Measurement of Vaginal Temperature by Radiotelemetry and Activity by Pedometers for the Detection of Estrus in Beef Cows

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MEASUREMENT OF VAGINAL TEMPERATURE BY RADIOTELEMETRY AND ACTIVITY BY PEDOMETERS FOR THE DETECTION OF ESTRUS IN BEEF COWS

BY

BETH L. KYLE

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

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ABSTRACT

Kyle, Beth L. M.Sc., The University of Manitoba, May, 1996. Measurement of Vaginal Temperature by Radiotelemetry and Activity by Pedometers for the Detection of Estrus in Beef Cows. Major Professors; Alma D. Kennedy and Julie A.Small.

Vaginal temperature (Tv) and daily activity were monitored in 24 beef cows during the breeding seasons of two consecutive years. Eight and 16 loose-housed, suckled beef cows were used during the first and second years, respectively. Vaginal temperature was measured continuously throughout the breeding seasons using radiotelemetry. Radiotransmitters were held in the vagina using a flexible, plastic anchor. To monitor daily activity, a pedometer was placed on the hind-leg of each cow in both years, and in year 2 a second pedometer was secured to a collar which was fitted around the neck. An additional pedometer study, using 12 unsuckled cows fitted with pedometers on the leg and on the neck, was conducted to determine the time interval from the detection of the indication of estrus activity by the pedometer (DEP) to ovulation. Pedometers were read twice daily. Detection sensitivity (DS) (correctly detected estrous periods ÷ total estrous periods) and detection accuracy (DA) (correctly detected estrous periods÷total estrous periods+false indictions of estrous periods) of both years combined for Tv were 89.4% and 77.8%, respectively. Pedometers attached to the leg had DS (%) of 62.5, 89.3, and 100 and DA(%) of 27.8, 69.4, and 82.4, for year 1, year 2, and the supplemental pedometer study, respectively. Pedometers attached to the neck had DS (%) of 76.7 and 92.9 and DA(%) of 41.1 and 76.5, for year 2 and the supplemental study, respectively. The time interval between DEP and ovulation was found to be 12±6 h. Results indicate that measurement of Tv and daily activity are effective means of detecting estrus in beef cows.

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Lastly, but prehaps most importantly, thanks to my family for providing me with unquestioning acceptance, support, and enthusiasm throughout all of my endeavors. Thank-you for always being there for me.

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List of Abbreviations

Abbreviation	Name
AI	Artificial Insemination
ET	Embryo Transfer
GnRH	Gonadotrophin Releasing Hormone
E2	Estradiol-17β
LH	Luteinizing Hormone
CL	Corpus Luteum
P4	Progesterone
LED	Light Emitting Diode
EOP	Endogenous Opioid Peptide
Tv	Vaginal Temperature
TE	Total Estrous Periods Studied
DE	Detected Estrous Periods
FP	False Positives
DS	Detection Sensitivity
DC	Detections which were Correct
DA	Detection Accuracy
SD	Standard Deviation
CV	Coefficient of Variation
DEP	Detection of the indication of estrus activity by the pedometer

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INTRODUCTION

Increased use of technologies such as artificial insemination (AI) and embryo transfer (ET), could dramatically improve reproductive efficiency within the beef industry. Artificial insemination is a common practice in modern dairy herd management. In comparison, AI has not been as readily adopted by the beef industry. Benefits of AI for beef producers include; the use of genetically superior bulls which may not otherwise be available at a low cost; ability to breed different subpopulations within the herd (ie. heifers and cows) to different bulls to produce offspring with desired traits for that group, ability to select from sires with histories of reproductive success rather than purchasing an unproven sire, elimination of initial purchase and maintenance costs of bulls, and to decrease the length of the breeding season, thereby increasing the uniformity of the calf crop at weaning. Knowledge and understanding of the benefits of using AI for beef cattle has increased over the past few years, resulting in a very gradual increase in the numbers of cows bred using this technology. Presently, AI tends to be used more in heifers than in cows, and on smaller, pure-bred operations (Cranfield and Howard,1994). Although no comprehensive surveys have been conducted, estimates of the amount of AI being used in the beef industry can be made by noting the sale of semen to beef operations. Percentage of the cow population that is bred with AI differs depending on geographic location and therefore herd size and management systems. It is estimated that 20% of the beef cow population in Eastern Canada, 6-8% in Central Canada, and 2-3% in Western Canada, are bred using AI.

The major deterrent for increased use of AI, and other technologies, continues to be difficulties in determining when to breed. A survey of Ontario beef producers indicated that difficulty with detecting estrus was a greater deterrent in using AI than the cost of semen, total AI costs, and lack of handling facilities (Cranfield and Howard, 1994). Observation for behavioural estrus is the method most often used for detecting estrus in both dairy and beef operations. Within the dairy industry only 50-60% of all estrous periods are detected (Barr, 1975). Effective detection of estrus using visual methods is labour intensive. A recent survey of Alberta dairy producers indicated that only 50.3% of dairy operations have a daily schedule for detecting estrus (Spicer et al.,1994). Detection can be expected to be equivalent or lower on beef operations as there is generally less time and opportunity for effective observations. In Eastern and Central provinces approximately 90% of beef producers also work off farm, which limits time available for effective detection of estrus. In Western Canada, with the tendency towards larger herd sizes and more extensive management practices, difficulties in viewing all cattle and in handling individual cows decrease opportunities for detecting estrus. Poor detection of estrus leads to breedings at the wrong time relative to ovulation which will result in increased costs through repeat breedings and/or disappointing conception rates. Poor results combined with higher than expected costs decreases the feasibility of using AI for many beef producers.

The development of a method of detecting estrus suitable for use in typical beef management systems would greatly aid in promoting the use of AI in the beef industry. Several devices have been developed for the dairy industry to aid in determining the best time to breed by indicating behavioural and/or physiological changes associated with estrus. Two methods which may be adapted for use in beef operations are the monitoring of daily locomotory activity using pedometers and the measurement of body temperature using radiotelemetry. Research measuring activity (Kiddy, 1977, Redden et al.,1993) and body temperature (Mosher et al.,1990, Redden et al.,1993), have shown promising results in dairy animals. The objective of the present study was to determine the effectiveness of these techniques for detecting estrus in loose-housed beef cows.

LITERATURE REVIEW

Estrous cycle in cattle

The estrous cycle of domestic cattle ranges in length from 17-25 days, on average a cow will cycle every 21 days. As with other mammalian females, the reproductive cycle is controlled through the interactions of the ovarian steroids, gonadotrophins from the pituitary, and gonadotrophin releasing hormone (GnRH) from the hypothalamus. The cycle consists of two major periods, the follicular and the luteal periods, which are characterised by specific fluctuations in the serum steroid hormone ratio (Hafez, 1987). Estrogens (E2), are produced by the granulosa cells of the developing follicle, reaching maximum levels during the pre-ovulatory period. The high circulating E2 levels stimulate the hypothalamus to increase the release of GnRH which, in turn, causes an increase in the release of the gonadotrophin, luteinizing hormone (LH), from the pituitary. There is a gradual increase in serum LH concentration during the four days prior to ovulation. This is followed by a rapid rise in LH, reaching maximum concentrations approximately 24h before ovulation. The surge in LH, which is 6-8h in duration, causes the follicle to rupture, resulting in ovulation. Serum E2 concentration begins to decline with the rise of LH and returns to basal levels approximately 8h before ovulation (Chenault et al.,1974). After the rupture of the follicle, a corpus luteum (CL) forms on the ovulatory ovary and begins producing high concentrations of progesterone (P4) within five to six days of ovulation (Schams et al.,1977). The luteal phase is approximately 16 d in duration. During this phase, one or two dominant follicles will form, in succession, which are large enough to produce significant amounts of E2. Follicles will atrophy without ovulating while the CL is producing high levels of P4. A number of events, primarily the release of prostaglandin (PGF2α) from the uterus, will cause the regression of the CL, marking the end of the luteal phase. With CL regression, serum P4 concentration drops and E2 rises as the pre-ovulatory follicle increases in size and the cycle repeats. Receptors for steroid hormones are located at numerous sites throughout the body and brain. Fluctuations in the ratio of steroid hormone concentrations in the blood, therefore, induce shifts in behaviour patterns and physiological functions.

Estrous Behaviour

The term estrus is used to describe the pre-ovulatory stage of the cycle during which behavioural changes occur which relate to the change in steroid hormones. During estrus most cows show an increase in the initiation of aggressive activity towards other cows, investigatory behaviour (sniffing, rubbing, chin resting, and body orientation) directed towards and received by other cows, mounting other cows, partial mounting and/or failed mounting by other cows, and standing to be mounted (Esslemont et al.,1980). Although all of these behaviours are seen at different stages of the cycle, and even during pregnancy, the frequency of occurrence is highest during estrus. For example, aggressive activity doubles during the immediate pre-ovulatory stage, and 79%

and 90% of cows mounting and standing to be mounted, respectively, are in estrus (Hurnik et al.,1975). Differences in the distribution of daily activities also occur before ovulation. During this time, there is a large increase in locomotory activity, with less time devoted to resting and eating activities (Hurnik et al.,1975, Phillips and Schofield,1990). The varying ratio of serum E2 and P4 concentrations acts to produce the sequence of behaviours which is seen throughout the estrous cycle. Estrous behaviour occurs when E2 from the growing follicle rises and the CL has begun regression, decreasing production of P4. When administered in isolation to ovariectomized cows, P4 inhibits estrous behaviour and E2 acts to strongly stimulate all sexual behaviours (Vailes et al.,1992). The ability of E2 to stimulate individual behaviours varies, depending on the level of P4 present. Therefore, peak frequencies of individual behaviours occur at slightly different times during estrus. Rates of aggressive activity and mounting without standing estrus reach peaks before mounting with standing estrus. Investigatory behaviours are equally high before and after standing (Esselmont et al., 1980). Mounting, in particular, is not as inhibited by P4 as other behaviours and can occur when concentrations of P4 and E2 in serum are high. (Valies et al.,1992). The standing reflex, however, is strongly inhibited by P4 even with the presence of high serum E2. Therefore, only when serum P4 reaches basal concentrations and E2 is maximal will standing estrus occur. During the estrous cycle, the standing reflex occurs almost exclusively within the period of 24±6 h before ovulation (Vailes et al.,1992). Standing to be mounted (ie, standing estrus), therefore, is the most reliable behavioural indication of ovulation. As well, because it occurs during a relatively specified period before ovulation, standing estrus is also the most accurate behavioural signal on which the timing of insemination may be based.

Visual Observations of Behaviour for Detection of Estrus

Standing estrus is the most reliable behavioural indication of ovulation. The duration of this estrous behaviour, however, has been found to range from 0 to 26h (Salisbury,1978). There are a number of factors which will influence the intensity and duration of estrus, and therefore the effectiveness of relying solely on visual observations for detecting estrus. There is evidence to suggest cows are more likely to express estrus behaviour overnight. This would decrease the opportunities for detecting estrus during normal observation periods. This may be a natural circadian rhythm or may be due to the decline in human activity overnight (Hurnik et al.,1975). Other studies, however, have noted high levels of estrus behaviour during the day (Fulkerson et al.,1983). The physical environment, particularly footing and available space, will affect the willingness of cows to mount (Phillips and Schofield,1990). The number of cows in estrus during the same period also affects the intensity and duration of estrus behaviour. If only one cow within a group is in estrus, standing behaviour may not be seen due to a lack of mounting interest by the non-estrual cows (Esselmont et al.,1980). When a number of cows are in estrus simultaneously, the number of mounts per cow and the duration of mounting activity increase. Humik et al. (1975) noted that mounts per cow increased from 11 to 53 when one and three cows, respectively, were in estrus. As well, definite differences exist among individual cows in their willingness to mount estrual cows, perhaps due to social rankings (Vailes et al.,1992). Therefore, the expression of estrous behaviour is dependent on more than the circulating steroid hormone ratio, and the effectiveness of visual observations for the detection of esturs will vary according to different circumstances.

Visual observations for estrous behaviour have been the most commonly used means of estrous detection in both dairy and beef operations. King et al.(1976) found that, subsequent to the first post-partum ovulation, estrus was observed for >94% of ovulations when behaviour was continuously video-recorded. Fulkerson et al.(1983) found that 83% of estrous periods could be detected during continuous observations for 12h per day. Lengthy observations and/or continuous video recording are not practical in typical management systems. As the time spent observing for standing estrus decreases the number of estrous periods detected also declines. Therefore, as well as problems with variation of the expression of estrus behaviours, there are problems with implementing an effective daily observation schedule. As stated in Spicer et al.(1994), only 50.3% of Alberta dairy producers had regularly scheduled observations times. Detection rates using visual observations for dairy cows are typically less than 60% (Barr, 1975).

Synchronization of Estrus

In order to decrease the amount of time necessary for detecting estrus and to shorten the breeding season, the hormone status of the cow can be manipulated to synchronize a large portion of the herd to be in estrus at one time. In the majority of beef operations, breeding of the entire herd is scheduled within a specified time during the year. Therefore, synchronization of estrus within the herd is an option to decrease difficulties in detecting estrus when breeding with AI. However, depending on which hormone is used for synchronization, there may be problems with conception or a wide range of days over which estrus may occur. Also, the stage of the cycle when synchronization is induced may affect the interval from synchronization to estrus (Reviewed in Odde, 1990). Therefore, depending on the management system, a precise means of detecting estrus is often still necessary even with current synchronization programs.

Methods of Detecting Estrus

Much time and research has been devoted to developing methods of detecting estrus which may be easily adopted into regular herd management and which yield detection rates superior to those achieved by visual observation for standing estrus. Detection aids are devices which are designed to detect the occurrence of behavioural and/or physiological changes which are associated with estrus. Due to the greater use of

AI in the dairy industry, detection aids have been developed to be adopted by dairy management. A number of the methods which have been tested for dairy cows would be difficult and impractical to use under typical beef management. Methods of detecting estrus which require frequent handling of cows and/or access to milk samples would not be applicable. Such methods include the measurement of milk progesterone (Walton and King,1986), milk temperature (Maatje and Rossing,1976), milk production (Walton and King,1986), manual reading of vaginal impedance (Kitwood et al.,1993), vaginal pH (Lewis and Newman,1984), and manual readings of body temperature (Lewis and Newman,1984, Walton and King,1986).

Methods which are applicable for beef, as well as dairy cattle, rely on indications of standing estrus. Such methods include mount detectors, teaser animal (with and without the use of marking devices), and tail paint. Fulkerson et al.(1983), reported detection rates of estrus in dairy cows of 50,88, and 80% using visual observations only, or in combination with hormone treated steers or tail paint, respectively. Zartman et al.(1983), found that although pressure sensitive dye-filled mount detectors always indicated standing estrus, several false indications of estrus occurred and a high percentage of replacement detectors were required. Williams et al.(1981) found similar mount detectors to have an accuracy of detection of only 29%. In a review by Lehrer et al.(1992), it was concluded that, although cost effective in dairy cows, this type of system was limited due the time requirements for the necessity of daily upkeep.

Two methods of detecting estrus which have been tested in dairy but not beef animals are the monitoring of activity using pedometers and the measurement of body temperature using radiotelemetry. The following two sections review the progress of development of these methods in dairy animals.

Measurement of Daily Activity for the Detection of Estrus

As has been found in other mammalian females, cows show measurable increases in locomotory activity during the pre-ovulatory period compared to other times of the estrous cycle. Pedometers are devices used to estimate the amount of locomotory activity. Farris (1954) was the first to use pedometers to quantify the difference in activity between the day of estrus and non-estrus days in cattle. The increase in activity was found to last for 24 h. A drop in activity, before and after estrus, was also noted. Mechanical pedometers, similar to those used by Farris, were designed originally for monitoring locomotory activity in humans. These pedometers are read manually and all calculations are performed by the herdsman. Subsequent results using this type of pedometer have been varied. Over 20 years after the initial pedometer study, Kiddy (1977) laid the ground work for the more recent use of activity measurement for detecting estrus in cows. In this study, pedometers were strapped to the ankle of the cow's rear leg and were read manually twice daily. Results were encouraging, with daily activity of cows housed in free-stalls shown to increase at estrus by two and three times the standard deviation (SD) of the mean of all non-estrus days in 98% and 93%, respectively, of 87 estrous cycles. An average increase of activity at estrus of 393% over non-estrus activity was found for this type of housing. Results for cows housed in comfort stalls indicated that activity on 72% and 98% of the estrus days was two and three times the SD of the mean of non-estrus days, respectively (39 estrous cycles studied). Activity increased 276% above activity of non-estrus days when the cows were housed in tie-stalls. Kiddy reported few problems with pedometer loss, malfunctions, or false readings of estrus. Williams et al. (1981), using similar pedometers attached to the forelegs of cows, found 74% and 68% of estrous periods were associated with activity increases of one and two times the SD of the mean of non-estrus activity, respectively. This was compared to a detection rate of 68% using traditional visual methods. This study showed that a combination of visual observation and pedometer detected increase in activity greater than twice the SD of the mean of non-estrus days would yield a 10% increase in the number of estrous periods detected with fewer false positives (FP) than by using pedometers alone. A high rate of pedometer loss and malfunction was reported, however. Lewis and Newman (1984), found that pedometer readings increased, on average, by 200% when cows were in estrus compared to non-estrus days. Maximum pedometer readings coincided with the day of estrus in 74% of the 55 estrous periods. Pedometers attached to the front leg of 10 Egyptian buffalo detected 85% of synchronized estrous periods when read once daily (Williams et al.,1986). This compares to 14% of estrous periods detected using visual methods in the same study. Redden et al. (1993), using pedometers attached to the hind-leg above the hock and read manually twice daily, measured activity in dairy cows during 26 estrous periods. Unlike the earlier manually read pedometers, the pedometers used in this study were electronic with a digital display. Of numerous criteria tested, a 50% increase in activity over a 15 day baseline gave the highest detection rate with the lowest number of FP. These criteria resulted in 80% of the estrous periods being detected with four FP. On average, activity at estrus increased 2.8 fold during the daytime period when cows were turned out for a 3h exercise period, but no increase was noted during the overnight period when cows were confined in tie-stalls.

An electronic pedometer or activity tag has been developed specifically for use in cows (Lui and Spahr, 1993). The activity tag contains an internal microprocessor for automatic calculations of variations in activity from the baseline. Activity tags can be set for a choice of thresholds which will modify the sensitivity of detection. Indications of movement are recorded as total activity during periods of 12 h duration. A new total is recorded every 2 h. Activity for the previous 12 h is compared to the same 12 h period of a set baseline. Attention is drawn to individual tags with activity counts exceeding the pre-determined criteria by flashing of a light emitting diode (LED) on the pedometer casing. By placing a magnet on the pedometer casing, a pattern of light flashes indicate the level of activity for each two h period during the previous 12 h. The duration of elevations in activity can be detected in this manner. As well as the visual display, data can be downloaded, radiotelemetrically, to a computer for a more detailed analysis of activity patterns.

As with the mechanical pedometer, results from studies with activity tags have shown considerable variation in the effectiveness of using pedometers for detecting estrus. Holdsworth (1982), compared mechanical pedometers and activity tags. Mechanical pedometer readings had to be double that of the previous day to indicate estrus. Doubling or tripling of activity was indicated by a LED display on the activity tag. Both mechanical pedometers and activity tags were read at AM and PM milkings. In this study, all types of pedometers, particularly the mechanical pedometers, were found to have a high percentage of loss, malfunction, and to be difficult to read. As well, a very low detection rate and a high occurrence of FP were encountered. Favero et al. (1984), studied results of activity tags placed on the rear leg, front leg, and the side of the neck in 12 synchronized cows. It was determined that estrus activity could be detected in all locations, but that placement on the legs (front or back), yielded better results because between-animal SD at estrus was higher for neck than leg readings. Pennington et al.(1986), used activity tags attached to the rear leg. Data was radiotransmitted to a computer twice daily as cows passed through the milking parlour. Values during estrus were compared to a baseline consisting of an average of the 5 previous readings for the same time period. Of the 26 estrous periods studied, 23 had pedometer readings above 100% of the baseline. In this study, twelve of the 14 behaviours normally associated with estrus, were found to occur during the 12 h time period when pedometers indicated an increase in activity. In Peter and Bosu (1986), a difference in performance of the activity tag was found to be dependent on the number of days post-partum on which estrus occurred. Fifty-seven percent, 91% and 93% of first, second, and third ovulations post-partum, respectively, were detected by the tags. Of the total of 91 estrous cycles studied, 76% were associated with a tag indication of estrus, while 35% were detected using visual methods. Based on activity tags visual display, Moore and Spahr (1991) found that 55% of all visually confirmed estrous periods were detected by tags and that four out of five indications of estrus activity by the tags were not associated with estrus. Problems with the internal microprocessor were considered the major cause for the poor results. Manual calculations of radiotelemetrically acquired data yielded a 73% detection rate. Tag data was lost for 43 visually observed estrous periods and 44% of the tags had to be replaced due to malfunction during the eight month study. The highest rates of accuracy and detection were achieved using criteria which consisted of a threshold of 2.0 fold (two-fold increase in activity required) and a 12h algorithm (activity during a 12h period compared to previous 12h periods). Pulvermacher and Maatje (1992), examined radiotelemetrically acquired activity tag data. They reported 45, 55, and 64% of estrous periods were detected when thresholds of 1.8, 2.0, and 2.2, respectively, were applied. In this analysis activity had to be elevated for at least four consecutive hours to be considered an estrus related increase. If only a two hour criterion was applied, detection rate increased considerably but this was associated with a large number of FP. Lui and Spahr (1993), monitored activity telemetrically during 66 estrous cycles in dairy cows. Whereas 58% of estrus were visually detected, 74% of estrous periods were detected using the activity tags and applying criteria including a 1.75 fold increase in activity for a duration of four hours over a two day baseline. Using these criteria 49 estrous periods were detected and 24 FP were found. In general, error rates were found to decrease with criteria involving increasing folds increase and duration. Jokhio (1994), analyzed activity data collected from free-stall and tie-stall dairy farms. A trend toward fewer days open and days to first service in most herds was found in herds using activity tags. The tags were found to increase operator awareness of cows which were potentially in estrus. Kennedy and Ingalls (1995) studied cows housed in tie-stalls with a 3h exercise period. For the period commencing 40 days post-partum, 74% (17/23) of estrous periods were detected using activity tags. False positives were a problem in this study. Even with the implementation of a false positive correction procedure, there was approximately one false positive for every correctly detected estrus. Visual detection in this study was 13% with two FP.

Despite the relatively large amount of research which has been conducted with pedometers, it is difficult to gain an overall assessment of the true potential of pedometers for detecting estrus, because of variations in management and reporting of results among trials. As the effectiveness of pedometers is based on the intensity of a behavioural response, differences in management (ie housing type, stocking density, floor type) would likely influence the results. As well, there has been no standard method of stating results, therefore it becomes difficult to compare studies. For example, some studies report a high incidence of FP readings while in others only a few FP are reported, or FP are not mentioned. Whether the variations are due to management differences, discrepancies in the calculation and/or expression of results, or a true fault of pedometers is at times unclear.

Despite the differences in procedures, some similarities across trials are apparent. Pedometers increase the number of estrous periods detected when compared to visual observations for estrus behaviour. Therefore, pedometers could provide an effective means of detecting estrus in cattle. The major drawback from using pedometers has been false positive readings of estrus. However, correction factors and/or common sense may reduce this problem. Activity on estrus days has been approximately two to four times the average activity of non-estrus days. Therefore, the criteria chosen as optimum in most studies generally included an increase in activity of 1.5 to 2.0 fold over an activity baseline of a prescribed number days.

Measurement of Body Temperature for the Detection of Estrus

Body temperature is affected by the stage of the estrous cycle in cattle. The most dramatic change in body temperature occurs during the period of time associated with estrus. During this period an increase in body temperature of approximately 0.3 to 1.0 °C lasting for 0 to 21 hours has been shown (Zartman et al.,1983, Mosher et al.,1990, Redden et al.,1993). This temperature peak has been found to coincided with standing estrus (Rajamahendren et al.,1989) and with the surge in LH (Rajamahendren et al.,1989, Mosher et al.,1990, Rajamahendren and Taylor, 1991). As well as the increase in body temperature at estrus, a less obvious decrease in temperature is seen during the 2-4 days preceding estrus (Maatje and Rossing 1976, Fordham et al. 1988, Redden et al. 1993). Lewis and Newman (1984), found vaginal temperature to be lowest during the day before ovulation, to rise for six days after ovulation, to remain steady during diestrus and to decline gradually prior to the next ovulation.

The exact cause of these temperature changes has not been firmly established. However, the large increase in locomotory activity during estrus is thought to contribute to the increase in temperature at that time. Walton and King (1986), found that the largest increases in temperature prior to ovulation were accompanied by visual signs of estrus. However, the increase in temperature has also occurred with no visual signs of estrus are observed (Fordham et al. 1988), and when activity was limited by housing type (Redden et al. 1993). This evidence, therefore, suggests a physiological mechanism independent, of increased activity.

The temperature change has been assumed to be related either directly or indirectly to the shift in the steroid hormone milieu. In rats and humans, there has been evidence linking the central control of body temperature and reproduction. In these species, a close association between temperature regulating neurons and GnRH neurons within the pre-optic area of the hypothalamus, has been found (Berglund and Simpkins, 1988 and Cagnacci et al., 1992). It has been established that the positive feedback action of estrogen on the LH surge generator, primarily located in the preoptic area of the hypothalamus, causes the pre-ovulatory surge in LH (Hafez, 1987). The presence of temperature sensitive neurons in the preoptic area of the hypothalamus suggests that the change in temperature during estrus might be related to stimulation of the hypothalamus by gonadal steroids. In many species, endogenous opioid peptides (EOP) act in an inhibitory manner on the release of gonadotrophin releasing hormone (GnRH) throughout most of the estrous cycle (Reviewed in Barb et al.,1991). There has been evidence to suggest that a decrease in EOP inhibition of GnRH, induced by gonadal steroids, causes the LH surge (Reviewed in Barb et al.,1991). Opioids are also thought to have a role in the centrally mediated control of body temperature (Rezvani et al.,1982). In rats, behavioural and physiological responses (including LH release and body temperature) to the opiate, morphine, were influenced by the stage of the estrous cycle at the time of morphine administration. Results indicated there was a decrease in the typical reactions to morphine on the afternoon of proestrus compared to the morning of proestrus and diestrus, for all responses measured (Berglund and Simpkins 1988). A similar decrease in opiate, and possibly EOP, inhibition may occur during the estrous cycle in cows.

Another possible explanation for the fluctuations in temperature during the cycle is the thermogenic effect of progesterone which has been well established in humans (Czaja and Butera, 1986 and McCarthy and Rockette, 1986). In cattle, a drop in temperature has been measured during the time when the production of progesterone from the CL declines prior to estrus (Redden et al. 1993). Cagnacci et al.,(1992), suggested that the effects of progesterone in women are not mediated directly through EOP activity.

Therefore, the pre-estrual decline in temperature and the peak in temperature occurring during estrus may be controlled by separate mechanisms.

In dairy cows the possibility of using the change in body temperature as a means of predicting estrus has been explored. Changes in milk, skin, rectal and vaginal temperatures have been studied. Measurement of milk temperature has been effective in detecting estrus in dairy cows (Maatje and Rossing,1976 and Ball et al.,1978). A commercial system is currently available for assessing the occurrence of estrus through milk temperature. Ear temperature was of no use in predicting estrus in cattle (Redden et al.,1993). Hurnik et al. (1985) found that skin temperature, detected by thermal infrared imagery, increased at estrus. However, a large number of FP were also found. Once daily manual readings of rectal (Ball et al.,1978, Walton and King, 1986) and vaginal temperatures (Lewis and Newman, 1984) were not very effective in detecting the estrual change in temperature. This could be due to fluctuations in body temperature resulting from diurnal and activity related patterns (Araki et al.,1987) or related to changes in ambient temperature (Lewis and Newman, 1984).

Aside from the variable success in effectively detecting the estrual rise in temperature, the methods described above would be difficult and/or impossible to implement into typical beef management. One method which holds promise as a practical means of detecting estrus in beef cows is the non-surgical placement of temperature radiotransmitters within the vagina. Zartman et al. (1983), used a plastic support anchor to hold radiotransmitters in place in 18 heifers for 107 days. Temperatures were received from the transmitters once daily at 6:45h. The temperature at estrus was found to be approximately 0.5°C above the average of the previous 5 mornings. The anchor was found to cause no problems relating to fertility or health of the heifers. Average days open and average number of services/conception were found to be higher in a control group compared to the heifers bred on the basis of the temperature peaks. Junge-Wentrup and Holtz (1984), using vaginal radiotransmitters, found that only a few individuals showed a slight increase in Tv near estrus and that there was no relationship between ovarian cycle and body temperature. Clapper et al. (1990), using a system similar to Zartman et al. (1983), recorded vaginal temperatures twice daily until close to expected estrus when hourly readings were taken. A temperature peak coincident with estrus was defined as an increase of≥0.3°C for at least 3 hours, above the temperatures measured at the same time of the day on the previous and subsequent day. With this definition, significant temperature peaks were found for all 10 cows studied. No FP were detected based on twice-daily temperature readings during the rest of the cycle. Vaginal temperature remained elevated for a range of 3 to 9 h. Using the same telemetry system, Mosher et al. (1990), monitored vaginal temperature continuously in nine post-pubertal dairy heifers. Vaginal temperature was recorded at 15-min intervals and later compiled into hourly means. An increase in temperature of ≥0.3°C for at least 3 above the mean of the temperatures taken on the three previous days during the same hour of the day was used to identify the temperature peak. With these criteria, 78% of all estrous periods were detected and no FP occurred. The range in duration of the increase in temperature was 4 to 21 h. Redden et al. (1993), monitored vaginal temperature in 13 lactating dairy cows continuously for an average of 67 consecutive days. The criteria used for identifying a temperature peak were a ≥0.3° C increase in temperature lasting at least three hours compared to the mean of the temperature of the four previous days during the same hour of the day. Using these criteria, 17 of 21 estrous periods were detected and three FP occurred. The mean maximal increase in temperature was 0.6±0.3° C and the mean duration of the temperature peak was 6.8±4.6 h.

Although there have been relatively few studies conducted, continuous monitoring of vaginal temperature may be a promising strategy for detecting estrus in cows. As shown by the studies with continuous monitoring of temperature, the peak may last for only a few hours and must be compared with a baseline of the average temperature for the same time of day to be effective in distinguished the slight rise in temperature associated with normal daily rhythms. As well, continuous monitoring by radiotelemetry is the only practical method for using body temperature to detect estrus in cows maintained in typical beef management systems.

Conclusion

To date, there has been no assessment of the effectiveness of using pedometers or vaginal transmitters for detecting estrus in beef cows. Although the evidence suggests these methods may be effective for dairy cows, results using dairy cows may not apply to beef cattle due to breed and/or management differences. For example, most beef cows would be suckling calves during the breeding period and the physiological responses to suckling differ from milking (Reviewed by Williams 1990). Perhaps due to high milk production, there is an increased occurrence of ovarian abnormalities in dairy cows compared to beef cows. This suggests that the endocrine system of beef cows is more stable than that of dairy cows, therefore hormone levels and behaviour would be affected (Jainudeen and Hafez, 1987). Hanzen et al. (1994), for example, found detection of estrus was higher for beef cows than for dairy cows. Differences in management, such as housing facilities and frequency of handling individual animals, also exist between beef and dairy operations. Therefore, specific testing of pedometers and temperature telemetry in beef cows would not only increase knowledge concerning these methods of detecting estrus, but would also determine if there are substantial differences in the practicality of using these methods for beef cows compared to dairy cows. The development of a method of detecting estrus which is practical and effective under beef management conditions could greatly increase the feasibility of incorporating AI into for beef reproductive management.

Manuscript 1 - Measurement of Vaginal Temperature By Radiotelemetry for the Detection of Estrus in Beef Cows

Abstract

Peaks in vaginal temperature (Tv) were assessed as predictors of estrus in loose-housed, suckled beef cows during the breeding seasons of two consecutive years. Vaginal temperature was monitored continuously, using radiotelemetry, in 8 and 16 beef cows during the first and second years, respectively. A flexible plastic anchor was used to retain the radiotransmitter within the cow vagina for the duration of each breeding season. Blood samples were collected twice weekly for analysis of serum progesterone to confirm the occurrence of estrus and ovarian status was checked weekly using transrectal ultrasonography. Visual observations for estrus behaviour were made for 20 min/h between 4:00h and 8:00h, and casually from 8:00h to 16:00h. Values for Tv collected at 4-minute intervals were available for a total of 47 estrous periods in the two years. A peak in Tv was defined as an increase in Tv of at least 0.4°C for 3 or more consecutive hours over the corresponding hourly means of the previous 4 days. During the two breeding seasons, a peak in Tv was found for 42 of the 47 confirmed estrous periods (detection sensitivity of 89.4%). Detection accuracy was found to be 77.8% (7 false positives). Mean maximal increase in Tv was 0.91°C and the average duration of the peak in Tv was 6.5±2.7h. A decline in Tv was observed a few days prior to estrus and a slight rise in Tv was noted to occur at mid-cycle. Detection sensitivity and detection accuracy for visual observations of standing estrus were found to be 53.2% and 52.1%, respectively. Continuous monitoring of Tv using radiotelemetry was found to be a sensitive and accurate means of predicting estrus in beef cows.

Introduction

Difficulty with detecting estrus impedes the use and success of artificial insemination (AI), embryo transfer, and methods to control ovulation that would greatly improve reproductive efficiency of beef cows. It has been known for some time that there are changes in body temperature associated with estrus in dairy cattle (Wrenn et al. 1958, Fallon 1959). In dairy cows, there is a depression of body temperature a few days before estrus (Lewis and Newman 1984, Redden et al. 1993). This is followed by a temperature increase which has been found to be closely associated with the onset of standing estrus (Rajamahendran et al.,1989, Rajamahendran and Taylor 1991) and the pre-ovulatory surge in luteinizing hormone (LH) (Rajamahendran et al.,1989, Mosher et al.,1990). The temperature peak can be relatively short-lived, lasting at times for only a few hours (Redden et al.,1993). Therefore, frequent temperature readings are necessary to consistently detect the peak. As well, handling of cattle may induce stress-related changes in body temperature (Mosher et al.,1990). Thus, remote temperature sensing is the only practical method for using the change in body temperature as a means of detecting estrus. Although studies have shown the effectiveness of using radiotelemetry for detection of the change in body temperature in dairy cows (Zartman et al.,1983, Mosher et al.,1990, Redden et al.,1993), the use of this technology in beef cows has not been tested. Remote temperature sensing is particularly practical for detecting estrus in beef cows, considering the handling and housing practices which are common to beef cattle management. The objective of the present study was to test the feasibility and effectiveness of using vaginal temperature (Tv), detected by radiotelemetry as a method of detecting estrus in loose-housed, suckled beef cows.

Materials and Methods

Animals and Management

Year 1

During March and April 1992, eight multiparous suckled Hereford-cross cows were fitted with radiotransmitters commencing from 21 to 56 days post-partum. Radiotransmitters remained in the vagina for 55 to 63 days. Study cows were housed in two pens containing 10 cows each. Pen 1 housed cows nursing only their own calves and pen 2 housed cows nursing their own plus an adopted calf. Three cows in pen 1 and five cows from pen 2 were used for the study. The indoor straw-bedded pens (11.6 m X 11.6m) were connected to outside yards (11.6 m X 8.8m). Cows were given free access to hay and water. Cows in pen 1 and pen 2 were given 1 kg and 2 kg grain per head, respectively, twice daily at 10:00h and 16:00h. As part of another experiment (Manuscript 2), cows were also fitted with pedometers above the hock on the hind-leg. Based on previous successful results, barn staff made visual observations for estrus (standing heat, mounting activity, vaginal discharge) for 20 min/h daily from 4:00h to 8:00h and casually (while performing other duties) from 8:00h to 16:00h. Beginning in April, cows were bred by AI based on visual observations.

Year 2

During March and April 1993, sixteen multiparous Hereford-cross suckled cows were fitted with radiotransmitters commencing from 25 to 62 days post-partum. Radiotransmitters remained in the vagina for 10 to 54 days. Cows were loose-housed in a group of 10 (cow nursing one calf) and six (cows nursing two calves) in the pens described for Yr. 1. Management was similar to Yr. 1, except grain feeding took place once daily in the AM. As part of another experiment (Manuscript 2), cows were fitted with pedometers above the hock on the hind-leg and on the neck. Cows were bred in April using AI based on Tv results, pedometer results (Manuscript 2), and/or visual observations.

Measurement of Vaginal Temperature

A radiotelemetric system (Wildlife Materials Inc., Carbondale, IL) consisting of identity coded temperature sensitive radiotransmitters, radio receiving antennae, a radio receiver and a 386 MB computer for data logging was used to collect Tv from all cows at approximately 4-minute intervals (Redden, 1992). The antenna was attached to the ceiling of the barn between the two pens containing the test cows. The distance between the antennae and the furthest pen boundary was 60 m. The time interval between consecutive radio transmissions was inversely related to temperature of the radiotransmitter. For each signal received, a record was made of the cow identity and the time of reception of the signal. Custom software was subsequently used to calculate the temperature of the radiotransmitter during the interval from the last received signal (Redden, 1992).

Radiotransmitters were encased in a support anchor (Figure 1) and were cold sterilized (benzalkonium chloride solution, 1:750, ml/ml) prior to insertion into the vagina to a depth of approximately 20 cm. Anchor finger lengths were between 25-30 mm and 30-35 mm for Yr. 1 and Yr. 2, respectively. The vulva area was washed thoroughly with iodine solution prior to anchor/transmitter insertion into the vagina. Hand insertion of the anchor encased transmitter was facilitated by use of lubricating gel, warming the anchor, and flattening of the anchor finger projections against the anchor body during passage through the external sphincter of the vagina.

Confirmation of Estrus

In Yr. 1, blood samples were obtained twice weekly by jugular venipuncture and were analyzed, in one assay, for serum progesterone (P4) by radioimmunoassay using the method of Abraham et al.(1971) as modified by Yuthasastrakosol et al.(1974) and Sheikheldin et al.(1988). Assay sensitivity and intra-assay coefficient of variation (CV) were .07 ng/ml and 15.6%, respectively. Samples collected twice weekly in Yr. 2 were analyzed using Coat-a-Count® kits (Diagnostic Products Corporation, Los Angles, CA).

Assay sensitivity and intra-assay coefficient of variation were 0.05 ng/ml and 6.5%, respectively. An estrus was considered confirmed if serum P4 was ≤1.0 ng/ml at the time of estrus and rose above 1.0 ng/ml within one week. Transrectal ultrasonography of the ovaries, using a real-time ultrasound scanner (Pie-Medical 450, Brampton, Ontario) and a 5.6 MHz rectal probe, was conducted weekly for further confirmation of ovarian status. This provided additional information concerning the cows reproductive status.

Calculations and Statistics

Temperature means for each hour were calculated from the 4-minute readings. Hourly means were compared to a baseline consisting of the average for the same hour calculated over the previous 4 days. An estrus was considered to have occurred if the hourly mean increased by 0.3° C above baseline for 3 or more consecutive hours (Redden et al.,1993). In Yr. 2, where AI was based on temperature, as well as other methods of detecting estrus, calculations were made daily between 8:00h and 10:00h when data for the previous 24h was examined. If an increase in Tv met the above criteria, cows were then bred using AI between 10:00h and 15:00h of the day on which the temperature increase was noted. In both years, further criteria were applied to the data on a post hoc basis. Baselines consisting of the previous two, three, four, or five days were tested. For each of these baselines, all temperature peaks meeting or exceeding a 0.3° C and a 0.4° C increase over the basal level and lasting for a minimum of 3 and 4 consecutive hours were determined. A temperature peak was considered to be associated with estrus if it coincided (within three days) with a P4 value of ≤1 mg/ml and the estrus was confirmed by ultrasound. A peak was termed a false positive if it occurred outside of the confirmed estrous periods. On a number of occasions, Tv was elevated above baseline for the required minimum period, declined below the required increase for a number of hours, and then met the criteria again. If such a pattern occurred, with the second peak occurring within 24 hours of the first, both peaks were considered part of the estrual increase. Similarly, if the same pattern occurred outside of a confirmed estrus period, with the second peak falling within 24 h of the first, both peaks were considered part of the same false positive.

Measures of the detection of estrus using Tv were calculated for each criteria using the following definitions and formulae:

TE=total number of P4 and ultrasonography confirmed estrous periods

DE=total number of Tv correctly detected estrous periods (increases in Tv corresponding with confirmed estrus)

FP=total number of false positive readings (increases in Tv not corresponding with confirmed estrous periods)

DS=Tv detection sensitivity as a percentage ((DE/TE)*100)

DC=Tv detections which were correct as a percentage ((DE/(DE+FP))*100)

DA=Tv detection accuracy as a percentage ((DE/TE+FP)*100)

Contrasts were used to compare Tv during specific periods of the estrous cycle. Students t-test was used to compare differences in the time interval between the onset of the temperature peak and AI for successful and failed breeds. Statistics were performed using SAS version 5.16 (1986).

Results

Radiotransmitter and Support Anchor Function

Difficulties with the telemetry system and/or computer system were minor and resulted in approximately 50 hours of missing data per cow during both years. Failure of the support anchor to hold the transmitter in place resulted in 297 hours of missing data for Yr. 1. The frequency of loss of the anchor and transmitter varied among cows in Yr. 1. For example, although transmitter loss occurred in six cows, 119 of the total missing hours of data occurred in one cow (X11). During Yr. 2, only one incident of transmitter loss occurred. Improvement of anchor function may have been due to the use of longer finger projections on the anchor in Yr. 2. During Yr. 2, however, a manufacturing error in the wax coating of the transmitters resulted in electrical failures and missed readings on a number of occasions. As a result, data was available for between zero to two estrous periods per cow rather than the expected three to four estrous cycles per cow. Breeding by AI was possible without removal of the radiotransmitter from the vagina. No ill effects to the health of the cows were noted due to the presence of the transmitters.

Detection of Estrus

Vaginal temperature results for years 1 and 2 were similar and have been combined for analysis. An example of Tv data for one cow (X43) is shown in graphical form in Figure 2. Increases in temperature exceeding criteria occurred 52, 73, and 94 days post-partum. The days of increased Tv were within periods in which the occurrence of ovulation was confirmed by P4 concentrations and ultrasound results.

Results for all criteria are given in Appendix I (Tables 1 and 2). Only estrous periods for which data was available for ≥5 days of baseline (n=42) were included when comparing criteria. Variation due to changes in criteria are apparent. Detection sensitivity decreased as the criteria for magnitude and duration of the peak increased. Generally, the detection sensitivity decreased with an increase in the days of baseline. The number of FP follows the same trends as the number of true estrous periods detected, decreasing with increasing requirement for magnitude and duration of the peak, and for the length of baseline. The decline in the number of FP is greater than the decrease in the number of detected estrous periods when the criterion for magnitude of Tv increase was raised from 0.3° C to 0.4° C. This suggests the magnitude of FP peaks is lower than that of estrual related peaks in Tv.

The percent correct, indicating the percent of the detected temperature peaks which were associated with estrus, is inversely related to the number of FP. Detection accuracy shows the effects of both detection sensitivity and the number of FP, and therefore gives the most practical overall assessment of criteria for detecting estrus. The criteria that maximized accuracy defined estrus as an elevation in Tv of ≥ 0.4° C for ≥ 3 consecutive h above the previous three days baseline. Results obtained for the DE, FP, DS, DC, and DA using the above criteria for Tv and using visual observations for estrus are presented in Table 1. All estrous periods with three days of baseline data available (n=47) were used for these calculations. Detection sensitivity was 89.4% using Tv compared to 53.2% for visual observations. Accuracy using Tv (77.8%) was higher than that found using visual observations (52.1%). However, the percentage of detected estrous periods which were correct was lower for vaginal temperature (85.7%) than for visual observations (96.2%), indicating there were fewer FP for the visual method. For the estrous periods detected using the above criteria, peaks in Tv at estrus had a mean ±S.D. duration of 6.5±2.7 h (range = 3 h to 14 h) and the mean ± S.D. of the maximum increase in Tv at estrus was 0.91±0.35° C. With this criteria, only seven FP occurred (two during Yr. 1 and five during Yr. 2). One false positive in Yr. 1 occurred 29 hours after a estrus related increase in Tv. Although the peak was defined as a false positive, because it did not occur within 24 hours of the first peak in Tv, it may have been associated with estrus and would not have led to a false breeding of the cow. The five FP detected during year 2 occurred in one cow (W111). This cow displayed temperature peaks meeting the criteria every four to eight days throughout the test period. Ovarian function, P4 profile, and early gestation appeared normal for this individual. In general, the increase in Tv for FP was lower than the increase of estrus related peaks, as can be seen by the sharp decrease in the number of FP when the requirement for peak magnitude was increased from 0.3° C to 0.4°C (Appendix I). False positives for cow W111, however, were similar to estrus related temperature increases in Tv with a mean maximum increase of 1.12±0.6°C and a duration of 6.2±2.3h. As noted by Junge-Wentrup and Holtz (1984), infections and/or the occurrence of mastitis dramatically changes the body temperature profile and obscures normal temperature patterns. Therefore, W111 may have had a chronic infection, causing frequent fluctuations in body temperature.

Hourly mean Tv for all estrous periods following cycles of 19-22 days (n=15) in length were plotted from the first hour Tv had returned to baseline at the previous estrus, through the estrous cycle to the last hour of the Tv peak (Figure 3). With the exception of 10 h associated with the estrus peak, the cycle was divided into seven periods of three days each. Overall, Tv during the three days before estrus was found to be significantly lower ($p<0.05$) when compared to all other three day periods. When calculated on an individual basis, this was also true for all but one estrus. Also based on individual cycles, Tv of the three days associated with the mid-point of the cycle were found to be significantly ($p<0.05$) higher when contrasted with the remainder of the cycle.

Figure 4 indicates the mean hourly Tv for all estrous periods detected (n=42) for seven days before and subsequent to the last hour for which Tv was elevated (met the criteria). Both the dramatic elevation in Tv at estrus and a tendency for Tv to be depressed prior to estrus are evident. Overall, Tv of the 48h period before the estrual increase (-3 d to -1 d) was found to be significantly ($p<0.05$) lower than for all other 48h periods. On an individual basis, this was true for 84% of the estrous periods studied.

Overall, there was also a significant difference in Tv between the periods of -3 d to -1 d and -7 d to -3 d. This was also true for 76% of the individual estrous periods.

Of the 47 estrous periods studied, 25 were associated with visual observations of standing estrus or high levels of mounting. Low levels of mounting were observed on five occasions, and no visual signs of estrus were found for 17 of the estrous periods. Eighteen of the 22 estrous periods with little or no visible behaviour were detected using Tv. In Yr. 2, AI was performed for 12 of the estrous periods detected by Tv. Of the seven resulting pregnancies, four were the result of AI based solely on Tv data.

There was a difference ($p<0.05$) in the interval from the onset of the temperature peak to the time of AI between cows which conceived and those which did not conceive in year 2. Successful breedings (n=7) took place 20±5 h after the first hour of the temperature peak, while breedings which did not lead to conception (n=5) were conducted 12±5 h after the temperature peak.

Discussion

The use of temperature telemetry for the detection of estrus was particularly sensitive (89.4%) for the beef cows of the present study. Using the same or comparable telemetric devices in dairy cows, Redden et al. (1993) and Mosher et al. (1990) were able to detect 81% and 78% of estrous periods, respectively. The detection criteria chosen in the three studies were similar. However, due to the apparently larger magnitude of the peak in Tv in beef cows, the criteria yielding the highest accuracy included a 0.4°C increase rather than a 0.3°C increase which was used in the other studies. It is likely that the large increase in Tv of beef cows at estrus contributed to both the excellent sensitivity and accuracy in detecting estrus.

The mean maximum increase in Tv at estrus was 0.91±0.35°C in beef cows but only 0.6±0.3°C in dairy cows (Redden et al. 1993). The dairy cows studied by Redden et al. (1993) were housed in tie-stalls for the majority of the day. If the increase in locomotor activity at estrus contributes to the temperature peak, as suggested by Walton and King (1986), loose housing of the beef cows in the present study may have contributed to the large increase in Tv at estrus. Duration of the Tv peak in the present study was comparable to that seen in dairy cows, taking into account the variations in criteria used (Redden et al. 1993, Mosher et al. 1990).

The changes in temperature which occurred during the estrous cycle were consistent among estrous periods studied (Figure 3). This suggests that fluctuations in temperature might be related to the hormonal milieu during particular phases of the estrous cycle. Central control of body temperature (Rezvani et al.1982) and the release of GnRH (Reviewed by Barb et al. 1991) have been found to be influenced by opioid peptide action in the pre-optic area of the hypothalamus. In rats, there was a decrease in typical behaviourial and physiological responses to an opiate on the afternoon of proestrus compared to the morning of proestrus and diestrus for all responses measured, including body temperature and LH release (Berglund and Simpkins 1988). The increase in body temperature at estrus, therefore, may be related to the initiation of the LH surge. A similar decrease in opiate, and possibly EOP, inhibition may occur during the estrous cycle in cows.

Another possibility for the fluctuations in temperature during the cycle is the thermogenic effect of progesterone which has been well established in humans (Czaja and Butera, 1986). In the present study, elevated and lowered temperatures were found during the luteal and pre-estrus periods, respectively, which corresponds with changes in P4 concentrations. Lewis and Newman (1984), found similar changes when measuring vaginal temperature in cattle once daily throughout the cycle. Cagnacci et al. (1992), suggested that the effects of progesterone in women are not mediated directly through EOP activity. Therefore, the changes in temperature seen during the cycle and at estrus may be controlled by separate mechanisms.

The estrual temperature peak and the LH surge are thought to occur synchronously during the pre-ovulatory period (Rajamahendran et al.,1989, Mosher et al.,1990). The LH surge has been found to be a reliable indicator of the timing of ovulation (Rajamahendran et al.,1989). In Yr. 2, AI was performed, in part, based on Tv results. Although calculated from a small sample size in this study, a difference in timing of AI which did and did not result in conception was found. These preliminary results suggest that AI should be done on the day following the peak in Tv. However, the temporal relationship between Tv and ovulation should be investigated further. Monitoring of Tv using radiotelemetry may prove to be an accurate method of predicting the time of ovulation and, therefore, could prove valuable in ensuring the correct timing of AI.

Although FP were not a major problem in this study, the pre-estrus decrease in Tv might be useful in distinguishing estrual and false positive peaks in Tv, as suggested by Redden et al. (1993). Due to the low number of FP in this study, a similar analysis to Redden et al. (1993) could not be made. For 84% of the estrous periods in the present study, Tv for the 2 d period preceding the day of estrus was found to be significantly lower than the Tv of the previous 4 d period. The pre-estral depression in Tv might be used as an early indicator of estrus, if it is found to be predictable in magnitude and duration, and if it is unique to the pre-estrus period.

Monitoring of vaginal temperature using radiotelemetry was found to be an effective means of detecting estrus in beef cows in the present study. Using a criteria of a 0.4°C increase in temperature for at least three consecutive hours over a three day baseline, detection sensitivity and detection accuracy were found to be 89.4% and 77.8%, respectively. This compares to a 53.2% and a 52.1% detection sensitivity and detection accuracy using visual observations for estrus behaviour. The results of the present study indicate that the monitoring of Tv by radiotelemetry is an effective and practical means of detecting estrus for beef cattle and its use could promote the use of AI within the beef industry.

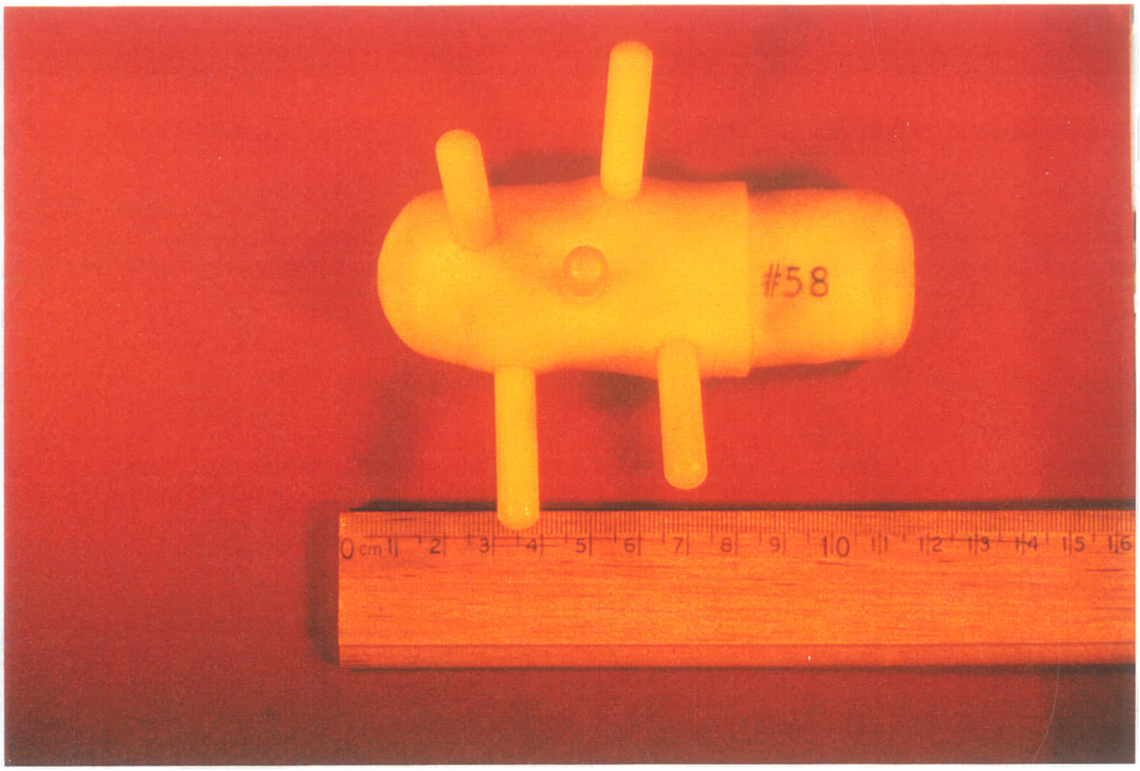

Figure 1 - Radiotransmitter encased in a vaginal anchor.

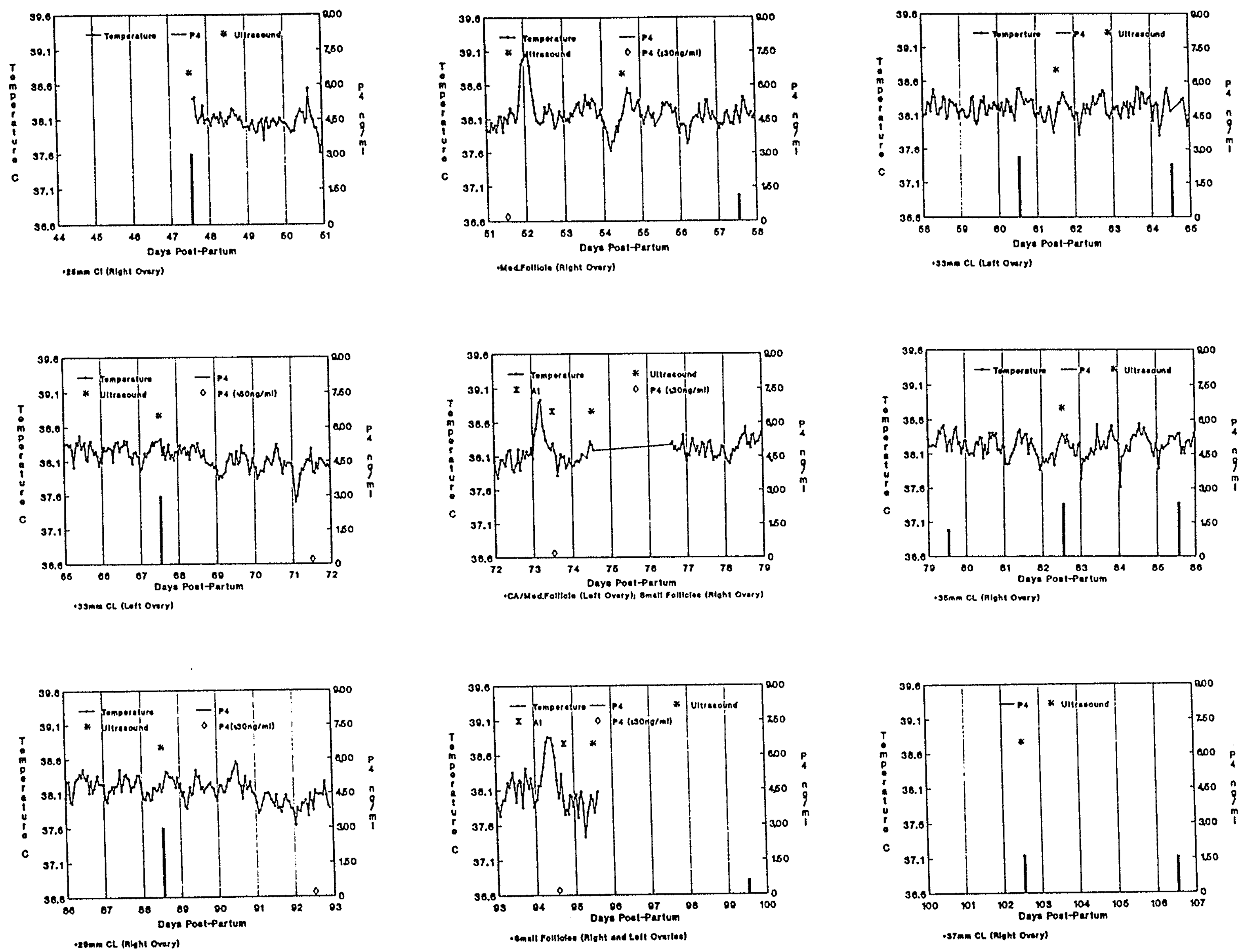

Figure 2 - Vaginal temperature, progesterone, and ultrasound data for cow X43 from days 44 to 107 post-partum. Estrus related peaks in vaginal temperature occurred on days 52,73, and 94. Artificial insemination occurred on days 73 and 94.

Table 1 - Detection sensitivity, Tv detections which were correct, and detection accuracy for vaginal temperature and for visual observations of behaviourial estrous periods(TE[a]=47).

	Vaginal Temperature[b]	Visual Observations
Detected Estrus	42	25
False Positives	7	1
Detection Sensitivity(%)	89.4	53.2
Correct Detections(%)	85.7	96.2
Detection Accuracy(%)	77.8	52.1

[a] TE=total confirmed estrous periods
[b]Criteria of 0.4°C increase in temperature for at least 3 consecutive hours over a 3 day baseline.

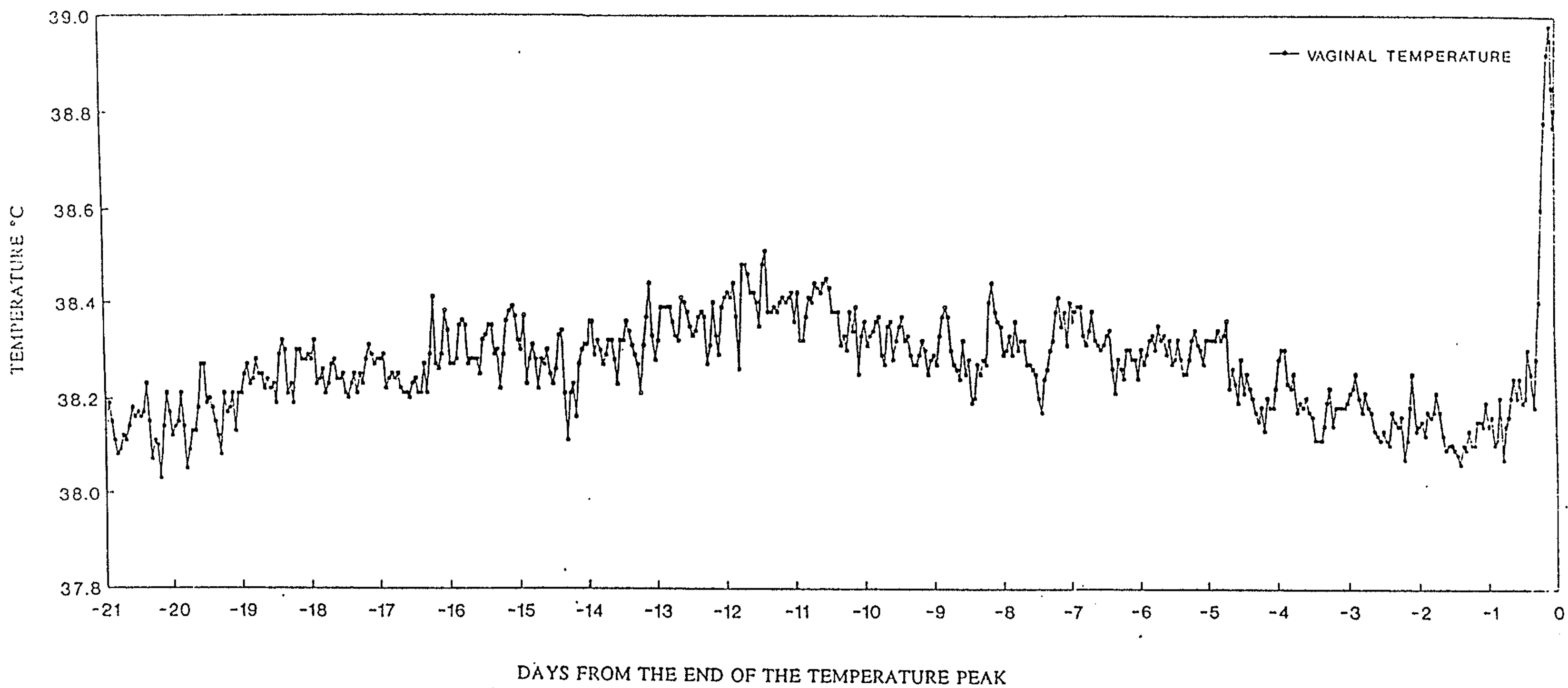

Figure 3 - Mean hourly vaginal temperature (Tv) for all estrous periods following cycles of 19-22 days (n=15) from the first hour Tv was below criteria to the last hour Tv met criteria.

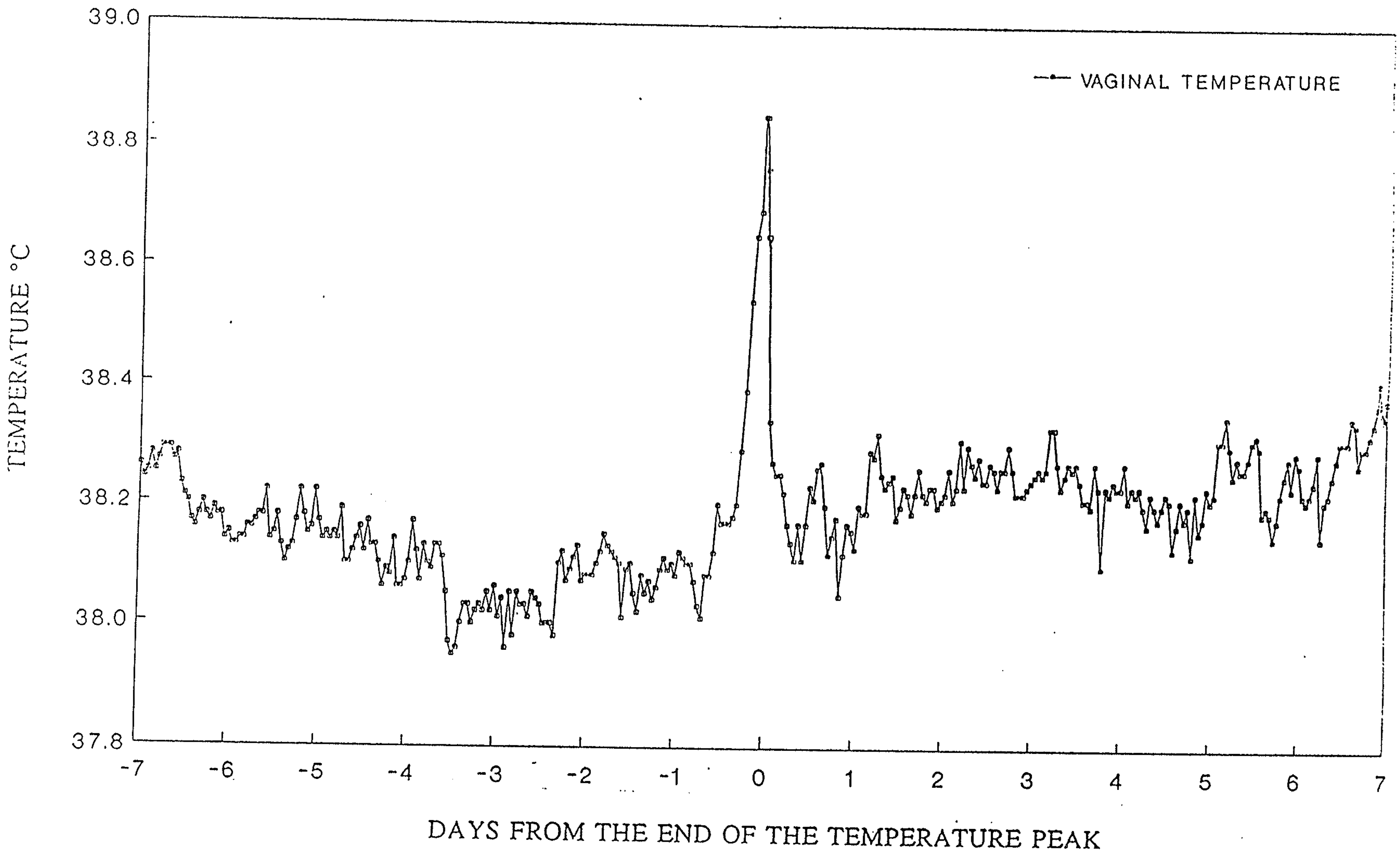

Figure 4 - Mean hourly vaginal temperature (Tv) for all estrous periods detected (n=42) for seven days before and subsequent to the last hour Tv met criteria.

Manuscript 2 - Measurement of Activity by Pedometers for the Detection of Estrus in Beef Cows

Abstract

Peaks in pedometer measured activity were assessed as predictors of estrus in suckled beef cows during the breeding seasons of two consecutive years. Activity of 8 and 16 cows was monitored in Year 1 and Year 2, respectively (Experiment 1). Additionally, 12 unsuckled beef cows were studied over a period ranging from 28 to 52 days (Experiment 2). A pedometer was placed above the hock on the hind-leg of each cow in all trials, and an additional pedometer was secured to a collar which was fitted around the neck of cows in Experiment 1 (Year 2) and Experiment 2. In both experiments, cows were loose-housed and pedometers were manually read twice daily. Blood samples for the analysis of serum progesterone and ultrasound scans of the ovaries were taken to confirm the occurrence of ovulation. In Experiment 1 visual observations for behavioural estrus were made for 20 min/h between 4:00h and 8:00h and casually between 8:00h and 16:00h. Using criteria which optimize detection accuracy (DA), pedometers attached to the leg had a detection sensitivity (DS) (%) of 62.5, 89.3, and 100.0 and DA (%) of 27.8, 69.4, and 82.4 for Experiment 1 (Year 1 and Year 2), and Experiment 2, respectively. Using criteria which optimize DA, pedometers attached to the neck had DS (%) of 92.9 and 76.7 and a DA (%) of 76.5 and 41.1, in Experiment 2 and Experiment 1 (Year 2), respectively. Visual detection DS (%) ranged from 68.8 to 36.7 and DA (%) ranged from 64.7 to 36.7. The difference in pedometer results using criteria which optimize DA and standard criteria of a 2.0 fold increase in activity over a 10 day baseline was minimal. Overall, pedometers were found to be an effective means of detecting estrus in beef cattle.

Introduction

Cows show varying levels of increased locomotory activity and decreased time spent feeding and resting prior to ovulation (Hurnik et al.,1975). Pedometers, which are mechanical and/or electronic devices used to measure locomotory activity, have been used as a means of quantifying changes in daily activity in dairy cows. Numerous studies have assessed the effectiveness of using pedometers to measure activity as a means of estrous detection. In dairy cows, pedometers have been shown to detect a high percentage of estrous periods (76% to 98%), compared to detection by visual observations alone (35% to 60%) (Kiddy 1977, Williams et al.,1981, Peter and Bosu 1986, Redden et al.,1993). The present study is the first to examine the feasibility and effectiveness of using pedometers for the detection of estrus in suckled (Experiment 1) and non-suckled (Experiment 2) loose-house beef cows.

Materials and Methods

Experiment 1 - Year 1

Animals and Management

Eight multiparous Hereford-cross suckled cows, 21 to 56 days post-partum, were used during the March-May breeding season in 1992. Study cows were housed in two pens containing 10 cows each. Pen 1 housed cows nursing only their own calves and pen 2 housed cows nursing their own plus an adopted calf. Three cows in pen 1 and 5 cows in pen 2 were used for the study. The indoor bedded pens (11.6 X 11.6 m) were connected with access to an outside yard (11.6 X 8.8 m). Cows were given free access to hay and water and were given grain (1 kg/head for pen 1 and 2 kg/head for pen 2) twice daily at 10:00h and 16:00h. As part of another study, all cows on test were fitted with vaginal radiotransmitters (Manuscript 1). Visual observations for estrus (standing heat, mounting activity, vaginal discharge) were made daily for 20 min/h from 4:00h to 8:00h and casually from 8:00h to 16:00h. Beginning in April, breeding using AI was performed based on visual observations of estrus.

Pedometers

Pedometers were attached to a hind-leg of each cow. The digital pedometers (Digi-Walker-L and Digi-Walker Mini,EM-201, Yamax Corp.,Yokohama) were modified for cow use by coating with rubber and attachment of foam backing using duct tape. Leg pedometers were secured to the leg above the hock with elastic straps and Velcro (Figure 5). Pedometer numeric values were recorded twice daily at feeding for a period of 67 d. Daytime (10:00h - 16:00h) and overnight (16:00h - 10:00h) pedometer activity values were calculated daily and used to generate daytime and overnight baseline means.

Confirmation of Estrus

Blood samples, obtained twice weekly by jugular venipuncture, were analyzed for serum progesterone (P4) by radioimmunoassay using the method of Abraham et al.(1971) as modified by Yuthasastrakosol et al.(1974) and Sheikheldin et al.(1988). An estrus was considered confirmed if serum progesterone was found to be ≤ 1.0 ng/ml at the time of estrus and then to rise above 1.0 ng/ml within one week. Transrectal ultrasonography of the ovaries, using a real-time ultrasound scanner (Pie-Medical 450, Sterne Medical Equipment, Brampton, Ontario) and a 5.6 MHz rectal probe, was conducted weekly for further confirmation of ovarian status. Diameters of corpora lutea and all visible follicles (>6mm) were measured and recorded.

Experiment 2

Animals and Management

Seven multiparous and five primiparous Hereford-cross beef cows were used as experimental animals in this experiment and as part of another study (Manuscript 3). Cows were loose housed in an indoor pen (24 m X 4.5 m), with access to an outside yard (6 m X 6 m). Cows were given free access to hay and water and were given 0.5 kg of barley daily. Eleven cows had calved just prior to the beginning of the experiment, and one cow had been open and cycling normally at the beginning of the study. Calves were removed within 24 hours of parturition as part of an experiment unrelated to this study.

Data were collected between January 20 and March 17, 1993. The time on test varied among cows from 28 to 52 days, depending on calving dates. Early post-partum cows were put on test between 3 and 14 days post-partum. Observations for estrus behaviour were not made on a routine basis. Synchronization of estrus using Estrumate® was performed in certain cows (see Manuscript 3).

Pedometers

As described in experiment 1, a pedometer was attached above the hock of a hind-leg of each cow. An additional pedometer was held in place on the neck by attachment to the side of a collar fitted with a steel weight to prevent the collar (and pedometer) from rotating on the neck (Figure 6). A slip buckle was used to secure the collar. Pedometer numeric values were recorded twice daily at 8:00h and 16:00h. Leg and neck pedometer daytime (8:00h - 16:00h) and overnight (16:00h - 8:00h) activity values were calculated daily and used to generate daytime and overnight baseline means.

Confirmation of Estrus

Blood samples were collected three times weekly from all cows and were tested for serum progesterone levels using Target® kits (BioMetallics Inc., Princeton, NJ). Transrectal ultrasonography of the ovaries, as described for Exp.1, was performed three times weekly. Additional blood samples and ultrasound data were taken at estrus (see Manuscript 3).

Experiment 1 - Year 2

Animals and Management

Sixteen multiparous Hereford-cross suckled cows, between 25 and 57 days postpartum, were used in the study. During the March-May breeding season in 1993, cows were loose-housed in groups of 10 (cows nursing one calf) and six (cows nursing two calves). Management was similar to Exp.1 (Yr.1), except grain feeding took place once daily in the AM. As part of another study, all cows on test were fitted with radiotelemetric vaginal transmitters (Manuscript 1). Visual observations for estrus (standing estrus, mounting activity, vaginal discharge) were made daily for 20 min/h from 4:00h to 8:00h and casually from 8:00h to 16:00h. In April, breeding using AI was performed based on results of vaginal temperature data, pedometer data, and/or visual observations of estrus.

Pedometers

Pedometers, as previously described, were attached to a hind-leg and neck of each cow. To improve attachment to the neck, the buckles on the neck collars were changed from slip buckles used in Exp. 2 to standard belt-type buckles. Leg and neck pedometer daytime (8:00h - 16:00h) and overnight (16:00h - 8:00h) activity values were calculated daily and used to generate daytime and overnight baseline means. During this study, pedometers were used to detect estrus for the purpose of timing of A.I.. Based on the results of Exp. 1 (Yr. 1) and Redden et al. (1993), the criteria used to indicate estrus were a 1.5 fold increase in activity over a 5 day baseline. If these criteria were exceeded at the AM pedometer reading, AI was performed in the PM of the same day. If the pedometer detected estrus was noted during the PM reading, AI was performed the following day.

Confirmation of Estrus

Blood samples were taken twice weekly for radioimmunoassay of serum progesterone using Coat-A-Count® kits (Diagnostic Products Corp., Los Angles, CA). Transrectal ultrasonography of the ovaries, as described previously, was performed once weekly.

Calculations and Statistics

Assessment of the effectiveness of pedometers in the detection of estrus for all experiments was based on examination of various criteria, post hoc. The criteria examined included variable lengths of baseline (2, 5, 10, and 15 d) and the fold increase in activity relative to baseline activity (1.5, 2.0, 2.5, 3.0).

Measures of the detection of estrus using pedometers were calculated for each set of criteria using the following definitions and formulae:

TE=total number of P4 and/or ultrasonography confirmed estrous periods

DE=total number of pedometer detected estrous periods (increases in pedometer values corresponding with confirmed estrous periods)

FP=total number of false positive readings (increases in pedometer values not corresponding with confirmed estrous periods)

DS=pedometer detection sensitivity as a percentage (DE/TE*100)

DC=pedometer detections which were correct as a percentage ((DE/(DE+FP))*100)

DA=pedometer detection accuracy as a percentage ((DE/TE+FP)*100)

Criteria showing the highest DA (which accounts for both DS and the number of FP) within each experiment and pedometer position were used for all further analysis. Only estrous periods with ≥15 days baseline were used in data analysis such that the incidence of FP could be adequately assessed. To reduce the incidence of FP, a correction was imposed which discounted an increase in activity if it occurred less than 15 days after an increase of greater magnitude (15 day correction). When the pedometer values met the criteria for two readings within 24 h of each other, both were considered part of the same estrus or false positive.

Paired t-tests were used to compare pedometer measured activity on estrus days to that of non-estrus days, within each experiment. Statistics were performed using SAS version 5.16 (1986).

Results

Pedometer Function and Testing

Incidence of pedometer loss and malfunction for each experiment and pedometer position are shown in Table 2. Pedometer detachment and malfunction occurred more frequently for leg pedometers in Exp. 1 than in Exp. 2. Pedometers attached to the neck had a lower incidence of malfunction and, after changing the buckle in Exp.2 (Yr. 2), showed a much reduced occurrence of detachment compared to leg pedometers. In a preliminary trial, pedometers placed on the leg or neck were tested by walking cows a standard distance with 3 replications. Pedometer numeric values were recorded after each replication. Pedometers on the leg and neck were found to register similar values when tested in this manner. Overall, pedometers were found to have a within pedometer coefficient of variation (CV) of 11.1% and a between pedometer CV of 20%. Activity, progesterone, and ultrasound results for cow X43 between 44 and 107 days postpartum are shown in graphical form in Figure 7. Increases in leg-measured activity exceeding criteria were observed 52, 73, and 94 days post-partum. The days of increased activity were within periods in which the occurrence of ovulation was confirmed by P4 concentrations and ultrasound results.

Detection of Estrus

Experiment 1 - Year 1

Pedometer data were available for 16 estrous periods in eight cows. Results for all criteria are given in Appendix II (Tables 1a and 1b). Overall, DS and DA were low for most criteria tested. The accuracy of detection remained relatively constant among the 1.5, 2.0 and 2.5 fold increases. Although there were no dramatic differences, the set of criteria with the highest DA (27.8%) was a 1.5 fold increase and either a 10 or 15 day baseline, using the 15 day correction. Detection sensitivity and DC for this set of criteria was 62.5% and 33.3%, respectively. Visual observations detected 11 of the 16 estrous periods with one false positive (DS=68.8%, DC=91.7%, DA=64.7%) (Table 4). The difference in activity between all estrus and non-estrus pedometer readings was significant ($p<.05$) (Table 3). Mean±SD estrus and baseline activity values were 2758±1864 and 1167±778, respectively (Table 3). Forty percent of the estrous periods were first detected during the daytime period and 60% during the overnight period. The percentage of estrous periods in which there were multiple consecutive indications of estrus by the leg pedometer for the magnitudes of increase of 1.5, 2.0, 2.5, and 3.0 fold were 70, 25, 14.3, and 0, respectively (Table 5).

Experiment 2

Leg and neck pedometer results were available for 14 estrous periods in 10 cows. Results for all criteria tested are given in Appendix II, Tables 2a and 2b for leg pedometers, and Tables 3a and 3b for neck pedometers. Detection sensitivity for leg pedometers was 100% for all but three criteria tested. Overall, there was a relatively low incidence of FP for both leg and neck pedometers, when the 15 day correction was employed. Highest DA (82.4%) for leg pedometers was achieved using a 2.5 fold increase and a 15 day baseline, and using the 15 day correction. Detection sensitivity and DC for these criteria were 100% and 82.4%, respectively (Table 4). Criteria yielding the highest DA (76.5%) for neck pedometers were a 2.0 fold increase and a 10 day baseline, with the 15 day correction. Detection sensitivity and DC for these criteria were 92.9% and 81.3%, respectively (Table 4). The difference between estrus and non-estrus readings for both leg and neck pedometers was found to be highly significant ($p<0.01$) (Table 3). The majority of estrus periods were first detected during the overnight period (87.5% for leg pedometers and 78.6% for neck pedometers). The percentage of estrous periods in which there were multiple consecutive indications of estrus by the leg pedometer for the magnitudes of increase of 1.5, 2.0, 2.5, and 3.0 fold were 57.1, 28.6, 14.3, and 15.4, respectively (Table 5).

Experiment 1 - Year 2

Data was available for 28 and 30 estrous periods for leg and neck pedometers, respectively. Results for all criteria are given in Appendix II, Tables 4a and 4b for leg pedometers and Tables 5a and 5b, for neck pedometers. Overall, results of criteria tested for leg pedometers showed high DS, whereas DS for neck pedometers was relatively low. As with the leg pedometers in Exp. 1 (Yr. 1), employment of the 15 day correction not only greatly decreased the number of FP for neck pedometers, but also decreased the number of estrous periods detected. Criteria yielding the highest DA (69.4%) for leg pedometers was a 2.0 fold increase in activity above a 10 day baseline, with the 15 day correction. Detection sensitivity and DC for these criteria were 89.3% and 75.8%, respectively (Table 4). Criteria giving the highest DA (41.1%) for neck pedometers was a 1.5 fold increase over a 15 day baseline. Detection sensitivity and DC for this criteria were 76.7% and 46.9%, respectively (Table 4). The difference in estrus vs all non-estrus readings for neck and leg pedometers was significant ($p<0.01$) (Table 3). Of estrus periods detected by leg pedometers, equal numbers were first detected in the daytime and in the overnight periods. Neck pedometer detected estrous periods occurred first during the daytime period 43.5% of the time and 56.5% of the time during the overnight period. The percentage of estrous periods in which there were multiple consecutive indications of estrus by the leg pedometer for the magnitudes of increase of 1.5, 2.0, 2.5, and 3.0 fold was 84.6, 56.0, 28.6, and 5.9, respectively (Table 5).

Twenty-four artificial inseminations were performed on the 16 cows during the study after either visual, vaginal temperature, or pedometer detection of estrus. Standing estrus and/or a high incidence of mounting activity were observed prior to only 42% (10/24) of the inseminations (Table 6). On six occasions, a very low incidence of mounting activity was observed (1-5 mounts) prior to AI, and on eight occasions no visual indications of estrus were detected by routine visual observations. Leg pedometers detected 100% (10/10) of the estrous periods for which there was a high visual indication of estrus, 83% (5/6) of the estrous periods for which there was a low visual indication of estrus, and 75% (6/8) estrous periods for which there was no visual indication of estrus. Twelve of the 24 inseminations resulted in pregnancy (Table 6).

Discussion

The present study is the first to examine the feasibility and effectiveness of the use of pedometers for the detection of estrus in beef cows. Manually read pedometers have previously been modified for attachment to the hind-leg of the dairy cow below (Kiddy 1977) and above (Redden et al.,1993) the hock. In these studies pedometer detachment from the leg and electronic malfunction were not considered to be significant problems. Williams et al. (1981), however, reported a high rate of pedometer loss and malfunction in dairy cows. In the present study, frequency of leg pedometer loss was not uniform for all cows and tended be high for specific cows. This may have been due to leg conformation and/or particular behaviour patterns of these individuals and/or their calves.

Pulling and chewing of the leg straps by calves was noted on several occasions and may have resulted in the higher occurrence of pedometer loss for cows in Exp. 1 compared to Exp. 2 (Table 2). The modification of neck collars in Exp. 1 (Yr. 2) greatly decreased the incidence of neck pedometer loss in comparison to Exp. 2. Electronic problems occurred mainly with the pedometer model Digi-Walker-L, which had not been used previously for cattle.

Pedometers placed on the neck did not detect estrus as well as leg pedometers within Exp. 2 and Exp. 1 (Yr. 2). Favero et al. (1984) also found placement of pedometers on legs was superior to placement on the neck for detection of estrus. In the present study, however, neck pedometers in both Exp. 2 and Exp. 1 (Yr. 2) were superior, based on DA, to leg pedometers in Exp. 1 (Yr. 1), and neck pedometers in Exp. 2 were slightly better than leg pedometers in Exp. 1 (Yr. 2). Therefore, neck pedometers may be of some use especially given the very low incidence of neck pedometer loss in Exp. 1 (Yr.2) with the modified buckles. Low occurrence of pedometer loss is particularly desirable for beef animals, where minimal handling of the cows occurs on a daily basis. Also, neck pedometers were in a better position on the cow to be read easily and safely. They remained cleaner and drier than leg pedometers , which also made them easier to read and perhaps less prone to damage. Further work with neck pedometers is necessary to demonstrate their effectiveness under different management systems.

The present experiments showed varying levels of effectiveness for pedometers as aids in detecting estrus. Although the management in Exp. 1 was identical between years, leg pedometer results obtained in Yr. 2 were superior to those of Yr. 1 (Appendix

II, Tables 1a,b and 2a,b). The use of the 15 day correction in Exp. 1 (Yr. 1) decreased the number of FP but also resulted in a substantial decline in the number of estrous periods detected, particularly when using a criterion of 1.5 fold increase in activity. This effect was not seen to the same degree in Exp. 1 (Yr. 2). This indicates that the magnitudes of leg pedometer FP and the estrual increases in activity were closer than for leg pedometers in Exp.1 (Yr. 1), compared to Exp. 1 (Yr. 2). Poor results in Yr. 1, therefore, may have been due to unexplained variations in baseline values. Little difference, however, can be seen in the leg pedometer SD between the two years. The difference between estrus and non-estrus readings for Exp.1 (Yr. 1) was found to be significant ($p<0.05$), but not highly significant ($p<0.01$) as in Exp. 2 and in Exp. 1 (Yr.2) (Table 3). Thus, it appears likely that poor detection in Yr. 1 was mainly due to a relatively wide variation in magnitude of estrual peaks.

Excellent DS and DA were obtained in Exp. 2 for both leg and neck pedometers. Exp. 2 was unique in that dry cows as opposed to suckled cows were used. Non-suckled cows may show stronger behavioural estrous periods sooner after parturition in comparison to suckled cows. Suckling is known to cause inhibition of pulsatile release of LH and to cause an increase in the length of post-partum anestrus in beef cows (Reviewed by Williams 1990). As well, the presence of calves in Exp. 1 may have led to greater variation in the day-to-day activity of the cows. A very obvious example of this was the large increase in activity seen for all cows, upon the removal of the calves for an overnight period for a milk yield test. Similar increases due to calf activity may have occurred at random intervals causing disruptions in the baseline, which would make the identification estrual peaks in activity more difficult.

The relative importance of detecting the maximum number of estrous periods possible and of obtaining a low number of FP will be reflected in the type of criteria chosen for individual situations. In the present experiments, criteria were chosen based on the goal of achieving high overall accuracy, which takes into account both the number of estrous periods detected and the occurrence of FP. Consistent changes in DS, DC, and DA are seen in all experiments when certain aspects of the criteria are changed. For example, implementation of the 15 day correction was highly effective in decreasing the number of FP, particularly at lower required fold increases in activity (1.5 and 2.0), without a large negative impact on DS.

The length of the baseline (days) did not have a large effect on overall accuracy of detection (Appendix II). Generally, however, a baseline of at least 10 days appears to be optimal. Interestingly, a 2 day baseline appears to give the greatest number of FP readings. This may be relevant to those using the Heat-Seeker® activity tag system which does calculations based on a two day baseline and for which the incidence of FP is high (Lui and Spahr, 1993, Kennedy and Ingalls, 1995).

In the present experiments, thresholds of 1.5, 2.0, or 2.5 were found to give the optimal DA. A threshold of between 1.5 and 2.5 has been used in many of the studies involving dairy cows under various management systems and using different pedometers (Pennington et al.,1986, Pulvermacher and Maaje, 1991, Redden et al.,1993). Therefore, the amount that activity increases at estrus must be fairly consistent among cows. The efficiency of pedometers in various situations, therefore, may relate more to the consistency of baseline values rather than the magnitude of the increase in activity at estrus.

One effect of manipulating the threshold was a change in the occurrence of the number of estrous periods with multiple consecutive indications of estrus by the pedometer. Multiple consecutive indications of estrus occurred at a greater incidence when a low threshold was used. The decrease in the multiple consecutive indications of estrus was most dramatic when the threshold was increased from 1.5 to 2.0 (Table 5). The question of when to AI relative to the time of the pedometer indication of estrus is complicated by these multiple consecutive indications. The initial rise in pedometer measured activity may be associated with estrus activity which occurs before standing estrus (ie. attempts to repel premature mounting and/or an increase in initiating activities towards other cows). As AI would normally occur based on the first reading exceeding criteria, pre-mature insemination could therefore result from having a low threshold. A detailed study of the sequence of behaviour and the timing of ovulation relative to pedometer measured activity would be beneficial.

During Exp. 1 (Yr. 2), 24 breedings using AI were performed based on pedometer results and/or visual observations of estrus behaviour. Based on the results of Exp.1 (Yr. 1) and of Redden et al.(1993), detection criteria of a 1.5 fold increase in activity above a 5 day baseline were applied to the pedometer data, during Exp. 1 (Yr. 2), to identify estrual peaks in activity. On only 10 occasions was there a high confidence in the occurrence of estrus by visual means (Table 6). Pedometers detected all 10 of these estrous periods. On fourteen occasions, there were either no obvious visual signs of estrus or a low incidence of mounting activity (1-5 mounts). Eleven of these 14 estrous periods were detected using leg pedometers. Seven of these eleven (64%) inseminations resulted in pregnancy, indicating the potential of pedometers for increasing breeding opportunities which would not have occurred when using visual observations alone (Table 6). However, the high occurrence of pedometer FP the criteria of a 1.5 fold increase in activity above a 5 day baseline caused difficulties in making breeding decisions based on pedometers alone. Relying completely on pedometers would have resulted in a large number of breedings on non-estrus days, which would have wasted time in handling cows unnecessarily and caused an increase in AI cost/conception. Had criteria of a 2.0 fold increase above a 10 day baseline, and the 15 day correction, been used to identify estrual activity peaks during the experiment, difficulties would have been greatly reduced as these criteria yielded only eight FP. Because of variability in sensitivity of pedometers among studies, even when cows were housed under similar management systems, the implementation of some form of visual observations may be advisable. The advantages of using both types of detection has been noted in previous studies. Williams et al. (1981) noted that combined use of pedometers and visual methods would yield a 10% increase over the number of estrous periods detected by pedometers alone, and fewer FP than by using pedometers alone. As noted by Jokhio (1994), pedometer indications of estrus can be used to draw attention to cows showing limited signs of estrus. Therefore, pedometers may be used to single out cows which have a high potential of being in estrus, without the added time expenditure of routine visual detection of all cows. The choice of whether to use a combination of methods or to rely solely on one method would depend on management constraints and the importance of a low number of services per conception (time available for detection program vs semen costs).

If using pedometers as the sole means of detecting estrus, the results from the present studies suggests that criteria of a 2.0 fold increase in activity above a 10 day baseline, and the 15 day correction would yield good results under most conditions, and might be considered as standard criteria. Differences in results amoung sets of criteria which optimize DA and standard criteria for each experiment are shown in Table 3. No dramatic differences in results are seen between the two sets of criteria. It is suggested, therefore, that these standard criteria may be used with optimum or close to optimum results when pedometers are used as the sole means of detecting estrus in situations with management similar to the present studies.

Overall, pedometers were found to greatly increase the awareness of the occurrence of estrous periods which would otherwise have been missed by routine observations for behaviour. Based on the results of the present study, pedometers have the potential to be an effective method of detecting estrus in beef cows.

Figure 5 - Pedometer (Digi-Walker-L) placed above the hock on the hind-leg of the cow

Figure 6 - Pedometers (Digi-Walker-L) placed on the hind-leg and secured to a collar on the neck.

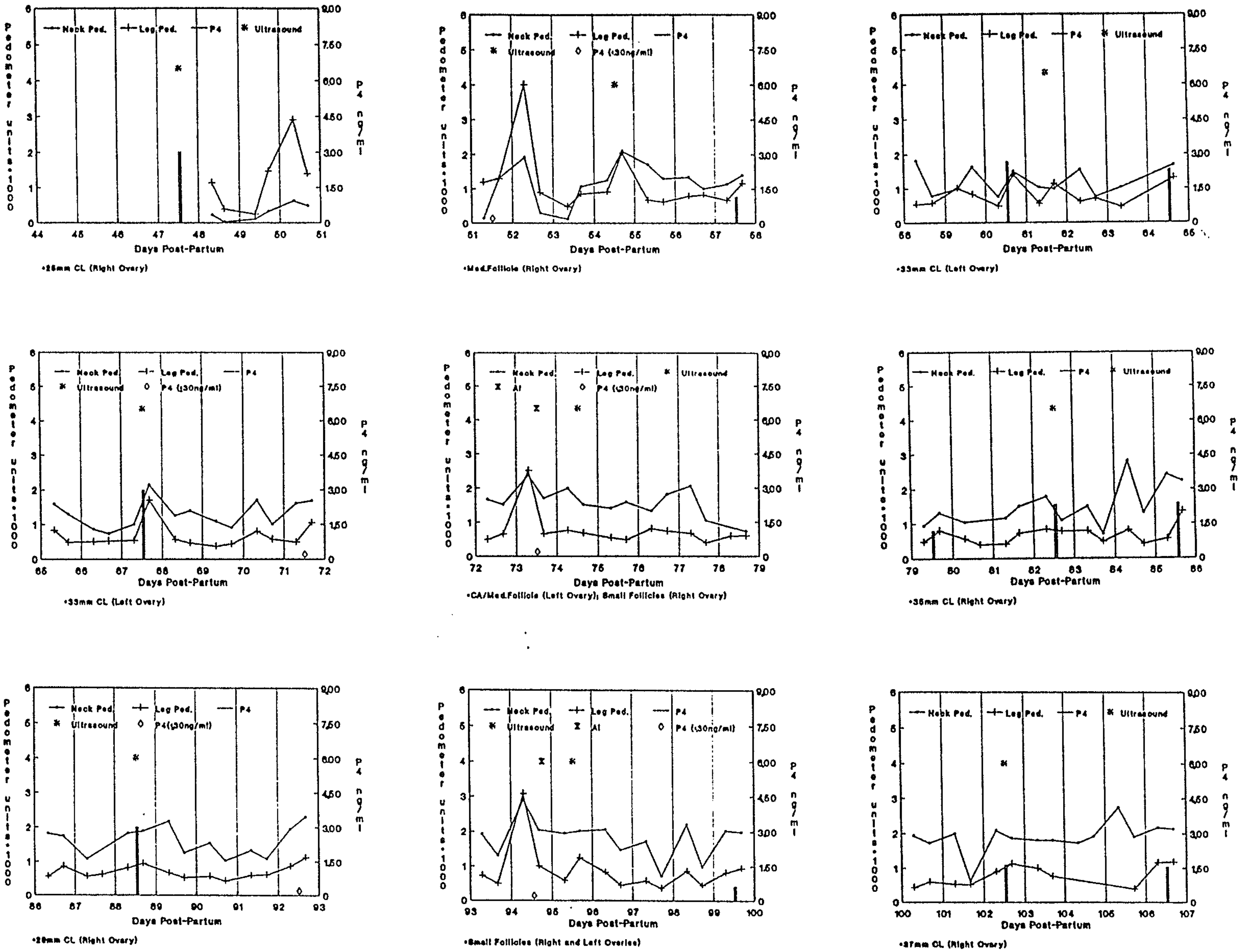

Figure 7 - Activity, progesterone, and ultrasound data for cow X43 from days 44 to 107 post-partum. Estrus related peaks in pedometer measured activity occurred on days 52, 73, and 94. Artificial insemination occurred on days 73 and 94.

Table 2 - Total and average per cow occurrence of pedometer loss and malfunction.

Experiment	Position	Loss Total	Loss Average	Malfunction Total	Malfunction Average
1 (Yr.1)	Leg	24	3.0	17	2.1
2	Leg	15	1.5	9	0.9
1 (Yr.2)	Leg	56	3.5	38	2.4
2	Neck	19	1.9	4	0.4
1 (Yr.2)	Neck	1	0.1	20	1.3

Table 3- Mean±SD for all estrus and non-estrus readings and mean±SE of difference between estrus and non-estrus readings for each experiment and pedometer position.

Exp.	Position	Estrus mean	Estrus SD	Non-estrus mean	Non-estrus SD	Difference[a] mean	Difference[a] SE
1 (Yr.1)	Leg	2758	1864	1167	778	*2356	727
2	Leg	4900	2100	1032	647	**3935	552
1 (Yr.2)	Leg	3277	1490	1084	656	**2337	246
2	Neck	6431	3607	1763	1213	**4931	929
1 (Yr.2)	Neck	3733	1826	2312	1483	**1721	217

[a]Difference between estrus and non-estrus readings were calculated using paired t-tests.

* Significant difference ($p<0.05$) in estrus and non-estrus readings within experiment

**Highly significant difference ($p<0.01$) in estrus and non-estrus readings within experiment

Table 4 - Detection sensitivity (DS)[a], detections which were correct (DC)[a], and detection accuracy (DA)[a] for criteria yielding highest detection accuracy, standard criteria[b] and visual observations[c] for all experiments and pedometer positions.

Experiment	Position	Criteria[d]	DS(%)	DC(%)	DA(%)
1 (Yr.1)	Leg	1.5:15	62.5	33.3	27.8
		2.0:10	50.0	29.6	22.9
		Visual	68.8	91.7	64.7
2	Leg	2.5:15	100.0	82.4	82.4
		2.0:10	100.0	66.7	66.7
		Visual	NA	NA	NA
1 (Yr.2)	Leg[e]	2.0:10	89.3	75.8	69.4
		Visual	39.3	100.00	39.3
2	Neck[e]	2.0:10	92.9	81.3	74.5
		Visual	NA	NA	NA
1 (Yr.2)	Neck	1.5:15	76.7	46.9	41.1
		2.0:10	53.3	55.2	37.2
		Visual	36.7	100.0	36.7

[a] See Materials and Methods for definitions of terms

[b] Standard criteria=2.0 threshold, a 10 day baseline and 15 day correction

[c] Standing estrus and/or high frequency of mounting activity

[d] Criteria=Magnitude of activity increase:days of baseline and 15 day correction

[e] Criteria yielding the highest DA was standard criteria

Table 5 - Percentage of estrous periods detected with leg pedometers where criteria were met for more than one consecutive pedometer reading. Results for all thresholds tested are given (10 day baseline).

Exp.	1.5	2.0	2.5	3.0
1 (Yr.1)	70.0	25.0	14.3	0
2	57.1	28.6	14.3	15.4
1 (Yr. 2)	84.6	56.0	28.6	5.9

Table 6 - Type of visual indication of estrus observed prior to each of the 24 breedings by AI in Exp.1 (Yr. 2), and the number of breedings showing each type of visual indication which were detected by leg pedometers and which resulted in pregnancy.

Visual Indication of Estrus	Number of Breedings by AI	Detected by Leg Pedometer	Pregnancy
High Indication[a]	10	10	4
Low Indication[b]	6	5	5
No Indication[c]	8	6	3

[a]Standing estrus and/or high incidence of mounting activity
[b]Low incidence of mounting activity (1-5 mounts)
[c]No visual signs of estrus seen during routine observations

Manuscript 3 - Timing of the Detection of the Indication of Estrus Activity by Pedometers Relative to Ovulation and the LH Surge in Beef Cows

Abstract

Pedometers have been found to be effective in detecting estrus in cattle. However, the interval from the detection of the indication of estrus activity by the pedometer (DEP) to ovulation has not been established. Twelve loose-housed, unsuckled beef cows, between 3 and 14 days postpartum, were fitted with pedometers secured to a hind-leg and neck for between 28 and 52 days. Pedometers were read twice daily at 8:00h and 16:00h. To monitor ovarian status, serum P4 levels were approximated using Target® kits and ultrasound scans of the ovaries were made 3 times weekly. At the DEP, or when behavioural estrus was noted, blood samples and ultrasound scans were made at 4h intervals between 7:00h and 23:00h until ovulation was confirmed by ultrasound scanning (disappearance of the dominant follicle). Blood samples taken during the estrual period were analysed for serum luteinizing hormone (LH) and estradiol-17β (E2). The DEP occurred before ovulation in all 16 of the estrous periods studied. Time from the DEP to ovulation was found to be 12±6h with a range of 5 to 25h. Time from the DEP to the peak in LH and E2 were found to be 1±4h and 6±6h, respectively. Results from this study indicate there is sufficient time after the DEP for effective AI, when pedometers are read twice daily.

Introduction

Pedometers have been shown to be effective aids for detecting estrus in dairy (Kiddy,1977 and Redden et al.,1993) and beef (Manuscript 2) cows. However, the correct timing of artificial insemination (AI) relative to the detection of the indication of estrus activity by the pedometer (DEP) has not been established. Maximum conception rates have been obtained when cows were bred between mid-estrus and the end of standing estrus, with good results up to 6h after estrus. On average, behavioural estrus begins approximately 30h and ends 12h before ovulation (Reviewed in Salisbury,1978 and Foote,1975). Increases in pedometer readings have been shown to correspond with behavioural estrus in dairy cows (Pennington et al., 1986). However, the temporal relationship between ovulation and DEP has not been directly measured. Information concerning the relationship between the DEP and physiological events, such as ovulation and the luteinizing hormone (LH) surge, which usually occurs 24h preceding ovulation, would provide a basis for the timing of insemination relative to the DEP.

The objectives of the present study were to provide basic information on the timing of the DEP relative to ovulation, when pedometers are read twice daily, and to examine LH and estradiol-17β (E2) levels during the time interval from the DEP until ovulation.

Materials and Methods

Animals and Management

Seven multiparous and five primiparous Hereford-cross beef cows were used as experimental animals. Cows were loose-housed in an indoor pen (24 m X 4.5 m), with access to an outside yard (6 m X 6 m). Cows were given hay and water ad libitum and 0.5 kg barley daily. Eleven cows had calved just prior to the beginning of the experiment and one cow had been open and cycling normally. Calves were removed within 24 hours of parturition as part of an experiment unrelated to this study. Data was collected between January 20 to March 17, 1993. The time on test varied among cows from 28 to 52 days, depending on calving dates.

Pedometers

As described in Manuscript 2, pedometers were attached to the neck and leg of each cow. Activity monitoring began between 3 and 14 days post-partum. Pedometers were read manually at 8:00h and 16:00h daily. Activity for the previous time period was calculated immediately after each reading.

Blood Sampling

Blood samples (20 ml) were obtained by jugular venipuncture from all cows three times weekly for serum progesterone (P4) measurement. Additionally, blood samples were collected at estrus for determination of LH and E2 concentrations in serum. The samples were collected at 4h intervals from 7:00h to 23:00h from the time of the detection of estrus (by pedometer and/or visual indications) until ovulation. Serum was stored at -20° C until analysis.

Ultrasound Scanning of the Ovaries

A real-time ultrasound scanner (Pie-Medical 450, Sterne Medical Equipment, Brampton, Ontario) and a 5.6 MHz rectal probe were used for transrectal ultrasonography of the ovaries. Diameters of corpora lutea and all visible follicles (>6mm) were measured and recorded three times weekly throughout the estrous cycle. During estrus, the ovaries were scanned at 4h intervals corresponding to the times for blood sample collection. Ovulation was indicated by the disappearance of the dominant follicle (Rajamahendran et al.,1989). Time of ovulation was estimated to be the mid-point between the final two ultrasound scans (Larsson, 1987).

Detection of Estrus

An estrus was assumed to have occurred if pedometer values indicated a 1.5 fold increase in activity over a baseline of the previous 5 readings for the same time period (i.e. AM or PM readings). Frequent ultrasound scanning and blood sampling were initiated at the next 4h sampling period after such an increase, if tri-weekly scanning and P4 values confirmed the possibility of estrus (ie. presence of a pre-ovulatory follicle and low progesterone). Visual observations for standing heat were not made at regular intervals. However, frequent scanning and blood sampling were initiated if estrus behaviour was noted, regardless of pedometer readings.

Estrous Synchronization

Estrus was synchronized in three cows by administration of two, 2 ml (35 mg) injections of the prostaglandin analogue, cloprostenol (Estrumate®) 10 days apart. Synchronization facilitated and guaranteed the opportunity for earlier blood sampling for these three cows. A subsequent natural estrus was studied in two of these cows.

Hormone Analysis

Sera from tri-weekly samples were determined to have either high, medium, or low P4 values using Target® kits (BioMetallics Inc., Princeton, NJ). Serum collected at estrus was analyzed for LH and E2 using radioimmunoassay procedures described by Evans et al.(1992) and Joseph et al.(1992), respectively. The sensitivities of the LH and E2 assays were 1 ng/ml and 1 pg/ml, respectively. Intra-assay coefficients of variations were 6% for the LH assay and 11% for the E2 assay.

Statistics

Basal LH and E2 values were determined by calculating the mean+standard deviation (x+SD) of all values obtained from each cow. The mean for all samples with values not exceeding x+SD was used as the basal hormone level (Schams et al.,1977). A hormone value that exceeded the basal hormone level by more than twice the SD of the basal hormone level was considered part of the estrus related peak. The end of the hormone peak was defined as the last hormone value meeting this criterion. The effects of estrus synchronization and follicle number on the ovulation interval were tested using Students t-tests. Statistics were performed using SAS version 5.16 (1986).

Results

All pedometer and hormone values for individual cows are provided in graphical form in Appendix III (Figures 1-12). One cow (A80), had an ulceration of the rectal wall and was omitted from the ultrasound scanning routine. Another cow (Y9) did not exhibit a detectable estrus. The remaining 10 cows displayed normal fluctuations in serum P4 values and ovarian function. A total of 16 estrus periods were studied in these cows.

Time of ovulation and the time of the end of the LH surge in relation to the time of the DEP for individual estrous periods are presented in Figure 8. Ovulation occurred subsequent to DEP for all 16 estrus periods studied. Fourteen of the 16 estrous periods (87.5%) were detected at the AM pedometer reading with ovulation occurring from a maximun of 7 to 23 hours subsequent to DEP. The two (12.5%) PM detected estrous periods occurred in one cow (UA11), with ovulation occurring from a maximum of 15 to 23 hours after DEP. Mean±SD interval in hours from the DEP to ovulation are presented in Table 7. Overall, DEP preceded ovulation by 12±6 hours, when the time of ovulation was estimated to be the mid-point between the final two ultrasound scans.

The three synchronized cows had a significantly longer interval from DEP to ovulation than non-synchronized cows ($p<0.05$). All synchronized cows had more than one large follicle. There was more than one large follicle (>10mm) on the ovaries of seven cows at estrus. On average, cows with multiple follicles were found to have a significantly ($p<0.05$) longer interval from the DEP to ovulation than those cows with one large follicle (Table 7).

Luteinizing hormone and E2 values are included in Appendix III. Elevated concentrations of LH and E2 in serum were detected for 8 and 7 of the estrous periods, respectively (Figure 8). The mean±SD time interval from the end of the hormone increase to the DEP and to ovulation are given in Table 8. The end of the LH and E2 increases preceded DEP by 1 and 6 hours, respectively and ovulation by 16 and 20 hours, respectively.

Additional pedometer results are reported in Appendix II. Details of these results are discussed in Manuscript 2.

Discussion

In all instances the DEP was found to occur before ovulation. This was expected as increased pedometer readings have been found to occur during behavioural estrus in synchronized dairy cows (Pennington et al.,1986) and behavioural estrus precedes ovulation (Salisbury, 1978). Because the lifespan of cattle sperm is approximately 30-48h and sperm requires approximately 6h after ejaculation to complete capacitation (Bazer et al.,1987), good conception rates can be achieved when AI is performed between 24 and 6h before ovulation (Salisbury,1978). In the present study, the time interval from the DEP to ovulation ranged from 25 to 5h, with an average of 12±6h. Successful timing of breeding, therefore, would be possible when using pedometers read twice daily. Results of the present study suggest that AI should take place as soon as possible after DEP to be within the recommended time period before ovulation.

In this study, the majority of pedometer detected estrous periods occurred overnight and ovulation occurred most frequently 7-11h after the 8:00h pedometer reading, between 15:00h to 19:00h (Figure 8). Similarly, Schams et al.(1977) found ovulation occurred mainly around the times of 12:00h and 20:00h. In the present study, therefore, once per day pedometer readings (8:00h) would have been sufficient to successfully time AI for the majority of the cows. However, other studies have indicated that there is no particular time of the day when the occurrence of estrus or ovulation is greater (Larsson, 1987). Therefore, more frequent pedometer readings would be recommended for optimizing conception rates.

Synchronization appeared to have an effect on the interval from estrus to ovulation. All synchronized cows plus one untreated cow (A91(1)) showed visual signs of estrus on the same evening. As the number of cows in estrus tends to increase the time that estrus behaviour is shown (Hurnik et al.1975), the longer interval for these cows may have been due to the effect of the number of cows in estrus. That is, estrus may have been initiated early in some of these cows due to the presence of other cows in estrus. As well, in seven of the ovulations studied, more than one large follicle (≥10mm) developed during estrus. In four of these cases there was a relatively long period between DEP and ovulation. Larsson (1987) found cows with two large follicles experienced a longer interval between the onset of estrus and ovulation than did cows with one large follicle.

As the peak in serum concentrations of LH occurs approximately coincident with the onset of estrus behaviour (Schams et al.,1977, Chenault et al.,1974), many LH peaks would have been missed in the present study where blood sampling usually commenced on the basis of DEP. The mean of 16±8h from the end of the LH peak to ovulation is in agreement with the interval of 19.5±6.4h determined in dairy cows by Rajamahendran et al.(1989). The duration of the LH peak is approximately 7 to 9 hours in dairy (Rajamahendran et al.,1989, Schams et al.,1977) and beef (Christenson et al.,1975) cows. Based on this information, it is predicted that the peak in LH would have occurred, on average, 24h before ovulation in the present study. In addition, DEP was found to occur 1h after the end of the LH peak. Thus the activity which caused the DEP would have occurred during the time that the LH peak occurred. This is in agreement with the accepted time intervals from the LH peak to the onset of estrus (Rajamahendran et al.,1989) and ovulation (Schams et al.,1977, Chenault et al.,1974). However, earlier blood sampling would be needed to provide a more reliable indication of the time interval from the peak in serum hormone concentrations to the pedometer indication of estrus.

In the present study ovulation was shown to occur subsequent to the DEP for all estrous periods studied, with a mean interval of 12±6h. This indicates that optimal timing of AI in beef cows is possible based on the results of pedometers read twice daily, and that breeding should take place at the time of DEP or within 6h of DEP.

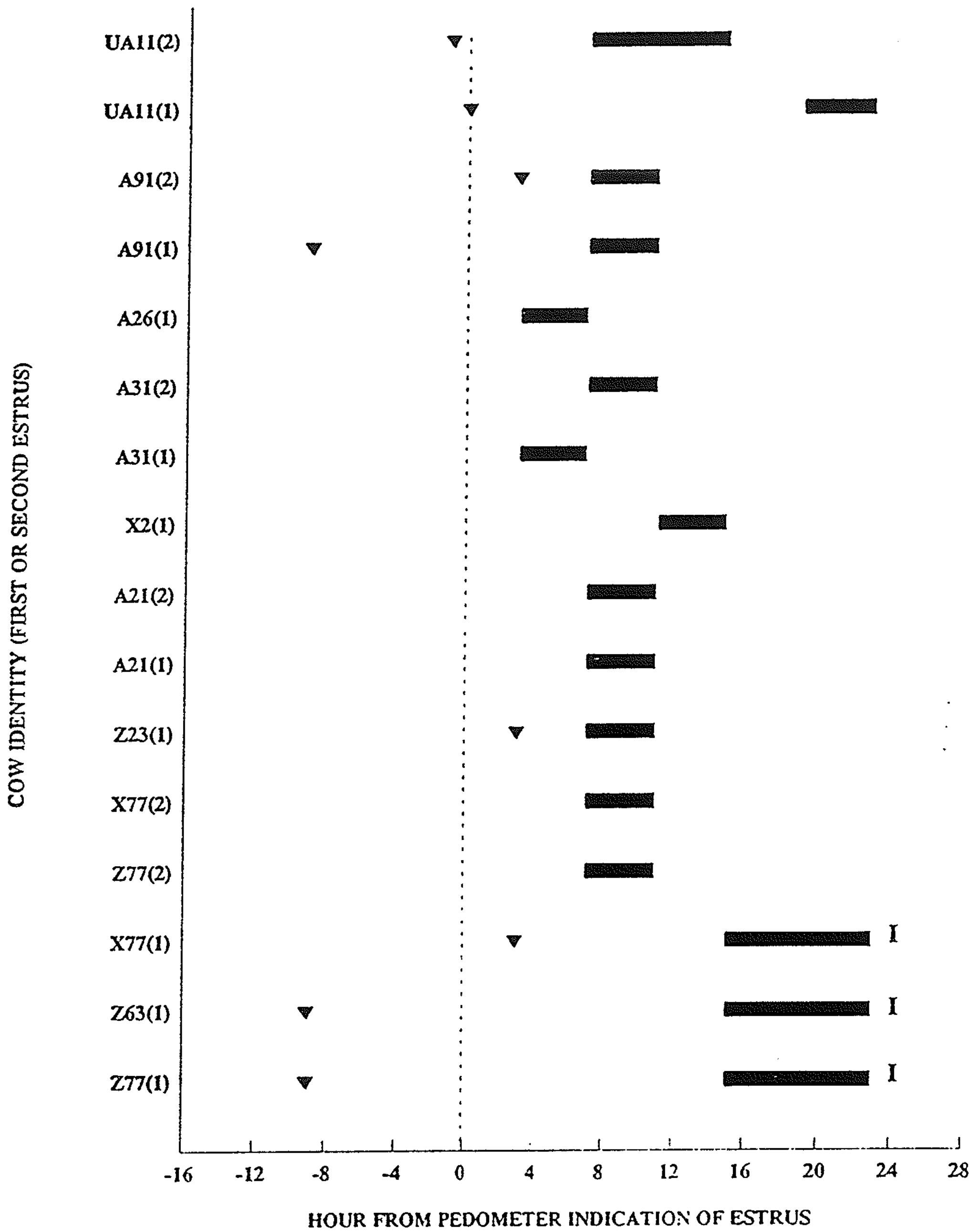

Figure 8 - Time of ovulation (▬) and the time of the end of the LH surge (▾) in relation to the time of the detection of the indication of estrus activity by the pedometer (0 hour) for natural and synchronized (I) estrous periods.

Table 7 - Interval between the detection of the indication of estrus activity by the pedometer (DEP) and ovulation for all estrous periods and for estrous periods preceded by multiple or single follicles.

	Interval(h)	
	Mean	SD
Multiple Follicles (n=7)	16	6
Single Follicle (n=9)	8	3
Total (n=16)	12	6

Table 8 - Interval from the end of pre-ovulatory hormone increases to the detection of the indication of estrus activity by the pedometer (DEP) and ovulation.

	Interval(h)			
	DEP		Ovulation	
	Mean	SD	Mean	SD
LH[1] (n=8)	1	4	16	8
E2[1] (n=7)	6	6	20	8

[1] Intervals were calculated from the last hormone value greater than twice the SD of the basal levels.

General Discussion

In the present studies, both pedometers and temperature sensing radiotransmitters were found to be effective aids for the detection of estrus in beef cows. Using radiotransmitters to monitor vaginal temperature (Tv), to identify a temperature increase of 0.4° C for at least three hours over a three day baseline, resulted in detection of 42 of a total of 47 estrous periods (detection sensitivity (DS)=89.4%), with seven FP (detection accuracy (DA)=77.8%). Use of visual observations for estrus yielded a DS of 53.2% and a DA of 52.2%. Five of the seven FP in Tv occurred in one cow (W111). Omitting this cow from the results yielded a DA of 87.2% for the remaining 23 cows in the study. The mean maximum Tv increase at estrus was 0.91° C. This was higher than the Tv increase found in dairy cows using the same telemetry system (Redden et al.,1993). Loose-housing of cows in the present study may have contributed to the large change in Tv observed during estrus.

As well as the dramatic change in Tv at estrus, an additional temperature pattern occurred throughout the estrous cycle. As found by Redden et al.(1993), there was a decline in Tv during the 3-4 days prior to estrus. In the present study, there was also a slight increase in Tv noted during the mid-point of the cycle. Lewis and Newman (1984), on the basis of Tv readings taken manually once daily, also noted a decline in temperature prior to estrus and a gradual increase in temperature towards the middle of the cycle. Progesterone (P4) has been found to have a thermogenic effect in other species (Czaja and Butera, 1986). Therefore, fluctuations in Tv observed during the estrous cycle in cows may be due to changes in P4 concentrations.

Previous studies have indicated that the increase in body temperature at estrus in cows is temporally related to the pre-ovulatory surge in LH (Rajamahendran et al.,1989, Mosher et al.,1990), and evidence in other species indicates that the surge in LH and the change in body temperature may be physiologically linked (Berglund and Simpkins, 1988). As the interval between the LH surge and ovulation has been found to be relatively constant among cows (Schams et al.1977, Chenault et al.,1974), continuous measurement of Tv may be used to improve conception rates by identification of the ideal time for AI.

The effectiveness of pedometers to detect estrus varied. In Exp. 2, using unsuckled cows, excellent results were obtained. Using criteria of 2.5 fold increase in activity above a 15 day baseline, and the 15 day correction, a DS of 100% was obtained and only three FP were found for leg pedometers (DA=82.4%). Physiological and behavioural differences due to the absence of calves may have contributed to the results observed in this experiment. Although management was similar in Yr. 1 and Yr. 2 of Exp. 1, a large variation in results was noted. In Yr. 1, using criteria of a 1.5 fold increase in activity above a 15 day baseline, and the 15 day correction, a DS of 62.5% and a DA of 27.8% were found for leg pedometers. For leg pedometers in Yr. 2, a DS of 89.3% and a DA of 69.4% were obtained using a 2.0 fold increase in activity above a 10 day baseline, and the 15 day correction. Reasons for the discrepancies in the results of the two years are unknown. The difference between estrus and non-estrus readings in Yr.1 was not as great as the difference in these values in Yr. 2. Poor results in Yr. 1, therefore, may have been due to a more moderate increase in activity at estrus. Visual DS for Yr. 1 was 68.8% with a DA of 64.7% (1 FP). In Yr. 2 a visual DS and DA of 39.3% was found (0 FP).

From the pedometer results of the present experiments, criteria of 2.0 fold increase in activity above a 10 day baseline, and the 15 day correction, could be used as standard criteria for cows under similar management. During Exp.1 (Yr.2), using criteria of a 1.5 fold increase in activity above a five day baseline, and the 15 day correction, and with the aid of visual detection methods, a high DS was obtained and no breedings by AI occurred outside of the P4 and ultrasound confirmed estrus periods. Use of standard criteria in this experiment would have yielded a DS of 89.3% and only eight FP. Using these criteria, pedometers alone could have been used to detect estrus in this instance.

In Manuscript 3, the detection of the indication of estrus by the pedometer (DEP) was found to occur before ovulation in all instances. An average time interval of 12 ± 6h with a range of 25 to 5h between the DEP and ovulation was found. This indicates that there is sufficient time between DEP and ovulation to allow for optimal timing of AI when pedometers are read twice daily.

In the present study, both pedometers and temperature transmitters were more sensitive for detecting estrus than were visual observations alone. In Yr. 2, a DS (%) of 89.4 and 89.3 and a DA (%) of 77.8 and 69.4 were found for Tv and activity data, respectively. Use of Tv, however, showed some advantages over pedometers. Vaginal temperature results were very consistent for both years of the study. The criteria which optimized DA was the same for each year individually and for both years combined. Although management was similar, variation in results and criteria which optimized DA were found between Yr. 1 and Yr. 2 for pedometers in Exp. 1. Overall, there was a much lower incidence of FP for temperature transmitters compared to pedometers, before the 15 day correction was applied to pedometer data. Because of the very low incidence of FP with temperature transmitters the use of the 15 day correction was not necessary, which aids in simplifying the Tv criteria. As well, there was a much lower incidence of radiotransmitter loss and daily maintenance required for temperature transmitters was low compared to pedometers.

Although the temperature transmitters proved to be an easier method of detecting estrus in this study, pedometers show a great deal of promise, as indicated by the results of experiments 2 and 3. Pedometers used in these studies are a much less expensive option for detecting estrus compared to the radiotelemetry system used for temperature monitoring. As well, although no negative effects on cow health or fertility were observed while using the vaginal anchors and temperature transmitters in this study, the long term effects have not been examined.

CONCLUSIONS

1. Continuous measurement of vaginal temperature using radiotransmitters was found to be a sensitive and accurate method of detecting estrus in loose-housed beef cows. For Yr. 1 and Yr. 2, a combined detection sensitivity (DS) of 89.6% and a detection accuracy (DA) of 77.8% were obtained using criteria of a 0.4° C increase in temperature for at least three hours over a three day baseline.

2. Overall, twice daily measurement of activity using pedometers was an effective method of detecting estrus in beef cows. Leg pedometers had a DS (%) of 62.5, 89.3, and 100.0 and a DA (%) of 27.8, 82.4, and 69.4 for experiments 1,2, and 3, respectively. Neck pedometers had a DS (%) of 92.9, and 76.7 and a DA (%) of 76.5 and 41.1 for experiments 2 and 3, respectively.

3. Standard criteria of a 2.0 fold increase in activity above a 10 day baseline, and with the 15 day correction employed, can be applied to yield good results for beef cows housed under management systems similar to those of the present experiments.

4. The time interval from the detection of the indication of estrus by pedometers to ovulation was 12±6h. Sufficient time after the detection of the pedometer indication of estrus is available for effective AI, when pedometers are read twice daily.

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APPENDICES

APPENDIX I - Results of criteria applied to temperature data (Table 1 and Table 2)

Table 1 - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*, detections which were correct (DC)*, and detection accuracy (DA)* for criteria including a temperature peak of > 3 hours duration and magnitudes of 0.3 C(a), 0.4 C(b), and 0.5 C(c) over various days of baseline (16 estruses studied (yr1), 26 estruses studied (yr2), 42 estruses studied (total)).

(a)

Baseline(day)		DE	FP	DS(%)	DC(%)	DA(%)
2	yr1	15	13	88.24	53.57	50.00
	yr2	26	18	100.00	59.09	59.09
	total	41	31	95.35	56.94	55.41
3	yr1	15	11	88.24	57.69	53.57
	yr2	25	14	96.15	64.10	62.50
	total	40	25	93.02	61.54	58.82
4	yr1	15	10	88.24	60.00	55.56
	yr2	24	14	92.31	63.16	60.00
	total	39	24	90.70	61.90	58.21
5	yr1	15	11	88.24	57.69	53.57
	yr2	24	15	92.31	61.54	58.54
	total	39	26	90.70	60.00	56.52

(b)

2	yr1	14	3	82.35	82.35	70.00
	yr2	24	6	92.31	80.00	75.00
	total	38	9	88.37	80.85	73.08
3	yr1	14	2	82.35	87.50	73.68
	yr2	24	5	92.31	82.76	77.42
	total	38	7	88.37	84.44	76.00
4	yr1	13	2	76.47	86.67	68.42
	yr2	24	5	92.31	82.76	77.42
	total	37	7	86.05	84.09	74.00
5	yr1	14	2	82.35	87.50	73.68
	yr2	23	5	88.46	82.14	74.19
	total	37	7	86.05	84.09	74.00

(c)

2	yr1	13	1	76.47	92.86	72.22
	yr2	23	4	88.46	85.19	76.67
	total	36	5	83.72	87.80	75.00
3	yr1	12	1	70.59	92.31	66.67
	yr2	21	4	80.77	84.00	70.00
	total	33	5	76.74	86.84	68.75
4	yr1	11	1	64.71	91.67	61.11
	yr2	22	3	84.62	88.00	75.86
	total	33	4	76.74	89.19	70.21
5	yr1	11	1	64.71	91.67	61.11
	yr2	20	3	76.92	86.96	68.97
	total	31	4	72.09	88.57	65.96

* See Manuscript 1 for definitions of terms.

Table 2 - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*, detections which were correct (DC)*, and detection accuracy (DA)* for criteria including a temperature peak of > 4 hours duration and magnitudes of 0.3 C(a), 0.4 C(b), and 0.5 C(c) over various days of baseline (16 estruses studied (yr1), 26 estruses studied (yr2), 42 estruses studied (total)).

(a)

Baseline(day)		DE	FP	DS(%)	DC(%)	DA(%)
2	yr1	15	2	88.24	88.24	78.95
	yr2	23	8	88.46	74.19	67.65
	total	38	10	88.37	79.17	71.70
3	yr1	13	4	76.47	76.47	61.90
	yr2	23	8	88.46	74.19	67.65
	total	36	12	83.72	75.00	65.45
4	yr1	14	4	82.35	77.78	66.67
	yr2	22	5	84.62	81.48	70.97
	total	36	9	83.72	80.00	69.23
5	yr1	14	4	82.35	77.78	66.67
	yr2	22	5	84.62	81.48	70.97
	total	36	9	83.72	80.00	69.23

(b)

2	yr1	14	1	82.35	93.33	77.78
	yr2	21	4	80.77	84.00	70.00
	total	35	5	81.40	87.50	72.92
3	yr1	11	1	64.71	91.67	61.11
	yr2	22	3	84.62	88.00	75.86
	total	33	4	76.74	89.19	70.21
4	yr1	11	1	64.71	91.67	61.11
	yr2	21	3	80.77	87.50	72.41
	total	32	4	74.42	88.89	68.09
5	yr1	11	1	64.71	91.67	61.11
	yr2	20	3	76.92	86.96	68.97
	total	31	4	72.09	88.57	65.96

(c)

2	yr1	9	1	52.94	90.00	50.00
	yr2	19	3	73.08	86.36	65.52
	total	28	4	65.12	87.50	59.57
3	yr1	9	1	52.94	90.00	50.00
	yr2	19	4	73.08	82.61	63.33
	total	28	5	65.12	84.85	58.33
4	yr1	7	1	41.18	87.50	38.89
	yr2	18	2	69.23	90.00	64.29
	total	25	3	58.14	89.29	54.35
5	yr1	7	1	41.18	87.50	38.89
	yr2	18	3	69.23	85.71	62.07
	total	25	4	58.14	86.21	53.19

* See Manuscript 1 for definitions of terms.

APPENDIX II - Results of criteria applied to pedometer data (Tables 1a and 1b, 2a and 2b, 3a and 3b, 4a and 4b, and 5a and 5b)

Table 1a - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data for experiment 1 using the 15 day criterion* (16 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	10	21	62.50	32.26	27.03
	5	10	23	62.50	30.30	25.64
	10	10	20	62.50	33.33	27.78
	15	10	20	62.50	33.33	27.78
2.0	2	9	18	56.25	33.33	26.47
	5	8	21	50.00	27.59	21.62
	10	8	19	50.00	29.63	22.86
	15	8	17	50.00	32.00	24.24
2.5	2	5	15	31.25	25.00	16.13
	5	6	12	37.50	33.33	21.43
	10	7	10	43.75	41.18	26.92
	15	5	13	31.25	27.78	17.24
3.0	2	4	13	25.00	23.53	13.79
	5	4	5	25.00	44.44	19.05
	10	3	5	18.75	37.50	14.29
	15	3	4	18.75	42.86	15.00

Table 1b - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data for experiment 1 (16 estruses studied).

Thresholds	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	14	71	87.50	16.47	16.09
	5	14	65	87.50	17.72	17.28
	10	14	54	87.50	20.59	20.00
	15	13	48	81.25	21.31	20.31
2.0	2	11	38	68.75	22.45	20.37
	5	10	33	62.50	23.26	20.41
	10	10	32	62.50	23.81	20.83
	15	9	25	56.25	26.47	21.95
2.5	2	7	22	43.75	24.14	18.42
	5	6	14	37.50	30.00	20.00
	10	7	14	43.75	33.33	23.33
	15	6	15	37.50	28.57	19.35
3.0	2	5	13	31.25	27.78	17.24
	5	4	5	25.00	44.44	19.05
	10	3	5	18.75	37.50	14.29
	15	3	4	18.75	42.86	15.00

* See Manuscript 2 for definitions of terms.

Table 2a - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data for experiment 2 using the 15 day criteria* (14 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	14	10	100.00	58.33	58.33
	5	14	8	100.00	63.64	63.64
	10	14	9	100.00	60.87	60.87
	15	14	9	100.00	60.87	60.87
2.0	2	14	7	100.00	66.67	66.67
	5	14	5	100.00	73.68	73.68
	10	14	7	100.00	66.67	66.67
	15	14	7	100.00	66.67	66.67
2.5	2	14	6	100.00	70.00	70.00
	5	14	4	100.00	77.78	77.78
	10	14	5	100.00	73.68	73.68
	15	14	3	100.00	82.35	82.35
3.0	2	13	5	92.86	72.22	68.42
	5	14	4	100.00	77.78	77.78
	10	13	3	92.86	81.25	76.47
	15	12	2	85.71	85.71	75.00

Table 2b - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data for experiment 2 (14 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	14	54	100.00	20.59	20.59
	5	14	38	100.00	26.92	26.92
	10	14	34	100.00	29.17	29.17
	15	14	25	100.00	35.90	35.90
2.0	2	14	34	100.00	29.17	29.17
	5	14	17	100.00	45.16	45.16
	10	14	11	100.00	56.00	56.00
	15	14	12	100.00	53.85	53.85
2.5	2	14	20	100.00	41.18	41.18
	5	14	8	100.00	63.64	63.64
	10	14	5	100.00	73.68	73.68
	15	14	3	100.00	82.35	82.35
3.0	2	13	16	92.86	44.83	43.33
	5	14	4	100.00	77.78	77.78
	10	13	3	92.86	81.25	76.47
	15	12	2	85.71	85.71	75.00

* See Manuscript 2 for definitions of terms.

Table 3a - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on neck pedometer data for experiment 2 using the 15 day criterion* (14 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	13	11	92.86	54.17	52.00
	5	13	12	92.86	52.00	50.00
	10	13	9	92.86	59.09	56.52
	15	13	9	92.86	59.09	56.52
2.0	2	13	8	92.86	61.90	59.09
	5	12	4	85.71	75.00	66.67
	10	13	3	92.86	81.25	76.47
	15	12	3	85.71	80.00	70.59
2.5	2	11	6	78.57	64.71	55.00
	5	10	2	71.43	83.33	62.50
	10	11	1	78.57	91.67	73.33
	15	10	1	71.43	90.91	66.67
3.0	2	7	4	50.00	63.64	38.89
	5	8	0	57.14	100.00	57.14
	10	8	0	57.14	100.00	57.14
	15	9	0	64.29	100.00	64.29

Table 3b - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on neck pedometer data for experiment 2 (14 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	14	52	100.00	21.21	21.21
	5	13	36	92.86	26.53	26.00
	10	14	34	100.00	29.17	29.17
	15	14	29	100.00	32.56	32.56
2.0	2	13	22	92.86	37.14	36.11
	5	12	10	85.71	54.55	50.00
	10	13	9	92.86	59.09	56.52
	15	13	10	92.86	56.52	54.17
2.5	2	12	11	85.71	52.17	48.00
	5	10	3	71.43	76.92	58.82
	10	11	1	78.57	91.67	73.33
	15	10	2	71.43	83.33	62.50
3.0	2	7	4	50.00	63.64	38.89
	5	8	0	57.14	100.00	57.14
	10	8	0	57.14	100.00	57.14
	15	9	0	64.29	100.00	64.29

* See Manuscript 2 for definitions of terms.

Table 4a - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data in experiment 3 using the 15 day criterion* (28 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	25	23	89.29	52.08	49.02
	5	26	22	92.86	54.17	52.00
	10	26	24	92.86	52.00	50.00
	15	26	21	92.86	55.32	53.06
2.0	2	26	16	92.86	61.90	59.09
	5	26	11	92.86	70.27	66.67
	10	25	8	89.29	75.76	69.44
	15	24	10	85.71	70.59	63.16
2.5	2	23	15	82.14	60.53	53.49
	5	21	7	75.00	75.00	60.00
	10	21	8	75.00	72.41	58.33
	15	21	8	75.00	72.41	58.33
3.0	2	17	14	60.71	54.84	40.48
	5	17	5	60.71	77.27	51.52
	10	17	2	60.71	89.47	56.67
	15	16	3	57.14	84.21	51.61

Table 4b - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were corrrect (DC)*,and detection accuracy (DA)* for all criteria tested on leg pedometer data for experiment 3 (28 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	26	134	92.86	16.25	16.05
	5	27	99	96.43	21.43	21.26
	10	26	87	92.86	23.01	22.61
	15	26	77	92.86	25.24	24.76
2.0	2	26	68	92.86	27.66	27.08
	5	26	33	92.86	44.07	42.62
	10	25	15	89.29	62.50	58.14
	15	24	17	85.71	58.54	53.33
2.5	2	23	32	82.14	41.82	38.33
	5	21	11	75.00	65.63	53.85
	10	21	8	75.00	72.41	58.33
	15	21	8	75.00	72.41	58.33
3.0	2	17	21	60.71	44.74	34.69
	5	17	6	60.71	73.91	50.00
	10	17	2	60.71	89.47	56.67
	15	16	3	57.14	84.21	51.61

* See Manuscript 2 for definitions of terms.

Table 5a - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on neck pedometer data for experiment 3 using the 15 day criterion* (30 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	19	31	63.33	38.00	31.15
	5	24	35	80.00	40.68	36.92
	10	23	28	76.67	45.10	39.66
	15	23	26	76.67	46.94	41.07
2.0	2	18	24	60.00	42.86	33.33
	5	17	18	56.67	48.57	35.42
	10	16	13	53.33	55.17	37.21
	15	13	11	43.33	54.17	31.71
2.5	2	10	17	33.33	37.04	21.28
	5	9	7	30.00	56.25	24.32
	10	10	6	33.33	62.50	27.78
	15	9	5	30.00	64.29	25.71
3.0	2	6	7	20.00	46.15	16.22
	5	6	3	20.00	66.67	18.18
	10	6	2	20.00	75.00	18.75
	15	5	0	16.67	100.00	16.67

Table 5b - Number of estruses detected (DE), number of false positives detected (FP), detection sensitivity (DS)*,detections which were correct (DC)*,and detection accuracy (DA)* for all criteria tested on neck pedometer data for experiment 3 (30 estruses studied).

Threshold	Baseline(d)	DE	FP	DS(%)	DC(%)	DA(%)
1.5	2	26	135	86.67	16.15	15.76
	5	27	100	90.00	21.26	20.77
	10	25	90	83.33	21.74	20.83
	15	24	77	80.00	23.76	22.43
2.0	2	21	59	70.00	26.25	23.60
	5	17	32	56.67	34.69	27.42
	10	16	19	53.33	45.71	32.65
	15	13	17	43.33	43.33	27.66
2.5	2	11	27	36.67	28.95	19.30
	5	9	7	30.00	56.25	24.32
	10	10	7	33.33	58.82	27.03
	15	9	6	30.00	60.00	25.00
3.0	2	6	13	20.00	31.58	13.95
	5	6	3	20.00	66.67	18.18
	10	6	2	20.00	75.00	18.75
	15	5	0	16.67	100.00	16.67

* See Manuscript 2 for definitions of terms.

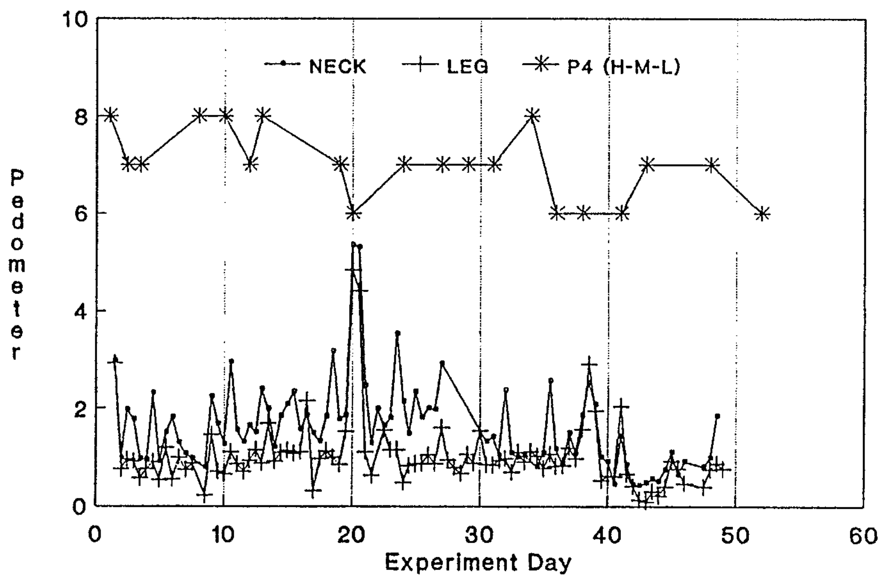
APPENDIX III - Pedometer and hormone data for individual cows in Manuscript 3

Figure (a) = All pedometer and progesterone data collected during the study.
Figure (b) = Pedometer, LH and E2 data collected during the five days surrounding the first ovulation studied.
Figure (c) = Pedometer, LH, and E2 data collected during the five days surrounding the second ovulation studied.

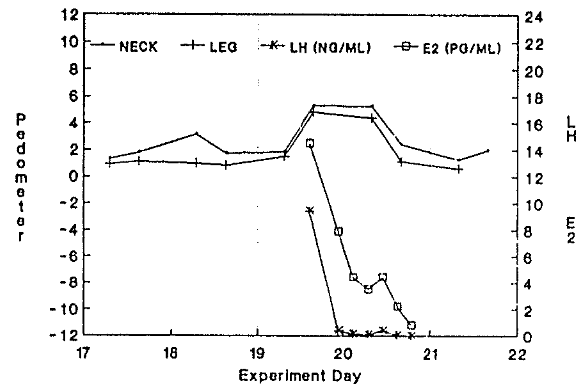
*Pedometer = Pedometer increments X 1000.
*Progesterone levels are reported as (H)igh=8000, (M)edium=7000, and (L)ow=6000.

Figure 1 (Cow AU11)

(a)



(b)



(c)

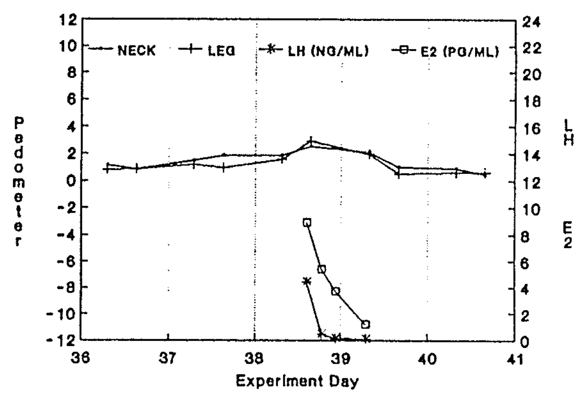
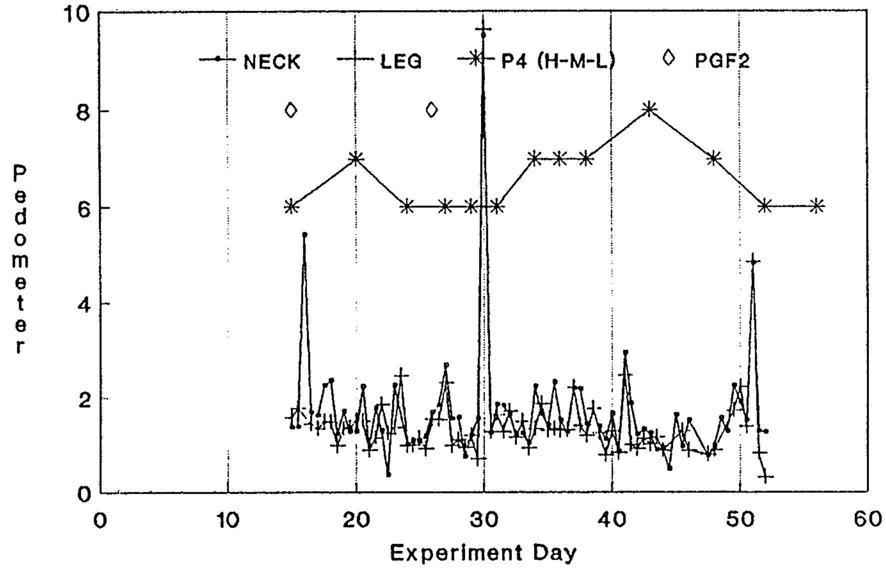
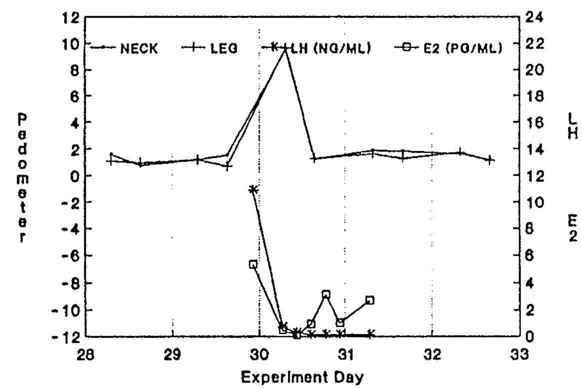


Figure 2 (Cow Z77)
(a)



(b)



(c)

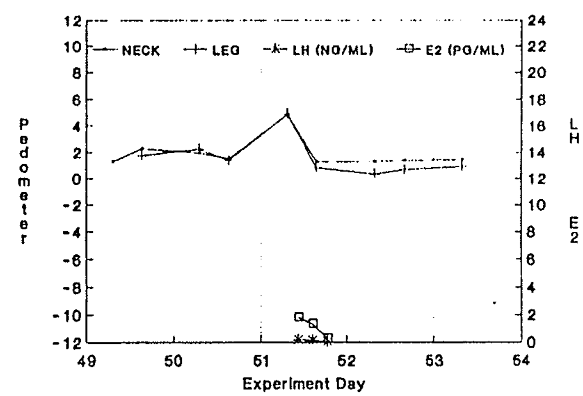
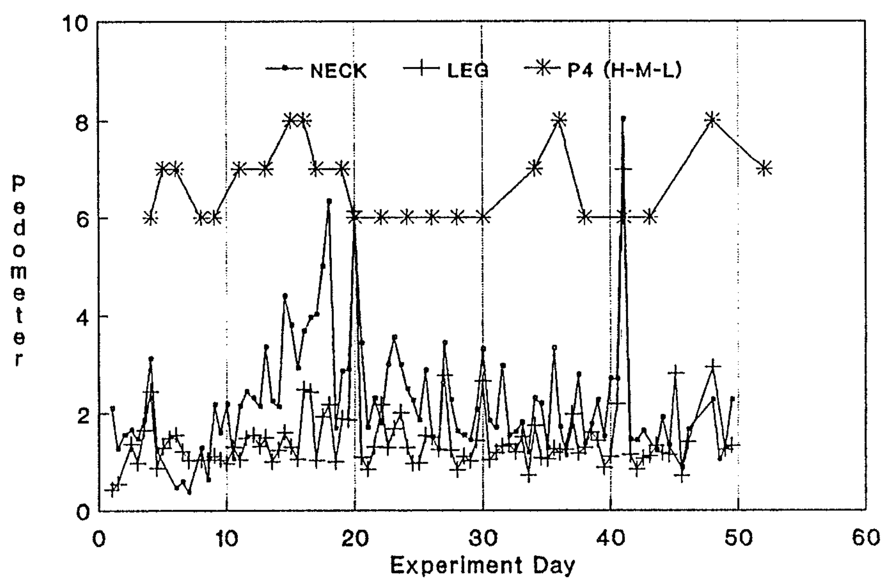
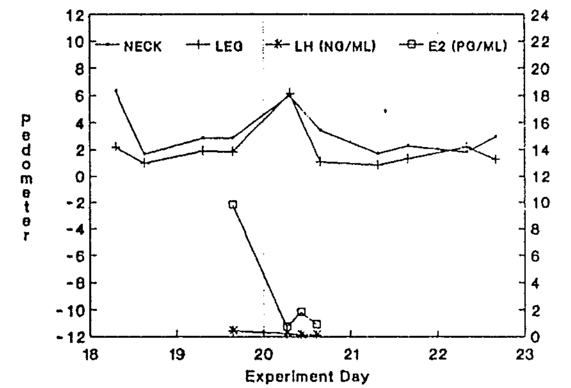


Figure 3 (Cow A21)

(a)



(b)



(c)

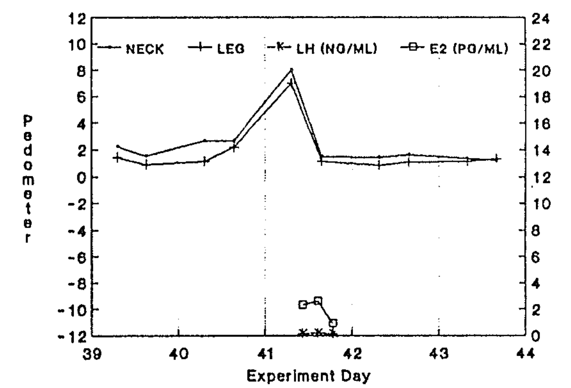
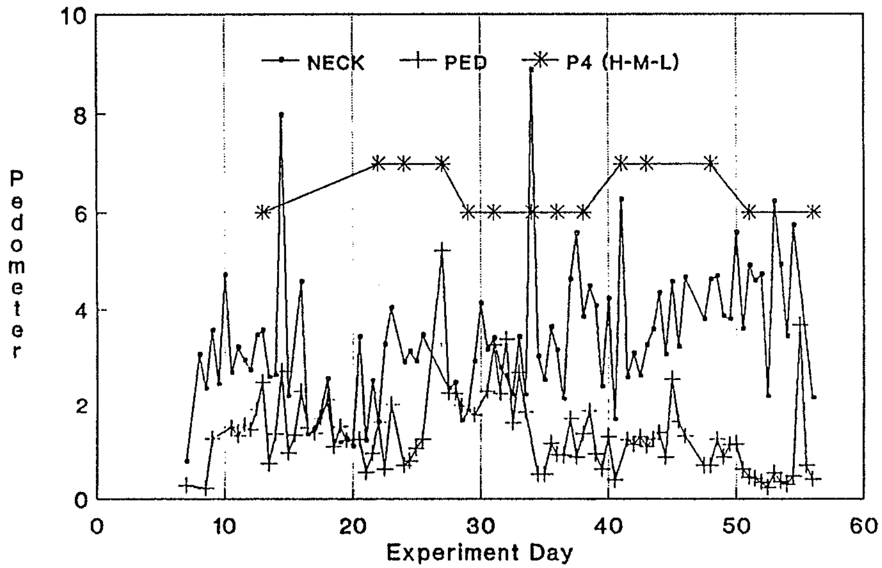
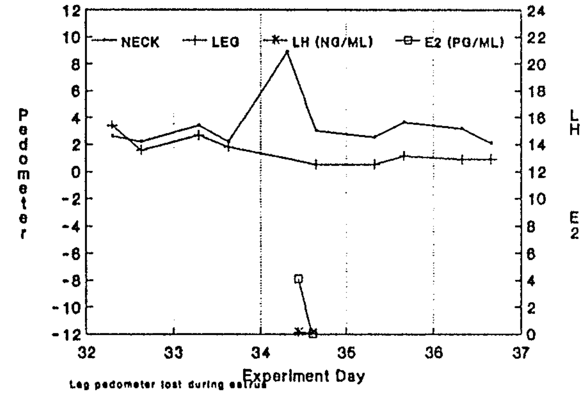


Figure 4 (Cow A31)

(a)



(b)



(c)

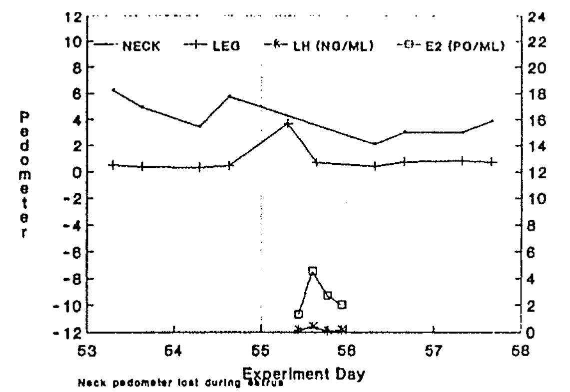
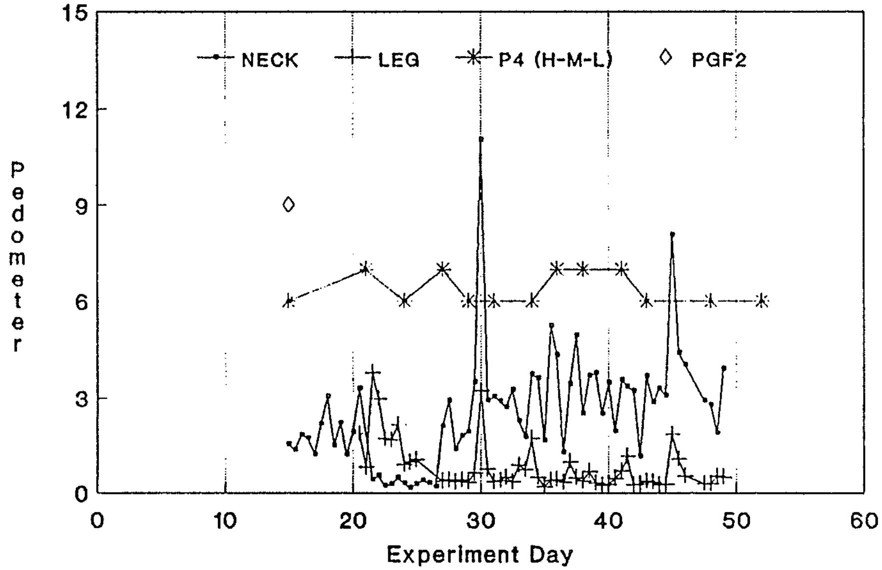
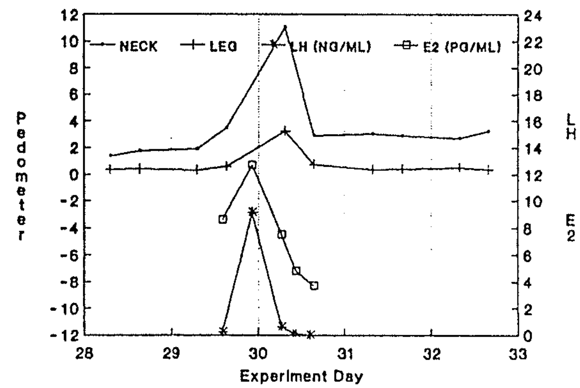


Figure 5 (Cow A91)
(a)



(b)



(c)

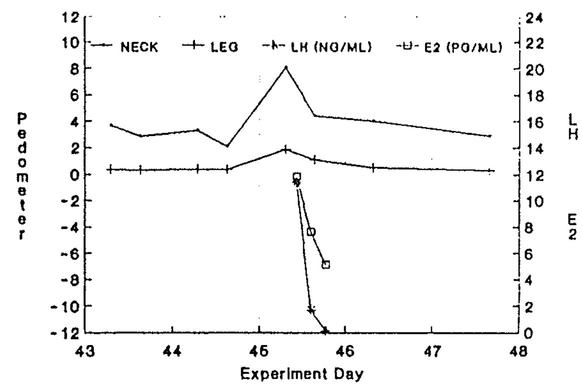
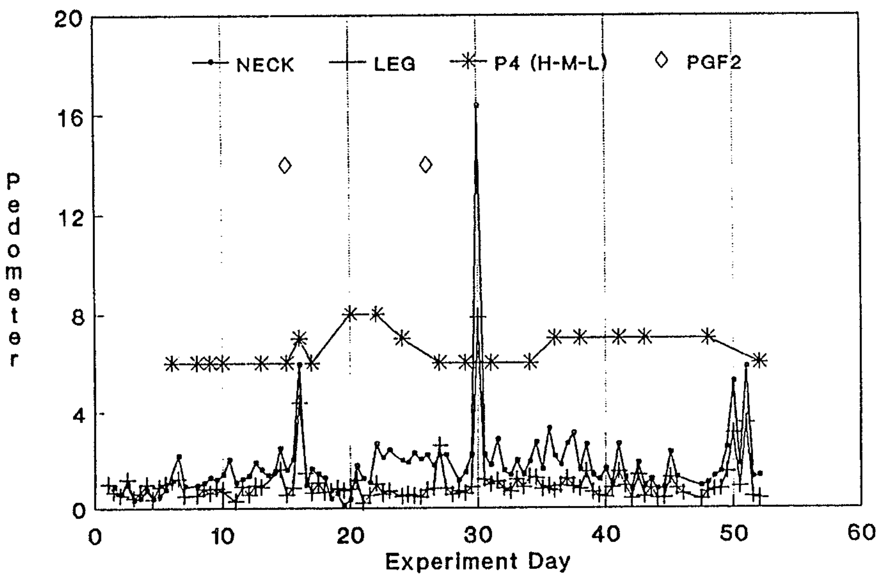
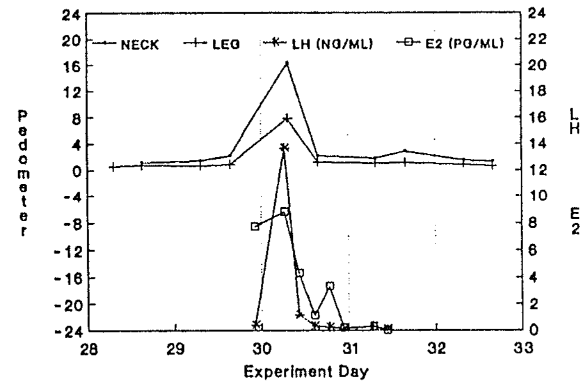


Figure 6 (Cow X77)

(a)



(b)



(c)

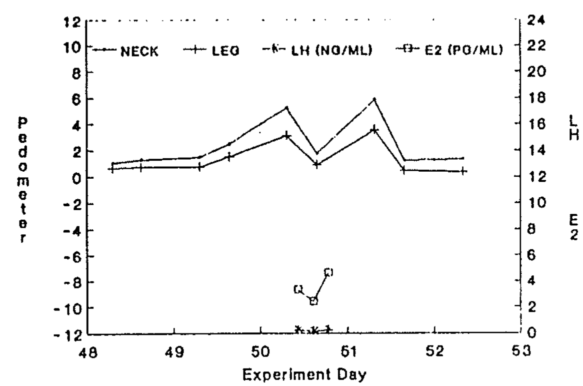
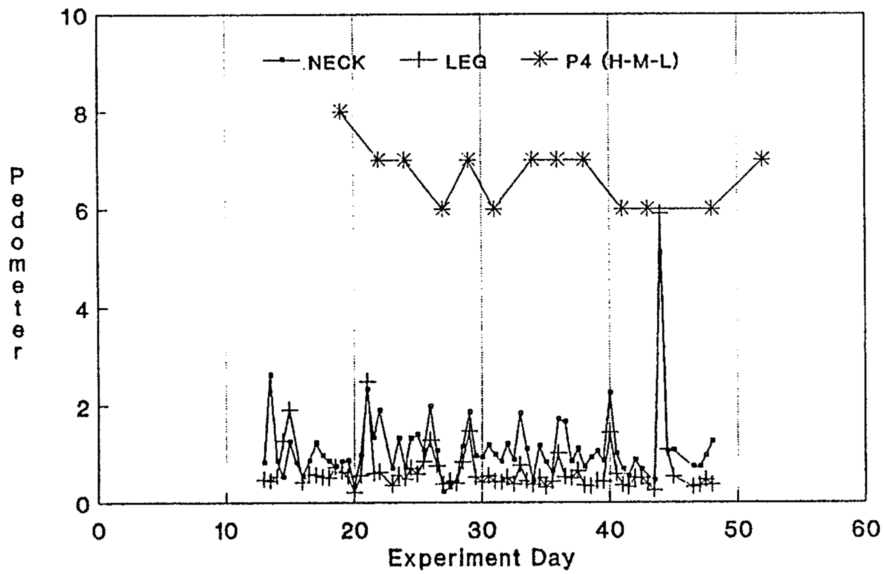


Figure 7 (Cow X2)
(a)



(b)

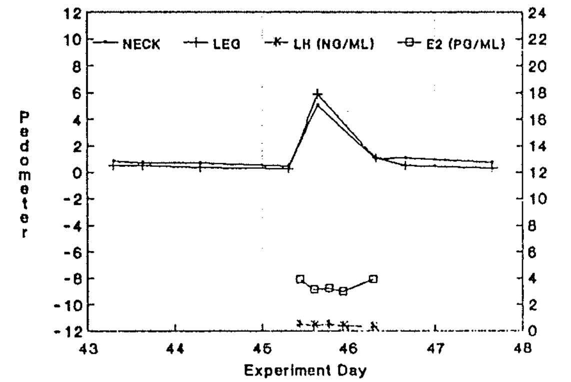
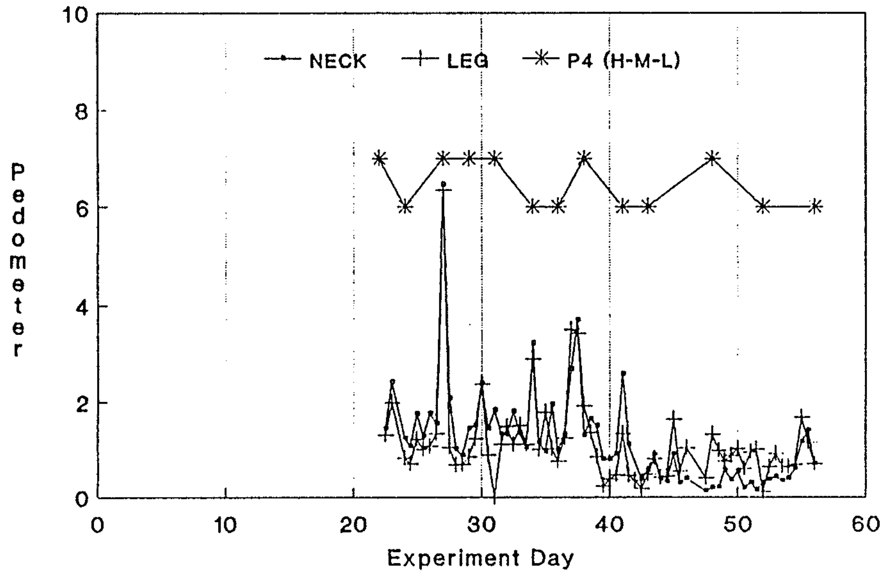
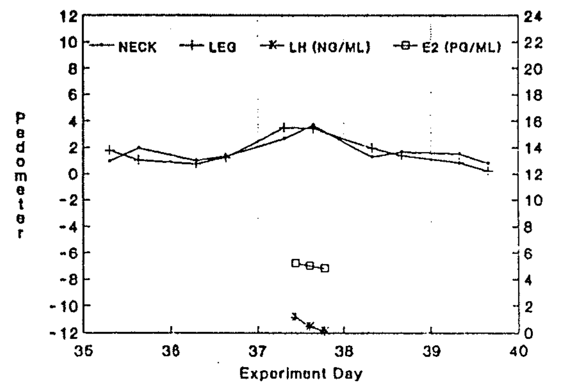


Figure 8 (Cow Z23)

(a)



(b)



(b)

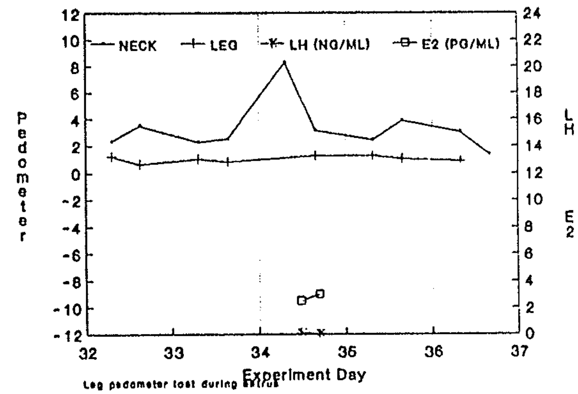


Figure 9 (Cow A26)
(a)

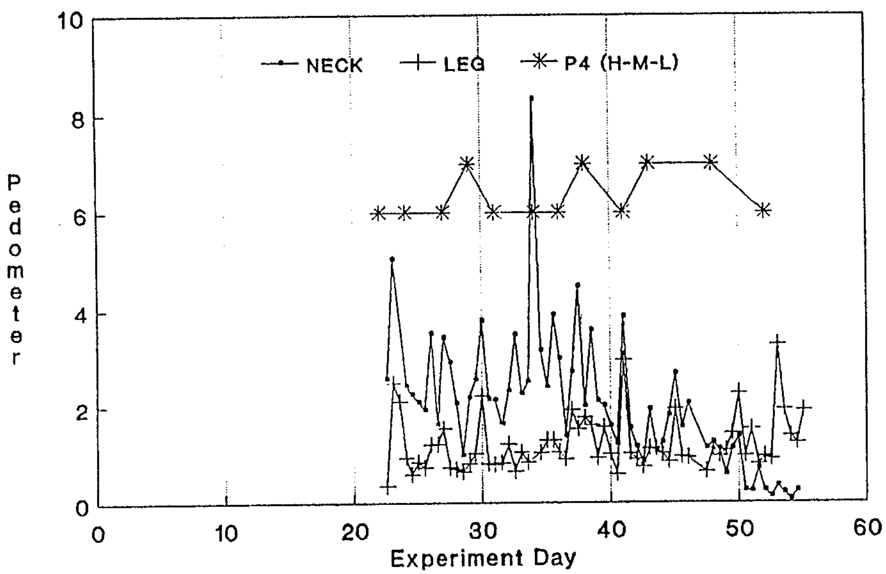
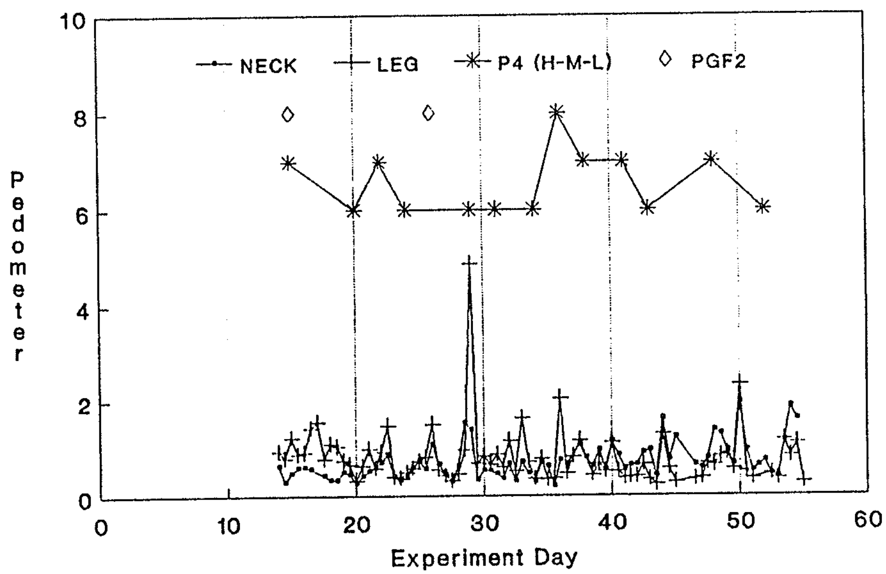


Figure 10 (Cow Z63)
(a)



(b)

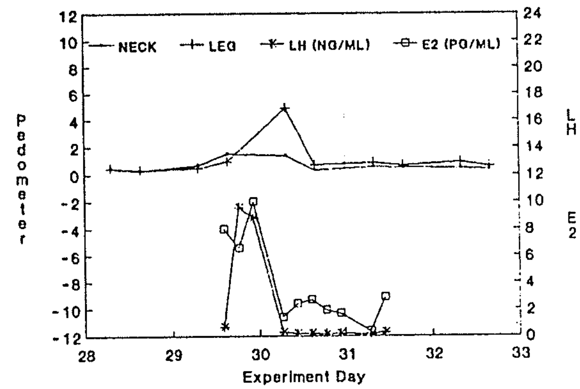


Figure 11 (Cow Y9)
(a)

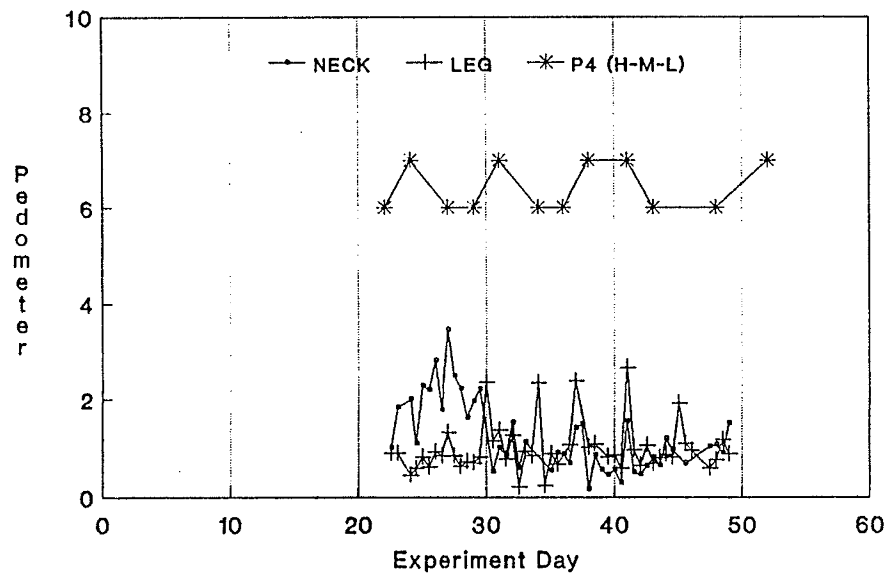


Figure 12 (Cow A80)
(a)