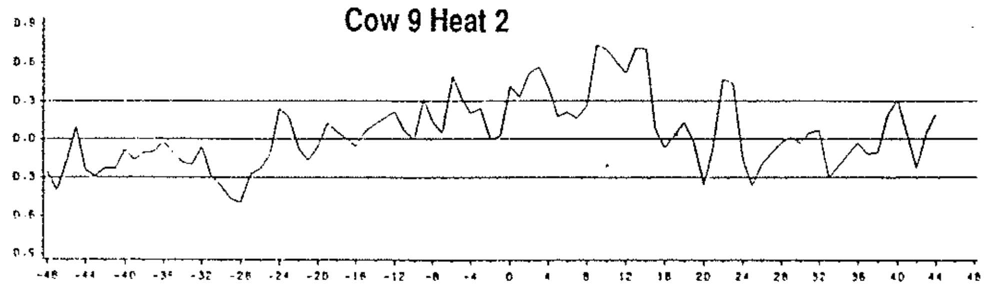


Time 0 = 0000 hr.

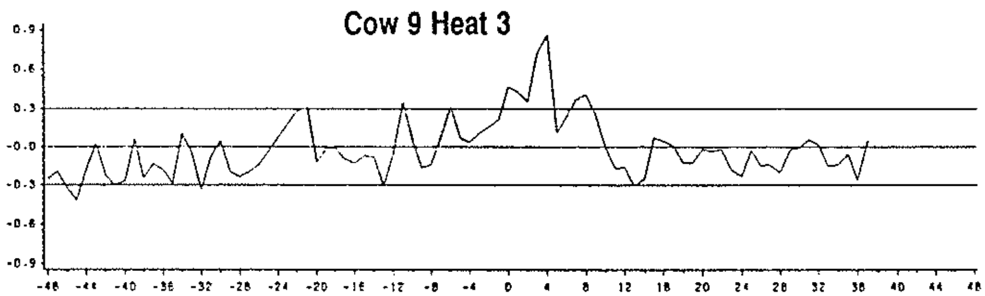


Time 0 = 1200 hr.

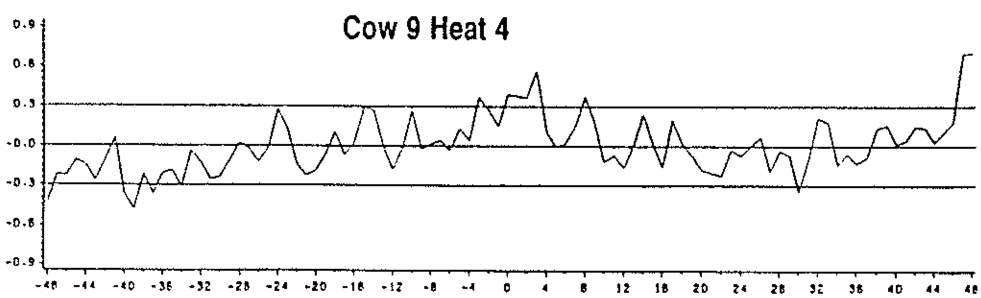
Change in Tv from Baseline (C)



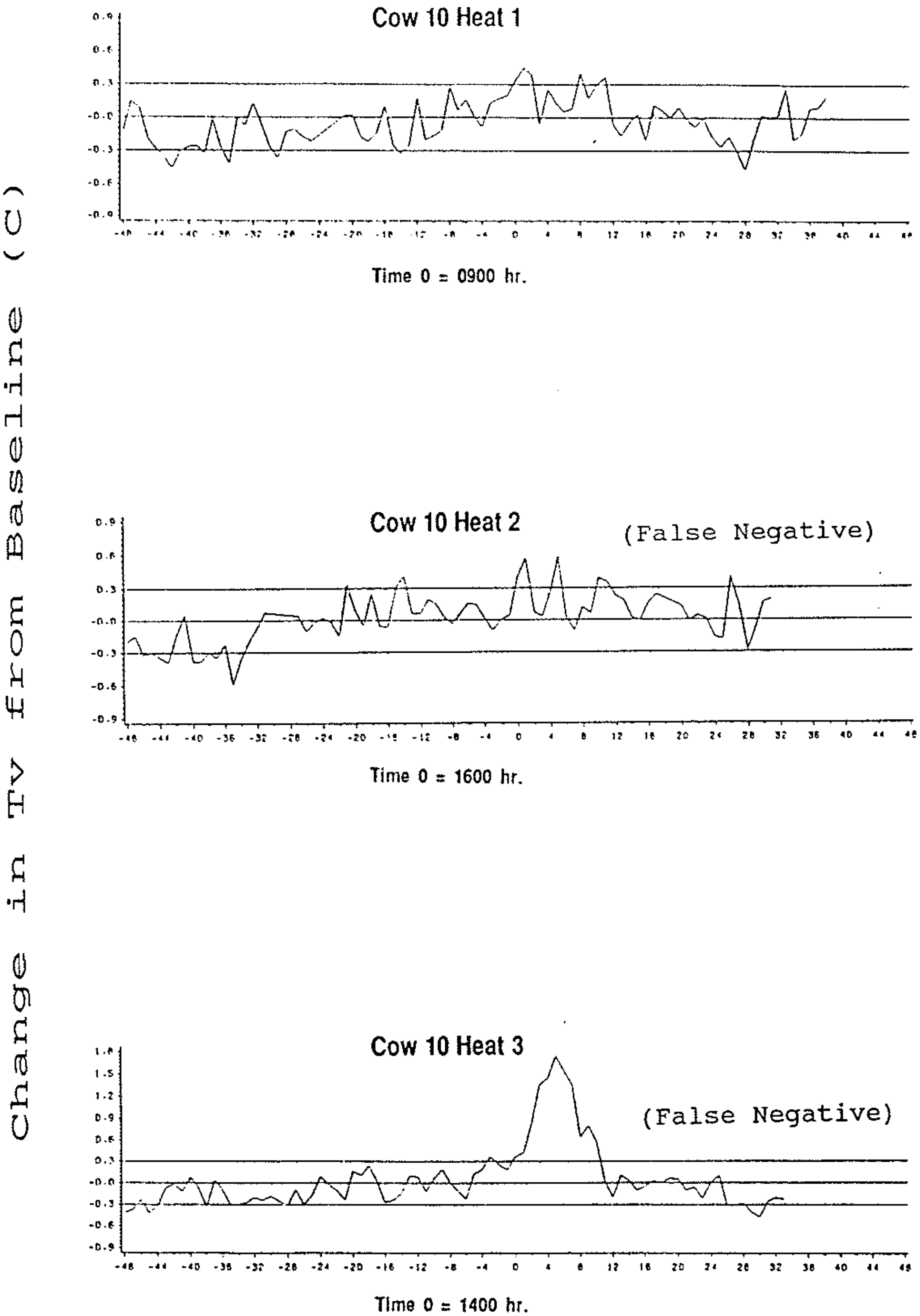
Time 0 = 0000 hr.



Time 0 = 1000 hr.

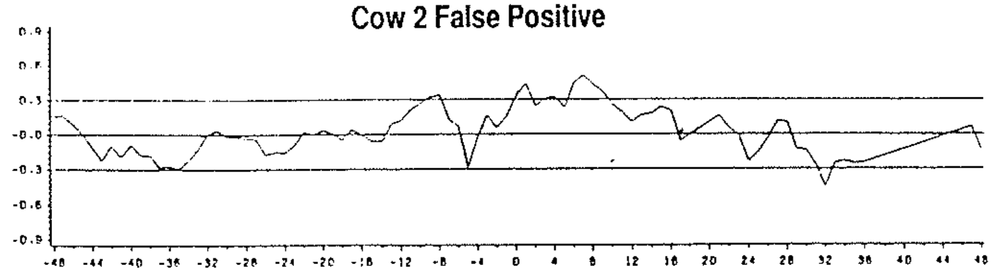


Time 0 = 1200 hr.


Change in Tv from Baseline (C)
Cow 10 Heat 1
Time 0 = 0900 hr.
Cow 10 Heat 2
(False Negative)
Time 0 = 1600 hr.
Cow 10 Heat 3
(False Negative)
Time 0 = 1400 hr.

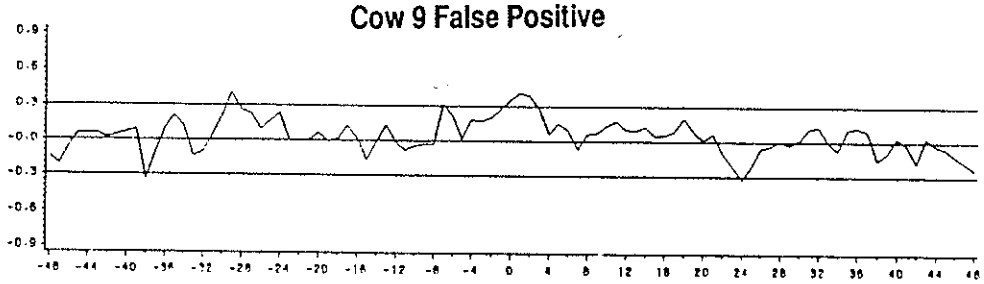
Change in Tv from Baseline (C)

Cow 2 False Positive



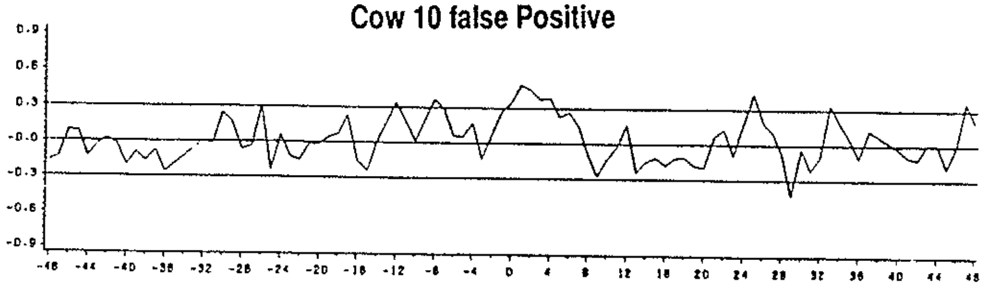
57 Days pp Time 0 = 1500 hr.

Cow 9 False Positive



105 Days pp Time 0 = 1000 hr.

Cow 10 false Positive



85 Days pp Time 0 = 2000 hr.

Appendix 5. Results of analysis of variance to test for differences in change in Tv from baseline surrounding the time of estrus.

Source of Variation	Degrees of freedom	Mean Square
Period	4	4.90
Cow	9	.37
Period X Cow	36	.24
Error	3405	.03