ARDI Final Report

Project 04-632
"Electronic Tracking of Cattle Identification (RFID) and Core Body Temperature"

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by

Dr. J. A. Small
Agriculture and Agri-Food Canada,
Nova Scotia Agricultural College
Truro

RESEARCHER(S):
Dr. J. A. Small, Agriculture and Agri-Food Canada Research Centre, Brandon MB
Dr. A. D. Kennedy, University of Manitoba, Winnipeg MB
Dr J. Singh, University of Saskatchewan (PhD student supervisor*)

PROJECT PARTNERS:
DataTic Technologies, Palo Alto, CA
Phase IV Engineering, Inc., Greely CO

PROJECT EMPLOYEES, STUDENTS OR CONTRACTORS:
Douglas Wey - Technical Assistant, University of Manitoba
Sherri Witherspoon - Technical Assistant University of Manitoba
Sylvia Kahane - Post-doctoral Fellow, University of Manitoba
Nels Thorsteinson - Bioinformatics student, University of British Columbia

Participants not-funded by ARDI
*Luis M. Pfeifer - Ph D student, University of Saskatchewan.
Seonaid MacDonnell - 4th year undergraduate student, Nova Scotia Agricultural College.

EXECUTIVE SUMMARY:
Seven experiments were conducted with beef heifers at the Agriculture and Agri-Food Canada Research Center, Brandon, MB to develop the application of a passive core temperature (Tc) monitoring system for cattle. The Tc monitoring system used magnetic, inductively coupled full duplex transponder boluses containing thermistors, a panel reader (transceiver) with ambient temperature (Ta) and relative humidity sensors, and acquisition software. The panel reader activated the transponder boluses from a distance of several meters, acquisitions occurred as an individual passed within 1 m of the panel reader, and the software recorded identification, reticulo-rumen temperature (Trr), Ta and humidity data in real-time.

Prior to these first studies in Canada the system had only been tested in the United States with large free-stall managed dairy cows that pass the panel reader upon entry and exit of the milking parlor

(twice daily). Our work discovered a way for the system to work in beef cattle, where daily activity is not entrained to the degree it is in dairy cattle, and housing is generally outdoors all year round, especially in western Canada. Two outdoor installation designs were developed where growing heifers would be self- motivated to pass the panel reader. Our work also developed the math and terminology to describe "self-motivated" monitoring, providing benchmarks for subsequent development of the system for loose housed cattle.

Transponders were administered per os to heifers housed in one of four pens, in outdoor shed-lot facilities, each with a panel reader co-located with the water bowl. Fencing was arranged within pens for water motivated (WM) acquisitions during experiments. 1 (Initial), 2 (Fever) and 3 (Photoperiod), or for either WM or activity motivated (AM) acquisitions during experiment 4 (Pen setup), In these first studies, we demonstrated that most heifers were monitored daily (Monitoring rate 100%), several times per day (Monitoring frequency 7.8 ± 0.5), mostly during the afternoon and evening rather than night and morning 6-h periods. Most Trr values (average 37.8 ± 0.2°C, range 22 to 42°C) were within the expected range for core temperature in cattle and extremes below normal were caused by drinking the cold (4°C) water. The transponder drop-out rate was 19% which could be overcome in most cases by administering a second bolus. The panel reader ambient temperature sensors were not reliable but did not impair the Trr monitoring function of the system. Pen setup for AM in contrast to WM acquisitions increased monitoring rate (MR) and monitoring frequency (MF) and Trr (by 1°C) in all periods of the day. Subsequent experiments endeavoured to apply Trr monitoring to reproductive management. Fencing was arranged for AM-motivated acquisitions during experiment 5 (estrus detection), WM- and AM-motivated acquisitions during experiments 6 (selection of poor candidates for fixed time artificial insemination) and 7 (forecast parturition).

Monitoring frequency and Trr were affected by subjecting cattle to an endotoxin (induced-fever), different photoperiod and pen management treatments, day of the estrus cycle and day prepartum. Cattle clinically ill were not motivated to pass the panel reader, but when encouraged to do so Trr detected the fever. Therefore, Trr monitoring to detect a febrile response in advance of clinical illness would be more advantageous. Fencing arranged within pens for activity motivated (AM-) rather than water motivated Trr monitoring improves the precision of Trr monitoring but the best ratio of cattle to panel readers is yet to be determined.

Subsequent experiments developed tests to apply Trr monitoring to detect pubescent estrus (Experiment 5), identify poor candidates for fixed time artificial insemination (TAI; Experiment 6) and forecast calving (Experiment 7). Although mean MF increased on the day of estrus a rise in mean Trr usually followed estrus, Trr monitoring , in its present form, to detect pubescent estrus in individual cattle was poor relative to twice-daily visual observation of estrous behaviour (accuracy less than 0.60) probably because as arranged the MF was insufficient to gather precise Trr data in all periods of day. The accuracy of the Trr tests to detect poor candidates for TAI ranged from 0.65 to 0.75, and agreement was fair between the number of forecasted and actual poor candidates for TAI (Table 6.1). Thus depending upon the cost of semen relative to the cost of days open either test could reduce breeding costs by reducing the number of poor candidates subjected to TAI. The accuracy of the Trr test to forecast calving was 0.88 within 2 d and 0.92 within 5 d.

This work has provided the foundation to continue development of this tracking and alerting system for application to cattle operations[1]. In all tests, comparing the current Trr to an average over the previous 2 to 4 d but not more than 5 d had the best results. Certainly a strategy to increase MF in all periods of day improved the detection of changes in reproductive function and requires more research, and research is also required to develop on-farm user friendly software for interpreting the Trr data captured. The prospects for application of this technology to farms already engaged in precision farming is excellent.

[1]This work lead directly to collaborative research by J Small with DVM Systems, LLC, Greely CO and Colorado State University, Boulder CO to develop the Trr monitoring for application to dairy cattle. Please refer to poject variances in Appendix I.

BACKGROUND AND OBJECTIVE:

Frequent daily monitoring of core body temperature (Tc) over many consecutive days detects estrus [1, 2], calving [3] and fever [4] where Tc deviates from normal over short periods of time. Once [5], or twice [6] or 4x [7] daily monitoring of Tc at a consistent time over several days has also detected peaks and nadirs in Tc around the time of estrus [5, 6], ovulation [7] and calving [3]. Manually measuring Tc (e.g. rectal or vaginal temperature) over several days is somewhat invasive and obviously of limited practical value. Early radio-frequency technology(RFID) used battery powered transponders to facilitate remote non-invasive monitoring of Tc at pre-programmed intervals, but the systems were also of limited practical value because of the cost and animal welfare issues with prolonged use. Lower cost RFID technology using passive transponders (no battery required) has been applied on-farm to uniquely identify farm stock as part of the protocols to ensure food safety. Since January 2005, all Canadian cattle that leave the farm must be identified with Canadian Food Inspection Agency (CFIA) approved RFID ear-tag. A new development for cattle management combines RFID and temperature sensing technology with magnetic boluses routinely used to protect against hardware disease. The passive Tc monitoring system (DVM Systems, LLC currently) uses magnetic, RFID transponder boluses containing thermistors, a panel reader (transceiver) and software to capture Tc in real-time as cattle pass the panel reader.

The passive Tc monitoring system can be used in addition to the CFIA ear-tag, and offers the cattle industry continuous, tamperproof, non-invasive monitoring of Tc and protection against hardware disease throughout the animal's lifetime. Although reticulo-rumen temperature (Trr) is an accurate measure of Tc, precision has been subject to variation caused by drinking water temperature [8] and heat of fermentation [9]; and perhaps the frequency and distribution of voluntary acquisitions throughout the day and over time [10]. However, the prospects for this technology to be used to enhance food safety and animal welfare are excellent, the impact will depend upon how well the monitoring system detects deviations in Tc caused by production diseases, calving or estrus.

Objectives:

The objective was to demonstrate the application of non-invasive passive monitoring of Trr for tracking Canadian cattle, and for enabling the farmer to detect early changes in animal health (eg. fever, or depressed feed and water intake) that relate to disease, and possibly accurate detection of estrus and the onset of calving.

PROJECT ACTIVITIES (PROCEDURES, RESULTS AND DISCUSSION)

Equipment reliability for Trr monitoring

Over 90,000 observations on 72 heifers were taken over 190 consecutive days in five experiments. The bolus failure rate was 19% [11], but in all but one case, a second bolus was administered and monitoring continued with minimal recording days lost. Regurgitation of RFID boluses can be a problem, but was never encountered in our studies, likely because the bolus specific gravity was that recommended for maximum retention rate [12]. It is possible that the initial batch of boluses had a higher than expected number of defective boluses, or, alternatively, handling of these initial boluses resulted in malfunction. No others have reported on the function of this particular RFID and Trr monitoring system. Additional trials are required to confirm that our 19% failure rate was not characteristic of all MaGiix RFID boluses. (Note: DVM Systems, LLC, Greely CO have taken over development of the system).

The ambient temperature and relative humidity measurements by the panel reader sensors were not reliable, but this did not influence the acquisition of transponder data in any way. It is noteworthy that the Trr monitoring system worked well when set-up outdoors during the cold conditions of

winter. Based on the 30-yr historical average, the conditions were typical of winter in southern Manitoba, and the experimental periods were also representative with minimum ambient temperature below -20°C on 20% of the days. This gave us confidence that the Trr monitoring system functioned reliably.

Application of Trr monitoring - (Experiments 1, 2, 3, 4)

Initial experiments were conducted over 82 d (Nov. 04 to Jan. 24, 49°N, mean ambient temperature -3.6 to 12.9 ± 1.6°C) to determine the effects of fever, photoperiod and pen setup on the rate and frequency with which heifers were monitored and the core (reticulo-rumen) temperatures (Trr) obtained with the MaGiix cow temperature monitoring system. Transponders were administered *per os* to 72 heifers (7.9 ± 0.5 mo of age and 283 ± 23 kg body weight) housed in one of four pens, in outdoor shed-lot facilities, each with a panel reader co-located with the water bowl. Fencing was arranged within pens for water motivated (WM) acquisitions during experiments. 1 (Initial), 2 (Fever) and 3 (Photoperiod), or for either WM or activity motivated (AM) acquisitions during experiment 4 (Pen setup). Over the course of Days 19 to 39, 32 heifers of similar body weight were selected for the fever study, and randomly assigned to receive saline on the first day and fever agent (Esherichia coli 055:B5 Sigma, St. Louis, MO) at 0900 on the following day, or vice versa. In December Lot 2 was equipped with time controlled lights to apply extended photoperiod treatment from Day 40 onward. The lights went on at 30 min before dusk and remained on until the completion of a 16 h photoperiod, and were staggered off over 30 min to simulate twilight. The lighting system has been described in detail elsewhere [13]. Lot 1 continued to receive natural winter short day (8 h) photoperiod only. A mixed ration (59% dry matter) was provided at 1500 daily. From Day 54 onward fencing was rearranged within one pen in each lot so that acquisitions could be independent of seeking water and, therefore, deemed activity motivated (AM).

Results: Overall, most heifers were monitored daily (Monitoring Rate 100% mode), several times per day (Monitoring Frequency 7.8 ± 0.5, mean ± sd), mostly during the afternoon and evening rather than night and morning 6 h periods, and Trr (37.8 ± 0.2°C, range 22º to 42°C) were usually lower for the afternoon than night.

A 2°C increase in mean Trr caused by endotoxin was detected when monitoring was scheduled rather than unscheduled. Detection of the thermogenic response to lipopolysaacharide (endotoxin released by Escherichia coli) suggests the system could be effective for subclinical detection of diseases. However, the system must be sensitive enough to detect an increase in Tc before clinical signs, because ill cattle did not pass the panel reader voluntarily. This application requires further development beyond the scope of the research proposed

Extended (16 h) in contrast to natural (8h) photoperiod increased evening monitoring rate and frequency, and increased morning Trr by 0.5°C. Compared with natural photoperiod, the extended photoperiod motivated more heifers to drink more frequently in the evening, and morning visits to the panel reader were not always associated with drinking.

Pen setup for AM in contrast to WM acquisitions increased ($P<0.05$) MR and MF and Trr (by 1°C) in all periods of the day. The AM acquisitions reduced the influence of drinking cold water on Trr, because visits to the panel reader were motivated by locomotory activities associated with the desire to feed, lie down or stand in a group, including, but not limited, to seeking water. the effects of drinking

These first studies provided proof of concept and very important information for applying the technology to cattle management. The calculations of ME, MR and MF were applied to all subsequent experiments.

Detection of Pubescent Estrus (Experiment 5)

This study evaluated Trr monitoring and 30 min twice-daily (8 am and 3 pm) visual observation for signs of estrus in beef heifers from January 27 to March 24th 2005. Blood samples were collected from heifers 5 to 10 d following observation of standing estrus for enzyme-immunoassay of plasma progesterone concentrations [14, 15] to confirm estrus (CE) with ovulation [16]. There were 144 heifers in one of four pens within one of two shed-lot facilities. In each facility the two pens of 18 heifers with Trr monitoring were collapsed to one pen of 36 heifers with one panel reader set-up for AM-acquisitions as described for Experiment 4 (natural and extended photoperiod AM-pen setup).

Experienced stockpersons used visual observations (2 x daily for 30 at each facility) to identify heifers in estrus. The Trr acquisitions were processed as described previously to determine ME, MR and MF for four 6 h periods night (0000 to 0559), morning (0600 to 1159), afternoon (1200 to 1759) and evening (1800 to 2359). Weather data were obtained from the weather station at the Brandon Airport (Environment Canada 2010; station number 5010480) located at a similar elevation (Table 5.1). Univariate analysis and chi square tests were first done to compare Trr monitoring performance between the two experiments. Subsequently, Trr values less than 37.5°C were deemed influenced by drinking water and discarded from the calculations of mean Trr. Data were then subjected to analysis of variance (PROC MIXED) to determine the effects of day of estrus and photoperiod on Trr and to identify criterion for developing a test for Trr detection of estrus. Data from heifers with complete Trr datasets and one or more progesterone confirmed estrus were used to test sets of criterion to detect estrus. The Trr detection of estrus was deemed correct when peaks or nadirs in Trr were coincident with a confirmed estrus (TP; true positive) or absent in the absence of a confirmed estrus (TN; true negative); and deemed incorrect when peaks or nadirs occurred more than 3 d away (FP; false positive), or were absent within 3 d (FN; false negative) of a confirmed estrus [17]. The Receiver Operating Curve Analysis (ROC), a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity) was used to select the best test and that was subjected to the kappa test of agreement [18].

Results:
The Trr monitoring was completed for 70 heifers (2 transponders failed), and 39.6% of these heifers had attained puberty before the start of the trial, and 51% exhibited one estrus and 13% exhibited two confirmed estruses (CE) during the study (Table 5.1). The Trr monitoring system function and Trr values were similar to that reported for Experiments 1 to 4 with most monitoring events occurring in the afternoon and evening consistently (Table 5.1). The study was conducted during the seasonal transition to Spring, and this may have influenced the behaviour of the NP-treated heifers to increase night and morning monitoring rates when compared to the EP-treated heifers (Table 5.1).

Based on the ANOVA, passive monitoring of Trr could be used to detect CE in pubescent heifers (Figure 5.1). Regardless of photoperiod treatment, the daily monitoring frequency was highest on the day of estrus which implied that MF detected the increase in locomotory behaviour of cattle in estrus. Secondly, photoperiod treatment and time contributed significant variation in mean daily Trr without interaction. The Trr were higher for EP than NP and overtime were lower around the day of estrous than other days of the estrous cycle. These observations agreed with others in that the higher Tc in mid-cycle cattle is in part due to the thermogenic effect of progesterone. It follows that the decrease prior to estrus is due to the removal of progesterone caused by prostaglandin induced regression of the corpus luteum. The ANOVA of more precise changes in Trr and MF around the time of CE are shown (Figure 5.2). The Trr means were higher for EP than NP mostly during the evening and also during the afternoon 1.5 d after estrus, and except -1 d before when it is lower, because Trr increased for NP at this time. The MF means were lower for EP than NP mostly during the morning and also during the evening following the day of CE.

Several sets of criterion were applied to determine a Trr test for pubescent estrus but all initial attempts resulted in either too many false positives or too many false negative tests (Figure 5.3). The data were extremely variable and without consistent Trr monitoring in all periods of day a short estrus period, typical of pubescent heifers, is likely to be missed. Therefore, Trr monitoring alone in its present form is unlikely to improve estrus detection in pubescent heifers significantly. However,

since this project was done new ways of evaluating the data have been learned and applied to this dataset before submission for publication in a peer reviewed journal.

Conclusion: The study provided good evidence of the application of Trr monitoring to detect estrus but further research is required to improve the precision of Trr data gathered in all periods of day.

Table 5.1 Description of heifers, reticular temperature (Trr) monitoring system function and ambient conditions during the study to evaluate Trr monitoring to detect pubescent estrus.

Item	Natural photoperiod (NP)	Extended photoperiod (EP)
No. of heifers monitored[1]	35	35
No. of heifers confirmed estrus before Jan 29	15	12
No. of heifers 1 (2) confirmed estrus after Jan 29	16 (6)	20 (3)
Interval pretest to 1st on test confirmed estrus (d)	27.7 ± 4.1	28.7 ± 4.9
Interval 1st to 2nd on test confirmed estrus (d)	18.6 ± 1.6	20.7 ± 2.2
Date of 1st on test confirmed estrus	11Feb ± 3.0	19Feb ± 3
Reticular temperature monitoring		
No. of monitoring events (ME)	16686	14893
Monitoring frequency (ME /d ± stdev)	7.7 ± 1.5	8.1 ± 1.2
Distribution of ME throughout the day		
Night(%)	15.6*a*	8.3*b*
Morning (%)	19.7*a*	12.2*b*
Afternoon (%)	29.7	33.6
Evening (%)	35.0*b*	45.9*a*
Monitoring rate (consecutive d with 1 or more ME:		
Night(%)	67.4*a*	34.5*b*
Morning (%)	58.8*a*	32.5*b*
Afternoon (%)	97.1	95.9
Evening (%)	97.6	97.4
Reticular temperature values:		
Mean Trr (± stdev)	38.40 ± 1.59	38.74 ± 1.16
Mode (Q1)	39.05 (38.38)	39.22 (38.56)
Range	28.05, 41.50	29.22, 43.33
Ambient conditions		
Natural photoperiod (h)	9.10 to 12.47	16[2]
Maximum ambient temperature (°C)	-6.7 ± 5.2	
Minimum ambient temperature (°C)	-17.0 ± 7.1	
Mean ambient temperature (°C) [3]	-11.9 ± 5.9	
Total snowfall (cm)	44.2± 2.8	
Snow on ground (cm)	36.3 ± 10.1	
Total precipitation mm (days with ppt)	22	

[1] Pens within treatment were collapsed to one pen and one panel reader set-up for activity-motivated acquisitions. Two heifers excluded because of transponder failure.
[2] Supplemental light treatment terminated March 10 (DYP 127).
[3] Feb 14 to 26; Mar 14 and 15th ambient temperatures fell below -20 C; snow storm (16.4 cm) Mar 1

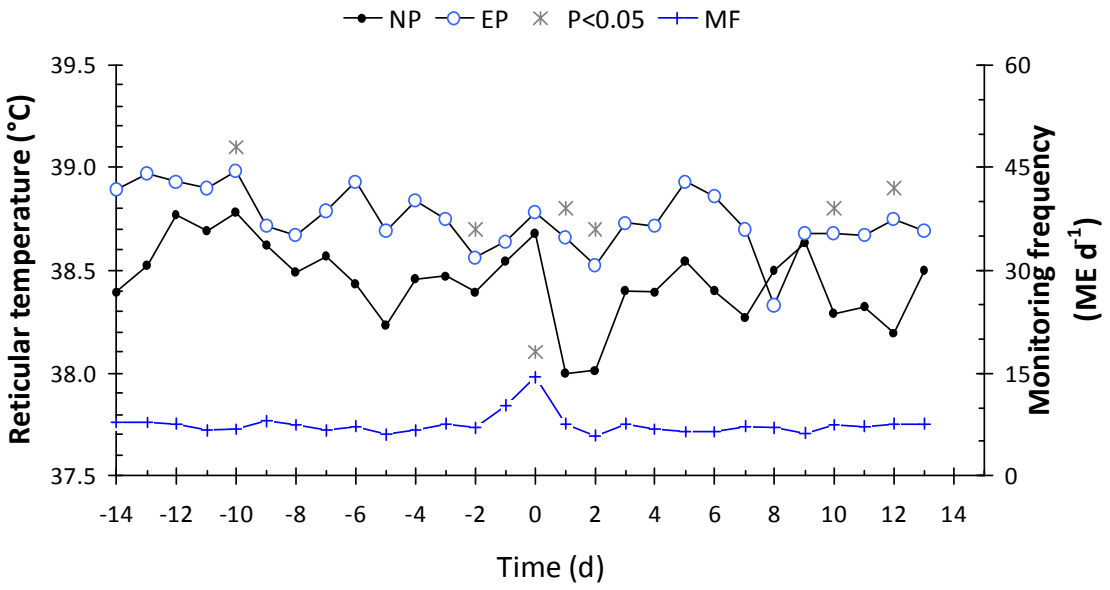


Figure 5.1. Least squares means for average daily reticular temperature (Trr) relative to the day of progesterone confirmed observation of estrus in beef heifers subjected to natural (NP) or extended (EP) photoperiod treatment. The Trr were lower for NP than EP (P<0.001) and higher at -10 than -2, , 1, 2, 10 and 12 d of estrus. Least squares means for the total daily Trr monitoring events (MF) were highest (P<0.001) at 0 d, and did not differ between NP and EP (P=0.865) regardless of d (P=0.727).

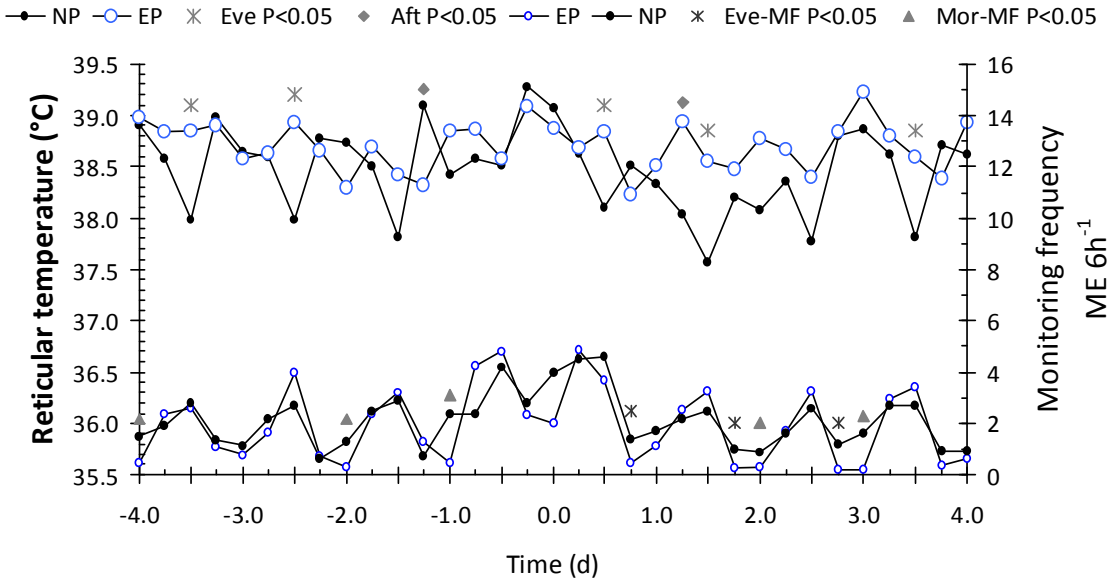


Figure 5.2 Least squares means reticular temperature (Trr) and monitoring frequency for morning, afternoon, evening and night periods relative to the morning of the day of progesterone confirmed observation of estrus in beef heifers subjected to natural (NP) or extended (EP) photoperiod treatment. Photoperiod x Time P<0.001 for Trr and MF (refer to text).

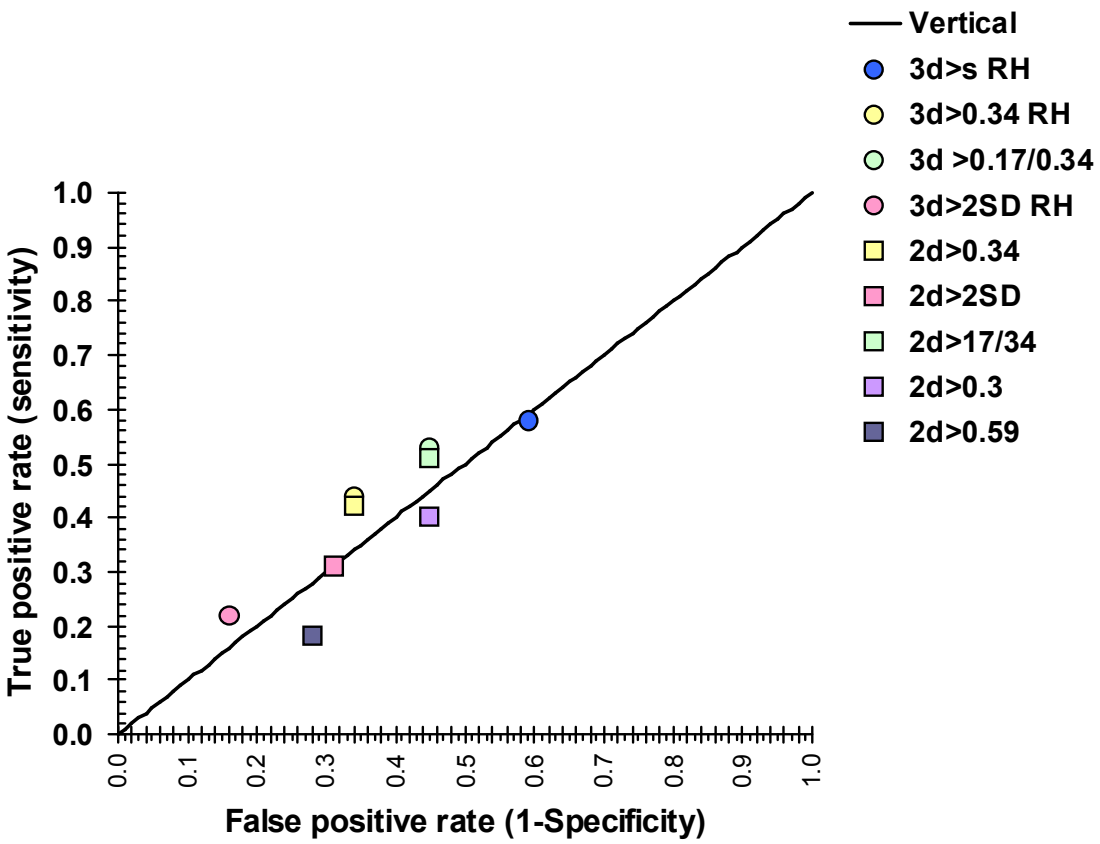


Figure 5.3 Receiver operating curve analysis of sets of criterion (2 and 3 d baseline and deviations 2 x standard deviation or the baseline, 0.34, 0.3 and 0.59 C above baseline). The maximum Trr within a period was used for these analysis. None of these tests were worth further development.

Trr detection of poor candidates for TAI (Experiment 6)

In this study Trr monitoring of heifers was done from April 14 to May 10, 2005. The objectives were to evaluate the use of Trr monitoring to predict the success of fixed-time artificial insemination (TAI) and to develop a test to detect poor candidates for TAI. At this time the heifers described in Experiment 1, 2, 3, 4 and 5 were yearlings (12.5 ± 0.5 mo., 421 ± 32 kg body weight, 5.4 ± 1.2 mm backfat thickness) and moved April 2 to one pen in an outdoor shed-lot with two panel readers. The heifers continued to receive a mixed ration (59% dry matter) at 1500 daily and received natural photoperiod only. Fencing was arranged for water motivated acquisitions by one panel reader, and activity motivated acquisitions by the other (Small et al. Can. J. Anim. Sci. 88:225-235, 2008). All heifers were administered estradiol benzoate (1 mg im) on the 8th d after the second of two doses of PGF (500 ug im; Estrumate) given 11 d apart, two groups were given progestin inserts (1/2 Cue-mate) removed concurrent with PGF (25 mg im; Lutalyse, 1100h, Day 0), and a third group was given a temperature recording device (HOBO ProV2) anchored in the vagina and programmed to record temperature (Tv) every 15 min from Day -7 to Day 7; all received pLH (12.5 mg im; Lutropin-V) concurrent with TAI 54 h after PGF (Day 0). Fertile bulls were placed with the heifers on Day 2 to Day 40 to service heifers that failed to conceive to TAI. On Day 35 real-time trans-rectal ultrasonography was used to determine the number pregnant to TAI, and on Day 120 veterinary palpations were done to determine the total number pregnant (Table 6.1).

The Trr acquisitions were processed as described previously to determine ME, MR and MF for four 6 h periods night (0000 to 0559), morning (0600 to 1159), afternoon (1200 to 1759) and evening (1800 to 2359). Univariate analysis and chi square tests were first done to determine Trr monitoring performance. Subsequently, Trr values less than 37.5°C were deemed influenced by drinking water and discarded from the calculations of mean Trr. Data were subjected to analysis of variance (ANOVA; PROC MIXED) to determine the effects of synchronization treatment on Trr and MF. The average Tv for each period were calculated and paired with the average Trr to determine the correlation between Tv and Trr. Data were then subjected to analysis of variance (PROC MIXED) to determine the effects of synchronization treatment and pregnancy on Trr and to identify criterion for developing a test for Trr detection of poor candidates for TAI. Finally, data from cattle with complete Trr datasets for at least 7 d before and following the third prostaglandin treatment were used to test sets of criterion to identify poor candidates for TAI. The ROC analysis was used to identify the best test and that was subjected to the kappa test of agreement.

Results: Transponder boluses failed in 8 heifers and Hobo dataloggers were expelled from 2 heifers. As observed previously there were fewer monitoring events (ME) during the night than other times of day, and mean Trr and MF were highest 2 and 3 d following prostaglandin treatment (Figure 6.1). Mean MF were higher for Group 3 than Group 1 (16.8 ± 0.9*a*, 18.1 ± 0.9*ab*, and 20.2 ± 0.7*b,* Groups 1, 2, 3 respectively $P<0.05$) and also mean afternoon and evening Trr before PG3 were 0.4° and 0.8°C higher than the other groups. This difference was likely due to progesterone suppression of estrus in progestin treated heifers. There were 30 heifers pregnant and 33 not-pregnant to TAI.

On average Trr were 0.47 ± 0.09°C higher than Tv ($P<0.01$) and the correlation between Trr and Tv ($N=766$) was 0.64 ($P<0.01$). However, the correlation was dependent upon stage of the estrous cycle and was highest (0.71; $N=180$) between PG3 and TAI. Current Trr and Tv were compared to the previous 3 d baselines for each period. For pregnant heifers, Tv decreased to nadir within 24 h of PGF and peaked above baseline at 58 h (Day 2) returning to baseline at 78 h (Day 3); for not-pregnant heifers peaks occurred 78 h to 90 h returning to baseline at 96 h (Day 4). Mean Trr was 0.3°C higher ($P<0.05$) for pregnant than non-pregnant heifers at 60 h (Day 2) and vice-versa at 114 h (Day 4). The number of Trr ME was greater ($P<0.05$) for pregnant than not-pregnant heifers at 30 h, 36 h and 60 h periods after PG (7.9 vs 12.8 ± 0.8 ME 6 h^{-1} at 60 h) (Figure 6.2). Based on the ANOVA, passive

monitoring of Trr could improve the efficacy of TAI in heifers by facilitating a management decision to re-inseminate or observe for return to estrus.

The next step was to develop a test for Trr to detect poor candidates for TAI. Pregnancy rates in heifers subjected to TAI vary from 28% to 55% [Small et al.] , in part due to premature estrus, failure to synchronize ovulation, and/or ovulation of follicles with poor fertility. The time between the increase in Tc and LH surge is more consistent than the drop in progesterone, increase estradiol and onset of behavioural estrus and LH surge. (Rajamahendran et al. 1989, Clapper et al. 1990, Mosher et al. 1990). Vaginal temperature (Tv) but not rectal temperature correlated with LH (Rajamahendran et al 1989). Therefore, our hypothesis was that cattle with no increase in Tc within 12 h of TAI are poor candidates for TAI ie. could be exempt from TAI .

Several combinations of criterion were used to define a peak in Tv and Trr, and Trr MF around the time of TAI. Tests were 6 baselines (2, 3, 4, 5 , 6, 7 d) and 6 possible deviations from baseline (current Tv or Trr is 0.2, 0.3, 0.4, 0.5, 0.6 °C or 2 x standard deviation from the average for that time over the previous 2 to 7 d), and 3 test times (51 h, 66 h and 72 h after PG3). True positive (TP) poor candidates for TAI were heifers that failed to conceive to TAI and were pregnant to natural service; true negative (TN) poor candidates for TAI were heifers determined pregnant to TAI and pregnant at veterinary palpation. A false positive (FP) test incorrectly classified a heifer as a poor candidate for TAI; a false negative (FN) test incorrectly classified a heifer as a candidate for TAI. The Receiver Operating Curve Analysis (Figure 6.3), a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity) determined the best tests to detect poor candidates for TAI where when the current Tv or Trr was 0.2° or 0.3°C above the average of the previous 3 d for that period. In otherwords, a heifer would be classified a poor candidate for TAI that afternoon (54 h) unless at least 1 peak in Tc occurred since PG3 (0 h); if the first test was positive then a heifer would be a poor candidate for AI the next morning (66 h) unless at least 1 peak in Tc occurred during the previous night and evening; if test 1 and 2 were positive then a heifer would be a poor candidate for AI the next afternoon (72 h) unless at least 1 peak in Tc occurred the previous morning. Eliminating heifers for TAI based on 1 test decreased the true positive rate whereas 3 tests increased the false positive rate. A 3 d baseline was best for Tv and Trr and although more poor candidates were identified when a peak in Tc was defined as an increase 0.3°C above 3-d baseline, an increase of 0.2°C reduced the number of heifers incorrectly deemed poor candidates for TAI and improved the positive predictive value of the test (Table 6.1). Improvement of the negative predictive value of the test around the time of TAI may be limited because of cattle may ovulate and conceive to TAI but fail to maintain pregnancy. Based on the ROC , MF did not qualify for further analysis probably because estrus was suppressed by progestin treatment and expression of estrus (increased activity) does not always occur before TAI.

Conclusion: The accuracy of the Tv and Trr tests to detect poor candidates ranged from 0.65 to 0.75, and agreement was fair between the number of forecasted and actual poor candidates for TAI (Table 6.1). Thus depending upon the cost of semen relative to the cost of days open either test could reduce breeding costs by reducing the number of poor candidates subjected to TAI.

Table 6.1 Sensitivity, specificity, accuracy, positive and negative predictive value of vaginal temperature (Tv) and reticular temperature (Trr) tests for poor candidates for fixed-time artificial insemination (TAI) and kappa measure of agreement between forecasted and actual poor candidates for TAI.

	Test criterion[1]					
	+0.2°C, 3 d, 2 times			**+0.3°C, 3 d, 2 times**		
	Synchronization group					
	Group 3		**1,2,3**	**Group 3**		**1,2,3**
	Tv	**Trr**	**Trr**	**Tv**	**Trr**	**Trr**
No. Heifers (N)	30	27	63	30	27	63
No. Pregnant (TP)	11	16	33	11	16	33
No. Not pregnant (TN)	16	10	27	14	7	19
Sensitivity (TP/TP+FN)	0.58	0.44	0.43	0.68	0.69	0.67
Specificity (TN/TN+FP)	0.85	0.91	0.90	0.73	0.64	0.63
Accuracy (TN+TP/N)	0.75	0.63	0.65	0.71	0.67	0.65
Positive predictive value[2]	0.69	0.88	0.83	0.59	0.74	0.67
Negative predictive value[3]	0.78	0.53	0.59	0.80	0.59	0.63
No. False positive (FP)	3	1	3	5	4	11
No. False negative (FN)	5	9	19	4	5	11
McNemars test of symmetry	0.479	0.011	<0.001	0.739	0.738	1.000
Kappa coefficient [4,5]	**0.40**	0.31	**0.31**	**0.37**	0.32	**0.30**
Lower 95% CL	0.06	0.02	0.11	0.03	-0.04	0.06
Upper 95% CL	0.74	0.59	0.51	0.70	0.67	0.54
Probability > Z (2-sided)	0.025	0.052	0.003	0.044	0.096	0.0173
PABAK [4,5]		0.25	**0.300**			
L95		-0.10	0.06			
U95		0.62	0.53			
Probability > Z (2-sided)		0.179	0.016			

[1] Deviation above baseline at 51 and 61 h after prostaglandin (refer to text)
[2] Positive predictive value = TP/(TP+FP_)
[3] Negative predictive value= TN/(TN+FN)
[4] PABAK , Prevalence adjusted bias adjusted kappa when the test for symmetry fails.
[5] Fair agreement when Kappa coefficient 0.21 to 0.40 and P<0.05.

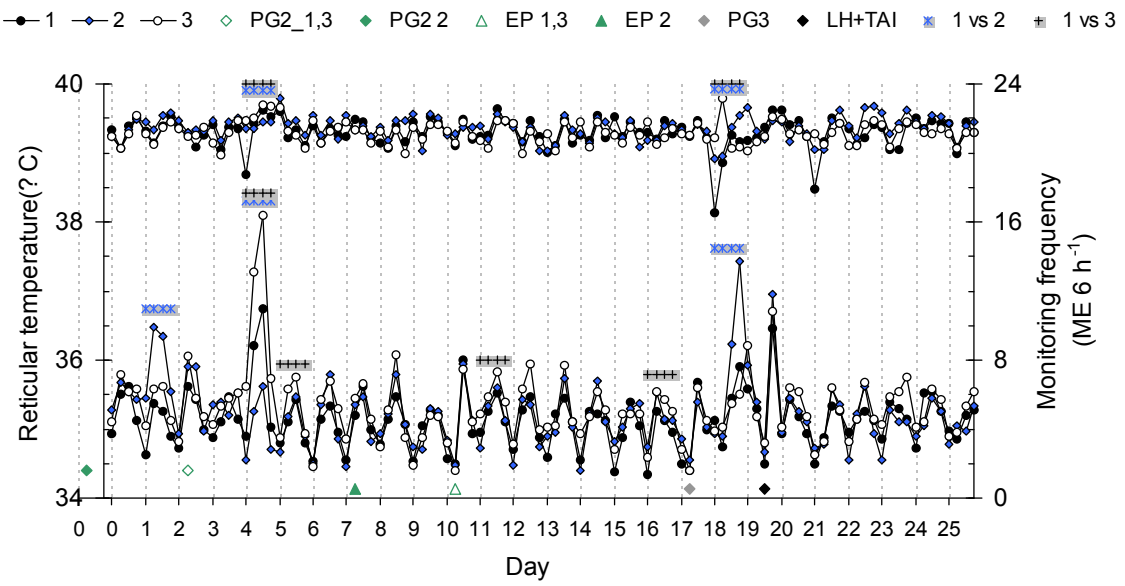


Figure 6.1. Least squares means for night, morning, afternoon and evening reticular temperature (Trr; top), and the frequency of passive monitoring of Trr (ME; bottom) in beef heifers subjected to synchronization protocols with (1,2) and without (3) estradiol and an intravaginal progesterone releasing device (CIDR) for fixed-time artificial insemination (TAI). All heifers were treated with prostaglandin F2-alpha (PG) and lutropin (LH) concurrent with TAI. * identify differences among treatments (P<0.05).

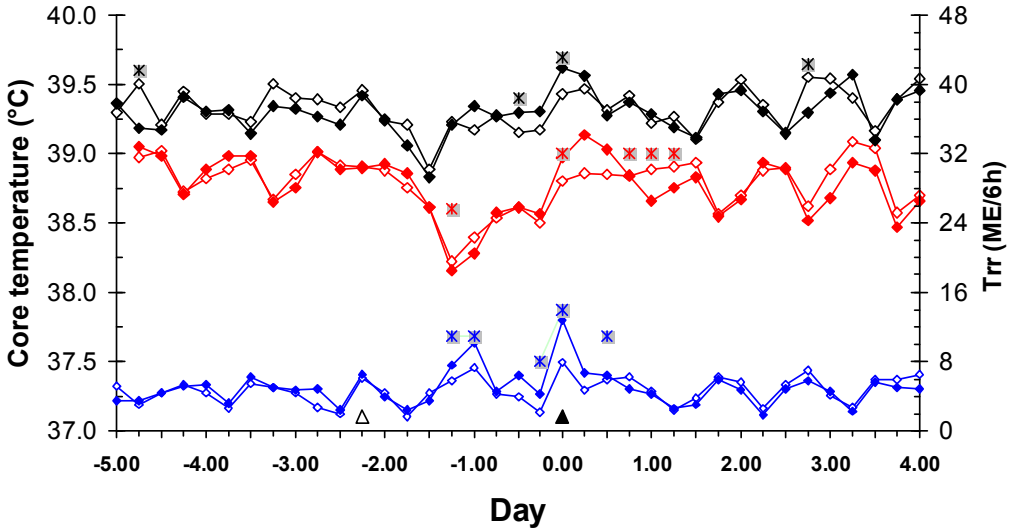


Figure 6.2. Least squares means for night, morning, afternoon and evening reticular temperature (Trr; top), vaginal temperature (Tv; middle) and the frequency of passive monitoring of Trr (ME; bottom) in beef heifers pregnant (solid symbols) and not-pregnant (open symbols) to fixed-time artificial insemination (TAI, afternoon Day 0). Time of treatments with PGF (open triangle) and LH concurrent with TAI (solid triangle) are shown. * pregnant differs from not-pregnant P<0.05.

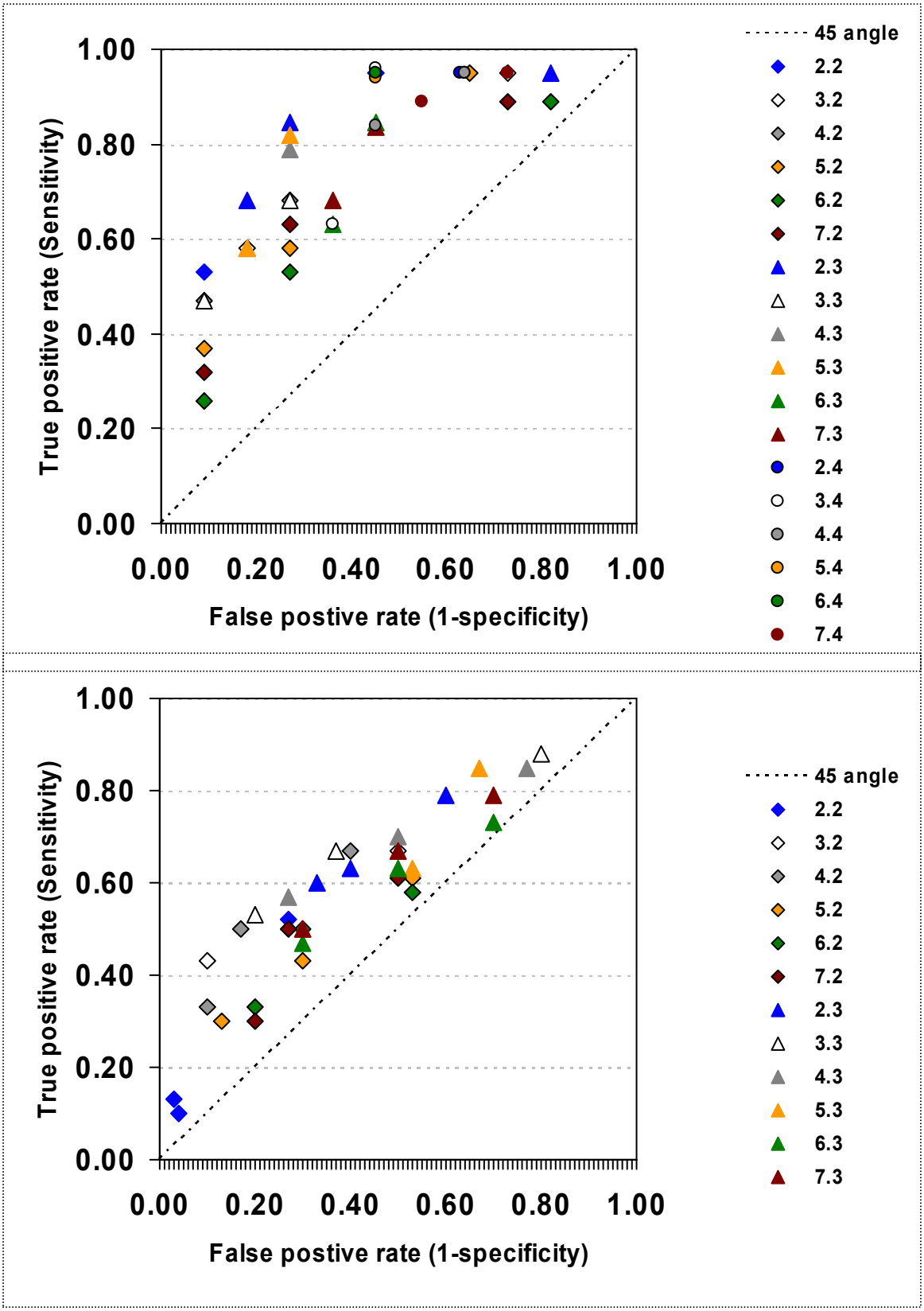


Figure 6.3. Receiver operating curve analysis of vaginal temperature (top panel) and reticular temperature (bottom) tests to detect poor candidates for TAI. The better the test the closer it falls to the upper left corner of the chart; the poorer the test the closer it is to the 45º line. The cluster in the upper right and lower left are the results when a test is done once or 3 times after PG3; best results were obtained with the 2 Test criterion (ie. Test 51 and 66 h after PG3).

Trr monitoring in beef cows to forecast calving (Experiment 7)

Objective: To determine if passive monitoring of reticulo-rumen temperature (Trr) can be used to detect the onset of calving in beef cattle.

Materials and Methods: The panel reader was set-up in the pre-calving shed/lot facility on February 4, 2006 for monitoring Trr each time heifers entered or exited the bedding area to feed, drink or lay down. Recording of acquisitions began February 6, 2006. The first study was conducted with 39 heifers pregnant to fixed-time artificial insemination (TAI) with semen from one sire on May 11, 2005. The 285 d expected due date was February 20, 2006. Heifers were given transponder boluses on January 27, 2006 and moved to the pre-calving facility. The second trial was conducted with 39 cows (and heifers) pregnant to natural service by bulls placed with cows following TAI. The cows were given transponder boluses on 27 March 2006 and observed for 16 d (to 12 April 2006). Cattle were given, once-daily in the afternoon, a total mixed ration that contained barley silage, chopped alfalfa brome-hay and a pelleted barley supplement containing vitamins A and E, minerals, and monensin (200 mg d; Rumensin).

The Trr acquisitions were processed as described previously to determine ME, MR and MF for four 6 h periods night (0000 to 0559), morning (0600 to 1159), afternoon (1200 to 1759) and evening (1800 to 2359). Experienced stockpersons used visual observations (4 x daily) of signs of parturition to determine when to move cattle from the study area to the calving facility. Weather data were obtained from the weather station at the Brandon Airport (Environment Canada 2010; station number 5010480) located at a similar elevation (409 m) to the pre-calving facility.

Univariate analysis and chi square tests were first done to compare Trr monitoring performance between the two experiments. Subsequently, Trr values less than 37.5°C were deemed influenced by drinking water and discarded from the calculations of mean Trr. Data were then subjected to analysis of variance (PROC MIXED) to determine the effects of parturition on prepartum Trr and to identify criterion for developing a test for Trr forecasting of parturition. Finally, data from cattle with complete Trr datasets for at least 10 d prepartum were used to test sets of criterion to forecast calving. The ROC analysis was used to identify the best test and that was subjected to the kappa test of agreement.

Results: Description of the cattle, Trr monitoring, calving and ambient conditions are shown (Table 7.1). Trr monitoring performance was similar between trials except that in trial 2 more observations were obtained at night and evening and fewer in the afternoon. In the first trial calving started earlier than predicted on February 5 (270 d gestation) and ended February 21 (286 d gestation). All calves were of normal birth weight (37 to 42 kg) and one set of twins was born. In the second trial 5 cows did not calve during the study period as predicted but were pregnant and served as "True negative" controls. During Trial 2, the average photoperiod was 3 h longer and ambient temperature (Ta) 16°C warmer than Trial 2.

Mean afternoon Trr were generally higher for Trial 2 than Trial 1, except at 6 d prepartum in Trial 1 Figure 7.1 (a). In both Trials mean Trr were the lowest 6 to 30 h prepartum. Mean daily Trr were greater for Trial 2 than Trial 1 except 1 d prepartum when Trr was lowest and MF highest in both trials (Figure 7.1 (b)). The differences in the distribution of acquisitions throughout the day and the higher afternoon Trr in Trial 2 compared to Trial 1 may have been due to the effect of increased photoperiod on cattle behavior as observed in Experiment 3, or warmer ambient temperatures. A separate ANOVA for Trial 2 determined mean Trr monitoring rates and mean Trr were similar ($P>0.10$) between prepartum cows (n=26) and heifers (n=8). However, in both trials, Trr decreased prior to parturition as reported for other measures of core temperature in prepartum cattle (lammoglia, Wrenn). Therefore, to forecast calving, sets of criterion were used to detect this decrease in Trr in individuals.

There were 27 cows with complete Trr datasets for 10 consecutive days; 22 cows that calved (true positive) and 5 cows pregnant that did not calve (true negative), and the average for night, morning, afternoon and evening (ME=803) were used to calculate am, pm and daily Trr baselines, respectively. Combinations of different criterion [2, 3, 4, 5 d baselines and various deviations from baseline] were tested for 5 days prepartum. Cattle handlers used visual signs with 85% sensitivity, 98% specificity, 96% accuracy and a kappa test measured excellent agreement (k=0.85; P<0.01) between observed and forecasted calving within 24 h. Receiver Operating Curve analysis determined the best Trr forecast of calving were when the current Trr, on two consecutive days, decreased with at least one ≥0.30°C below the average of the previous 2 d. When this test was restricted to calving within 2 d (Test 7) calving was forecasted with 54% sensitivity, 96% specificity, 88% accuracy and moderate agreement (k=0.55; P <0.01) between observed and forecasted calving. Restricting this test to within 5 d of calving (Test 23) improved sensitivity (62%), specificity (100%), accuracy (92%), and agreement strength was substantial (k=0.72; P<0.01). This is proof of concept that passive monitoring of Trr can be used to forecast parturition and could benefit farmers by helping with their labor management around calving

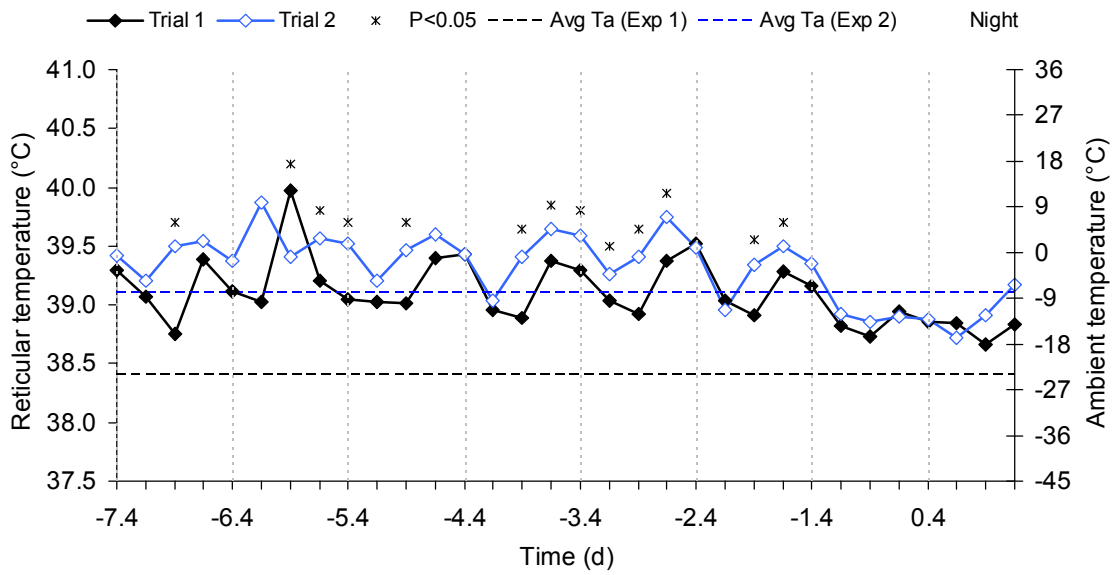
Conclusion: The accuracy Trr tests to forecast calving ranged from 0.88 to 0.92, and agreement with actual calving was substantial when forecasted within 5 d. Therefore, Trr can be used to forecast parturition and could benefit farmers by helping with their labor management around calving.

Table 7.1. Description of cattle and ambient conditions during the study to evaluate Trr monitoring to forecast parturition.

	Experiment 1	Experiment 2
Start date	06Feb	27Mar
No. pregnant	34	39
Duration of Trr monitoring (d)	15	16
Reticular temperature (Trr): No. monitoring events	2624	2293
Mean (± stdev)	38.42 ± 1.67	38.80 ± 1.46
Mode (Q1)	39.17 (38.38)	39.05 (38.69)
Range	26.39, 40.36	28.72, 40.44
Trr monitoring frequency (ME /d ± stdev)	8.4 ± 4.3	7.2 ± 4.4
No. of different periods of day (mean ± stdev)	2.68 ± 0.93	3.00 ± 1.01
Night(%)	9.2*b*	19.8*a*
Morning (%)	25.7	27.4
Afternoon (%)	49.9*a*	29.6*b*
Evening (%)	14.7*b*	23.1*a*
No. calved	34	34
First parity % (n)	100	21 (8)
Calving date (mean ± stdev)	14Feb ± 3	02Apr ± 5
Unassisted births % (unobserved %)	88 (50)[1]	91 (76)
Calf sex ratio (% Male)	62	65
Birth weight (kg) Male calves	36.8 ± 3	44.0 ± 5
Female calves	34.7 ± 4	42.0 ± 5
Period of day when calving occurred % (n):		
Night (0000 to 0559 h)	17.6 (6)	11.8 (4)
Morning (0600 to 1159 h)	38.2 (13)	32.3 (11)
Afternoon (1200 to 1759 h)	32.3 (11)	47.1 (16)
Evening (1800 to 2359 h)	11.8 (4)	8.8 (3)
Ambient conditions (mean ± stdev):		
Natural photoperiod (h)	10.1 ± 0.3	13.1 ± 0.3
Maximum ambient temperature (°C)	-8.7 ± 6.2	6.2 ± 3.6
Minimum ambient temperature (°C)	-20.6 ± 7.2	-3.4 ± 2.3
Mean ambient temperature (°C)	-14.7 ± 6.4	1.4 ± 2.0
Total snowfall (cm)	14.8 ± 1.5	1.8 ± 0.5
Snow on ground (cm)	28.2 ± 1.1	11.4 ± 4.6
Total precipitation mm (days with ppt)	10.2 ± 1.1 (9)	7.0 ± 3.5 (3)

[1] 2 stillbirths, one set of twins.
a-b means in rows with different letters are different (P<0.05)

(a)



(b)

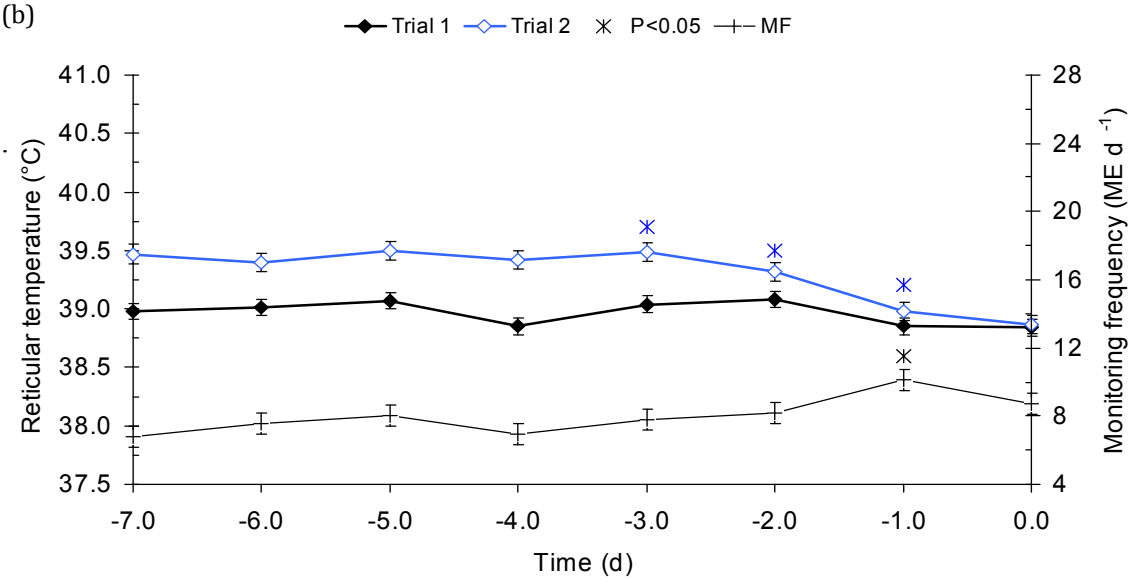


Figure 7.1 (a) Least squares means reticular temperature (Trr) in prepartum heifers (Trial 1) and cows and heifers (Trial 2). Afternoon Trr were generally higher for Trial 2 than Trial 1, except at 6 d prepartum in Trial 1. In both Trials mean Trr were the lowest 6 to 30 h prepartum. The average ambient temperature (Ta) during Trial 1 (cold) and Trial 2 (warm) are shown as dashed lines. (b) Least squares means Trr in prepartum heifers (Trial 1) and cows and heifers (Trial 2) and monitoring frequency (MF). Mean daily Trr were greater for Trial 2 than Trial 1 except 1 d prepartum when Trr was lowest and MF highest in both trials.

REFERENCES:

[1] Royal MD, Darwash AO, Flint APF, Webb R, Wooliams JA, Lamming GE, 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 70, 487–501.

[2] Lucy MC, 2001: Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 84, 1277–1293.

[3] Pryce JE,Royal MD, Garnsworthy PC, Mao IL 2003. Fertility in the high-producing dairy cow. Livestock Production Science 86 (2004) 125–135.

[4] Rozzi,P Miglior,F, Hand,KJ J 2007. A total merit selection index for Ontario organic dairy farmers. Dairy Sci 2007; 90(3): 1584-1593.

[5] Plaizier JCB, King GJ, Dekkers JCM, Lissemore K (1997) Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation. J Dairy Sci 80:2775-2783.

[6] Dekkers JCM, Ten-Hag HH, Weersink A. 1998. Economic aspects of persistency of lactation in dairy cattle. Livest Prod Sci 53(3): 237-252.

[7] Dobson H. Smith RF, Royal MD, Knight CH, Sheldon IM 2007. The high-producing dairy cow and its reproductive performance. Reprod. Dom. Anim 42:17-23.

[8] Roche JF 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim Reprod Sci 96:282-296.

[9] Fredeen AF, AstatkieT, Jannasch RW, Martuin RC 2002. Productivity of grazing Holstein cows in Atlantic Canada. J Dairy Sci. 85: 1331-1338.

[10]Ambrose DJ, Kastelic JP, Corbett R, Pitney PA, Petit HV, Small JA, Zalkovic P. 2006. Improved Embryo Survival and Calving Rates in Lactating Dairy Cows Fed a Diet Enriched in a-Linolenic Acid. J. Dairy Sci. 89: 3066-3074.

[11]Petit HV, Small JA, Palin MF, Giguère A, Santos, G.T.D. 2007. Estrous synchronization, ovarian function and expression of interferon stimulated protein, and milk yield in primiparous dairy cows fed flaxseed Can. J. Anim. Sci. 87: 342-352.

[12]Pursley JR, Mee OM, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2A and GnRH. Theriogenology 1995; 44:915-923.

[13]Redden KD, Kennedy AD, Ingalls JR and Gilson TL. 1993. Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. J. Dairy Sci. 76: 713-721.

[14]Kyle BL, Kennedy AD, Small JA. 1998 Measurements of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. Theriogenology 49:1437-1449.

[15]Lammoglia MA, Bellows RA, Short RE, Bellows SE, Bighorn EG, Stevenson JS, Randel RD. 1997. Body temperature and endocrine interactions before and after calving in beef cows. J. Anim. Sci. 75(9): 2526-2534.

[16]Brown-Brandl TM, Yanagi TJr, Xin H, Gates RS, Bucklin RA, Ross, GS. 2003. A new telemetry system for measuring core body temperature in livestock and poultry. Appl. Eng. Agric. 19(5): 583-589.

[17]Wrenn TR, Bitman J, Sykes JF 1958. Body temperature variations in dairy cattle during the estrous cycle and pregnancy. J Anim Sci 17: 1071-1076.

[18]Piccione G, Caola G and Refinetti R. 2003. Daily and estrous rhythmicity of body temperature in domestic cattle. BMC Physiology 3:7-15.

[19]Rajamahendran R, Robinson JA, Desbottes S., Walton, J. 1989. Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels and ovulation in dairy cows. Theriogenology 31:1173-1182.

[20]Small JA, Kennedy AD, Kahane SH. 2008. Body temperature monitoring with passive transponder boluses in beef heifers. Can. J. Anim. Sci. 88:225-235.

[21] Del Vecchio RP, Sutherland WD, Connor LM. A solid phase enzyme-immunoassay for progesterone determination in plasma. Can J Anim Sci 1995; 75:525-529.

[22] Colazo, M. G., Ambrose, D. J., Kastelic, J. P. and Small, J. A. 2008. A comparison of two enzymeimmunoassays to a radioimmunoassay for measurement of progesterone concentrations in bovine plasma, whole milk and skim milk. Can. J. Vet. Res. 72:32-36.

COMMUNICATIONS STRATEGY:

Two conference presentations and two scientific publications remain to be completed. A presentation on forecasting calving is scheduled for May 4-5, and pending assistance from students would expect next year to have one on estrus detection; manuscripts for scientific publication will follow conference presentations. Based on the work funded by ARDI the research has expanded to application to Dairy Cattle, and 3 large projects in Canada and USA.

PUBLICATIONS:

Peer reviewed Scientific
Small, J. A., Kennedy, A. D. and Kahane, S. H. 2008. Body temperature monitoring with passive transponder boluses in beef heifers. Can. J. Anim. Sci. 88:225-235.

Conference proceedings
MacDonnell, S. and Small, J. A. 2011. The use of reticulo-rumen temperature (Trr) monitoring in beef cows to forecast calving. Proceedings of the Canadian Society of Animal Science Annual Meeting May 4-5, Halifax NS.

Small J. A.,Kennedy A. D., Pfeifer L. M., Singh J. 2009. Predicting the success of fixed-time AI from passive monitoring of body temperature in beef heifers. Joint Annual Meeting of the Canadian and American Societies of Animal Science and American Dairy Science Association Montreal QC July 6-11. J. Anim. Sci. Vol. 87, E-Suppl. 2/J. Dairy Sci. Vol. 92, E-Suppl. 1 pg 582
http://adsa.asas.org/meetings/2009/abstracts/0581.PDF

Extension presentations:

Small, J. A. 2008. Electronic monitoring of body temperature in heifers. Fridays @ 3 Seminar Series Nova Scotia Agricultural College, Truro, NS, January 11.

Small, J. A. 2007. Body temperature monitoring with transponder boluses in beef heifers. AAFC-BRC Scientists Seminar Series Brandon, MB, April 12.

Small, J. A. 2006. Cow/calf production systems. Crops, Cows and Conservation. Winter science Workshop, Brandon, Jan 27, 2006. (available on DVD)

Reports
Small, J. A. 2005. Read rates of radio-frequency identification (RFID) boluses in beef heifers (Datatic, Inc Dec 2005)

Kennedy, A. D. and Small, J. A. 2005.Electronic Tracking of Cattle Identification (RFID) and Core Body Temperature. (ARDI Project 04-632; submitted Dec 16, 2005)

APPENDIX I

PROJECT VARIANCES:

2010 - S. MacDonnell, 4th year undergraduate student completed the analysis of the data to forecast parturition.
2007 - A. D. Kennedy retired, although Bioinformatics data were received there was no longer the expertise to interpret them. Therefore, J. A. Small developed programming using the Statistical Analysis System (SAS).
2007 - J. A. Small transferred from AAFC-Brandon to AAFC-Kentville/Truro
2006 - DataTic Technologies no longer replied to communications.

The project variances have delayed publication of the results as full scientific papers. However, studies to develop the application of the Trr monitoring system had only been conducted in the United States (CO; 42ºN) with large free-stall managed dairy cows that pass the panel reader upon entry and exit of the milking parlor (twice daily). Dr Small discovered a way for the system to work in beef cattle, where daily activity is not entrained to the degree it is in dairy cattle, and housing is generally outdoors all year round, especially in western Canada. At the AAFC-Brandon (49ºN), Dr Small developed two outdoor installation designs where growing heifers would be self- motivated to pass the panel reader. Dr Small also developed the math and terminology to describe "self-motivated" monitoring, providing benchmarks for subsequent development of the system for loose housed cattle. In these first studies, Dr Small demonstrated that most heifers were monitored daily (Monitoring rate 100%), several times per day (Monitoring frequency 7.8 ± 0.5), mostly during the afternoon and evening rather than night and morning 6-h periods. Most Tb acquisitions (average 37.8 ± 0.2°C, range 22 to 42°C) were within the expected range and extremes below normal were caused by drinking the cold (4°C) water. The circadian rhythm in monitoring frequency and Tc were affected by subjecting cattle to an endotoxin (induced-fever), different photoperiod and pen management treatments. Dr Small also reported a 19% drop-out rate for the boluses which could be overcome in most cases by administering a second bolus. The panel reader ambient temperature sensors were not reliable but did not impair the Trr monitoring function of the system. The first four experiments provided the foundation for Dr Small to continue development of this tracking and alerting system for cattle. Subsequently, Dr Small developed tests to apply Trr monitoring to detect pubescent estrus, identify poor candidates for fixed time artificial insemination (TAI) and forecast calving.

This work has provided the foundation to continue development of this tracking and alerting system for application to cattle operations. In fact this work lead directly to collaborative research with DVM Systems, LLC, Greely CO and Colorado State University, Boulder CO to develop the Trr monitoring for application to dairy cattle.