Detection of ovulation with passive monitoring of reticulo-rumen temperature (Trr)

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Background:

Fertility and Ovsynch

Fertility in dairy cattle has declined as milk yields have increased rapidly over the past 50 y [1, 2]. Failure to conceive to first service and maintain pregnancy reduced profitability of conventional [3] and pasture-based (organic) [4] dairy farms by prolonging the calving interval. Longer intervals to first service were economical in cows that maintained high milk production beyond peak yield (high persistency) and a calving interval of 13 mo [5]. Constraints to fertility are postpartum anestrus, poor oocyte/embryo quality, uterine infection, and clinical problems [6]. Cows that did not ovulate within 45 d postpartum, compared to those that did, required 2-fold more services per conception, and 3-times more days to conception [7]. In addition to anestrus, the most prevalent problem is accurate estrus detection (<60%) and correct timing of AI [8]. Synchronization protocols to facilitate first service on appointment (TAI) without estrus detection reduced days to first service [9].

The Ovsynch protocol for TAI [10] has been adopted for reproductive management of dairy herds world-wide. The protocol consists of two treatments of GnRH given im 9 days apart, prostaglandin F2-alpha (PGF) im on the 7th day, and TAI 16 to 18 h after the second GnRH treatment. However, cows were more likely to conceive to TAI when Ovsynch started on the 5th to 12th day of the estrous cycle [11]. Presynchronization with two treatments of PGF given im 14 d apart, and Ovsynch started 12 d after the second PGF, improved TAI pregnancy rates [12]. Therefore, the knowledge of when (or if) cows ovulated during the voluntary waiting period could be used to improve dairy fertility to TAI.

Core temperature and ovulation

The time between the increase in core temperature (Tc) and LH surge is more consistent than the drop in progesterone, increase estradiol and onset of behavioural estrus and LH surge [13, 14, 15]. Manually measuring Tc, once, twice and 4-times daily, at a consistent over several days detected peaks and troughs in Tc around the time of estrus [16,17 and ovulation [13] but this is somewhat invasive and obviously of limited practical value. Early radio-frequency technology used battery- powered transponders to facilitate remote non-invasive monitoring of Tc at pre-programmed intervals to detect estrus [18,19, but the systems were of limited practical value because of the cost and animal welfare issues with prolonged use. Lower cost radio-frequency technology using passive transponders (no battery required) has been applied on-farm uniquely identify farm stock as part of the protocols to ensure food safety. Since January 2005, all Canadian cattle that leave the farm must have the Canadian Food Inspection Agency (CFIA) approved radio-frequency identification (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. This 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. In addition, the magnet could facilitate easy retrieval of transponders from offal at the abattoir.

The cow temperature monitoring system (Phase IV Engineering, Boulder CO) uses magnetic, inductively coupled full duplex transponder boluses containing thermistors, a panel reader (transceiver) and acquisition software. The panel reader activates the transponder boluses from a distance of several meters, acquisitions occur as an individual passes within 1 m of the panel reader, and the software records identification and reticulo-rumen temperature (Trr).

Trr monitoring in beef heifers

The first experiments in beef heifers (7 to 14 mo) gave us confidence in the potential for Trr monitoring to be valuable in routine cattle management [20, 21]. One monitoring event was defined as the average of acquisitions (between 37.5º C and 43.0ºC) in one minute, and 4x daily monitoring as the average of all ME for 6 h periods of day. The daily Trr monitoring rate [100 (number of days with acquisitions between divided by the number of monitoring days)] was 100% when acquisitions were coincident with an entrained behaviour (feed delivery at 1500 h) and boluses were not regurgitated. The mean Trr values were approximately 1ºC higher when dissociated from drinking, and fever was detected when monitoring was scheduled [20]. Furthermore, Trr was approximately 0.5ºC higher and correlated (r=0.80) with vaginal temperature (Figure 1;21], and vaginal temperature, but not rectal temperature correlated with LH [13].

Trr monitoring in dairy cattle
Initial experiments with Trr monitoring in dairy cattle began in 2009 at a commercial dairy farm (Charles Hill and Sons, Onslow, NS). Cows housed in free-stalls are milked twice daily (0300 and 1500) in a double-12 herringbone type parlor. The Trr acquisitions occur as cows pass individually through either of two parlor exit alleys.

The first objective is to determine the sensitivity, specificity, positive and negative predictive value of Trr monitoring to detect ovulation. Milk samples were taken twice weekly for progesterone analysis [22] to determine the true number of ovulations in Trr monitored cattle. Progesterone analysis is in progress. To date based on ovulation confirmed by pregnancy, the results have provided proof of concept that Trr monitored following the morning and afternoon milking detects ovulation in dairy cows. The sensitivity, specificity and accuracy were similar to detection with telemetric monitoring of Tv [18] when ovulation was known. The negative predictive value was strong and therefore the Trr test for ovulation could identify anovular cattle.

The second objective is to validate the Trr detection of ovulation and test the application to improve fertility to Ovsynch. To achieve this goal more cattle with Trr monitoring are required. It is proposed that the experiment with 72 cows at the Hill Farm,

be replicated with 800 cows at a large commercial dairy (Shelton Farm, Greely, CO). This would require the participation of Colorado State University to ensure adherence to protocol and accuracy of data. At the Shelton Farm, cows are milked four-times daily for the first 35 d and three-times daily thereafter, and a panel reader acquires Trr as cows pass individually through the entrance to the double-30 herringbone type milking parlor. It is unknown if the proportion of aberrant Trr (due to drinking and/or activity) will differ by timing acquisitions before or after milking. At the Hill Farm, cows are held unrestrained in the milking stalls for at least 10 min before passing a panel reader. Indeed, the twice-daily Trr monitoring rate was 94% over 90 d. However, the greater frequency of milking at the Shelton Farm may minimize the influence of aberrant acquisitions on Trr monitoring.

Objectives:

(1) to validate Trr detection of ovulation in dairy cattle
(2) to determine the application to improve pregnancy rate to Ovsynch

Methodology:

General:

In addition to the cited studies monitoring Tc, the protocol for validating Trr detection of ovulation at the Hill Farm is based primarily on a study to validate the use of ultrasonography to identify anovular dairy cows [23] and a study of progesterone defined stages of the estrous cycle in relation to TAI pregnancy rate [24].

Cows grouped by calving date (weekly) and parity (primiparous and multiparous) will be given a transponder *per os* within 24 h of calving. The unique ID of the transponder bolus will be linked to the cow's visual tag and birth date to ensure accurate collection of health records (treatments for postpartum diseases, observations of standing estrus, AI services, pregnancy and milk yield).

Cows with Trr monitoring will be subjected to the Ovsynch protocol (GnRH on Days 0 and 9, PGF on Day 7 and TAI on Day 10 64-66 h after PGF) following the voluntary waiting period. At the Hill Farm, cows observed in standing heat between 60 and 75 days in milk (DIM) are inseminated, otherwise cows are subjected to the Ovsynch protocol starting at 74 days in milk (DIM). At the Shelton Farm cows, are subjected to the Presynch-Ovsynch protocol [12] beginning at 72 DIM (to be confirmed). Two doses of PGF are given 14 d apart and Ovsynch started 12 d after the second PGF.

Milk or blood samples for progesterone analysis will be taken on Days -12, -5, 0, 7, and 10. The true stage of the estrous cycle on Day 0 will be determined from tissue progesterone concentrations on Days -12, -5, and 0, and from ultrasonographs of the ovaries on Day 0 [23, 24]. Synchronization of luteolysis (PGF responder rate) will be

determined from tissue progesterone concentrations on Days 7 and 10. Synchronization of ovulation for TAI will be determined from tissue progesterone concentrations on Days 10 and 17. At the Shelton Farm, cows are administered GnRH 7 d before pregnancy determination to prepare non-pregnant cows for resynchronization.

Procedures specific to the Hill Farm:
Cows that become eligible from the previous Fri to Thu will start Ovsynch on the following Tuesday (PGF the following Tuesday). All GnRH and PGF treatments will be administered at 1700 (following milking), and TAI will be done on Fridays at 0900 (following milking). All TAI will be done by the herd reproduction manager who will also maintain records in the Farm database (SCOUT). Pregnancy determinations will be done by the Hill Farm veterinarian no earlier than 35 d after TAI. The GnRH and PGF treatments and ultrasonography will be done by Agriculture and Agri-Food Canada staff. All milk samples for progesterone analysis will be taken during the afternoon milking. The TAI are scheduled from September 24th to December 10th. Non-pay operating costs after panel reader installation estimated at $220 per cow at Hills.

True ovulations:

Progesterone concentrations equal to or greater than 1.0 ng/mL in serum or will be deemed indicative of luteal function. Progesterone concentrations will be assigned to one of two categories indicative of CL function: non-luteal (<1 ng/mL) and luteal (≥1 ng/mL).

The true number of ovulations and day of the estrous cycle before Ovsynch will be determined from tissue progesterone concentrations and ultrasonographic examination of the ovaries. Cows with luteal progesterone concentrations in one or more samples on Days -12, -5, and 0 will be classified cyclic, and those with all non-luteal progesterone concentrations will be classified anovular. In addition to progesterone, stage of the estrous cycle will be identified from ultrasonographic determination of the prevalence and diameter of CL, and follicles ≥8 mm on Day 0. Cows with progesterone ≥1 ng/mL on Day 7 and <1 ng/mL on Day 10 will be deemed PGF responsive (synchronized luteolysis).

True ovulation rate following TAI will be determined from tissue progesterone concentrations; (<1 ng/mL on Day 10 and ≥1 ng/mL on Day 17) and pregnancy. True ovulations will also be determined from cows that conceive to AI following observation of standing heat.

In beef heifers, Trr and Tv detected an increase in core temperature 2.5 d following estradiol/progesterone treatment to synchronize follicular wave emergence (unpublished results). An increase in Trr may occur following the first GnRH treatment, but additional ultrasonography (Day 7 and 10) to verify the concurrence with ovulation is not necessary to meet the objectives.

Expected outcomes [based on 11, 12, 23-25]:

- 84% of cows cycling after 2nd PGF;
- 64% ovulate to the 1st GnRH administered without regard to day of the estrous cycle
- 80% ovulate following the 2nd GnRH
- 32 to 42 % TAI pregnancy rate depending upon preovulatory follicle size; up to 52% with successful presynchronization

Statistical Analysis

Acquisitions will be downloaded twice weekly to determine Trr monitoring rate. Trr detection of ovulation will be done retrospectively. Different combinations of criterion to define Trr baseline and deviation from baseline will be subjected to Receiver Operating Curve Analysis to identify the set of criterion with the best compromise between sensitivity and specificity for detection of ovulation. The strength of agreement (based on kappa test) between the gold standard and Trr monitoring will demonstrate the use of Trr monitoring to select cows most likely to respond to the Ovsynch protocol, and secondly to identify cows that did not respond to the Ovsynch protocol.

Literature Cited:

[1] Royal MD et al. 2000.Anim Sci 70, 487–501.
[2] Lucy MC, 2001: J Dairy Sci 84, 1277–1293.
[3] Pryce JE et al. 2004. Livest Prod Sci 86:125–135.
[4] Rozzi,P et al. 2007. Dairy Sci 90: 1584-1593.
[5] Dekkers JCM et al 1998. Livest Prod Sci 53(3): 237-252.
[6] Dobson H. et al. 2007. Reprod. Dom. Anim 42:17-23.
[7] Taylor VJ et al. 2003. Theriogenology 59:1661-1677.
[8] Barr HL, 1975. J Dairy Sci. 58:246.
[9] Pursley JR et al 1995. Theriogenology 44:915-923.
[10] Pursley JR. 2007. In: Proceedings of the AABP annual meeting, 40:44–9.
[11] Vasconceles, J. L. M., et al. 1999.Theriogenology 52: 1067–1078.
[12] Moreira F, et al. 2001. J Anim Sci 2001;84:1646–1659.
[13] Rajamahendran R et al. 1989. Theriogenology 31:1173-1182.
[14] Clapper JA, et al. 1990. Anim. Reprod. Sci. 23:89-.
[15] Mosher MD, et al. 1990. Anim. Reprod. Sci. 23:99-.
[16] Wrenn TR et al. 1958. J Anim Sci 17: 1071-1076.
[17] Piccione G et al. 2003. BMC Physiology 3:7-15.
[18] Redden KD et al. 1993. J. Dairy Sci. 76: 713-721.
[19] Kyle BL et al. 1998. Theriogenology 49:1437-1449.
[20] Small JA et al. 2008. Can. J. Anim. Sci. 88:225-235.
[21] Small JA et al. 2009. JAM09 Montreal QC July 6-11.
[22] Colazo, M. G. et al. 2008. Can. J. Vet. Res. 72:32-36.
[23] Silva E. et al 2007. J. Dairy Sci. 90:3255-3262.
[24] Cartmill J. A. et al. 2001. J Dairy Sci. 84:1051-1059.
[25] Ambrose DJ et al. 2006. J. Dairy Sci. 89: 3066-3074

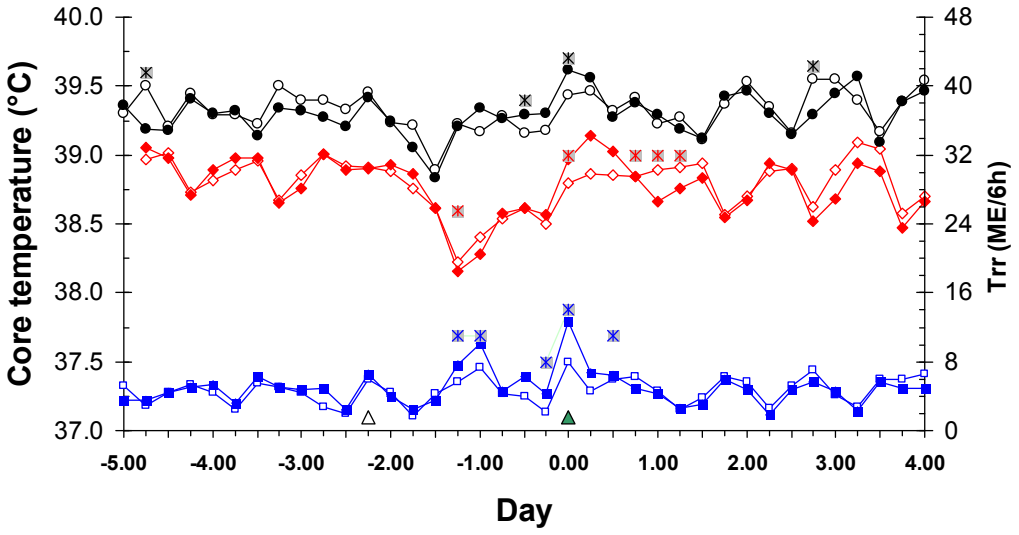

Figure 1. Least squares means for reticular temperature (Trr, circles), vaginal temperature (Tv, diamonds) and frequency of voluntary Trr monitoring in beef heifers determined pregnant (n=33; solid markers) and not pregnant (n=30; outline markers) to TAI. Time of prostaglandin F2-alpha (outline triangle) and LH (solid triangle) treatments are shown. Astericks (*); pregnant differs from not-pregnant $P<0.05$. Trr was the average of ME during four periods of day beginning 0000; Tv was logged every 15 min and the average taken for the four periods of day corresponding to Trr.

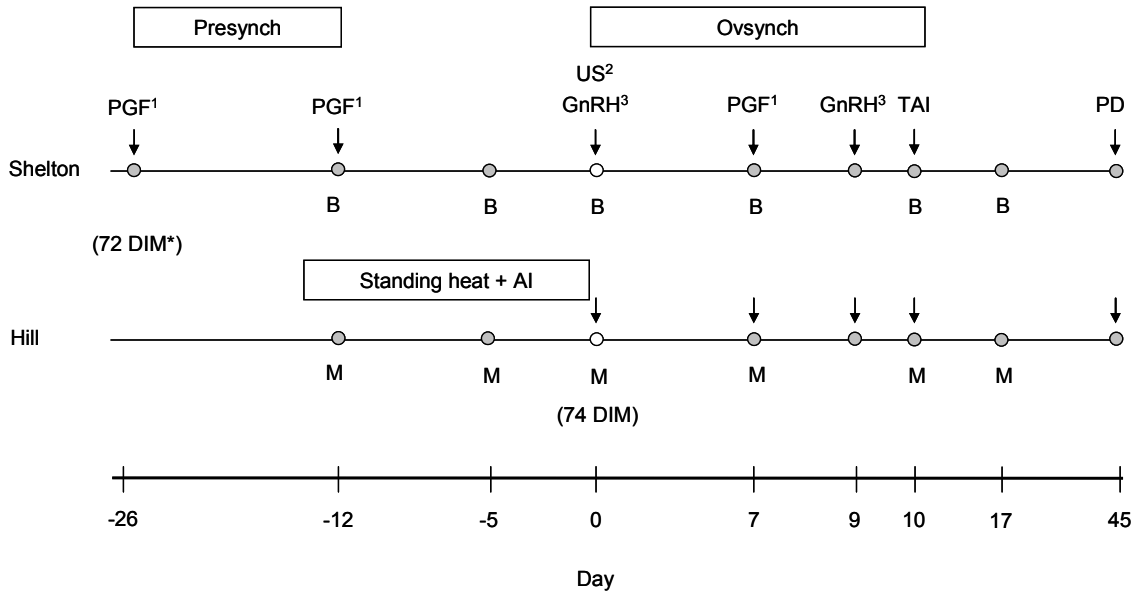

Figure 2. Treatment days (circles) and sampling schedules are shown for dairy cows following the voluntary waiting period (DIM, days in milk) at the Hill Farm and Shelton Farm. Indicated, as specified in the methodology are days of ultrasonographic (US) examinations of ovarian structures for CL and follicle development (open circles), veterinary palpation for pregnancy determination (PD), and blood (B) or milk (M) sampling for progesterone analyses.

[1] 500 ug cloprostenol im (Estrumate; Schering Plough Animal Health, Pointe Claire, QC)
[2] ultrasonography (Aloka SSD 500 with 7.5 mHz linear array transducer; ISM Inc, Edmonton, AB) of ovarian structures; open circles
[3] 100 µg gonadotropin releasing hormone im (Cystorelin; Merial Canada Inc., Baie D'Urfé, QC)
[4] fixed-time artificial insemination 64 to 66 h after PGF
[5] pregnancy determination by veterinary palpation
[6] blood sample for progesterone analysis
[7] milk sample for progesterone analysis
DIM* start date for Presynch-Ovsynch to be confirmed at Shelton Farm.