Changes in Rumen Temperature, Vaginal Temperature and Drinking Behaviour throughout the Estrous Cycle in Tie-Stalled Dairy Cattle

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by Suzan R. Mathew

In Partial Fulfillment of the

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

ABSTRACT

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The relationship between vaginal temperature, rumen temperature and drinking behaviour throughout the estrous cycle was studied in tie-stalled dairy cattle. In experiment 1, 4 first calf Holstein dairy heifers (71 to 161 d postpartum) that were fitted with rumen cannulae were monitored for a continuous 3 month period. Radiotelemetric temperature radiotransmitters were held in the vagina and rumen temperatures were monitored by temperature radiotransmitters placed in weighted plastic jars tethered in the rumen. Milk samples for progesterone analysis were taken during the morning milking 4 times weekly to identify periods of CL regression. The heifers were synchronized using a program called Ovsynch. An intramuscular injection of GnRH (100 μg) was administered at a random stage of the estrous cycle. The heifers then received an intramuscular injection of $PGF_{2\alpha}$ (500 μg) seven days later, and a second GnRH injection 48 h after that. The heifers were synchronized for estrous cycles 1 and 3, while estrous cycle 2 was not synchronized. The frequency and duration of drinking and feeding behaviours were recorded visually at 1 min intervals for 4 h after the provision of feed from the day of $PGF_{2\alpha}$ (day 60) to the day of insemination (day 63) in estrous cycle 3. All heifers were artificially inseminated (AI) 24 h after the second GnRH injection of cycle 3. In experiment 2, 26 Holstein cows, aged 3 to 6 yrs, were allocated to 3 trials lasting 20 days each. Cows in each trial were synchronized by either the Ovsynch protocol (GPsyn) (trial 1, n = 4; trial 2, n = 5; trial 3, n = 4) or

double $PGF_{2\alpha}$ administered 14 days apart (Psyn) (trial 1, n = 5; trial 2, n = 4; trial 3, n = 4). Watering behaviour was measured continuously in 15 cows in trials 1 and 2 (trial 1, n = 7; trial 2, n = 8) by monitoring changes in waterpipe temperature during periods of waterbowl activation. Vaginal temperature was monitored for Psyn cows in trials 2 and 3 as in experiment 1. Rumen temperature was monitored in 2 Psyn cows in trial 3 as in experiment 1. Visual observation of standing heat was performed after $PGF_{2\alpha}$ (day 15) in Psyn cows for trials 1 and 3 for a daily 3 h period in an outdoor exercise paddock. Psyn cows in trials 1 and 3 were bred 2 to 5 h after observation of standing heat, while Psyn cows in trial 2 underwent timed insemination 74 to 77 h after $PGF_{2\alpha}$. GPsyn cows in all trials were inseminated 26 to 29 h after the second injection of GnRH of the Ovsynch protocol. Milk samples for progesterone analysis were taken during the morning milking 4 times weekly as in experiment 1.

Milk progesterone analysis indicated 13 periods of low progesterone indicative of CL regression in experiment 1, of which vaginal temperature data (VagT) was available for 8 periods and rumen temperature (RumT) was available for 7 periods. Eight increases in VagT were detected during the 8 periods of low progesterone (true positives) and 20 false positives were detected. Five increases in RumT were detected during the 7 periods of low progesterone (true positives) and 26 false positives were detected. In experiment 2, eight periods of low progesterone (below 5 ng ml^{-1}) were detected. There were 8 increases in VagT that coincided with these periods of low progesterone (true positives), and 5 false positives were found overall. There were 2 increases in RumT that coincided with these periods of low progesterone (true positives), and 3 false positives were found overall. RumT does not appear to reflect

changes throughout the estrous cycle as well as VagT, as there were fewer true positives and more false positives. The magnitude of the RumT increase was similar between experiments at 0.5 ± 0.09°C in experiment 1 and 0.6 ± 0.08°C in experiment 2. The magnitude of the VagT increase was also similar between experiments at 0.3 ± 0.03°C in experiment 1 and 0.3 ± 0.07°C in experiment 2. The low magnitude of the true positive increase in VagT was persistent despite differences in exercise routine, days postpartum and hormone synchronization method. Six of 8 Psyn cows had a true positive increase in VagT one day before or the day of insemination in experiment 2, which corresponded to day of estrus. The 2 true positive increases in RumT in experiment 2 occurred on the same day as the true positive increase in VagT, and corresponded to day of estrus as well. The pregnancy and calving rates were 1 out of 4 for experiment 1. Pregnancy rates in experiment 2 were 1/4 for GPsyn and 2/5 for Psyn in trial 1, 4/5 for GPsyn and 2/4 for Psyn in trial 2 and 1/4 for GPsyn and 1/3 for Psyn in trial 3. Feeding and drinking behaviours were lower on the day before and the day of AI than on the 2 previous days of the cycle in experiment 1 when behaviours were monitored for a 4 h period daily. Watering behaviour was assessed by continuous electronic monitoring of waterpipe temperature. In experiment 2, GPsyn cows in trial 2 exhibited decreased watering behaviour on the day of $PGF_{2\alpha}$ followed by a compensatory increase on the following two days that preceded ovulation. There was no indication of changes in watering behaviour over time in Psyn cows. Watering behaviour did not appear to reflect changes throughout the estrous cycle as well as VagT or RumT.

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LIST OF ABBREVIATIONS

Abbreviations	Name
AI	Artificial Insemination
GnRH	Gonadotropin-Releasing Hormone
$PGF_{2\alpha}$	Prostaglandin-$F_{2\alpha}$
LH	Leuteinizing Hormone
FSH	Follicle-Stimulating Hormone
CL	Corpus Luteum
VagT	Vaginal Temperature
RumT	Rumen Temperature
SD	Standard Deviation
FP	False Positive
TP	True Positive
EDR	Estrus Detection Rate
Psyn	Double $PGF_{2\alpha}$ synchronization program
GPsyn	GnRH - $PGF_{2\alpha}$ synchronization program
EDO	Expected Day of Ovulation
WD	Watering Duration
WDa	Adjusted Daily Watering Duration

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INTRODUCTION

Intensive management practices have dramatically increased dairy herd size, and thus less time is available for conscientious observation of estrus. In fact, up to 40% of estruses are missed due to poor detection methods (Kristula et al. 1992). Technology is being used as a way to improve estrus detection rates, essentially replacing the human observer with an artificial one. Some of the first devices used for estrus detection involved the use of encapsulated dyes, which were released when an estrual cow was mounted. The technology later evolved to include pressure-sensitive transmitters that recorded the time and duration of mounting activity and sent the data to a computer. Other estrus detection aids involved measuring changes in locomotor activity (e.g. pedometers). Devices that measure either mounting or locomotor activity appear to be the most commercially viable, particularly since they are fitted externally.

The use of other methods of estrus detection (such as measurement of vaginal conductivity or body temperature) has been limited to research purposes likely due to their more invasive natures. Monitoring changes in body temperature (particularly vaginal temperature) has proven to be a reliable estrus detection method. Peaks in body temperature have been recorded on the day of observed estrus in both naturally cycling cattle (Redden et al. 1993; Kyle et al. 1998) and those synchronized with $PGF_{2\alpha}$ (Rajamahendran et al. 1989). Body temperature sensing could be made more commercially viable if the monitoring devices could be placed internally only once, and then work both continuously and accurately for the lifetime of the animal. An obvious site of placement would be the rumen as a balling gun could be used to internalize the

device. However, there have been no studies of rumen temperature throughout the estrous cycle.

A reduction in feed intake at estrus resulting in decreased milk yield of dairy cattle has been documented (Walton and King 1986; Schofield et al. 1991). It is likely that along with reduced feeding there is a reduction in the drinking of water at estrus, but this has never been studied. The main objectives of this study were to determine the usefulness of rumen temperature and drinking behaviour as either estrous or ovulation predictors in synchronized dairy cattle when compared to vaginal temperature.

LITERATURE REVIEW

Estrus is defined as the period of time when the female is receptive to the advances of the male and will stand for the purpose of mating (Bearden and Fuquay 1992). It is a phenomenon with both behavioural and physiological components. There is no exposure to bulls in commercial dairy herds, so the behaviours characteristic of proestrus, estrus and metestrus are exhibited towards other cows. True estrus is standing to be mounted (standing heat). A proestrus cow will exhibit such behaviours as resting its chin on another cow, following a cow in estrus, having her genitalia sniffed by another cow, increased vocalizing, body rubbing and head butting (Albright and Arave 1996). A proestrus cow may also mount cows in estrus. The physiological components of estrus involve changes in hormone levels, an increase in physical activity such as walking, and localized increases in internal body temperature.

Hormonal Regulation of Estrus

In normally cycling cattle, an estrous cycle is an approximately 21 d period of time during which growth of the ovarian follicle and subsequent ovulation of the oocyte occurs. The estrous cycle is divided into 4 periods. Estrus is the period of standing heat and is reported to last between 12 to 18 h (Bearden and Fuquay 1992). Metestrus immediately follows, with ovulation occurring 10 to 12 h after estrus. Corpus luteum (CL) formation and growth then takes place and lasts for 3 to 4 d. Diestrus lasts 10 to 14 d and is characterized by intense production of progesterone by the CL. Proestrus is a 3 to 4 d period where rapid follicular growth occurs before estrus. Hormones from the

hypothalamus, anterior pituitary, ovaries and uterus govern this follicular development. Gonadotropin-Releasing Hormone (GnRH) released from the neurosecretory cells of the hypothalamus enters the blood vessels of the anterior pituitary (Bearden and Fuquay 1992). GnRH then governs the production of Follicle-Stimulating Hormone (FSH) and Leuteinizing Hormone (LH). It is estradiol 17-ß that is responsible for the manifestation of estrus. The thecal cells of the corpus luteum (CL) of the previous cycle produces progesterone when under the influence of LH. This progesterone is responsible for inhibition of estrus during the non-fertile period (luteal phase) of the estrous cycle. Progesterone also exerts a negative feedback on the hypothalamus to reduce the production of GnRH, and consequently FSH and LH during the luteal phase of the estrous cycle. Estradiol 17-ß conversely exerts a positive feedback toward GnRH to increase the preovulatory pulse frequencies of FSH and LH. Immediately preceding estrus, an LH surge lasting 6 to 12 h triggers stigma formation on the dominant Graafian follicle resulting in ovulation 24 to 30 h later (Hafez 1993).

If no pregnancy occurs, the cells in the uterus produce the hormone-like substance Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) that acts on the CL to induce luteolysis (CL destruction) at the end of diestrus (Hafez 1993). This regression of the CL stops the production of progesterone and thus removes the inhibitory effects of progesterone on gonadotropin release, which induces rapid follicular growth during proestrus (the follicular phase of the estrous cycle) leading to the next estrus.

Methods of Estrus Detection

Complex interactions between the reproductive hormones and external factors cause the expression of those behaviours indicative of estrus in cattle. Intensive management practices have dramatically increased dairy herd size, and less time is available for conscientious observation of estrus. Thus alternative methods which require minimal time and labour are needed to accurately ascertain when an animal is in estrus. The methods already in use measure changes in the hormone profiles of both milk and plasma, external factors such as mounting or increased activity, or internal factors such as body temperature. Novel methods are being developed to further ascertain estrus with minimal physical or financial input.

Progesterone

The role of progesterone has been reviewed by Bearden and Fuquay (1992). Progesterone is secreted from the thecal cells of the CL and transported in the bloodstream. The levels of progesterone reflect the lifespan of the CL throughout an estrous cycle. Since progesterone exerts a negative feedback on gonadotropin release, the presence of high levels in the body indicates that ovulation has not yet occurred. Conversely, low levels of progesterone indicate CL regression and removal of the negative feedback. Thus final follicular growth and subsequent estrus and ovulation are imminent. Therefore progesterone analysis can determine the approximate time of estrus and ovulation in normally cycling cattle. Progesterone is found in plasma, saliva and milk. Milk progesterone has been used in lactating dairy cows since milk samples are usually easier to obtain than plasma. While whole milk samples contain similar levels of

progesterone as plasma, defatted milk samples generally contain only one quarter of those levels (Gao et al. 1988). However the reported correlations between plasma and whole milk ($r = 0.68$) and plasma and defatted milk ($r = 0.72$) were significant. Minimal milk progesterone levels that indicate CL regression range from 1 ng ml^{-1} (Redden et al. 1993; Kyle et al. 1998), to 4 ng ml^{-1} (McLeod et al. 1991) to 5 ng ml^{-1} (Gao et al. 1988). Thus milk progesterone can be used to successfully ascertain CL function.

Mount Detectors

The ability to determine if or when a cow was mounted during standing heat, without the need for frequent visual observation, led to the creation of mount detection devices. The complexity of the detectors range from devices that release dye when pressure is put on the them (e.g. KaMaR®), to light-emitting sensors (e.g. HotFlash®), to devices that store and transmit signals to a computer detailing the time and duration of mounting activity (e.g. HeatWatch®). Williamson (1972) achieved a 98% estrus detection rate (EDR) with the KaMaR® devices, compared to 89% with 24 h visual observation, and only 56% with twice daily visual observation. Likewise, Pennington and Callahan (1986) reported an EDR of 94% using a heatmount device as compared to 61% with visual observation performed 3 times daily. Later studies compared KaMaR® devices and HotFlash®. Both KaMaR® and HotFlash® were able to detect 76 to 79% of estruses in dairy cows, which was markedly higher than the 47% of estruses detected by androgenized cattle with chinmarkers (Gwazdauskas et al. 1990). However, Phatak (1999) reported that KaMaR® devices had higher EDR (67 to 80%) than both HotFlash®

(42 to 50%) and chinmarkers (50%) in freestall dairy herds. Problems associated with these tailhead-mounted devices are that they do not detail precisely when the cow was mounted. The devices also tend to fall off easily and must be replaced.

HeatWatch® (DDx Inc., Boulder, CO) was able to detect between 90 and 100% of estruses in dairy cows and heifers, compared to 58 to 65% detected by visual observation (Piggot et al. 1996). The results were very similar in beef cows and heifers, where detection with HeatWatch® ranged from 85 to 97% (Borger et al. 1996; Stevenson et al. 1996a; Mathew et al. 1999), vs. 66 to 73% by visual observation (Borger et al. 1996; Stevenson et al. 1996a). While these devices give more detailed information on mounting activity, they are still vulnerable to loss by falling off, and must have the batteries checked and replaced regularly.

Activity Monitors

Another distinct estrus behaviour in both beef and dairy cattle is increased physical activity, such as walking. Activity (as measured using pedometers) has been reported to increase 163% on the day of estrus compared to the 3 days preceding estrus and the 3 days following estrus (Moore and Spahr 1991). Lewis and Newman (1984) reported a 2.25 fold increase in pedometer activity readings for the 2 days before estrus and the day of estrus. Redden et al. (1993) found a 2.3 to 2.8 fold increase in activity on the day of estrus. Pedometers have been able to detect 72 to 74% of predicted periods of estrus based on increased activity (Liu and Spahr 1993; Kiddy 1977), while Redden et al. (1993) found an 80% EDR. Pedometers were also able to detect 57% of first

postpartum estruses which are usually undetectable by visual observation (19% EDR) (Peter and Bosu 1986). In the same trial, the EDR for the second and third postpartum estruses were 91 to 93 %, respectively. This contrasted sharply with the visual observation EDR for the same period (37% and 79% for the second and third postpartum estruses, respectively). Using an activity threshold of 1.5 to 2 times above the baseline, Kyle et al. (1996) detected 90 to 94% of estruses in beef cows.

While pedometers considerably improve EDR compared to twice or thrice daily visual observations, the underlying problem of false positives (FP) (approximately 1 per period of low progesterone coinciding with estrus) (Kennedy and Ingalls 1995) prevents pedometers from being used as the sole indicator of estrus as they can contribute to unnecessary inseminations.

Vaginal Conductivity

Measuring the changes in electrical resistance of vaginal tissue is another method of estrus detection. At estrus high levels of estrogen increase blood flow to the reproductive tract, causing increased vaginal tissue hydration. This hydration causes a spike in the electrical conductivity of the tissue, which can be measured by placement of electrodes within the vagina or insertion of a probe (Smith et al. 1989). The electrical resistance (1/conductivity) of vaginal tissue dropped at estrus (Schofield et al. 1991), which coincided with the LH surge (Canfield et al. 1989), low levels of progesterone and high levels of estrogen (Lewis et al. 1989). While this method of estrus detection is moderately successful, the amount of labour involved makes it prohibitive for very large

herds (e.g., taking measurements several times daily, proper sterilization between uses, etc.).

Body Temperature Sensing

A change in body temperature is a more subtle indicator of estrus. Hurnik et al. (1985) measured skin temperature by a once daily thermal infrared scanning of the gluteal region and found that temperature increases occurred in 93% of dairy cows on the day of estrus. However, the method was flawed due to the effect of ambient temperature on the skin, causing an unacceptable 33% FP rate and an overall EDR of only 78%. Redden et al. (1993) considered the use of skin temperature (in conjunction with vaginal temperature) as an indicator of thermoregulatory status (to remove those FP in core temperature caused by fever or ambient conditions), but skin temperature was not useful as an indicator of estrus per se.

Milk temperature in dairy cattle was also considered as a possibility for temperature measurement; this approach avoided insertion of thermistors or temperature radiotransmitters internally. Milk temperature was usually measured using a thermocouple placed within a teat cup during milking. Initially, milk temperature sensing seemed promising, as Ball et al. (1978) reported an 86% EDR and a 3% FP rate using a criterion based on a 0.1° C temperature increase over a 15 d baseline. Fordham et al. (1988) then reported a 70 to 73% EDR with an 11% FP rate using a criterion of 0.2°C increase over a 3 d baseline. The results were less promising when the method was tested on farm. With the best criterion available (elevated milk temperature of at least

0.3°C above a 5 d baseline), McArthur et al. (1992) reported in only a 50% EDR and an 81% FP rate with twice daily measurement. Therefore little research is now done on milk temperature as an indicator of estrus.

A promising site for temperature sensing has been the vagina. Vaginal temperature declined gradually for a few days just before estrus and then increased abruptly on the day of estrus (Bitman et al. 1984; Redden et al. 1993; Kyle et al. 1998). Vaginal temperature, as measured by radiotelemetric temperature sensing devices or thermometers, rose 0.3 to 1.0° C at estrus (Rajamahendran et al. 1989; Clapper et al. 1990; Mosher et al. 1990; Redden et al. 1993; Kyle et al. 1998). Clapper et al. (1990) found that lactating dairy cows had elevated temperatures lasting 8.2 ± 3.5 h when measurements were taken hourly. With measurements taken every 15 min, Mosher et al. (1990) found the duration of the temperature spike to be 11.1 ± 5.9 h in post-pubertal dairy heifers. When the technology became available for more frequent temperature measurements (every 4 min), it became possible to measure the duration of the temperature elevation more accurately. Redden et al. (1993) found a 6.8 ± 4.6 h temperature elevation in lactating dairy cows, while Kyle et al. (1998) found a 7.2 ± 2.6 h temperature elevation in beef cows. It is therefore necessary to monitor vaginal temperature continuously, so as to ensure that the commencement and the termination of the temperature elevation are not missed and the cows are not bred too late. Measuring vaginal temperature for estrus detection has resulted in an 81% EDR in lactating dairy cows, compared to only a 66% EDR by once daily visual observation (Redden et al.

1993). A 93% EDR was found in beef cows by using vaginal temperature, compared to a 56% EDR by 4 times daily visual observation (Kyle et al. 1998).

Rajamahendran et al. (1989) found that a rectal temperature peak commenced 22 to 27 h before ovulation in dairy cows. This elevation occurred simultaneously with the LH surge and the onset of estrus. Mosher (1990) found a similar timeframe of 21 to 23 h from the vaginal temperature, LH and estradiol peaks to ovulation in dairy heifers. Clapper et al. (1990) reported a vaginal temperature peak approximately 17.2 ± 4.5 h after the LH surge and onset of estrus in lactating dairy cows.

Although measuring changes in vaginal temperature can be useful for predicting estrus, the costs associated with both initial start-up and maintenance are high. As well, the temperature radiotransmitters can be lost and must be sterilized after every use. The costs and labour involved make this method of estrus detection somewhat prohibitive in large commercial herds. A novel location for temperature sensing would be the rumen. Potentially, a temperature radiotransmitter with a lifelong battery could be inserted into the rumen using a balling gun. Rumen temperature could be monitored by radiotelemetry for the lifetime of the animal. This would avoid loss of the temperature-sensing devices and would reduce maintenance costs such as battery replacement. There have been no studies of rumen temperature throughout the estrous cycle.

Patterns in Drinking Behaviour

Drinking behaviour of dairy cows had a highly repeatable diurnal pattern, with peaks around noon and sunset and a nadir after midnight; most periods of drinking

behaviour occurred within 3 to 4 h of milking and after feeding (Basarab et al. 1996). Daily water intake is very high (50 to 80 kg per cow) in dairy cows (Castle and Thomas 1975; Gorewit et al. 1989; Nocek and Braund 1985). It is also known that water intake is positively correlated with milk yield and dry matter intake (Castle and Thomas 1975; Murphy et al. 1983). Milk yield was reduced by up to 35% on the first milking of the day of estrus and was followed by a compensatory increase in milk yield at the afternoon milking (Walton and King 1986; Schofield et al. 1991). Feed intake has also been reported to decrease by up to 15% on the day of estrus (Walton and King 1986). Because water intake is positively correlated with milk production and feed intake, it seems likely that water intake would be reduced at estrus. However, no research has been done on this subject.

Ovarian Follicular Growth

Growth and development of the female germ cell begins in the cortex of the ovary. Each germ cell, or oocyte, is enclosed in a tissue sac called the ovarian follicle (Jones 1991). The maturation of the ovarian follicle occurs in 4 stages. The primary or primordial follicle consists of an oocyte encapsulated by a single layer of granulosa cells (Bearden and Fuquay 1992). All ovarian follicles present on the ovary originate from a pool of these primary follicles. Initial recruitment of follicles from this pool occurs at puberty. In cycling cattle, groups of primary follicles are recruited in a series of waves during a span of one estrous cycle. Two to four waves per cycle have been detected by ultrasound, with most cows having 2 or 3 waves (Sirois and Fortune 1988; Ginther et al.

1989). It is usually only the dominant follicle which emerges from the last wave that persists to ovulation. The first wave of either a 2 or 3 wave cycle begins on the day of estrus. The second wave begins 9 or 10 d later (Adams et al. 1993). The third wave (for those cows with long luteal periods) begins 7 d later (Sirois and Fortune 1988). From each wave only one follicle will become dominant and undergo further stages of development. The other follicles have their growth halted and become subordinate. Pulsatile release of FSH from the anterior pituitary occurs 1 to 2 d before a new wave starts (Adams et al. 1992a), and this drives follicular recruitment (Lee et al. 1983). This also promotes development of the primary follicle into a secondary follicle, which is characterized by the addition of a second granulosa layer. The tertiary follicle develops a fluid filled antral cavity between the granulosa layers. The follicle present at the final stage of growth is termed a Graafian follicle and can be 12.5 to 16.5 mm in diameter (Adams et al. 1992b; Ginther et al. 1989) before it is ready to ovulate. The nonovulatory waves last approximately 2 weeks, which includes a 6 d long growth phase before becoming atretic 7 d later (Greenwald and Roy 1994). Increased progesterone secretion from the CL caused by GnRH release and inhibin release from the ovarian granulosa cells act to suppress FSH (but not LH) release. This causes the eventual atresia of the nonovulatory follicular wave during the luteal phase of the estrous cycle (Thatcher et al. 1993; Savio et al. 1993). The wave containing the Graafian follicle emerges after CL regression, thereby causing the removal of the progesterone negative feedback on gonadotropin release. After attainment of LH receptors on the Graafian follicle, LH and FSH pulses induce final follicular growth before ovulation (Adams et al. 1992a).

Synchronization of Estrus

Observation of standing heat in naturally cycling cows is required on a daily basis since every cow cycles at a different time. With the use of such exogenous reproductive hormones as GnRH and $PGF_{2\alpha}$ estrus can be confined to a period of under 5 d per cycle for an entire herd. As up to 40% of standing heats are missed due to inefficient heat detection practices (Kristula et al. 1992), hormone synchronization can reduce the time required for observation of standing heat without sacrificing pregnancy rates due to missed heats.

$PGF_{2\alpha}$ and GnRH

$PGF_{2\alpha}$ is a hormone-like substance secreted from the uterus that acts to regress a functional CL and thus reduce progesterone levels to remove the negative feedback effect on FSH and LH release. This causes the final maturation of the Graafian follicle and allows ovulation to occur 2 to 5 d after $PGF_{2\alpha}$ administration. This confines estrus detection to an approximately 4 d period per estrous cycle. A single injection of $PGF_{2\alpha}$ has been reported to induce CL regression in 85 to 95% of cows with CL that were at least 7 d old (Heuwieser et al. 1997). This agrees with a study by Archibald et al. (1994) that had a 70 to 89% EDR with a single injection of $PGF_{2\alpha}$. When the stage of CL development is unknown, two injections of $PGF_{2\alpha}$ administered 7 or 14 d apart can synchronize CL regression. The first injection would regress any mature CL present on the ovary. The second injection would regress those CL that were previously immature

and unresponsive. Multiple injections of $PGF_{2\alpha}$ appear to be beneficial as Kristula et al. (1992) found a 5 d reduction in days to first service and a 30% higher pregnancy rate with weekly doses of $PGF_{2\alpha}$.

GnRH is a peptide secreted from the neurosecretory cells of the hypothalamus that acts to cause pulsatile releases of FSH and LH from the anterior pituitary. GnRH induces increased LH pulse frequency within 2 to 3 h when a Graafian follicle is present on the ovary. This terminates the follicular growth induced by FSH and triggers ovulation within 24 h (Lee et al. 1983). Single injections of GnRH have been reported to increase conception rates by 50% in dairy cows with slow involution of the reproductive tract and decrease time from parturition to first estrus by 6 d in normally cycling cows (Foote and Riek 1999).

GnRH does not appear to have a benefit when used in a $PGF_{2\alpha}$-based synchronization program. Various protocols have been tried to determine which order and timing of GnRH and $PGF_{2\alpha}$ could improve pregnancy rates / AI while minimizing time spent on observation of standing heat by creating a synchrony in CL regression and time to ovulation within the herd. This did not appear to be the case as Twagiramungu et al. (1992) and Stevenson et al. (1988; 1996b) reported either no improvement in conception rates or even a reduction in conception rates when GnRH was used in conjunction with $PGF_{2\alpha}$ rather than $PGF_{2\alpha}$ alone. Thus a correct hormone protocol has not yet been established to confine estrus to a small window of time.

Ovsynch

Ovsynch is a synchronization protocol that involves both GnRH and $PGF_{2\alpha}$ that is used to synchronize ovulation rather than estrus. The protocol involves an injection of GnRH at any point in the estrous cycle. $PGF_{2\alpha}$ is administered 7 days later. A second GnRH injection is given within 30 to 48 h after the $PGF_{2\alpha}$ injection. The animals are then inseminated 16 to 24 h after the final GnRH injection. Timed insemination is necessary for the Ovsynch method since only 30% of cows exhibit estrus (Pursley et al. 1995a). Pursley et al. (1995a) found that this protocol confined ovulation to an 8 h period, 24 to 32 h after the final GnRH injection. This meant that AI would be performed 14 h before ovulation or up to 10 h after ovulation.

Ovsynch synchronized ovulation well in mature cows, as 18 of 20 of cows studied by Pursley et al. (1995b) ovulated after the first GnRH injection, 100% underwent CL regression after the $PGF_{2\alpha}$ and 100% ovulated within the 8 h window after the second GnRH injection. Ovsynch worked less effectively in heifers. Pursley et al. (1995b) found that only 13 of 24 of heifers ovulated after the first GnRH injection, and CL regression and ovulation occurred at the expected time in only 18 of 24 heifers. Synchronization failure in heifers could be due to immature dominant follicles not having enough LH receptors to respond to GnRH, therefore no ovulation occurs and no CL is available when $PGF_{2\alpha}$ is administered (Pursley et al. 1995b). Unfortunately, Ovsynch treated dairy cows had conception rates from 37 to 39% (Pursley et al. 1997a; Pursley et al. 1997b). Dairy heifers achieved only a 35% conception rate with Ovsynch compared to a 74% conception rate with $PGF_{2\alpha}$ administered every 14 d (Pursley et al. 1997b). However, Ovsynch appeared to be more successful in beef rather than dairy cows. In

beef cows, Ovsynch produced a 54% conception rate (Geary et al. 1998). The extra costs involved with 3 hormone injections per cycle, as well as the lower than expected conception rates limit the widespread use of the Ovsynch protocol. While Ovsynch does not appear to benefit herds with good estrus detection practices, it could be advantageous to herds where estrus detection is poor.

Summary and Hypotheses

The regulation of the estrous cycle is governed by a complex set of interactions between the hormones secreted by the hypothalamus, anterior pituitary, ovaries and uterus. Understanding these interactions is crucial to successful reproductive efficiency. Manipulation of these hormonal interactions to reduce calving interval has met with limited success as with protocols using GnRH and $PGF_{2\alpha}$.

Physical devices used to detect changes in vaginal temperature, mounting activity or physical activity at estrus have proven to be successful, but costs associated with initial purchase and continual upkeep prohibit widespread use of these methods. The rumen could prove to be an ideal site to measure changes in temperature as insertion of the temperature sensor would not require surgical implantation. Rumen temperature might also provide insight into changes in feeding and drinking behaviours at estrus. However there have been no studies of rumen temperature throughout the estrous cycle. The main objectives of this study were to determine the usefulness of rumen temperature and drinking behaviour as estrous or ovulation predictors in synchronized tie-stalled dairy cattle when compared to vaginal temperature.

MANUSCRIPT 1 -

CHANGES IN THE RELATIONSHIP BETWEEN RUMEN TEMPERATURE AND VAGINAL TEMPERATURE IN FIRST CALF DAIRY HEIFERS THROUGHOUT THE ESTROUS CYCLE

ABSTRACT

The relationship between rumen temperature and vaginal temperature during the estrous cycle were studied in tie-stalled first calf dairy heifers. Vaginal and rumen temperatures were monitored continuously during a 3 month period by radiotelemetry in 4 first calf Holstein dairy heifers (71 to 161 d postpartum) that were fitted with rumen cannulae. To measure body temperature for individual cows a temperature radiotransmitter was placed in a plastisol anchor which had finger-like projections that held it in the vagina. Rumen temperature was monitored by a temperature radiotransmitter placed in a weighted plastic jar within a nylon bag that was tethered in the rumen. The heifers were synchronized using a program called Ovsynch. An intramuscular injection of GnRH (100 μg) was administered at a random stage of the estrous cycle. The heifers received an intramuscular injection of $PGF_{2\alpha}$ (500 μg) seven days later and a second GnRH injection 48 h after that. The heifers were synchronized for estrous cycles 1 and 3, while estrous cycle 2 was not synchronized. All heifers were artificially inseminated (AI) 24 h after the second GnRH injection of cycle 3. Milk samples for progesterone analysis were taken during the morning milking 4 times weekly throughout the experiment. The first day of vaginal temperature sensing was designated day 1. The frequency and duration of drinking and feeding behaviours were recorded visually at 1 min intervals for 4 h after the provision of feed from the day of $PGF_{2\alpha}$ (day 60) to the day of insemination (day 63) in estrous cycle 3. The pregnancy and calving rates in this experiment were 1 out of 4. Progesterone analysis indicated 13 periods of low progesterone indicative of CL regression. Vaginal temperature data was available

for 8 of these periods and rumen temperature was available for 7 periods. Eight increases in vaginal temperature were detected during the 8 periods of low progesterone (true positives) and 20 false positives were detected. The magnitude of the true positive vaginal temperature increase was 0.30 ± 0.03°C. Five increases in rumen temperature were detected during the 7 periods of low progesterone (true positives) and 26 false positives were detected. The magnitude of the true positive rumen temperature increase was 0.51 ± 0.09°C. Feeding and drinking behaviours were lower on the day before and the day of AI than on the 2 previous days of the cycle. Rumen temperature was not as effective as vaginal temperature for predicting changes during the estrous cycle in tie-stalled dairy heifers.

INTRODUCTION

It is becoming increasingly difficult to allocate the necessary time for daily observation of estrus, particularly in large dairy herds. Since up to 40% of standing heats are missed due to poor heat detection (Kristula et al. 1992), non-visual methods for detecting estrus are needed for successful inseminations.

Body temperature is known to be a reliable indicator of estrus in both dairy and beef cattle. Vaginal temperature (VagT) has been shown to decrease gradually 2 to 3 d before estrus, then rises by greater than 0.3°C above the baseline on the day of estrus (Bitman et al. 1984; Redden et al. 1993; Kyle et al. 1998). Since the duration of the increase in VagT has been reported to be as short as 8 h (Clapper et al. 1990; Redden et al. 1993; Kyle et al. 1998), continuous monitoring of VagT is necessary to accurately

detect the commencement and termination of the elevated temperature at estrus. Measuring changes in VagT can be useful for predicting estrus. However, the costs associated with both initial start-up and maintenance are high. As well, the temperature radiotransmitters can be lost and must be sterilized after every use. The costs and labour involved make this method of detecting estrus somewhat prohibitive.

To make temperature sensing commercially viable, temperature radiotransmitters need to be retained inside the body and function for the lifetime of the animal. The vagina is not an appropriate site for this, as the devices can be expelled and cannot be retained indefinitely. The rumen could be a site for monitoring temperature, as there is less chance of expulsion. There is no data, however, to indicate that a change in rumen temperature occurs at estrus. Placement of a temperature-sensing device in the rumen (via use of a balling gun) would avoid the need for surgical implantation, and the temperature radiotransmitter could be retained indefinitely. One temperature-sensing system is already commercially available (HTI Technologies, Inc., St. Petersburg, FL). There have been no previously published studies of rumen temperature throughout the estrous cycle in dairy or beef cattle. The main objectives of this study were to determine the usefulness of rumen temperature and drinking behaviour as ovulation predictors in synchronized tie-stalled first-calf dairy heifers when compared to vaginal temperature.

MATERIALS AND METHODS

Animals and Management

Four tie-stalled first calf Holstein heifers ranging from 71 to 161 d postpartum (115 ± 19 d) were studied at the Glenlea Research Station Dairy Unit at the University of Manitoba. The heifers were on an unrelated trial and were being fed 4 rotating trial diets consisting of barley, canola meal (with or without the addition of lignosulfates, then heat treated at one of 2 different temperatures), sunflower seeds, tallow, beet pulp and molasses with vitamin and mineral premixes. The heifers were fed once daily between 12:00 h and 12:30 h and had free access to water. The heifers were housed in tie-stalls except when they were exercised from 9:00 h to 12:00 h daily in an adjacent outdoor paddock (60 m x 40 m). The heifers were exercised in an indoor pen when outdoor temperatures were below –25°C. Since Ovsynch often inhibits estrus there was no conscientious observation of standing heat for these heifers. All procedures were in accordance with the guidelines outlined by the Canadian Council on Animal Care.

Synchronization

The day VagT monitoring started was designated day 1. Synchronization began 8 days after the commencement of vaginal temperature monitoring to allow for inspection of a temperature baseline that was not influenced by hormone manipulation. The Ovsynch method (Pursley et al. 1995a) was used to induce ovulation twice (35 days apart) to help increase pregnancy rates in this herd. A natural estrus was expected midway between the two synchronized estrous cycles. A GnRH analogue (100 μg

Factrel®, Ayerst Laboratories Inc., St. Laurent, QC) was administered intramuscularly on day 9 (day 1 was the start of VagT monitoring) to increase FSH and LH pulse frequency to induce ovulation in the heifers with a large Graafian follicle. A prostaglandin ($PGF_{2\alpha}$) analogue (500 μg Estrumate®, Coopers Agropharm Inc., Ajax, ON) was administered intramuscularly 7 days later to regress any CL present on the ovary. GnRH was administered again 2 days later to induce ovulation. The GnRH analogue for estrous cycle 3 was administered on days 53 and 62. The $PGF_{2\alpha}$ analogue was administered on day 60. AI was performed on day 63. The time of injections and AI was at 14:00 h. Table 1 depicts a timeline indicating days of milk sampling, injections and time of AI.

Measurement of Vaginal and Rumen Temperature

Temperature radiotransmitters were inserted into the vagina on day 1 of the experiment. Temperature radiotransmitters were inserted into the rumen (via existing rumen cannulae) on day 5 in Janice, Muffin and Puff. Rumen temperature monitoring began on day 7 in Una because of a malfunctioning radiotransmitter. It was necessary to sedate one heifer (Puff) with 1.75 ml xylazine i.m. (Rompun®) (Bayvet Inc., Concord, ON) to insert the vaginal temperature radiotransmitter. Any temperature radiotransmitters that were expelled from the vagina were re-inserted as soon as possible. The radiotelemetry system (Wildlife Materials Inc., Carbondale, IL.) consisted of temperature sensitive radiotransmitters, an antenna, a radio receiver and an IBM-compatible personal computer that recorded the data. Temperature influenced the frequency in which radio signals were sent; the higher the temperature, the more frequent

the signals. Signals were received at approximately 4 min intervals. The receiver recorded cow identity and time that a signal was received. A custom designed program converted the time between signals into a temperature. Each temperature radiotransmitter was fitted within a plastisol (F.H. and Sons Manufacturing Ltd., Rexdale, ON) anchor with finger-like projections (34 mm) to keep the temperature radiotransmitter securely in the vagina (Redden et al. 1993). The fitted temperature radiotransmitter was bathed in Germex® (N-alkyl dimethyl benzyl ammonium chloride at 11.5%) (1:80 dilution factor) for 1 hour prior to insertion. The external genitalia and surrounding skin of the vagina were washed first with a Hibitane® (chlorhexidine gluconate) solution and then washed with iodine. The fitted temperature radiotransmitter was lubricated with mineral oil and was inserted into the vagina by pressing the finger-like projections against the plastisol anchor and pushing it through the external vaginal sphincter into the vagina to a distance of 10 to 15 cm. The rumen temperature radiotransmitter for each animal was contained in a plastic bottle (50 mm x 115 mm) filled with gravel for weight. Each bottle was placed in a cotton rumen digesta bag that was tethered to the rumen plug by a string.

Observation of Behaviour

To examine if changes in behaviour occurred during the periods of low milk progesterone coincident with estrus and ovulation, visual observation of time spent eating, drinking, standing and lying down were made. Observations were made at 1 min

intervals for the 4 h period immediately after feed was offered on days 60 to 63 of the trial.

Progesterone Analysis and AI

Milk samples for progesterone analysis were taken from each heifer during the morning milking 4 times weekly (Mon., Wed., Fri., Sun.) throughout the experiment so that sampling occurred on the day of or the day after the hormone injections and AI. Milk progesterone was quantified using a radioimmunoassay as described by Tekpetey et al. (1987). CL regression was assumed to have occurred if milk progesterone levels fell below 5.0 ng ml^{-1} for 2 or more consecutive samples. The heifers were inseminated at 126 to 210 d postpartum by one herdsman at 14:00 h on the day after the second GnRH injection (day 63) using thawed semen from one bull. Pregnancy was confirmed by rectal palpation at 41 days after AI and calving rates were determined at the time of delivery.

Calculations and Statistical Analysis

Analysis of the behaviour data was done by General Linear Model procedures (SAS Institute Inc., 1988) to determine the effect of day, hour and day x hour on behavioural patterns prior to and on the expected day of ovulation (EDO) of the second synchronized cycle. The error term was day by animal where day was a repeated measure.

Hourly vaginal and rumen temperature means were calculated and a set of criteria was applied to vaginal (VagT) and rumen (RumT) temperature values in order to detect those increases throughout the study that coincided with low levels of progesterone. The original testing criterion was developed by Redden et al. (1993) and was an increase in VagT of 0.3°C compared to the mean temperature for the same hour of the day (1 h) for the previous 3 d (the 0.3C-1W-3D criterion). This increase in VagT had to be sustained for a minimum of 3 consecutive hours. An expanded set of criteria based on changes in absolute temperature or standard deviation were also tested for temperature increase identification. The aim was to find the set of criteria that detected the most increases in VagT that were associated with low levels of progesterone (true positives = TP), while detecting the fewest increases in VagT not associated with low progesterone (false positives = FP). This involved calculating the height and duration of a sustained temperature increase in VagT during a specific time of day on one specific day, then comparing it to a baseline of that same time of day over the previous few days. If the temperature increase occurred during the follicular stage of the estrous cycle when progesterone levels were low then the increase could be associated with impending estrus or ovulation. If more than one increase in VagT occurred during a period of low progesterone the first increase in VagT would be designated as the TP because day of estrus and day of ovulation were not confirmed in this experiment. Those increases in VagT that appeared 12 h after the TP were considered a FP even though progesterone levels were low. The temperature increase was first calculated by an absolute temperature formula:

Formula 1: Mean temperature for a window of time on a specific day – Mean temperature during that same window of time for the previous X days

The absolute temperature increases tested were 0.2°C, 0.25°C and 0.30°C. The windows of time tested were 1 h, 3 h, 6 h, and 12 h. Windows of time were tested sequentially over 24 h (i.e. 1:00 h to 7:00 h, 2:00 h to 8:00 h, etc. for 24 consecutive 6 h windows of time). The baseline days (X) were 1 d, 2 d, 3 d and 4 d.

Another attempt to detect VagT increases with a minimum of FP involved a comparison of an increase in temperature to a multiple of the standard deviation of the baseline. The temperature increase was calculated by using a standard deviation formula:

Formula 2: (Mean temperature for a window of time on a specific day - Mean temperature for that same window of time for the previous X days) / Standard deviation of the temperature during that same window of time for the previous X days.

The windows of time and baseline lengths were the same as for the absolute temperature formula. The temperature threshold ratios tested for the standard deviation formula were 1.5 to ≥ 4.0 fold increases at 0.5 fold intervals.

The increase in RumT was measured by the absolute temperature formula where the absolute temperature increases tested were 0.50°C, 0.75°C and 1.00°C. The windows of time tested were 1 h and 3 h and the baseline days (X) were 2 d, 3 d, 4 d and 5 d. The temperature increase calculated by the formulae for both VagT and RumT had to be maintained for at least 3 h to meet the criteria. Those increases in RumT that appeared 12 h after the TP were considered a FP even though progesterone levels were low.

RESULTS

The animals appeared to have no health problems associated with temperature radiotransmitter use. Difficulties with the telemetry system resulted in 122 h of lost vaginal data and 91 h of lost rumen data per cow. Insertion of a replacement rumen temperature radiotransmitter in one heifer (Puff) was delayed between days 25 and 31 due to technical difficulties. The vaginal temperature radiotransmitter for Una was expelled and re-inserted on 2 occasions, but was not re-inserted after expulsion on day 31 of the trial as retention of the device in the vagina appeared impossible. It was observed that insemination was difficult in two heifers (Janice and Muffin) due to the presence of the vaginal temperature radiotransmitter. However, insemination was accomplished without removal of the temperature radiotransmitter. Milk progesterone assay sensitivity and intra-assay coefficient of variation were 0.05 ng ml^{-1} and 7.9%, respectively. Muffin was the only heifer to become pregnant, and the calving rate in this experiment was 1 heifer out of 4.

There were 13 periods of low progesterone based on progesterone analysis. Vaginal temperature data was available for 8 of these periods of low progesterone (Janice, n = 3; Puff, n = 2; Muffin, n = 2; Una, n = 1). The progesterone profile for Una from days 33 to 66 is listed in appendix 1. The vaginal and rumen temperature graphs for Janice, Una, Muffin and Puff from days 1 to 32 are given in Figs. 1A, 2, 3A and 4A, respectively. The vaginal and rumen temperature graphs for Janice, Muffin and Puff from days 33 to 66 are given in Figs. 1B, 3B and 4B, respectively. The results of the vaginal absolute temperature testing criteria are listed in Table 2. Use of a 12 h window of time kept the number of FP low at 0.3°C with 3 to 11 FP overall. This meant that a FP could occur every 17 days (once an estrous cycle) to 50 days (less than once every 2 estrous cycles). However, these criteria yielded only 5 to 6 TP increases in VagT. Lowering the temperature threshold to 0.2°C (12 h window of the day) yielded 7 TP increases in VagT, and this was associated with 11 FP, or a FP once every 17 days. For each of the 6 h, 3 h and 1 h windows of time there was a set of criteria that yielded 8 TP increases in VagT (the maximum possible) with 20 to 23 FP. The best criterion in this experiment was a 0.2°C VagT increase within a 3 h window of time over a 3 d baseline (the 0.2C-3W-3D criterion). This set of criteria yielded 8 TP and 20 FP (a FP once every 10 days or twice an estrous cycle) for VagT. Over half (11/20) of these FP occurred when progesterone was high. The others (9/20) occurred when progesterone was low or beginning to rise. The day and duration of the 8 detected TP increases in VagT are listed in Table 3.

Several S.D. criteria found 8 TP increases in VagT, however there were also 42 to 105 FP. This meant that a FP occurred every 2 to 5 days throughout the estrous cycle. Therefore the S.D. criteria was disregarded as a useful testing method in this experiment.

The best VagT increase criterion (0.2C-3W-3D) was tested on RumT data. Only 2 possible TP increases in RumT (TP) were found during the 7 periods of low progesterone and 115 FP (a FP every 2 days throughout the estrous cycle) were found. The 0.3C-1W-3D criterion (Redden et al. 1993) yielded 4 TP increases in RumT and 80 FP (a FP every 3 days). The results of the rumen absolute temperature criteria are given in Table 4. The criterion chosen as the best was a 0.5°C RumT increase using a 3 h window of the day over a 5 d baseline (the 0.5C-3W-5D criterion). Five TP increases in RumT and 26 FP (a FP every 8 days) were found with this set of criteria. The day and duration of the 5 TP increases in RumT are listed in Table 5.

Both VagT and RumT appeared to vary on both a daily basis and between periods of high progesterone and low progesterone. Since an injection of GnRH has been shown to induce ovulation after 24 to 32 h (Pursley et al. 1995a), ovulation was expected to occur on days 19 and 63 with the Ovsynch method in this study. Therefore these days are termed the expected day of ovulation (EDO) even though ovulation was not measured in this experiment. Circadian patterns in VagT appeared in only two heifers (Janice and Una). Janice had a regular short-term drop in temperature of between 0.2 to 0.4°C near midnight (Fig. 1B). Una had a similar drop in VagT that occurred at late evening or early morning (Fig. 2). When progesterone levels were high,

the circadian pattern in both heifers remained consistent. The TP increase in VagT was depicted as either a short-term surge in temperature as seen in Janice on day 7 (Fig. 1A), or the lack of a short-term drop in temperature from noon to 14:00 h that had been detected on previous days, as seen in Janice on day 18 (Fig. 1A).

Certain TP increases in VagT were preceded by depressions in VagT that appeared to coincide with the fall in progesterone levels. This occurred 6 to 7 d before the TP increases in VagT seen in Muffin on day 19 (Fig. 3A), and for 2 to 3 d before the TP increases in VagT seen in Puff on day 19 (Fig. 4A). Two of the TP increases in VagT occurred prematurely, without the aid of $PGF_{2\alpha}$-induced CL regression. The TP increases in VagT observed in Janice on day 7 (Fig. 1A) and Una on day 16 (Fig. 2) began before $PGF_{2\alpha}$ administration on the afternoon of day 16. Three of the remaining 6 TP increases in VagT (Janice, days 18 (Fig. 1A) and 63 (Fig. 1B); Muffin, day 63 (Fig. 3B)) appeared to be the response to both $PGF_{2\alpha}$ and GnRH, occurring within 11 h of GnRH. Thus they were considered hormonally synchronized. The progesterone profile of one TP indicated hormonal synchronization (Muffin, day 19 (Fig. 3A)) yet the TP did not occur until 32 h after GnRH. The other 2 TP increases in VagT (Puff, days 19 (Fig. 4A) and 63 (Fig. 4B)) occurred 30 to 31 h after GnRH, during a prolonged period of low progesterone commencing 1 to 2 days before $PGF_{2\alpha}$ administration. This did not appear to be a response to the exogenous hormones, so they were considered to have occurred naturally. A radiotelemetry system power failure on days 39 to 41 prevented monitoring of the natural estrus expected during that time in 3 of the heifers. The period of low progesterone between days 44 to 46 in Muffin (Fig. 3B) was not associated with

a TP increase in VagT. However the TP increase in VagT may have occurred on day 47 when VagT data was unavailable.

The baseline was defined as the 3 d average temperature of the 3 h window of time immediately preceding the TP increase in VagT. The VagT baseline before the 8 TP increases in VagT was 38.00 ± 0.23°C. The magnitude of the 8 TP increases in VagT was 0.30 ± 0.02°C. The magnitude of the 20 FP increases in VagT was 0.31 ± 0.03°C. There was no difference in the magnitude of the TP and FP increases in VagT (P = 0.38). The duration of all 8 TP increases in VagT was 9.1 ± 1.6 h. The interval from GnRH administration to the TP increase in VagT was 20.5 ± 4.7 h (n = 6). The hormonally synchronized TP had an interval of 15.5 ± 5.5 h from time of GnRH administration to the TP increase in VagT (n = 4). The naturally occurring TP had an interval of 30.5 ± 0.5 h from time of GnRH administration to the TP increase in VagT (n = 2). Differences between baseline temperature, magnitude and duration of the TP increase in VagT were compared in two groups; pregnant versus open heifers and periods of low progesterone occurring on schedule according to the Ovsynch protocol (n = 6) versus periods of low progesterone occurring prematurely (n = 2) (Table 6). The magnitude of the TP increase in VagT was greater in the scheduled periods of low progesterone than in the premature periods of low progesterone (P = 0.02).

Distinct circadian patterns in RumT appeared in all 4 heifers as a short-term drop in RumT (greater than 2°C) at early afternoon followed by a rapid rise within 3 to 4 h. Then RumT fluctuated several times within a 1.5°C range for the rest of the day. An example of the circadian pattern is given in Fig. 1A. This pattern remained consistent

during periods of both high progesterone and low progesterone in all heifers. The TP increase in RumT in Janice appeared as a lower magnitude short-term temperature drop (approximately 1°C) from noon to 14:00 h on day 18 compared to a 2°C drop at the same time on previous days (Fig. 1A). The other TP increases in RumT were not readily apparent, such as on day 63 in Janice (Fig. 1B). Four of 5 TP increases in RumT began on the same day as the TP increases in VagT. One TP increase in RumT occurred prematurely, without the aid of $PGF_{2\alpha}$-induced CL regression on day 16 in Una (Fig. 2). Two of the remaining 4 TP increases in RumT (Janice, days 18 (Fig. 1A) and 63 (Fig. 1B)) appeared to be the response to both $PGF_{2\alpha}$ and GnRH, and so were considered hormonally synchronized. The other 2 TP increases in RumT (Puff, days 20 (Fig. 4A) and 64 (Fig.4B)) did not appear to be responses to the exogenous hormones, and so were considered to have occurred naturally.

The RumT baseline before the 5 TP increases in RumT was 37.65 ± 0.34°C. The magnitude of 5 TP increases in RumT was 0.50 ± 0.05°C. The magnitude of the 26 FP increases in RumT was 0.61 ± 0.15°C. There was no difference in the magnitude of the TP and FP increases in RumT ($P = 0.26$). The duration of the 5 TP increases in RumT was 6.6 ± 0.5 h. The interval from GnRH to the TP increase in RumT was 25.0 ± 9.9 h ($n = 4$). The hormonally synchronized TP had an interval of 9.5 ± 9.5 h from time of GnRH administration to the TP increase in RumT ($n = 2$). The naturally occurring TP had an interval of 40.5 ± 3.5 h from time of GnRH administration to the TP increase in RumT ($n = 2$). The magnitude of the hormonally synchronized TP increases in RumT was 0.45 ± 0.04°C and 0.57 ± 0.08°C for those occurring naturally.

A large increase in VagT of approximately 4°C was observed on days 28 and 29 in Muffin, but was not accompanied by any corresponding change in RumT (Fig. 3A). As well, Muffin displayed both VagT and RumT FP temperature increases as a response to $PGF_{2\alpha}$ around noon on day 60 (Fig. 3B).

The proportion of the daily observation period spent on the activities of feeding, standing only and drinking behaviours were significantly influenced by day of observation, hour of observation and the day x hour interaction (Table 7). The day x hour interactions are shown in Fig. 5. The percentage of time spent on drinking behaviour was approximately 15 to 20% across hours and days, except on both the day of GnRH and the following day (EDO) when the percentage of time spent on drinking behaviour was reduced to under 5% in hour 4.

There was a uniformly high percentage of time spent feeding in hour 1 for all 4 days at approximately 65 to 80%. Less time was spent feeding in hour 2 (35%) except on day 1 when it took up nearly 60% of the time. While time spent feeding varied during hours 3 and 4, and it was particularly low on day 3, hour 4 and day 4, hours 3 and 4. When the percentages of time spent on drinking and feeding behaviours were low the heifers were seen to spend the available time either standing on the EDO (day 4) or lying down on the day of GnRH (day 3).

DISCUSSION

This study was the first to document that vaginal temperature transmitters interfered with the ease of AI. It was feared that difficulty with the insemination of

Janice and Muffin might have had a deleterious effect on pregnancy rates. However, this problem did not appear since Muffin was the one of four heifers to become pregnant.

The progesterone profiles suggested that only Janice (Fig. 1B) and Muffin (Fig. 3B) were hormonally synchronized by Ovsynch in the final estrous cycle as progesterone levels fell within 3 days of $PGF_{2\alpha}$ on day 60. CL regression induced by $PGF_{2\alpha}$ and ovulation induced by GnRH was confirmed in Muffin as she did become pregnant. Because progesterone profiles were similar in these two heifers after $PGF_{2\alpha}$ it is likely that CL regression occurred in Janice as well. Synchronization failure during the final estrous cycle in Puff (Fig. 4B) appears to have resulted from premature natural CL regression as indicated by low progesterone levels 1 to 4 days before $PGF_{2\alpha}$. However, response to the 2nd GnRH was possible since there was no longer a progesterone-induced negative feedback effect to inhibit follicular maturation and impending ovulation. Ultrasound or rectal palpation confirmation of CL development and ovulation was not performed in this study so use of the term ovulation is speculative. The 1 of 4 pregnancy rate was not unexpected as conception rates of 37 to 39% were reported with Ovsynch (Pursley et al. 1997a; Pursley et al. 1997b) in herds of over 100 to 300 cows.

The magnitude of the TP increase in VagT of 0.3°C in this study was less than half the magnitude reported by Redden et al. (1993) (0.6°C), Kyle et al. (1998) (0.9°C) and Rajamahendran et al. (1989) (1.0°C). A low VagT threshold was necessary to detect the small increases in VagT, and the low threshold was likely the cause of increased detection of FP. In this study we chose to stress detection rate at the expense of a high FP rate. Redden et al. (1993) reported only 3 FP overall with an 81% EDR in

dairy cattle while Kyle et al. (1998) reported less than 10 FP with an 88% EDR in naturally cycling beef cattle. In contrast, the best criterion in this experiment yielded a 100% EDR with 20 FP, or 2 FP per estrous cycle. The magnitude of the VagT and RumT increases did not differ between TP and FP, making the task of distinguishing between them difficult. Costs associated with extra and possibly erroneous AI would prohibit use of this criterion as a sole indicator for timing inseminations. It is not possible to explain why a certain criterion combination detects more VagT increases than another combination. Variables such as diet, barn temperature or some other unknown variable beyond our control could be factors in the accuracy of a specific set of criteria.

Ovsynch allows for the expression of estrus in only 30% of cows (Pursley et al. 1995a). Estrus occurs after CL regression when progesterone levels are low and estradiol is high (Vailes et al. 1992). It is speculated that the second GnRH in the Ovsynch protocol can terminate estradiol production before high (threshold) levels are reached, but after a sufficient level of Graafian follicle maturation has occurred (Pursley et al. 1995b). Therefore, estrus would be inhibited, but a functional oocyte could be ovulated and a functional CL could be produced. It is possible that the levels of estrogen needed to induce estrus are also required to induce a larger magnitude VagT increase. This could explain the low magnitude of the TP increases in VagT in this experiment, compared to studies where cycles were synchronized with $PGF_{2\alpha}$ (Rajamahendran et al. 1989) or unsynchronized (Redden et al. 1993; Kyle et al. 1998). It was not possible to collect VagT data during the natural estrous cycle in this experiment, so a comparison of

the magnitude of TP increases in VagT in synchronized and natural estrous cycles could not be made.

Commencement of the TP increase in VagT relative to the second GnRH varied greatly in this study. When the estrous cycle appeared to be hormonally synchronized there was a mean interval of 16 h from the second GnRH to the TP increase in VagT (days 18 and 63 in Janice; days 19 and 63 in Muffin). When the estrous cycle appeared to proceed naturally, there was a mean interval of 31 h from the second GnRH to the TP increase in VagT (days 19 and 63 in Puff). The preovulatory LH pulse occurs within 3 h of GnRH, and the timing of this LH pulse is coincident to onset of estrus, estradiol peak and VagT increase (Rajamahendran et al. 1989; Mosher et al. 1990). However, Clapper et al. (1990) reported an increase in VagT 17 h after the LH surge and onset of estrus. Three of the 4 VagT increases that were effectively synchronized by Ovsynch occurred within 8 h of the expected LH peak. This is in agreement with Rajamahendran et al. (1989) and Mosher et al. (1990). The TP increase in VagT in Muffin on day 19 occurred 28 h after the expected LH peak and so is more in agreement with Clapper et al. (1990). Therefore it is likely that these 4 VagT increases represent onset of estrus. The time of the 2 TP increases in VagT in Puff correspond to the expected time of ovulation, which Pursley et al. (1995a) states is 24 to 32 h after GnRH. Since this heifer was not hormonally synchronized the time of the TP relative to GnRH appears to be irrelevant.

Normal daily fluctuations in RumT were greater than what was recorded in VagT. The daily drop in RumT appeared to occur at the time feeding and drinking behaviours commenced. In this experiment drinking behaviour was highest during the 2

h immediately after feeding commenced, then fell considerably when feeding stopped (Fig. 5). Within 10 min of commencement of water consumption, Cunningham et al. (1964) reported a decline in RumT of 5 to 14°C and a return to previous temperatures within 2 h. In this experiment the drop in RumT likely reflected water consumption. It is unclear as to whether rumination or simply lowered rates of water consumption caused the rapid increase in RumT 3 to 4 h after commencement of feeding. This 2 h period of time had fewer instances of feeding and drinking but more instances of lying down (Fig. 5), which would suggest rumination. Unfortunately, rumination was not measured in this experiment. It is very likely that a reduction in drinking behaviour resulted in the TP increase in RumT since drinking behaviour was markedly lower on the day of the second GnRH injection and the EDO (the days of the TP increase in RumT) compared to the previous 2 days (Fig. 5).

Commencement of the TP increase in RumT relative to GnRH administration also varied greatly. The heifer that did become pregnant (Muffin) had no TP increase in RumT. The hormonally synchronized TP increases in RumT in Janice occurred at 0 h and 19 h after the second GnRH injection in estrous cycles 1 and 3, respectively. These times corresponded to the time of the TP increase in VagT in previous studies (Rajamahendran et al. 1989; Mosher et al. 1990; Clapper et al. 1990). It is likely, therefore, that the time of the TP increase in RumT corresponded to the onset of estrus. The timing of the 2 TP increases in RumT in Puff averaged 40 h after the second GnRH injection, which more closely corresponded to the expected time of ovulation. However, Puff was not considered synchronized for these cycles as the TP followed a prolonged

period of low progesterone that commenced 1 to 2 days before $PGF_{2\alpha}$. Thus the time relative to GnRH is likely irrelevant. The absence of a decline in RumT in Muffin on day 45 might have been estrus related, but it is unclear since not all of the corresponding VagT data was available. Since the best RumT criterion only detected 5 TP increases in RumT while VagT detected 8 increases, it appears that RumT does not consistently reflect changes in the estrous cycle as well as VagT. As well, there are many more FP increases in RumT that would also make it an impractical indicator of stage of the estrous cycle.

The large VagT peak in Muffin on days 28 to 30 was not related to estrus and was not a response to an injection. It was most likely due to an infection. However, it was surprising that this large VagT peak was not associated with an increase in RumT. Cunningham et al. (1964) reported that while RumT decreased over 5°C when cattle drank cold water, the temperature response in the rectum was minimal at less than 1°C. It thus appears that temperature variations can be very localized and it is possible that the large VagT peak in Muffin was a reflection of a localized vaginal infection.

Both feed intake and milk production are often reduced at estrus (Walton and King 1986), but there have been no studies on drinking behaviour at estrus. Our results demonstrate that time spent drinking and feeding was reduced on the day of GnRH and the EDO. The reduction in time spent drinking and feeding was compensated by increased time spent lying down on the day of the second GnRH and increased standing activity on the EDO. Increased walking activity has been found in both dairy and beef cows at estrus (Lewis and Newman 1984; Liu and Spahr 1993; Kyle et al. 1996). It is

likely that the standing behaviour is the only available expression of physical activity motivation at estrus in tie-stalled cows. The increased physical activity seen on the EDO (compared to the increased lying down behaviour observed the day before) may have contributed to the increases in VagT observed. Time spent lying down during estrus and ovulation has not been previously studied. Our results suggest that the increased standing activity on the EDO may be preceded by a period of lesser activity than usual. This may be why a baseline of 48 h or less is recommended when attempting to detect increased activity at estrus using pedometers (Liu et Spahr 1993). There was no heat stress in this study as the period of observation occurred in early March, and there were no changes in management routine on any day of observation. Thus it is likely that changes in behaviour were related to changes in the estrous cycle rather than environmental factors.

CONCLUSION

Rumen temperature was tested to determine its effectiveness in measuring changes in the estrous cycle. The rumen was chosen as a site because of the ease of transmitter insertion compared to placement in the vagina. A reduction in time spent drinking may have contributed to the increases in rumen temperature that occurred on the day of suspected estrus and the EDO. The low magnitude of the increases in VagT in this study was associated with a high rate of false positives compared to previous studies. However, the number of FP increases in RumT was much higher. Eight of 8 and 5 of 7 periods of low progesterone were associated with increases in VagT and

RumT, respectively. It does not appear that RumT is as effective as VagT in reflecting changes in the estrous cycle.

Table 1. Timeline detailing days of milk sampling for progesterone analysis (P4), commencement and termination of vaginal temperature (VagT) and rumen temperature (RumT) monitoring, hormone injection schedule and AI for 4 heifers in experiment 1. All hormone injections were given at 14:00 h.

Day 1 – VagT starts
Day 2 -
Day 3 -
Day 4 -
Day 5 – RumT starts (n = 3)
Day 6 -
Day 7 – P4, RumT starts (n= 1)
Day 8 – P4
Day 9 - GnRH
Day 10 – P4
Day 11 –
Day 12 – P4
Day 13 -
Day 14 – P4
Day 15 – P4
Day 16 – $PGF_{2\alpha}$
Day 17 – P4
Day 18 - GnRH
Day 19 – P4
Day 20 -
Day 21 – P4
Day 22 – P4
Day 23 -
Day 24 – P4
Day 25 -
Day 26 – P4
Day 27 -
Day 28 – P4
Day 29 – P4
Day 30 -
Day 31 – P4
Day 32 -
Day 33 – P4
Day 34 -
Day 35 – P4
Day 36 – P4
Day 37 -
Day 38 – P4
Day 39 -
Day 40 – P4
Day 41 -
Day 42 – P4
Day 43 – P4
Day 44 -
Day 45 – P4
Day 46 -
Day 47 – P4
Day 48 -
Day 49 – P4
Day 50 – P4
Day 51 -
Day 52 – P4
Day 53 - GnRH
Day 54 – P4
Day 55 -
Day 56 – P4
Day 57 – P4
Day 58 -
Day 59 – P4
Day 60 - $PGF_{2\alpha}$
Day 61 – P4
Day 62 - GnRH
Day 63 – P4, AI
Day 64 – P4
Day 65 -
Day 66 – P4, VagT and RumT end

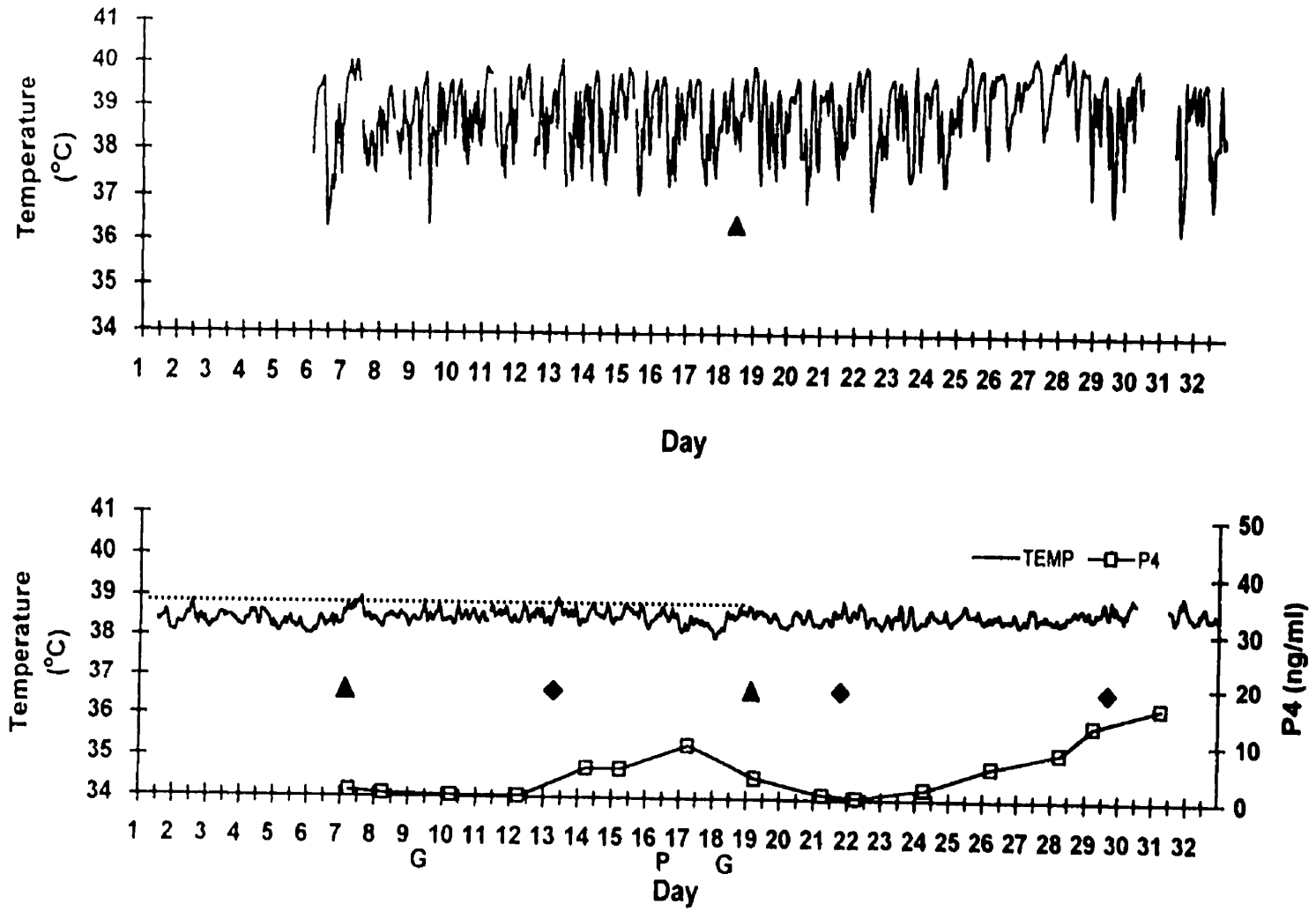


Fig. 1A. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Janice for days 1 - 32 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

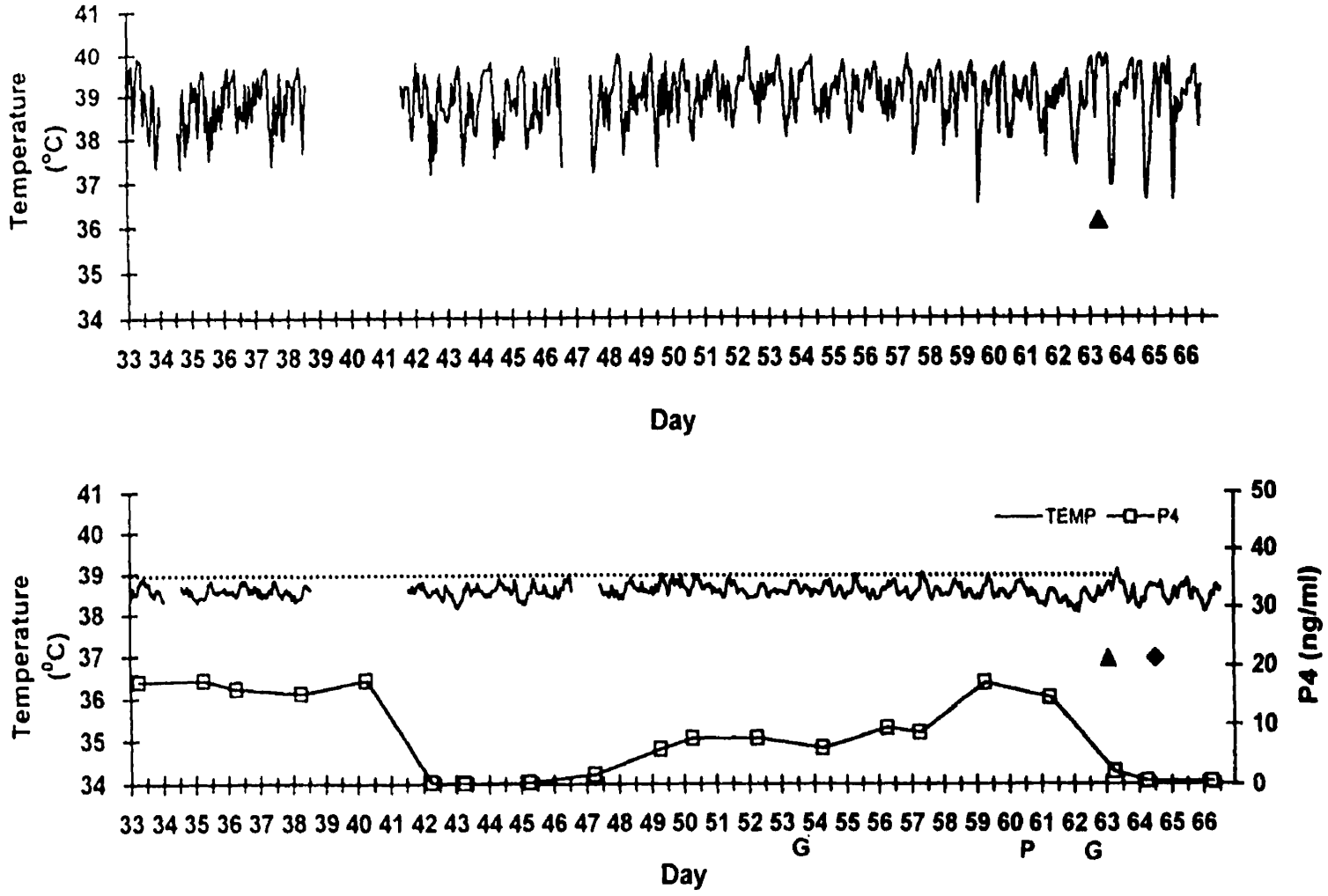


Fig. 1B. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Janice for days 33 - 66 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

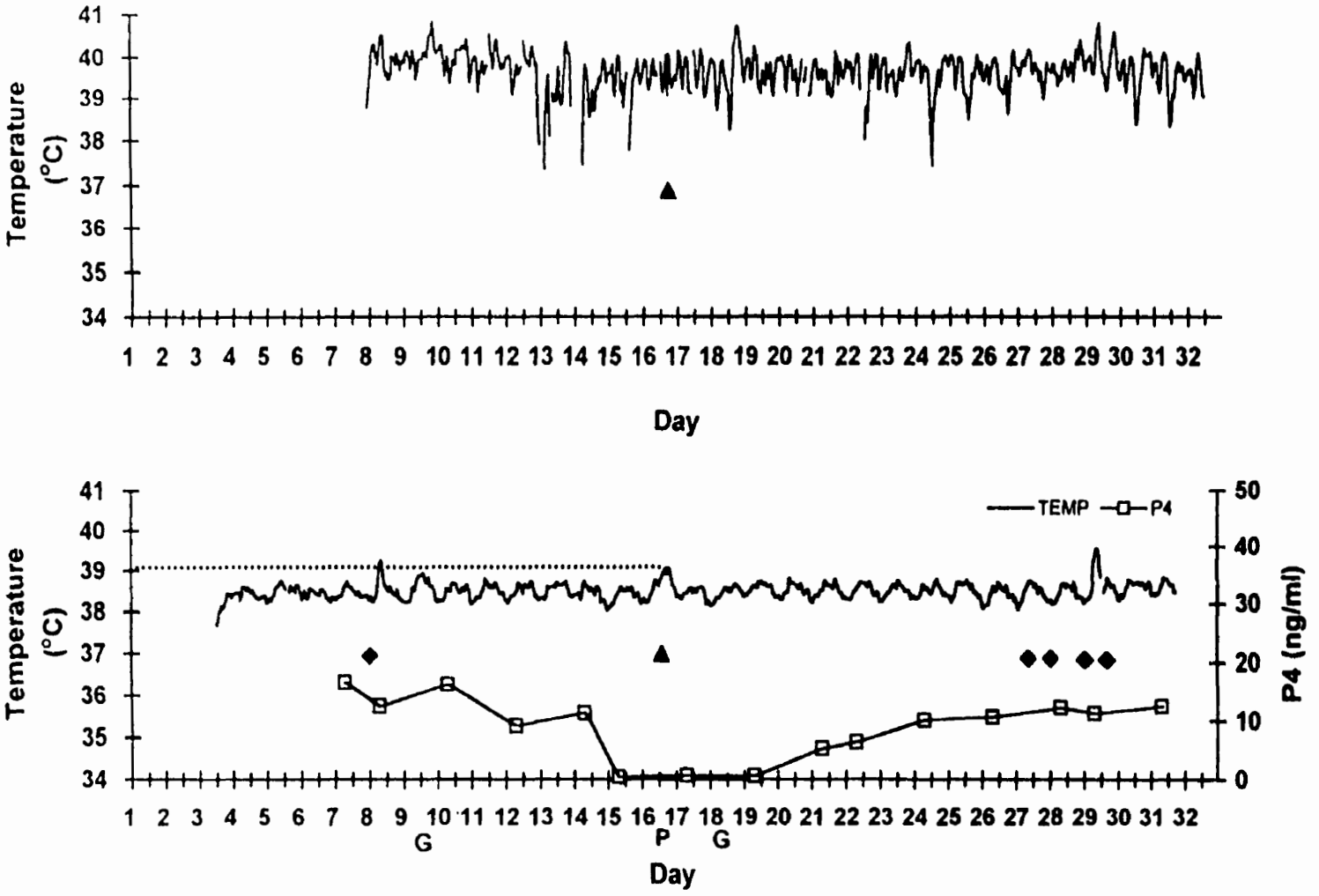


Fig. 2. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Una for days 1 - 32 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

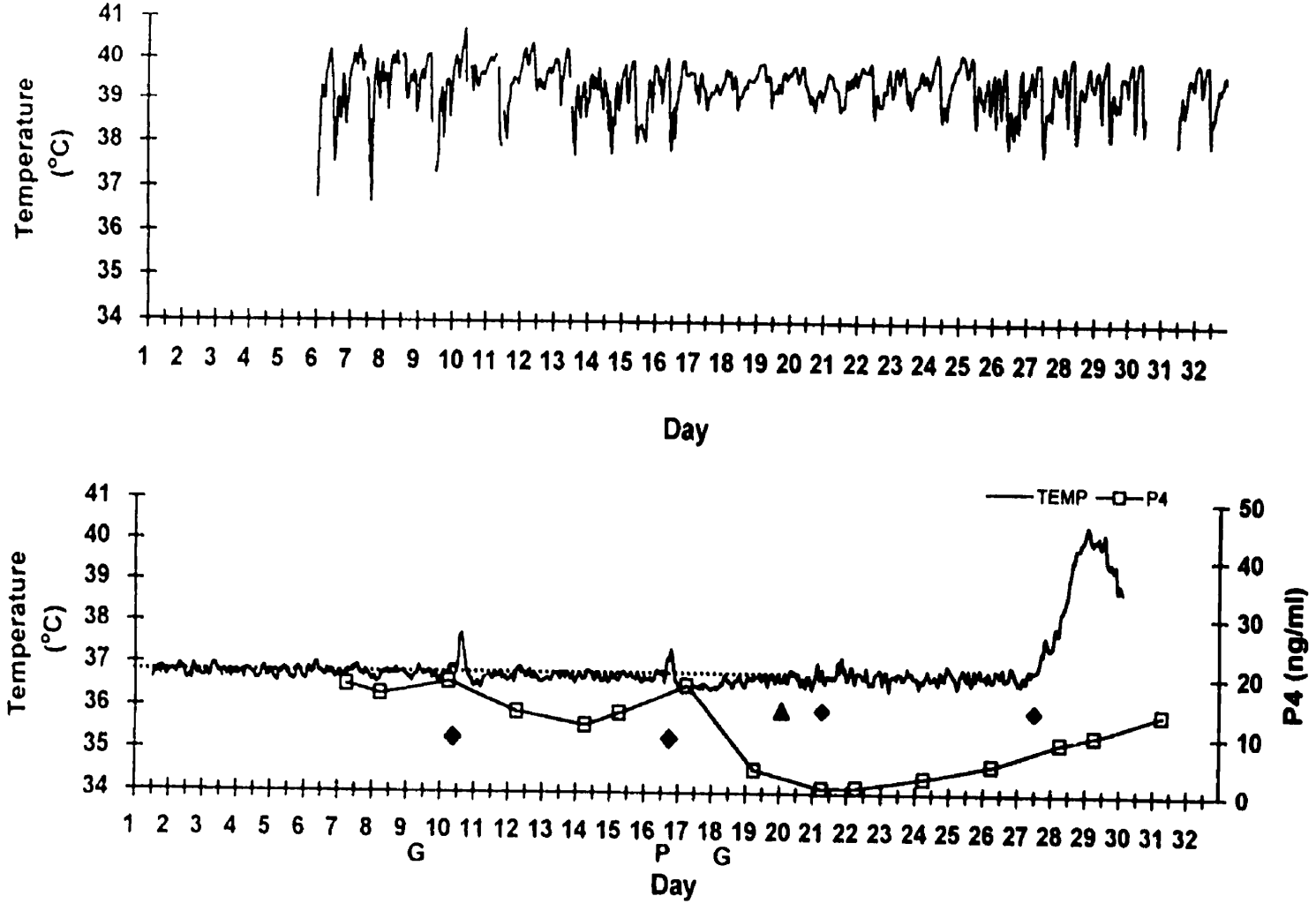


Fig. 3A. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Muffin for days 1 - 32 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

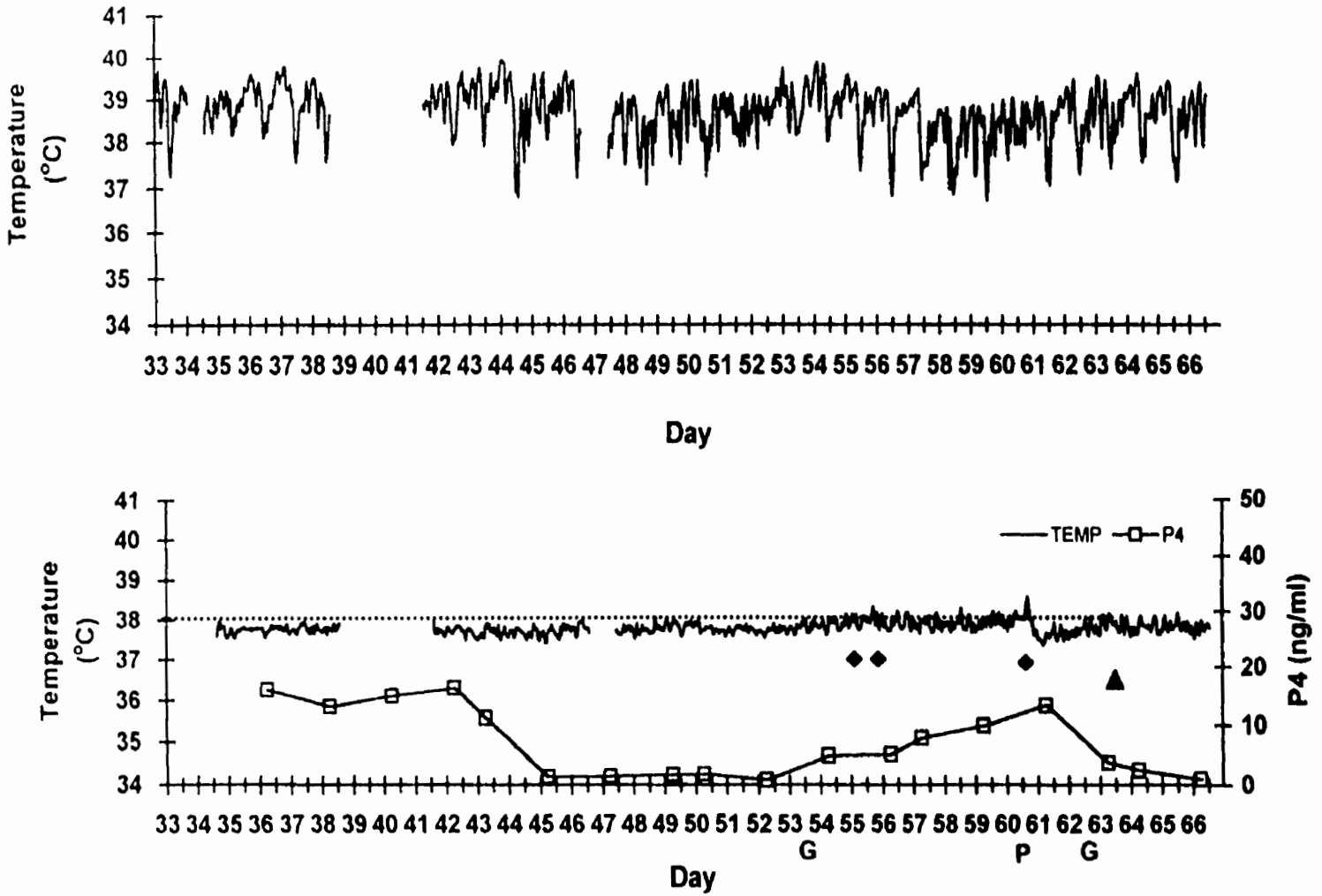


Fig. 3B. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Muffin for days 33 - 66 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

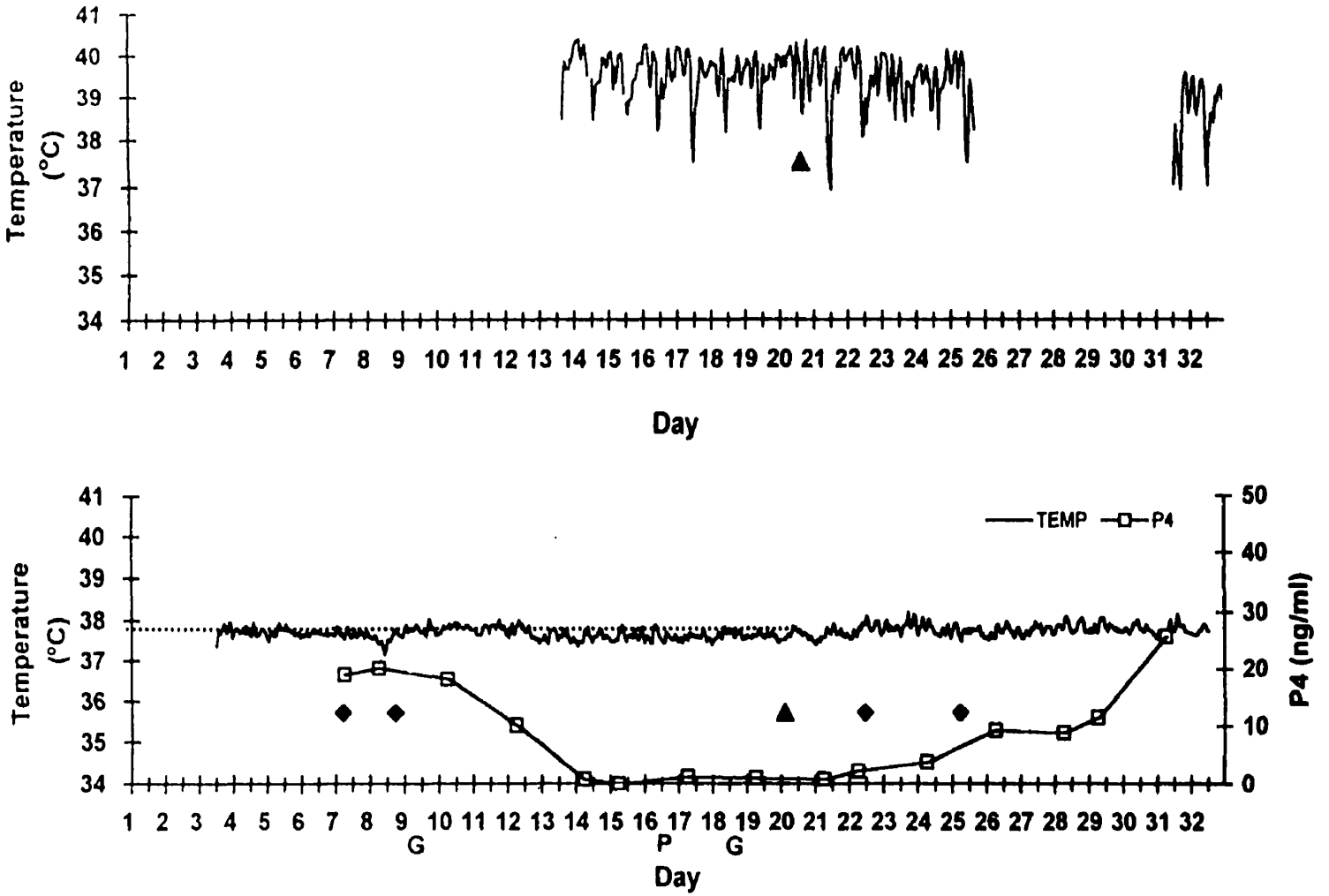


Fig. 4A. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Puff for days 1 - 32 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = day of PGF injection G = day of GnRH injection

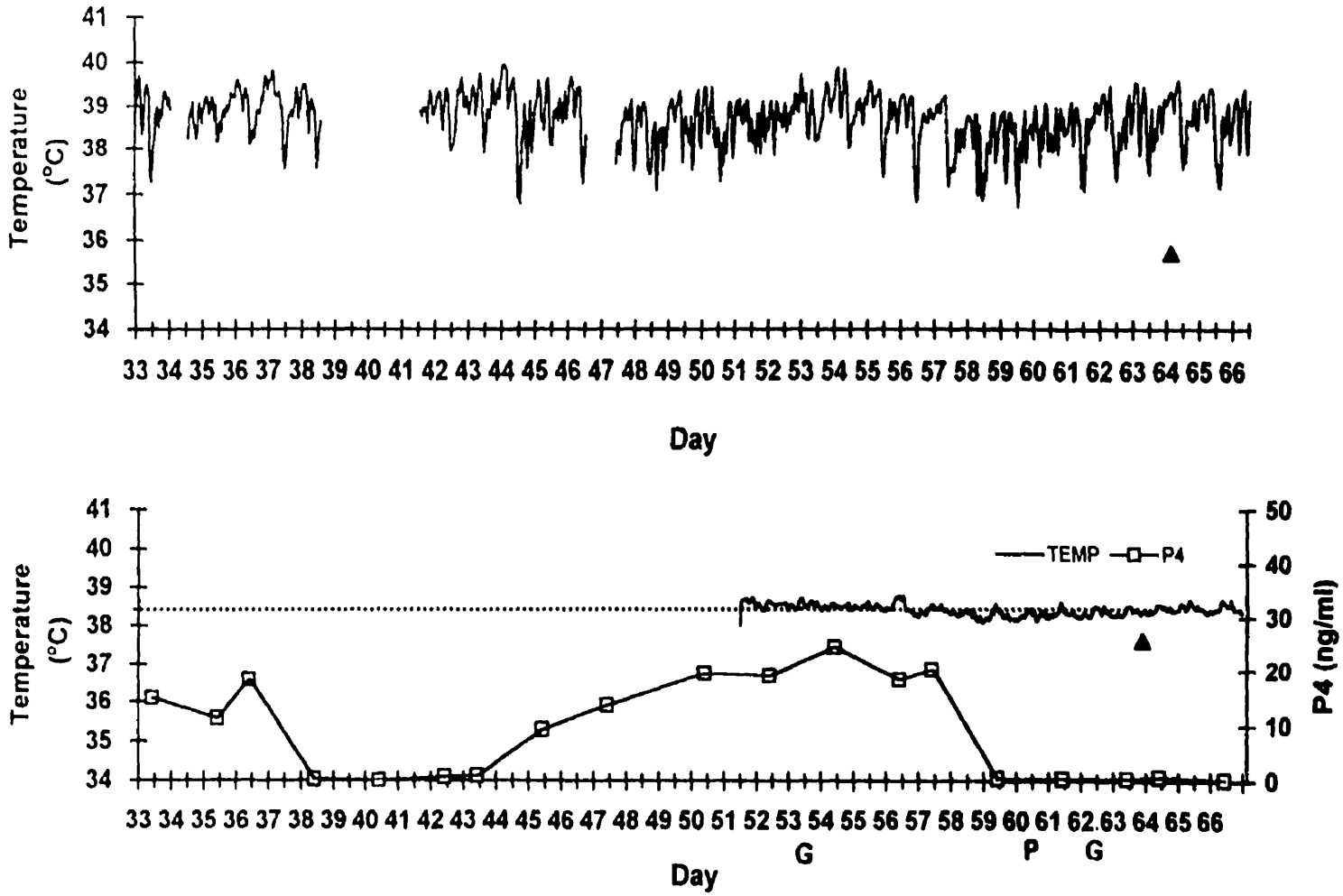


Fig. 4B. Rumen temperature (upper graph), vaginal temperature and progesterone (lower graph) of Puff for days 33 - 66 of the trial. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds indicate false positives in vaginal temperature. Rumen and vaginal temperatures are hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P =day of PGF injection G = day of GnRH injection

Table 2. Criteria for detection of increases in vaginal temperature 48 h after GnRH administration in experiment 1 based on changes in absolute temperature.

		Absolute Temperature Criterion		
Window Length	**Baseline Duration**	**0.30 (TP/FP)**	**0.25 (TP/FP)**	**0.20 (TP/FP)**
12h	*4day*	(6,10)	(6,17)	(6,26)
	3day	(5,11)	(6,20)	(6,25)
	2day	(5,3)	(5,8)	(7,11)
	1day	(6,4)	(6,6)	(6,11)
6h	*4day*	(5,4)	(6,9)	(6,15)
	3day	(5,6)	(5,11)	(8,22)
	2day	(6,12)	(7,13)	(7,18)
	1day	(6,8)	(6,9)	(6,14)
3h	*4day*	(5,10)	(6,17)	(6,26)
	3day	(6,10)	(7,14)	(8,20)*
	2day	(6,11)	(6,14)	(7,17)
	1day	(5,12)	(6,15)	(6,23)
1h	*4day*	(4,7)	(6,13)	(8,23)
	3day	(5,7)**	(5,11)	(7,18)
	2day	(5,8)	(5,12)	(6,20)
	1day	(5,19)	(5,22)	(6,27)

* Best criterion

** Criterion used by Redden et al (1993)

TP = True Positive: temperature increase when progesterone below 5 ng/ml

FP = False Positive: temperature increase when progesterone above 5 ng/ml

Table 3. Day of occurrence and duration of true positive (TP) vaginal temperature increase in experiment 1 after administration of GnRH when progesterone levels were below 5 ng ml^{-1}.

Animal	Day of Trial	Time of TP Vaginal Temperature Increase	Time from GnRH Injection to Beginning of TP Vaginal Temperature Increase (h)
Janice	Day 7	0:00 h – 15:00 h	NA
	Day 18 to 19	23:00 h – 4:00 h	9
	Day 63	0:00 h – 11:00 h	10
Muffin	Day 19 to 20	22:00 h – 5:00 h	32
	Day 63	1:00 h – 10:00 h	11
Puff	Day 19 to 20	20:00 h – 12:00 h	30
	Day 63 to 64	21:00 h – 2:00 h	31
Una	Day 16	13:00 h – 18:00 h	NA
		Mean ± S.E.	20.5 ± 4.7

Table 4. Criteria for detection of increases in rumen temperature 48 h after GnRH administration in experiment 1 based on changes in absolute temperature.

		Absolute Temperature Criterion		
Window Length	Baseline Duration	0.50 (TP/FP)	0.75 (TP/FP)	1.00 (TP/FP)
3h	*5day*	(5,26)*	(4,10)	(2,5)
	4day	(5,27)	(4,6)	(1,4)
	3day	(4,30)	(3,9)	(2,2)
	2day	(4,46)	(3,10)	(2,2)
1h	*5day*	(4,34)	(3,8)	(1,4)
	4day	(4,29)	(3,10)	(1,3)
	3day	(4,34)	(2,8)	(1,4)
	2day	(4,34)	(2,8)	(1,5)

* Best criterion

TP = True Positive: temperature increase when progesterone below 5 ng/ml

FP = False Positive: temperature increase when progesterone above 5 ng/ml

Table 5. Day of occurrence and duration of true positive (TP) rumen temperature increase in experiment 1 after administration of GnRH when progesterone levels were below 5 ng ml^{-1}.

Animal	Day of Trial	Time of TP Rumen Temperature Increase (h)	Time from GnRH Injection to Beginning of TP Rumen Temperature Increase (h)
Janice	Day 18	14:00 h – 20:00 h	0
	Day 63	9:00 h – 16:00 h	19
Puff	Day 20	10:00 h – 15:00 h	44
	Day 64	3:00 h – 11:00 h	37
Una	Day 16	17:00 h – 23:00 h	NA
		Mean ± S.E.	25.0 ± 9.9

Table 6. Baseline temperature, magnitude and duration of the true positive (TP) VagT increase between heifers that did (n = 1) or did not become pregnant (n = 3) and between those periods of low progesterone that occurred on schedule according to the Ovsynch protocol (n = 6) versus those periods that occurred prematurely (n = 2) in experiment 1.

	Pregnancy Results		Period of Low Progesterone		
	Pregnant	Open	Scheduled	Premature	Probability
VagT Baseline (°C)	37.77 ± 0.03	38.38 ± 0.05	38.40 ± 0.05	37.87 ± 0.29	0.36
Magnitude of TP vagT Increase (°C)	0.3	0.3 ± 0.05	0.4 ± 0.01	0.3 ± 0.03	0.02
Duration of TP vagT Increase (h)	9	8.0 ± 3.01	10.0 ± 5.01	8.8 ± 4.22	0.77

Table 7. Least square means and level of significance for the four cattle activites observed after the provision of feed

	Day (Activity as Percentage)					Hour (Activity as Percentage)					Level of Significance (P = probability)		
Activity	1	2	3	4	SE	1	2	3	4	SE	Day	Hour	DxH
Drinking	18	16	10	9	2.2	13	17	10	13	1.9	0.0024	0.0406	0.0292
Eating	44	41	36	30	2.4	72	40	21	19	3.5	0.0181	0.0001	0.0047
Standing Only	17	19	23	36	1.9	15	37	17	26	3.4	0.0008	0.0003	0.0192
Lying Down	21	25	31	25	4.2	1	6	53	42	5.7	0.7102	0.0001	0.0377

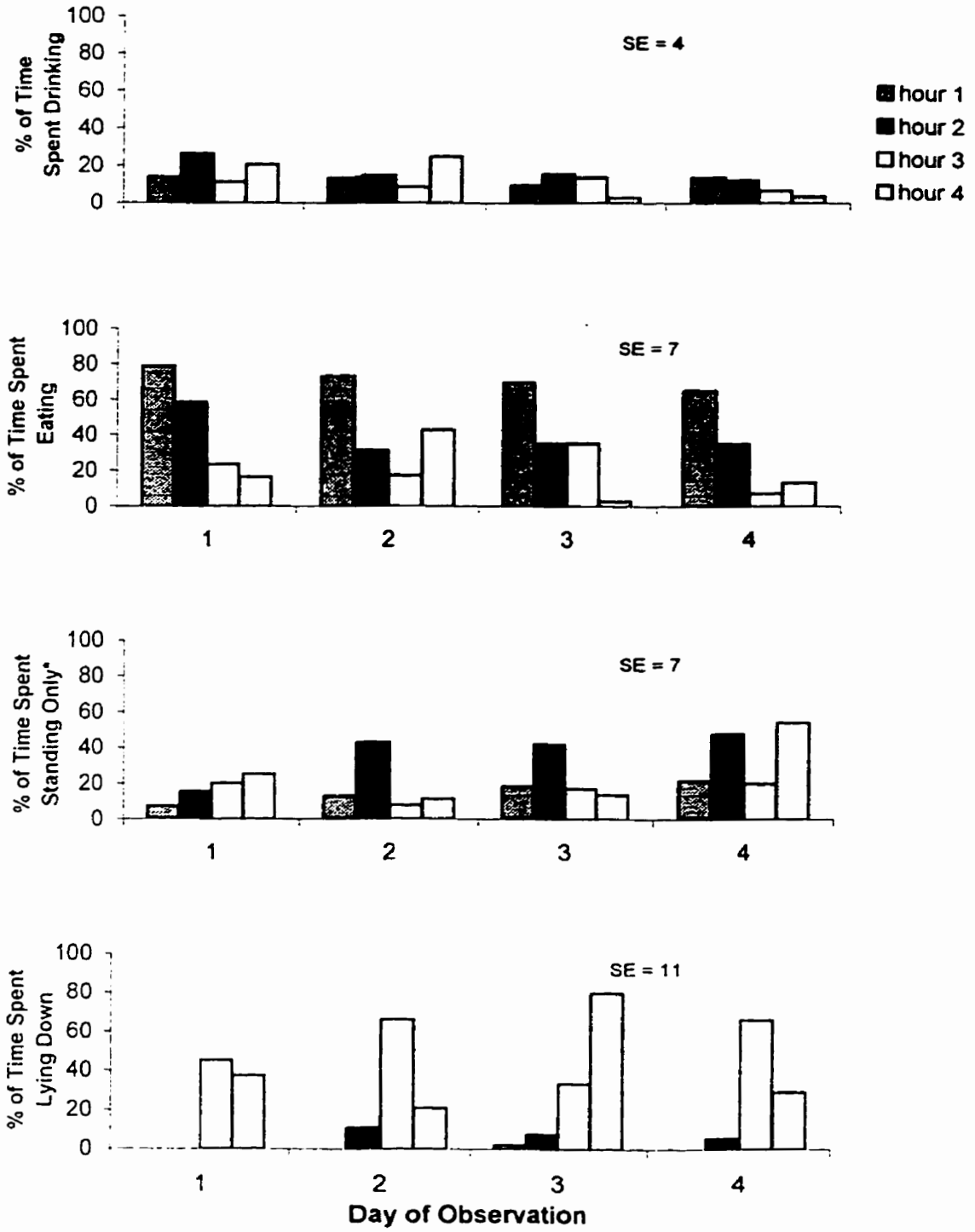


Fig. 5. Behaviour for hours 1 - 4 post-provision of feed on four days around the time of ovulation inducement. PGF and GnRH were administered 5 - 10 minutes before the end of hour 4 of behavioural observation. * % Standing Only does not include time of standing and eating or standing and drinking.

MANUSCRIPT 2 –

CHANGES IN THE RELATIONSHIP BETWEEN WATERING BEHAVIOUR, VAGINAL TEMPERATURE AND RUMEN TEMPERATURE IN TIE-STALLED DAIRY COWS THROUGHOUT THE ESTROUS CYCLE

ABSTRACT

Holstein cows (n = 26), aged 3 to 6 yrs, were allocated to 3 trials lasting 20 days each where watering behaviour, vaginal temperature and rumen temperature were measured. Cows in each trial were synchronized by either double $PGF_{2\alpha}$ administered 14 days apart (Psyn) (trial 1, n = 5; trial 2, n = 4; trial 3, n = 4) or the Ovsynch protocol (GPsyn) (trial 1, n = 4; trial 2, n = 5; trial 3, n = 4). With the Ovsynch protocol, an intramuscular injection of GnRH (100 μg) was administered at a random stage of the estrous cycle. Seven days later the cows received an intramuscular injection of $PGF_{2\alpha}$ (500 μg) and a second GnRH injection followed 48 h later. Watering behaviour was measured continuously in 15 cows in trials 1 and 2 (trial 1, n = 7; trial 2, n = 8) by monitoring changes in waterpipe temperature during periods of waterbowl activation. Vaginal temperature (VagT) was monitored continuously in Psyn cows of trials 2 and 3 by a radiotelemetric device inserted into the vagina. Rumen temperature (RumT) was monitored continuously by radiotransmitters tethered in the rumen of 2 rumen-cannulated Psyn cows in trial 3. Visual observation of standing heat was performed daily between 9:00 h to 12:00 h after $PGF_{2\alpha}$ (day 15) in Psyn cows for trials 1 and 3. Psyn cows in trials 1 and 3 were bred 2 to 5 h after observation of standing heat, while Psyn cows in trial 2 underwent timed insemination 74 to 77 h after $PGF_{2\alpha}$. GPsyn cows in all trials were inseminated 26 to 29 h after the second injection of GnRH. Pregnancy rates were 1/4 for GPsyn and 2/5 for Psyn in trial 1, 4/5 for GPsyn and 2/4 for Psyn in trial 2 and 1/4 for GPsyn and 1/3 for Psyn in trial 3. Eight periods of low progesterone (below 5 ng ml^{-1}) were detected by milk progesterone analysis in the 8 cows fitted with vaginal

radiotransmitters. Eight increases in VagT coincided with these periods of low progesterone (TP) and 5 FP were found. For the 2 occasions where RumT was available, there were 2 increases in RumT that coincided with the periods of low progesterone (TP) and 3 FP were found. The magnitude of the TP increases in VagT and RumT were 0.3 ± 0.07°C and 0.6 ± 0.08°C, respectively. Six of 8 Psyn cows had a TP increase in VagT one day before or the day of insemination, which corresponded to day of estrus. The 2 TP increases in RumT occurred on the same day as the TP increase in VagT, and thus corresponded to day of estrus as well. The number of watering episodes and duration of watering behaviour per day was 16.5 ± 1.1 and 78.1 ± 6.3 min, respectively. There was no indication of changes in watering behaviour over time in Psyn cows. In trial 2, GPsyn cows exhibited decreased watering behaviour on the day of $PGF_{2\alpha}$ followed by a compensatory increase on the following two days that preceded ovulation. Watering behaviour did not appear to reflect changes throughout the estrous cycle as well as did VagT or RumT.

INTRODUCTION

Body temperature, activity and mounting activity have been measured as indicators of estrus. However, there has been little research done on feeding behaviour and watering behaviour at estrus. Feed intake (Walton and King 1986) and milk production (Schofield et al. 1991; Walton and King 1986) are reported to decrease at estrus. Thus it is likely that water intake decreases as well. The main objective of this experiment was to examine watering behaviour in dairy cows before and during estrus.

Watering behaviour was examined individually by continuous electronic monitoring of individual cow watering devices. The second objective was to compare watering behaviour and pregnancy rates in cows synchronized with either Ovsynch or $PGF_{2\alpha}$ administered 14 days apart. The final objective was to examine changes in rumen temperature during the estrous cycle in comparison to vaginal temperature.

MATERIALS AND METHODS

Animals and Management

Twenty-six Holstein cows, from ages 3 to 6 yrs, were studied at the Glenlea Research Station Dairy Unit at the University of Manitoba. Three trials were conducted using 9 cows from November to December 1996 (152 ± 7.1 d postpartum), 9 cows from January to February 1997 (72 ± 3.7 d postpartum), and 8 cows from March to April 1997 (57 ± 6.4 d postpartum). Once daily at 12:00 h to 12:30 h the cows were fed 2 kg long hay and a silage based total mixed ration consisting of grass silage, standard 16% grain and sunflower seeds. The cows had free access to water using individual waterbowls. Cows in trials 1 and 3 were exercised outdoors from 9:00 h to 12:00 h daily, during which time the cows could be observed for standing heat. Cows in trial 2 were not exercised due to cold weather. All procedures were in accordance with the guidelines outlined by the Canadian Council on Animal Care.

Reproductive Management

Two synchronization treatments were used. Treatment 1 (GPsyn) was the Ovsynch protocol as described in experiment 1 and was used for 13 cows (trial 1, n = 4; trial 2, n = 5; trial 3, n = 4). Treatment 2 (Psyn) involved two injections of $PGF_{2\alpha}$ (500 μg Estrumate®, Coopers Agropharm Inc., Ajax, ON) given 14 d apart to the other 13 cows (trial 1, n = 5; trial 2, n = 4; trial 3, n = 4). In each trial, day 1 was the day of the first $PGF_{2\alpha}$ in the Psyn cows. GPsyn cows received their first GnRH injection on day 8. Both the GPsyn and Psyn cows received $PGF_{2\alpha}$ on day 15. GPsyn cows received their second GnRH injection on day 17. All injections were given at 10:00 h during trials 1 and 2. Injections during trial 3 were given at 14:00 h. Fig. 6 depicts the experimental procedures performed on both groups of cows in each trial. Visual observation for standing heat was performed from days 15 to 20 of trials 1 and 3. Cows in trial 2 were not observed for standing heat because they were not exercised. Casual observations of estrus by the staff for clear vaginal mucus discharge and restlessness in the evenings were also performed. Psyn cows in trials 1 and 3 were inseminated on the day standing heat was observed after the daily exercise period (12:30 to 15:30 h). GPsyn cows for all 3 trials were bred on day 18 at 26 to 29 h after the 2nd GnRH injection, (ie. 74 to 77 h after the $PGF_{2\alpha}$). The timing of insemination in trial 2 was the same for Psyn and GPsyn cows (day 18, 77 to 78 h after the $PGF_{2\alpha}$) because the lack of exercise prevented observation of standing heat. Composite milk samples were taken 4 times weekly (Mon., Wed., Fri., Sun.) from each cow after the morning milking for the entire test period, so that sampling occurred on the day of, or the day after the hormone injections and AI. Milk

progesterone was measured using a radioimmunoassay as described in Tekpetey et al. (1987). CL regression was considered confirmed if milk progesterone levels fell below 5.0 ng ml^{-1} for 2 or more consecutive samples. Pregnancy was confirmed by rectal palpation at 37 to 48 d after AI and calving rate was confirmed by the delivery of a live calf.

Measurement of Vaginal and Rumen Temperature

In trials 2 and 3 vaginal and rumen temperatures were monitored only in Psyn cows. The Psyn cows in trials 2 and 3 were fitted with vaginal temperature radiotransmitters and monitored by the radiotelemetry system (Redden et al. 1993) as described in experiment 1. The GPsyn synchronized cows were fitted with a sham device consisting of a syringe tube fitted in the plastisol anchor but no temperature radiotransmitter. All anchors had medium length (24 mm) finger-like projections. As well, two of the Psyn cows in trial 3 had rumen temperature monitored by insertion of temperature radiotransmitters through the rumen cannulae as described in experiment 1.

Measurement of Watering Behaviour

Watering behaviour, or the act of water delivery device activation for the purpose of drinking, was monitored in all cows throughout the entire experiment by continuously recording the temperature of the water pipe leading to the waterbowl. Water pipe temperature changed soon after cattle initiated or terminated waterbowl activation. Each tie-stall was equipped with an individual waterbowl and the pipe temperature was

monitored using an insulated copper and constantan thermocouple. The thermocouple was laid along the individual water pipe and the bulb was taped to the metal connection of the waterbowl (Fig. 7). In trials 1 and 2, thermocouple temperatures were recorded using a Molytek® strip-chart recorder (Molytek Inc., Pittsburgh, PA.) which recorded temperature every 15 s. In trial 3, the thermocouple temperature was recorded using an 8-channel input board (Omega Engineering Inc., Stamford, CT) attached to a personal computer, which ran a private datalogging program that recorded temperature every 30 s to a disk file. Water pipe temperature data was recorded from days 10 to 17 in trial 1 and days 11 to 18 in trial 2. This included the day of $PGF_{2\alpha}$ injection for both the Psyn and GPsyn cows, and the day of the final GnRH injection for the GPsyn cows.

Pre-trial tests were done where the water nozzle was pushed for a given length of time (10 s, 30 s, 1 min, 2 min, 3 min and 10 min) for each pipe. Based on these results, watering behaviour was assumed to have started when the pipe temperature indicated a rapid and noticeable increase above baseline temperature. Watering behaviour was assumed to have stopped as soon as the pipe temperature began to decrease towards the baseline temperature.

Calculations and Statistical Analysis

Vaginal and rumen temperatures were analyzed using the criteria developed in experiment 1. General Linear Model procedures (SAS Institute Inc., 1988) were used to determine differences between pregnant and nonpregnant cows with regard to mean baseline, magnitude and duration of the increase in VagT, and interval from $PGF_{2\alpha}$ to the

beginning of the increase in VagT. All means are expressed with their respective standard error. The mean VagT baseline for 118 to 142 h before $PGF_{2\alpha}$ and for 2 to 56 h after $PGF_{2\alpha}$ (prior to the beginning of the TP increase in VagT) was calculated. The difference between pre- and post- $PGF_{2\alpha}$ was calculated and compared by paired T-tests (SAS Institute Inc., 1988) for those with or without an apparent gradual decline in VagT subsequent to $PGF_{2\alpha}$. The number of watering episodes and watering duration (WD) per 3 h period of the day were calculated and analyzed for differences between periods of the day using the General Linear Model procedures (SAS Institute Inc., 1988). The daily WD (min/d) differed in trials 1 and 2 and this was adjusted to account for differences in milk yield between trials. Adjusted daily WD (WDa) was calculated by the following calculation:

WDa = WD/average milk yield = min/d/kg.

Results were analyzed by GLM procedures using the model:

WDa = synchronization method, cow (synchronization method), day, synchronization method x day interaction, and day x cow (synchronization method). The error term for synchronization method was cow (synchronization method). Data for each trial was analyzed separately since trial 1 cows were exercised while trial 2 cows were not. Additionally, in trials 1 and 2 the above analysis was performed for WDa calculated from midnight to noon, and WDa calculated from noon to midnight. Paired T-tests (SAS Institute Inc., 1988) were used for each trial to compare the period of time before and after $PGF_{2\alpha}$ (days 11 to 15, or days 11 to 13, or days 14 and 15 alone compared to days 16 to 17 together or each day alone) within a synchronization method.

Paired T-tests (SAS Institute Inc., 1988) were used to compare the period of time before and after $PGF_{2\alpha}$ (days 11 to 15 compared to days 16 to 17) between cows within a synchronization method that did or did not become pregnant. The model was WDa = pregnancy, cow (pregnancy), day, pregnancy x day interaction, and day x cow (pregnancy). The error term for pregnancy was cow (pregnancy). Day was a repeated measure in both situations.

RESULTS

One GPsyn animal in trial 1 had vaginal mucus discharge on the evening of day 16, the night before the second GnRH injection. She was inseminated the next afternoon. One Psyn cow (Ellymay) in trial 3 did not express estrus after the second $PGF_{2\alpha}$ and so was not bred. Ellymay was not included in pregnancy and calving rates, but her results were included in the VagT analysis. Therefore there were 8 cows available for VagT analysis. The overall pregnancy rate was 11 out of 25 cows (44 %). The pregnancy rates of both the GPsyn and Psyn cows are listed in Fig. 6. Six of 13 (46 %) GPsyn cows and 5 of 12 (42 %) Psyn cows became pregnant. The overall calving rate was 9 of 25 cows (36 %). The calving rate was 4 of 13 for GPsyn cows and 5 of 12 for Psyn cows. There was a 33% pregnancy loss for GPsyn cows. The pregnancy rate for those Psyn cows in trial 2 bred by timed insemination was 2 of 4 (50%). The pregnancy rate for those Psyn cows in trials 1 and 3 bred by visual detection of estrus was 3 of 8 (38%). The milk progesterone assay sensitivity and intra-assay coefficient of

variation was 0.06 ml $^{-1}$ and 6.3 %, respectively, in trial 1; 0.08 ml $^{-1}$ and 9.4 % in trial 2 and 0.08 ml $^{-1}$ and 8.9 % in trial 3.

Only Psyn cows were being monitored for changes in VagT. The failure of one temperature radiotransmitter resulted in 68 h of lost data. Also, radiotransmitter signals could not be received when trial 3 cows were exercised outdoors. VagT results were collected for all 8 Psyn cows in trials 2 and 3. These values as well as RumT values for 2 Psyn cows are given in Appendix 2. Each cow had a period of low progesterone before the TP increase in VagT.

As in experiment 1, there was a discernable circadian pattern in VagT with a drop of approximately 0.2°C between midnight to 06:00 h in 4 cows. An example is given in Fig. 8. This pattern was inconsistent in the other cows (Fig. 9). The daily VagT was 37.9 ± 0.10°C with a range between 37.3 to 38.5°C. A gradual decline in VagT appeared in 3 cows approximately 1 to 3 days before the TP increase in VagT, and this time period corresponded to the fall in progesterone induced by $PGF_{2\alpha}$. The difference in VagT pre- and post- $PGF_{2\alpha}$ tended to be significant ($P = 0.15$) between those cows that did have a decline (37.9 ± 0.03 vs. 37.8 ± 0.05, pre and post, respectively) and those that did not (37.9 ± 0.08 vs. 38.0 ± 0.12, pre and post, respectively). Examples of the VagT baseline with or without the decline are given in Figs. 10 and 8, respectively.

All the absolute temperature criteria from experiment 1 were applied to VagT data. In an attempt to maximize the TP increases in VagT, a temperature threshold of 0.15°C was also included. The results of the absolute temperature criteria testing are listed in Table 8. Eight TP increases in VagT were found (the maximum possible) with

the absolute temperature criteria, but these were accompanied by FP every 5 to 6 days. When the standard deviation criteria from experiment 1 were applied, 8 TP increases in VagT were found and the number of FP were kept low when the 4 d and 3 d baselines were used. The results of standard deviation criteria testing are listed in Table 9. The best criterion was an increase in VagT of 2 S.D. for a 3 h window of time over a 4 d baseline (the 2SD-3W-4D criterion). This criterion yielded 8 TP increases in VagT and 5 FP (a FP every 25 days). The day and duration of the TP increase in VagT found with the 2SD-3W-4D criterion are listed in Table 10. Examples of the TP increase in VagT are given in Figs. 8, 9 and 10.

The magnitude and duration of all 8 TP increases in VagT was 0.3 ± 0.07°C and 7.8 ± 1.8 h, respectively. The magnitude of FP increases in VagT was 0.3 ± 0.09°C, and the magnitude of TP and FP VagT increases did not differ ($P = 0.18$). The interval from $PGF_{2\alpha}$ to the beginning of the TP increase in VagT for all 8 cows was 41.1 ± 6.9 h. Whether or not a cow got pregnant or was exercised had no effect ($P > 0.12$) on these parameters. The results are given in Table 11.

A consistent circadian pattern was seen in the RumT of both cows with a drop of approximately 3 °C found at noon followed by a climb in temperature within 3 to 4 h. The daily RumT was 38.2 ± 0.33°C with a range between 34.4 to 39.8°C. Five different criteria were applied to the RumT data for the two cows in trial 3. The 0.3C-1W-3D criterion (Redden et al. 1993) yielded 1 of 2 possible TP increases in RumT and 3 FP. The 0.2C-3W-3D criterion yielded 1of 2 possible TP increases in RumT and 4 FP. The 0.5C-3W-5D rumen criterion from experiment 1 and the 2SD-3W-4D criterion yielded 2

of 2 possible TP increases in RumT and 3 FP (a FP every 9 days). The TP increases in RumT occurred at 16:00 h to 20:00 h on day 17 for Muffin and at 11:00 h to 15:00 h on day 17 for Janice. An example of a TP increase in RumT is given in Fig. 9.

The magnitude and duration of the TP increases in RumT were 0.6 ± 0.08°C (n = 2) and 4.0 h, respectively. The hours from $PGF_{2\alpha}$ administration to the TP increase in RumT was 47.5 ± 2.7 h. The magnitude of FP increases in RumT was 0.9 ± 0.15°C (n = 3). The magnitude of the TP and FP increases in RumT did not differ (P = 0.17).

In trial 2, there were TP increases in VagT the night before timed insemination in 3 of 4 Psyn cows. The other Psyn cow (Whitney) had the TP increase in VagT on the day of $PGF_{2\alpha}$, 3 d before timed insemination. In trial 3, three of 4 Psyn cows expressed estrus and had a TP increase in VagT on the day of estrus.

Technical difficulties limited the ability of the thermocouples to measure changes in water temperature for every cow, particularly in trial 3. Therefore, watering behaviour data was available for only 15 cows in trials 1 and 2 (8 GPsyn, 7 Psyn). The start of a watering episode on the Molytek® was depicted as a rapid heating up of the pipe, or a visible increase from an even baseline. The end of a watering episode was depicted as a pipe temperature decrease, or a somewhat rapid and smooth return to baseline (Fig. 11). The Molytek® printouts varied among thermocouples. Certain thermocouples had little variations in baseline so even watering episodes of short duration could be easily detected (Fig. 12). However, some thermocouples had a more variable baseline so short duration watering episodes as in Fig. 12 would be impossible to distinguish from baseline (Fig. 13). The shortest duration of watering behaviour

depended on the thermocouple. Watering episodes of 30 s duration could be measured by thermocouples with smooth baselines. Watering episodes of 1 min duration could be measured by thermocouples with more variable baselines. If a deflection stopped less than halfway when returning to baseline it was called one watering episode (Fig. 14). If the deflection stopped more than halfway when returning to baseline it, and the subsequent episodes, were considered multiple watering episodes (Fig. 15).

Cows in trial 1 had more watering episodes/day (20.1 ± 1.0, range of 1 to 46) than cows in trial 2 (12.9 ± 0.7, range of 1 to 31) (P = 0.02). There was no effect of time of day on number of watering episodes in either trial 1 (P = 0.69) or 2 (P = 0.78).

The duration of a single watering episode was less (P = 0.02) in trial 1 (3.0 ± 1.8 min, range of 0.3 to 7.2 min) than in trial 2 (7.5 ± 3.3 min, range of 0.6 to 21.6 min). There was no significant effect of time of day on WD (min/3 h period) in trials 1 (P = 0.34) and 2 (P = 0.21). Therefore time of day was not included in further statistics. The daily WD was less (P = 0.01) in trial 1 (59.5 ± 2.9 min/d, range of 17.3 to 123.4 min) than in trial 2 (96.7 ± 7.2 min/d, range of 25.3 to 235.2 min). This difference in WD between trials was eliminated by correcting for milk yield in that the overall daily WDa did not differ (P = 0.40) between trial 1 (2.0 ± 0.2 min/d/kg) and trial 2 (2.3 ± 0.2 min/d/kg). The daily WDa for GPsyn cows did not differ (P = 0.70) between trial 1 (2.3 ± 0.3 min/d/kg) and trial 2 (2.6 ± 0.3 min/d/kg). The daily WDa for Psyn cows did not differ (P = 0.65) between trial 1 (1.8 ± 0.2 min/d/kg) and trial 2 (2.0 ± 0.2 min/d/kg).

There was no effect of synchronization method (P = 0.58), day of trial (P = 0.36) or the interaction (P = 0.50) on WDa in trial 1. There tended to be an effect of day of

trial (P = 0.13) on WDa in trial 2, but synchronization method (P = 0.39) and the interaction (P = 0.50) had no effect. Fig. 16 shows the daily WDa for GPsyn and Psyn treatment groups in trial 2. The WDa for GPsyn cows in trial 2 was lower before $PGF_{2\alpha}$ administration (days 11 to 15) than after $PGF_{2\alpha}$ administration (days 16 and 17 together, P = 0.05; day 16 alone, P = 0.09; day 17 alone, P = 0.05). Also, the WDa for the GPsyn cows on day 15 alone was lower than days 16 and 17 together (P = 0.01), day 16 alone (P = 0.02) and day 17 alone (P = 0.02). The WDa for Psyn cows in trial 2 did not differ (P > 0.20) between the period before $PGF_{2\alpha}$ administration (days 11 to 15) versus the period after $PGF_{2\alpha}$ administration (days 16 and 17). There was no effect of synchronization method (P = 0.82), day of trial (P = 0.50) or interaction (P = 0.50) on the A.M. WDa in trial 2. There tended to be effects of synchronization method (P = 0.08), day of trial (P = 0.14) but not interaction (P = 0.50) on the P.M. WDa in trial 2 (Fig. 17). The trial 2 WDa for the GPsyn cows on days 11 to 15 was lower than on days 16 to 17 in the P.M. (P = 0.02). The WDa on day 15 tended (P = 0.06) to be lower than days 16 and 17 together in the P.M. The P.M. WDa for the Psyn cows in trial 2 did not differ (p > 0.25) between the periods pre- and post- $PGF_{2\alpha}$.

There was no effect of pregnancy (P = 0.39), day of trial (P = 0.44) or the interaction (P = 0.50) on WDa of Psyn cows in trials 1 and 2 combined. There tended to be an effect of pregnancy (P = 0.06) on WDa of GPsyn cows in trials 1 and 2 combined, but no effect of day of trial (P = 0.47) or the interaction (P = 0.50) (Fig. 18).

DISCUSSION

The pregnancy rate with GPsyn (6 of 13) was similar to experiment 1 (1 of 4) and the 37 to 39% reported by Pursley et al (1997a; 1997b) with herd sizes of over 300 cows. The disparity in GPsyn pregnancy rates between trials was likely not due to timing of insemination. Pursley et al. (1998) reported that insemination at 0 h, 8 h, 16 h, 24 h and 32 h after GnRH did not adversely affect pregnancy rates since insemination would occur before expected ovulation. However, insemination after 32 h would cause the introduction of sperm into the reproductive tract after ovulation was expected to occur. In this situation the earliest the capacitated sperm could reach the oocyte is 8 to 16 h after ovulation, where the lifespan of the oocyte is only 20 to 24 h long (Hafez 1993). The highest pregnancy rates (4 of 5) occurred in trial 2 when GPsyn cows were inseminated 29 to 30 h after the second GnRH injection. This is later than the 24 h recommended by Pursley et al. (1995a; 1995b) but was still within the 32 h timeframe. Insemination was performed 26 to 29 h after GnRH in trials 1 and 3, which was also within the 32 h timeframe. More likely, the low pregnancy rates (1 of 4) in trials 1 and 3 could have been due to problems in reproductive status. Some cows in trial 1 were more than 152 d postpartum, and some cows in trial 3 were less than 57 d postpartum when each trial began. This may have contributed to the low pregnancy rates in these trials. However the 1 of 4 pregnancy rate is in keeping with the literature at 37 to 39% (Pursley et al. 1997a; Pursley et al. 1997b) when the size of the test groups in this experiment are considered. The embryonic losses in GPsyn cows in trial 2 support the findings of Pursley et al. (1998) where a 20% loss was reported with the use of Ovsynch. The timing of insemination (before 32 h post-GnRH) was thought to be ideal. It is possible

that insemination 29 to 30 h post-GnRH is acceptable in terms of conception, but may predispose the cow to embryonic loss. The fertilization of an aging oocyte caused by late insemination can contribute to early embryonic loss (Bearden and Fuquay 1992). These 2 GPsyn cows with embryonic losses returned to estrus 2 months after this experiment ended, were inseminated upon visual observation of standing heat, and successfully calved. The results of the present study indicated that Ovsynch had no adverse effect on pregnancy rates in this herd. Ovsynch can be a reproductive management strategy in certain situations, particularly for cows maintained in tie-stalls where detection of estrus is difficult.

A pregnancy rate of 50 to 70% can be expected in dairy cows undergoing $PGF_{2\alpha}$ synchronization (Elmarimi et al. 1983; Seguin et al. 1983; Pankowski et al. 1995) where estrus detection is employed. The 5 of 12 Psyn pregnancy rate in this experiment was probably low because of once daily visual observation. Four of the Psyn cows had timed insemination and 2 conceived; this differed somewhat from previous literature which found low pregnancy rates with timed insemination (King et al. 1982). Once daily visual observation was applied to 8 cows and only 3 conceived. Dransfield et al. (1998) reported highest conception rates if AI occurred between 4 to 12 h after onset of estrus, and this timing of AI is not possible with once daily detection of estrus. Also, cows at high days postpartum were included in the study, suggesting either past poor reproductive soundness or poor estrus detection. Interestingly, in trial 1 the cows at high days postpartum had normal appearing progesterone profiles, suggesting that they

were cycling normally. Thus the reason for failure to catch now or previously is unknown.

It is unclear why the standard deviation testing criteria fared better than the absolute temperature testing criteria for identifying increases in VagT in this experiment compared to experiment 1. One possibility is the use of Psyn in this experiment. However, Rajamahendran et al. (1989) found increases in rectal temperature of 1°C in $PGF_{2\alpha}$ synchronized cows. Other factors such as age, barn temperature, exercise regime or some unknown factor may also be responsible. Also, the inconsistency in the presence of a circadian pattern in VagT for Psyn cows could have contributed to the success of the standard deviation criteria.

In some cows there was a tendency for a decline in VagT to occur after $PGF_{2\alpha}$, which resembled a decline observed by Kyle et al. (1998) in naturally cycling beef cows. The decline in VagT coincides with the decline in progesterone that occurs before estrus and ovulation. Measuring the decline in VagT in naturally cycling cows could aid in narrowing the period of observation of standing heat to under 5 days of an estrous cycle without the need for hormone synchronization.

The magnitude of the TP increase in VagT in Psyn cows was 0.3 °C. This was lower than the 0.6 to 1.0° C mean magnitude recorded by Redden et al. (1993) in this herd and Rajamahendran et al. (1989) in $PGF_{2\alpha}$ synchronized cows, respectively. The VagT increases were uniformly low despite variations in age, exercise regime and days postpartum. In six of the 8 Psyn cows the increase in VagT began between 46 to 56 h after $PGF_{2\alpha}$, which indicates a level of synchronicity with CL regression. Also since all

the 3 Psyn cows in trial 3 had TP increases in VagT on the day of standing heat, it appears that the increase in VagT was coincident with estrus. One Psyn cow (Ellymay) did not express standing heat during the daily observation period. The occurrence of a TP increase in VagT in this cow suggests she had a silent heat. Another cow (Whitney) was already in a period of low progesterone at the time of $PGF_{2\alpha}$ and would therefore be unresponsive to the hormone. Five of the 8 Psyn cows had progesterone below 5 ng ml^{-1} from the onset of VagT monitoring to the time of the TP increase in VagT. Four of these cows were in trial 3 where the average days postpartum was 57 d. Resumption of follicular activity has been reported to occur 20 to 30 d after parturition, but since estrus rarely occurs at the first postpartum ovulation they are considered silent heats (Hafez 1993). It is possible that these cows had begun a fertile estrous cycle when the experiment commenced and so could not respond to $PGF_{2\alpha}$. However, one of these cows with persistent low progesterone did become pregnant. In this situation ovarian activity might have been resumed naturally or was potentiated by $PGF_{2\alpha}$.

The two TP increases in RumT were detected by both an absolute temperature criterion (0.5C-3W-5D) and a standard deviation criterion (2SD-3W-4D) on the day of estrus in both cows. Since it is known that a drop in RumT is related to the drinking of water (Cunningham et al. 1964) it seems likely that both Psyn cows consumed less water on the day of estrus and expressed it as a TP increase in RumT. Unfortunately we were unable to collect watering behaviour data for trial 3.

Correction for milk yield resulted in no differences in WD between trials 1 and 2 although water consumption was achieved with significantly fewer watering episodes in

trial 2. The reason for this difference in watering behaviour is not apparent. The lowest WDa in trial 2 occurred on day 15, the day of $PGF_{2\alpha}$, and was followed by an increase in WDa in GPsyn cows on days 16 and 17. This 2 d period also coincided with the rapid fall in progesterone due to induced CL regression. It is possible that the tendency for a decline in VagT a few days subsequent to $PGF_{2\alpha}$ is the result of increased watering behaviour at this time causing a general cooling of the body. Conversely, a reduction in watering behaviour on the day of the $PGF_{2\alpha}$ injection could contribute to an immediate increase in VagT sometimes seen in response to $PGF_{2\alpha}$. The days when estrus was likely to occur (days 16 and 17) were not associated with decreased watering behaviour as was expected considering feeding and milk production decreased at estrus (Walton and King 1986). It is possible that the use of $PGF_{2\alpha}$ to synchronize cows induces watering behaviours not reflective of natural cycles. For example, the low watering behaviour on day 15 may be related to very rapid CL regression, more rapid than what would occur naturally. The increased watering behaviour on days 16 and 17 might then be compensatory behaviour as suggested by Walton and King (1986) where milk production was decreased on the morning of estrus, then increased in the afternoon.

CONCLUSION

The pregnancy rate in trial 2 was higher than in trials 1 and 3. Ovarian activity may not have fully resumed in cows in trial 3 before synchronization began, while cows in trial 1 may have had previous reproductive problems which may have reduced pregnancy rates. The low magnitude of the TP increase in VagT was consistent across

differences in age, exercise routine and days postpartum. $PGF_{2\alpha}$ administration was always followed by a TP increase in VagT, and a TP increase in RumT where measured. In many cases these increases occurred on the confirmed days of estrus. Measurement of both VagT and RumT were 100% effective in identifying confirmed estruses. Watering behaviour did not change over time in $PGF_{2\alpha}$ synchronized cows. Several Ovsynch synchronized cows in trial 2 exhibited a decrease in watering behaviour on the day of $PGF_{2\alpha}$, then underwent a possible compensatory increase in watering behaviour on the 2 days following $PGF_{2\alpha}$. Overall, watering behaviour was not as effective an indicator of changes throughout the estrous cycle as were VagT and RumT.

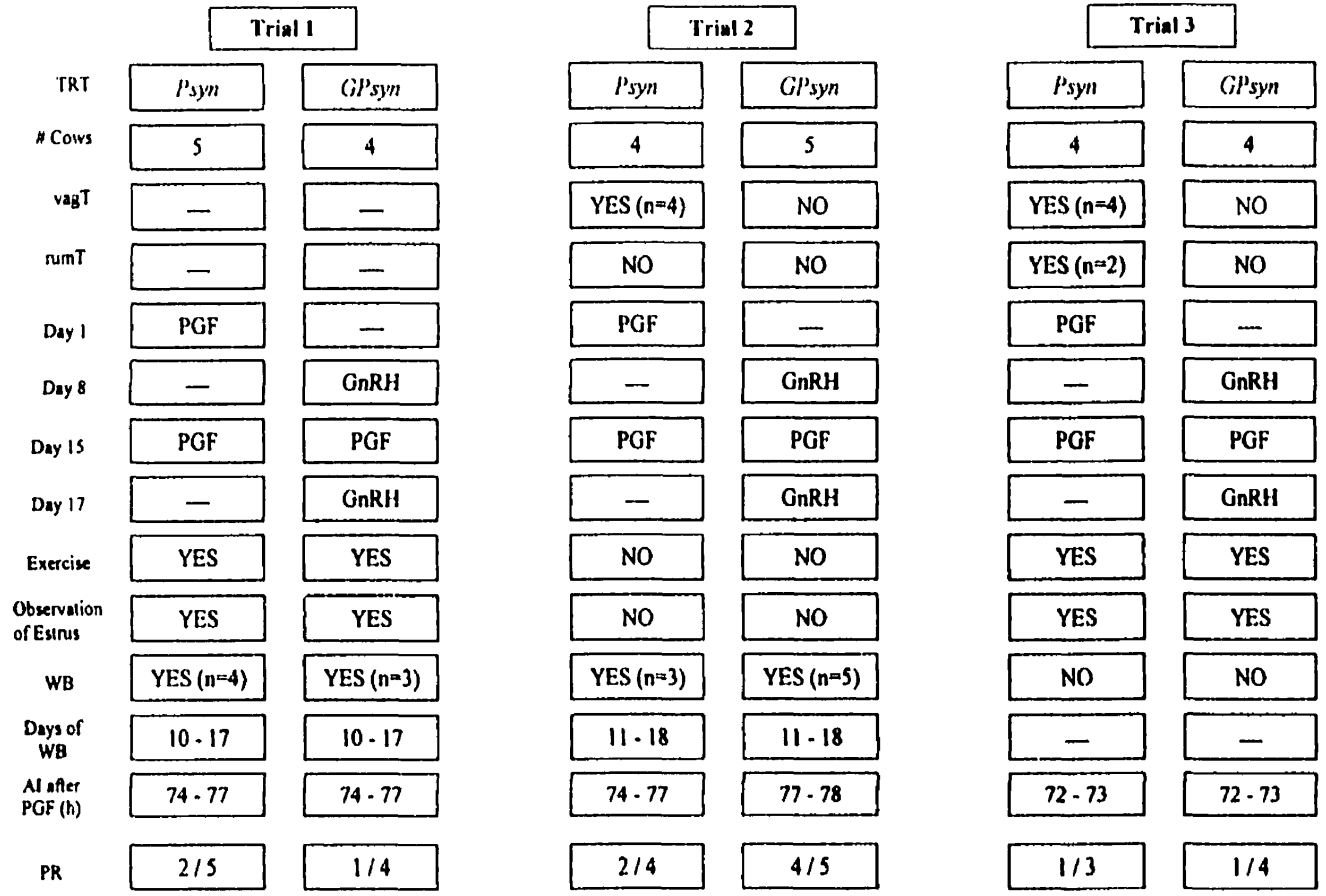


Fig. 6. Experimental protocol for 26 cows divided into 3 trials and 2 synchronization methods for experiment 2. WB = Watering behaviour PR = Pregnancy Rate

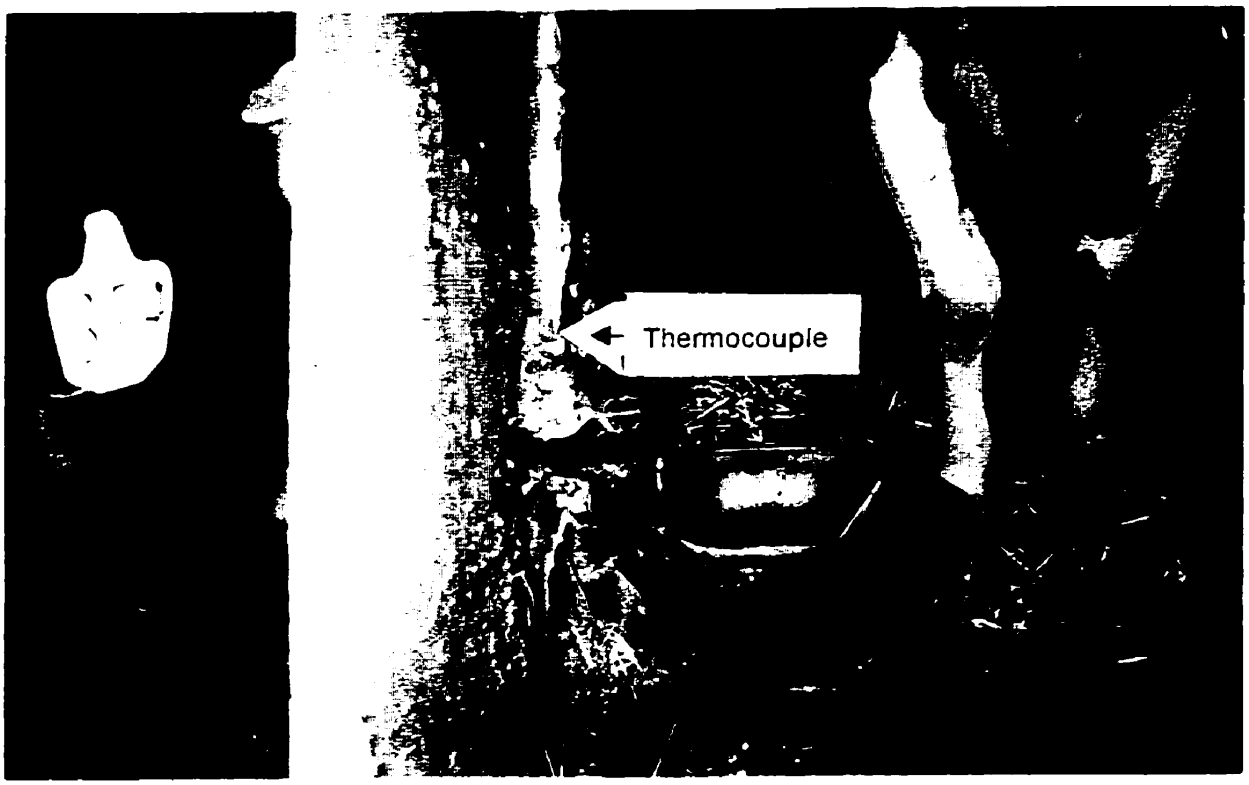


Fig. 7. Thermocouples placed on junction between waterpipe and waterbowl

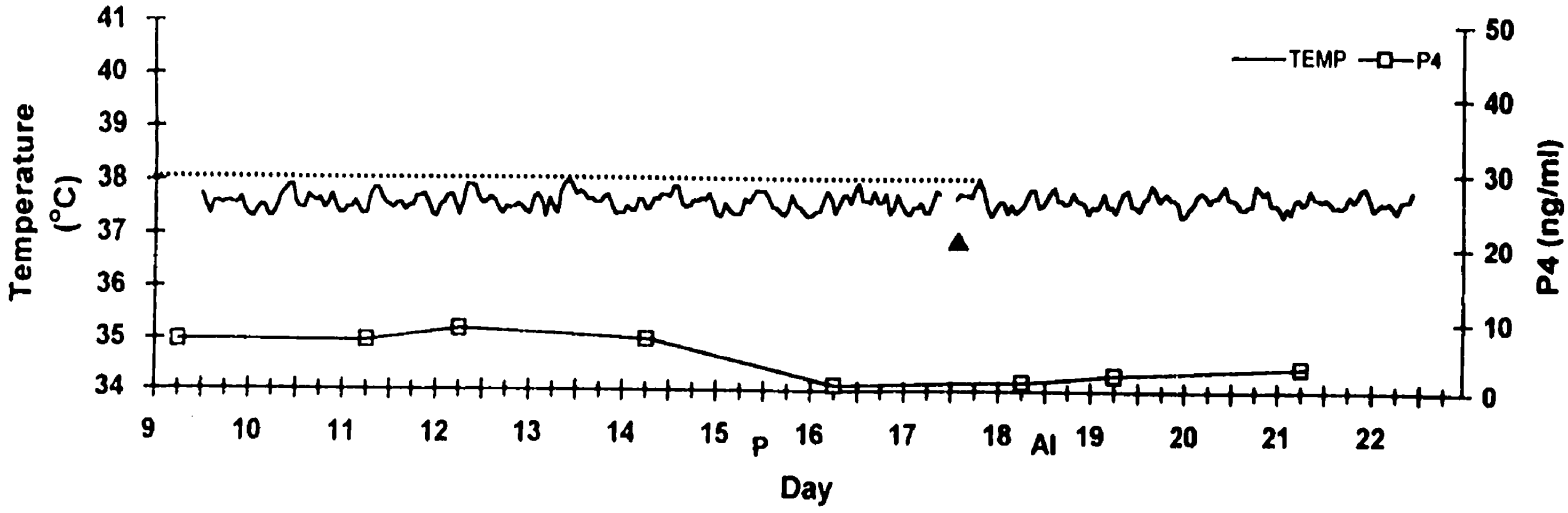


Fig. 8. Vaginal temperature and progesterone of Hannah for experiment 2. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds represent false positives. Vaginal temperatures are based on hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point representing maximum vaginal temperature on the day of a true positive. P = PGF injection AI = Day Bred

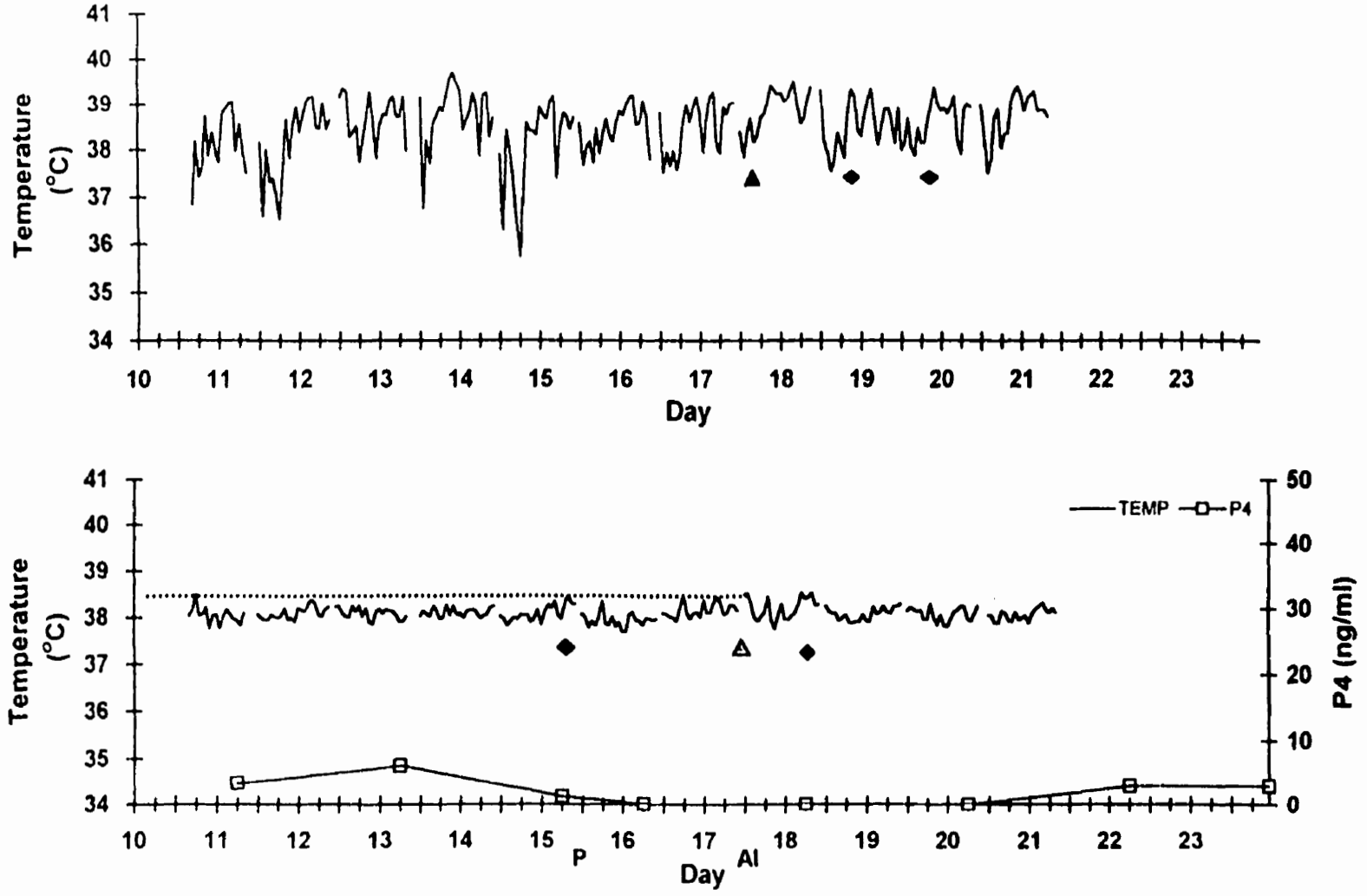


Fig. 9. Rumen temperature (upper graph), Vaginal temperature and progesterone (lower graph) of Muffin for experiment 2. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds represent false positives in rumen and vaginal temperature. Rumen and vaginal temperatures are based on hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point presenting maximum vaginal temperature on the day of a true positive. P = PGF injection AI = Day bred

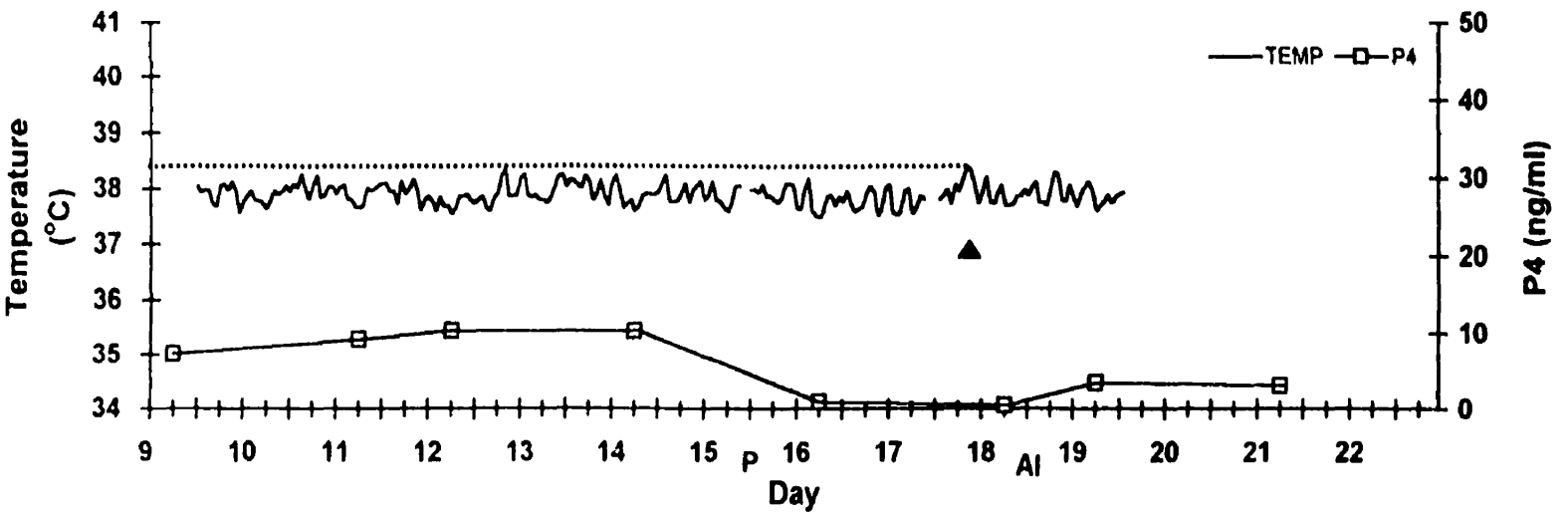


Fig. 10. Vaginal temperature and progesterone of Mavis for experiment 2. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds represent false positives. Vaginal temperatures are based on hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point representing maximum vaginal temperature on the day of a true positive. P = PGF injection AI = Day Bred

Table 8. Criteria for detection of increases in vaginal temperature after PGF administration in experiment 2 based on changes in absolute temperature.

		Absolute Temperature Criterion			
Window Length	Baseline Duration	0.30 (TP/FP)	0.25 (TP/FP)	0.20 (TP/FP)	0.15 (TP/FP)
12h	*4day*	(1,1)	(1,1)	(1,1)	(4,2)
	3day	(1,2)	(1,2)	(1,2)	(4,3)
	2day	(1,2)	(1,2)	(1,3)	(4,6)
	1day	(1,5)	(2,5)	(3,6)	(4,8)
6h	*4day*	(1,0)	(1,0)	(2,3)	(6,7)
	3day	(1,0)	(2,1)	(4,1)	(6,8)
	2day	(1,4)	(3,5)	(4,8)	(6,12)
	1day	(1,8)	(3,10)	(4,10)	(6,13)
3h	*4day*	(1,2)	(3,3)	(5,5)	(7,15)
	3day	(2,1)	(3,2)	(6,3)	(7,15)
	2day	(2,4)	(4,6)	(6,11)	(8,18)
	1day	(2,10)	(4,13)	(6,16)	(8,20)
1h	*4day*	(3,1)	(6,3)	(6,4)	(7,9)
	3day	(3,0)	(6,5)	(6,7)	(7,13)
	2day	(4,4)	(5,6)	(6,12)	(7,19)
	1day	(4,9)	(5,9)	(6,10)	(7,22)

TP = True Positive: temperature increase when progesterone below 5 ng/ml
FP = False Positive: temperature increase when progesterone above 5 ng/ml

Table 9. Criteria for detection of increases in vaginal temperature after PGF administration in experiment 2 based on variations of standard deviation.

		Standard Deviation Criterion					
Window Length	Baseline Duration	4+ (TP/FP)	3.5 (TP/FP)	3 (TP/FP)	2.5 (TP/FP)	2 (TP/FP)	1.5 (TP/FP)
12h	*4day*	(4,0)	(5,1)	(6,2)	(7,3)	(8,8)	(8,12)
	3day	(5,1)	(6,1)	(6,4)	(8,6)	(8,11)	(8,19)
	2day	(6,12)	(6,16)	(6,20)	(8,23)	(8,28)	(8,32)
6h	*4day*	(1,4)	(2,4)	(3,5)	(7,5)	(8,6)	(8,14)
	3day	(4,5)	(4,5)	(6,6)	(6,7)	(8,9)	(8,15)
	2day	(6,12)	(6,15)	(6,20)	(6,23)	(7,30)	(7,38)
3h	*4day*	(2,1)	(2,1)	(3,2)	(5,4)	(8,5)*	(8,12)
	3day	(4,1)	(5,2)	(7,5)	(7,6)	(8,10)	(8,16)
	2day	(4,8)	(4,9)	(6,13)	(7,25)	(8,28)	(8,35)
1h	*4day*	(0,1)	(0,1)	(1,1)	(1,1)	(3,2)	(4,5)
	3day	(0,1)	(0,1)	(1,1)	(3,1)	(3,2)	(5,12)
	2day	(2,1)	(3,1)	(3,5)	(5,9)	(5,13)	(6,21)

* Best criterion

TP = True Positive: temperature increase when progesterone below 5 ng/ml

FP = False Positive: temperature increase when progesterone above 5 ng/ml

Table 10. Day of occurrence and duration of true positive (TP) vaginal temperature increase in experiment 2 after administration of $PGF_{2\alpha}$ when progesterone levels were below 5 ng ml^{-1}.

Animal	Day of Trial	Time of TP Vaginal Temperature Increase	Time from $PGF_{2\alpha}$ Injection to Beginning of TP Vaginal Temperature Increase (h)
Hannah	Day 17 to 18	17:00 h – 1:00 h	55
Whitney	Day 15 to 16	12:00 h – 5:00 h	2
Ritz	Day 17 to 18	15:00 h – 4:00 h	53
Mavis	Day 17	18:00 h – 22:00 h	56
Ellymay	Day 16	11:00 h – 14:00 h	20
Tawny	Day 17	12:00 h – 15:00 h	46
Muffin	Day 17	10:00 h – 13:00 h	44
Janice	Day 17 to 18	19:00 h – 2:00 h	53
		Mean ± S.E.	41.1 ± 7.0

Table 11. Baseline temperature, magnitude and duration of the true positive (TP) vagT increase and time from PGF to TP vagT increase between cows that did (n = 3) or did not become pregnant (n = 5) and between cows that were (n = 4) or were not (n = 4) exercised in experiment 2.

	Pregnancy Results			Exercise Regime		
	Pregnant	Open	Probability	Exercise	No Exercise	Probability
VagT Baseline (°C)	37.93 ± 0.13	37.84 ± 0.11	0.64	37.88 ± 0.05	38.06 ± 0.29	0.43
Magnitude of TP vagT Increase (°C)	0.3 ± 0.05	0.3 ± 0.13	0.72	0.3 ± 0.11	0.2 ± 0.04	0.35
Duration of TP vagT Increase (h)	6.7 ± 3.18	9.8 ± 2.43	0.47	5.0 ± 1.13	10.5 ± 2.86	0.12
Interval from $PGF_{2\alpha}$ to vagT Increase (h)	51.7 ± 2.97	38.5 ± 12.40	0.42	40.7 ± 7.18	41.5 ± 13.22	0.96

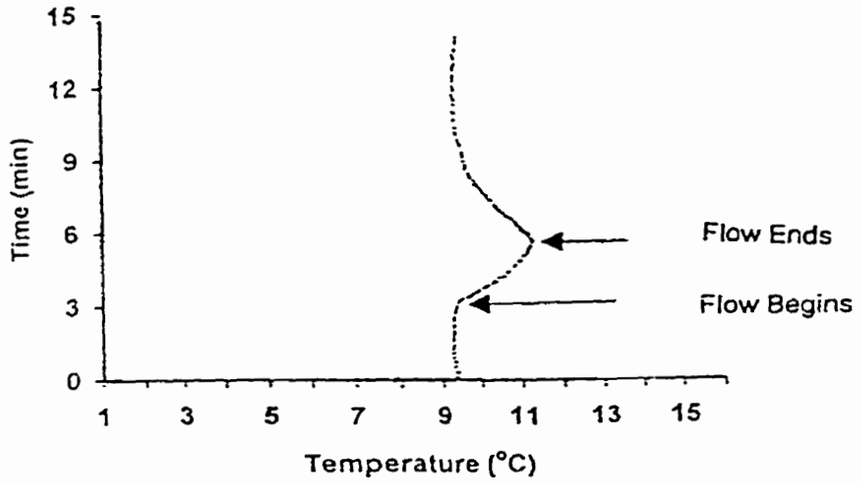
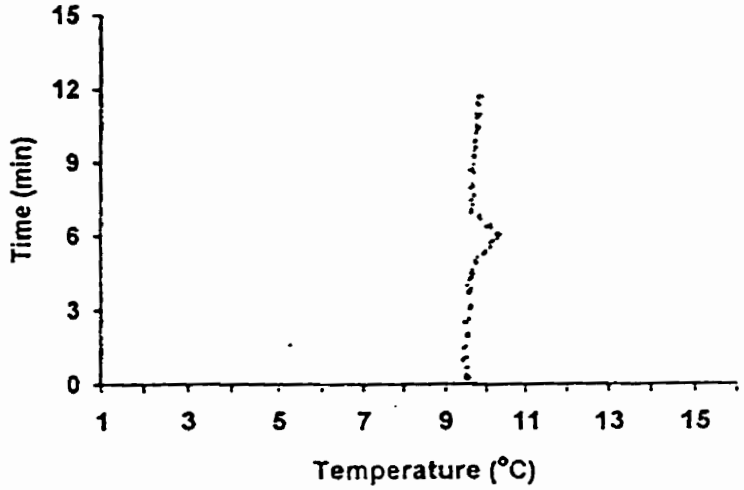
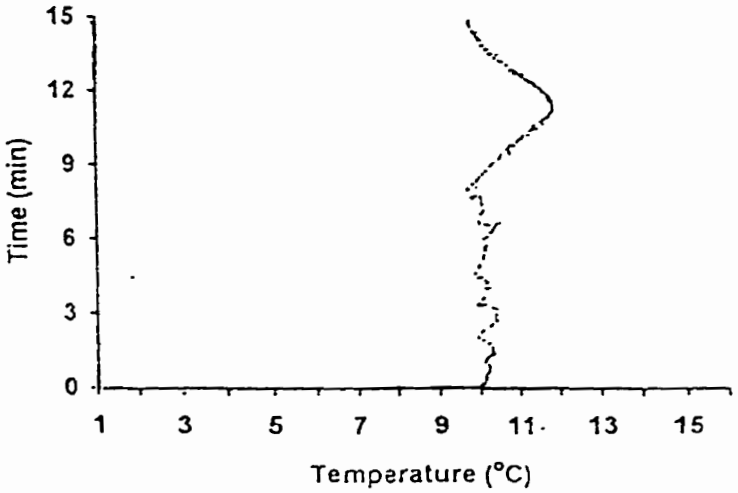


Fig. 11. Depiction of waterflow considered as one watering episode as measured by the Molytek® recorder.

12

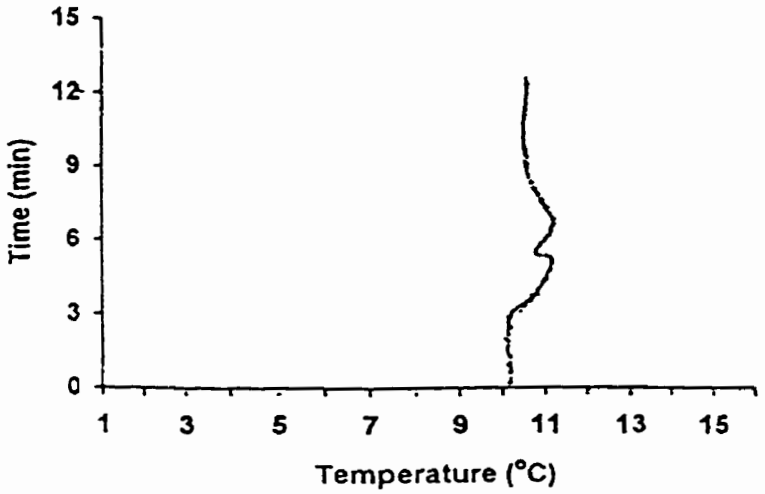


13

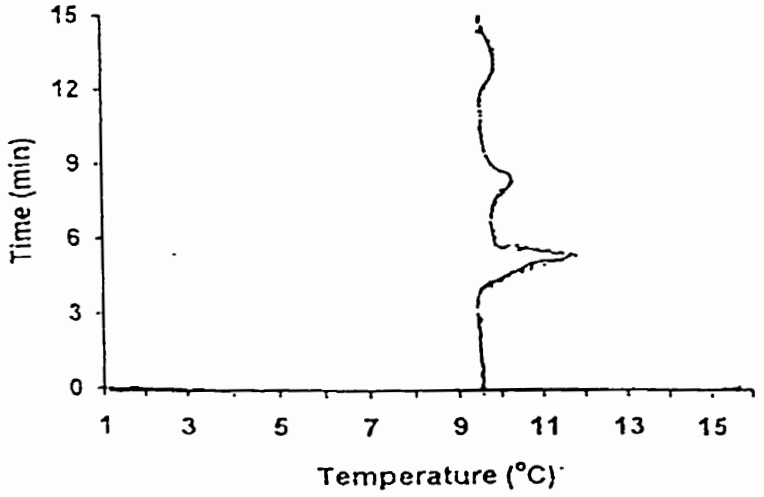


Figs. 12 and 13. Depiction of waterflow with a smooth baseline (Fig. 12) or a wavy baseline (Fig. 13) considered as a short drink as measured by the Molytek® recorder.

14



15



Figs. 14 and 15. Depiction of waterflow considered as one watering episode (Fig. 14) or as 2 separate watering episodes (Fig. 15) as measured by the Molytek® recorder.

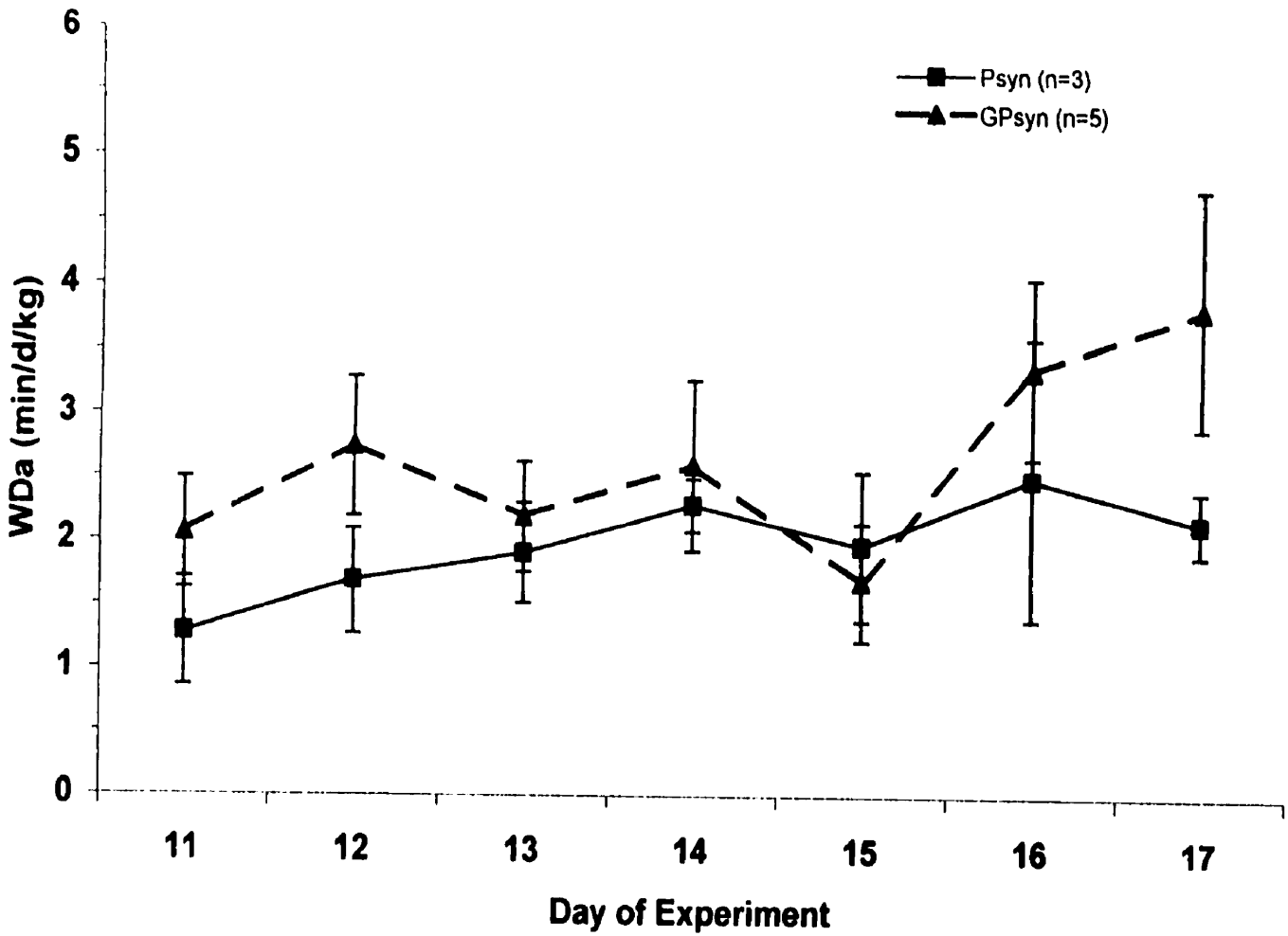


Fig. 16. Daily duration of watering behaviour adjusted for milk yield (WDa) for GPsyn and Psyn cows in trial 2.

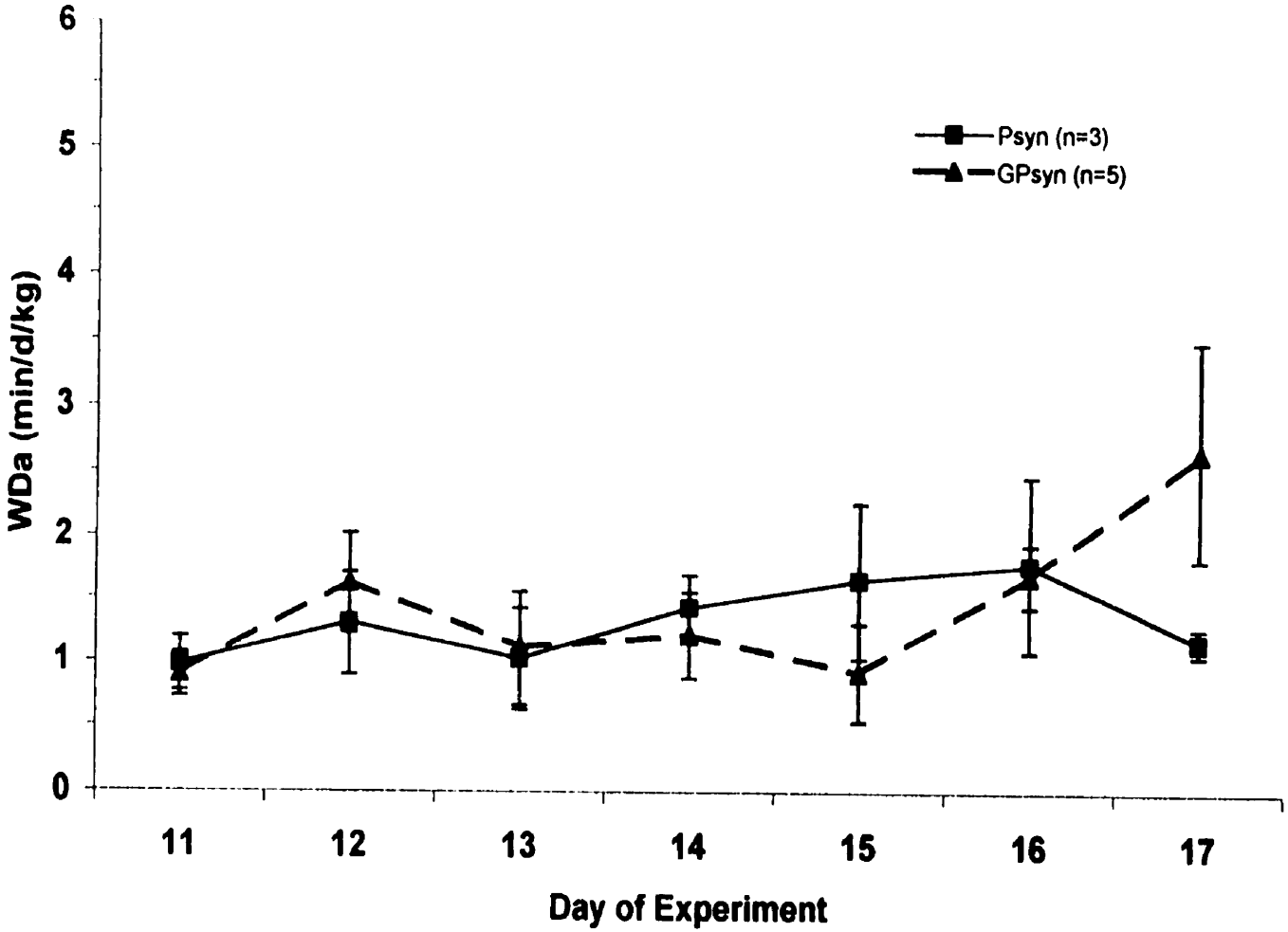


Fig. 17. Duration of watering behaviour adjusted for milk yield (WDa) for GPsyn and Psyn cows in the P.M. (12:00 h - 24:00 h) of trial 2.

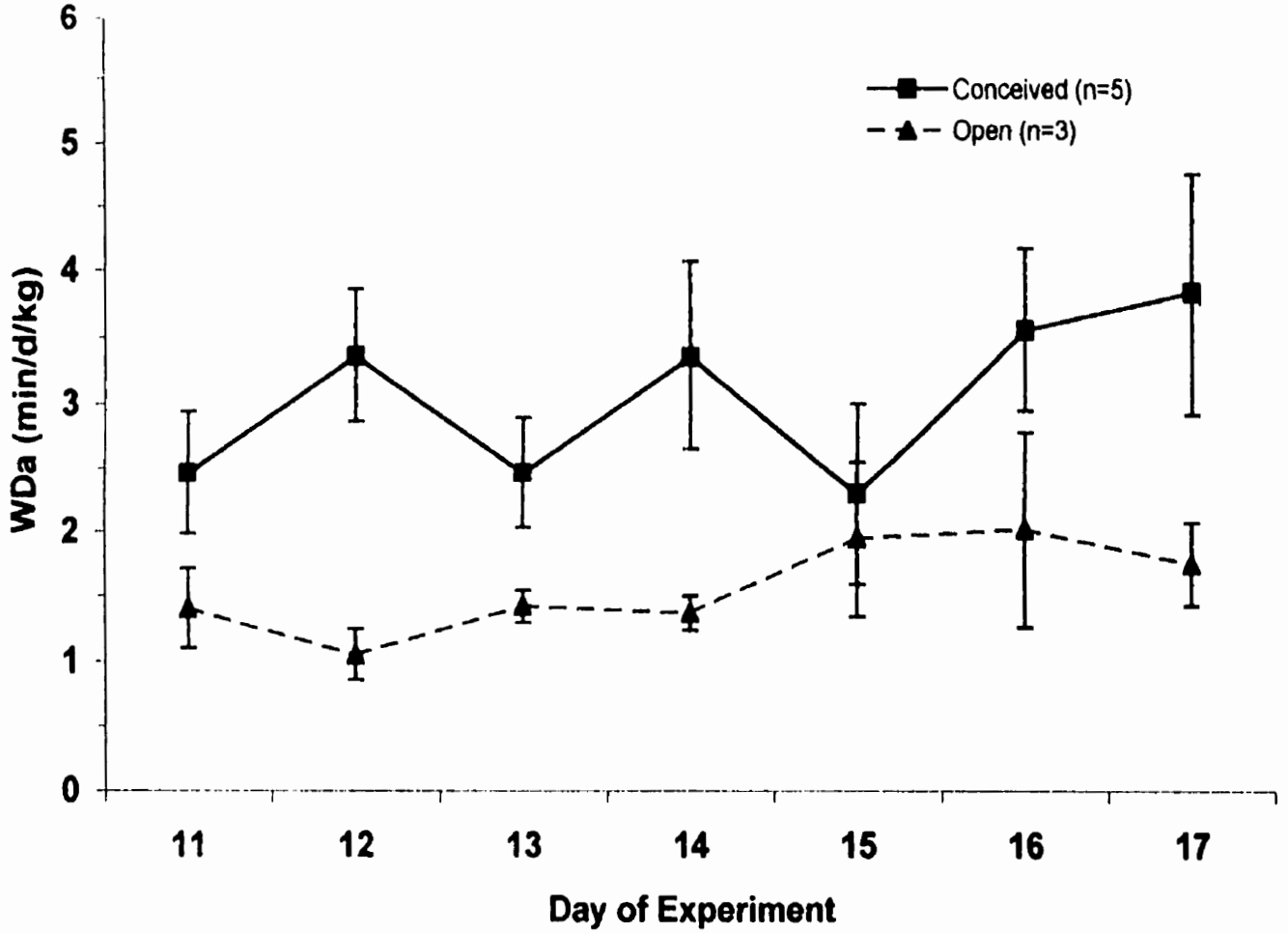


Fig. 18. Daily duration of watering behaviour adjusted for milk yield (WDa) for GPsyn cows in trials 1 and 2 combined that either got pregnant or remained open.

GENERAL DISCUSSION

The overall pregnancy rate of 7 /17 (41%) in the Ovsynch synchronized cows from both experiments was in keeping with those (37 to 39%) reported by Pursley et al. (1997a; 1997b). In tie-stalled conditions where both the expression and observation of estrus is difficult, a 41% pregnancy rate (without the need for estrus detection) is quite acceptable. The pregnancy rate in the $PGF_{2\alpha}$-synchronized cows was higher under timed insemination rather than visual observation of standing heat. This indicates that a single observation period for estrus detection may be inadequate for this herd. Therefore, increasing the number of exercise periods daily would be beneficial in increasing the chances of detecting estrus at the optimal time for breeding purposes if the cows are to be synchronized with $PGF_{2\alpha}$.

The magnitude of the TP increase in VagT in experiment 2 was identical (0.3°C) to that of experiment 1 where Ovsynch was used. This was contrary to the theory postulated in experiment 1 that Ovsynch inhibited the magnitude of the VagT increase by premature termination of estradiol production. Rajamahendran et al. (1989) reported a TP increase in VagT of greater than 1°C in $PGF_{2\alpha}$ synchronized cattle. The magnitude of the VagT increase was not different between TP and FP in both experiments, or between cows that did or did not conceive in experiment 2. It is possible that the low magnitude of the TP increase in VagT could be a herd effect, but Redden et al. (1993) reported VagT increases of 0.6°C with the same herd five years previously. However, since VagT was not studied in naturally cycling cows (no exogenous hormones given),

as was the case for Redden et al. (1993), it was not possible to make a direct comparison concerning the herd.

In the present studies, drinking behaviour did not appear to be as accurate as VagT or RumT in detecting ovulation (experiment 1) or estrus (experiment 2). Furthermore, RumT did not appear to be as accurate as VagT. Only 5 TP increases in RumT were associated with the 7 periods of low progesterone in experiment 1, whereas 8 of 8 TP increase in VagT were detected in both Ovsynch heifers in experiment 1 and $PGF_{2\alpha}$ synchronized cows in experiment 2. As well, the number of FP increases in RumT was high. Unfortunately, the small data set prevents the making of assumptions on the true effectiveness of RumT on monitoring changes throughout the estrous cycle.

The reduction in drinking behaviour reported on the expected days of estrus and ovulation in experiment 1 agreed with previous reports where decreased feed intake and milk yield occurred on the day of estrus (Walton and King 1986; Schofield et al. 1991). Since the Ovsynch method used in experiment 1 is known to inhibit estrus, $PGF_{2\alpha}$ synchronization, in addition to Ovsynch, was used in experiment 2 to allow for more pronounced behaviours to be expressed at estrus (e.g., a change in watering behaviour). It should be noted that the observation period for drinking behaviour in experiment 1 was only a 4 h period after feeding, whereas 24 h recordings were available in experiment 2. It is possible that experiment 1 did not have a reliable representation of drinking behaviour at estrus and ovulation. Also, Walton and King (1986) reported that decreased morning milk yield on the day of estrus was followed by a compensatory increase in afternoon milk yield. This may have happened in certain GPsyn cows of

experiment 2, where the $PGF_{2\alpha}$-induced reduction in watering behaviour (day 15) was followed by a compensatory increase on days 16 and 17, the days before ovulation. It is possible that the increased watering behaviour at estrus only occurs in cows synchronized by Ovsynch, as this pattern was not observed in Psyn cows. Further research is required to examine watering behaviour of naturally cycling dairy cows. It is also possible that the method for measurement of watering behaviour used in experiment 2 was not accurate in monitoring changes throughout the estrous cycle. Continuous video surveillance may be a better system for monitoring drinking behaviour. Also, since VagT and RumT appeared to have been affected by hormone synchronization, further studies need to be performed on naturally cycling cows.

CONCLUSION

Once daily visual observation of estrus in $PGF_{2\alpha}$ synchronized cows appeared to be inadequate in this study, contributing to a pregnancy rate that was lower than that of Psyn cows under timed insemination and in variance with the literature. Ovsynch produced pregnancy rates of between 25 to 80% in these studies, which was in agreement with previous studies. However, the incidence of pregnancy loss and cost of additional hormone usage could be detrimental in situations where herd pregnancy rates are not initially poor. The results from both studies indicate that increases in VagT consistently occurred around the time of estrus in all test animals. However, increases in rumen temperature at estrus did not occur in all animals and there were many FP increases in RumT. Thus VagT appeared to be a more reliable method of measuring

changes throughout the estrous cycle than RumT. More research has to be conducted to determine if rumen temperature is accurate enough to be considered for commercial purposes. Watering behaviour did not differ over time in $PGF_{2\alpha}$ synchronized cows. Watering behaviour increased two days before expected day of ovulation in Ovsynch synchronized cows monitored 24 h daily, but appeared to decrease on the day before and day of ovulation in Ovsynch heifers monitored only 4 h daily. Thus the length of the daily observation period and method of hormone synchronization are important factors in determining drinking behaviour throughout the estrous cycle. Therefore further studies in drinking behaviour should be done with naturally cycling cows. Drinking behaviour does not appear to be as reliable an indicator of changes throughout the estrous cycle as VagT or RumT in synchronized cattle.

CONCLUSIONS

1. Eight increases in VagT were detected during 8 periods of low progesterone (coincident with CL regression) in Ovsynch-synchronized heifers in experiment 1, and eight increases in VagT were detected during 8 periods of low progesterone in $PGF_{2\alpha}$-synchronized cows in experiment 2. Only 5 increases in RumT were detected during 7 periods of low progesterone in experiment 1, and 2 increases in RumT were detected during 2 periods of low progesterone in experiment 2. The FP rate between VagT and RumT was similar in experiment 1, but the FP rate of RumT in experiment 2 was almost 3 times higher than that of VagT. Overall, RumT does not appear to be as reliable an indicator of changes throughout the estrous cycle as VagT.

2. The magnitude of the TP increase in VagT was the same between experiments at 0.3°C. Also, the magnitude of the TP increase in RumT was similar at 0.5°C in experiment 1 and 0.6°C in experiment 2. Thus the increases in VagT and RumT are not influenced by differences in hormone synchronization method, age or exercise regime. However the low magnitude of the VagT increase resulted in an increased incidence of FP compared to earlier studies.

3. The TP increases in both VagT and RumT occurred on the expected or actual day of estrus in both experiments by those heifers and cows that were hormonally synchronized by either the Ovsynch or $PGF_{2\alpha}$ synchronization methods.

4. Pregnancy rates for Ovsynch was 1 of 4 in experiment 1, and 6 of 13 in experiment 2, which was in keeping with pregnancy rates of earlier Ovsynch studies. The pregnancy rate of 5 of 12 for $PGF_{2\alpha}$ synchronized cows in experiment 2 was lower than expected, particularly for inseminations based on a single 2 to 3 h daily visual observation period.

5. Drinking behaviour observed during a daily 4 h observation period appeared to decrease the day before and day of AI (coincident with expected estrus) compared to the previous 2 d in experiment 1 in Ovsynch synchronized heifers. The opposite occurred in Ovsynch synchronized cows in trial 2 of experiment 2, where continuous electronic monitoring of watering behaviour indicated a compensatory increase on the 2 days following a decrease on the day of $PGF_{2\alpha}$. The difference between experiments may have been due to differences in length and method of behaviour observation. $PGF_{2\alpha}$ synchronized cows in experiment 2 experienced no changes in watering behaviour over time. Thus watering behaviour does not appear to be as reliable an indicator of changes throughout the estrous cycle as either VagT or RumT.

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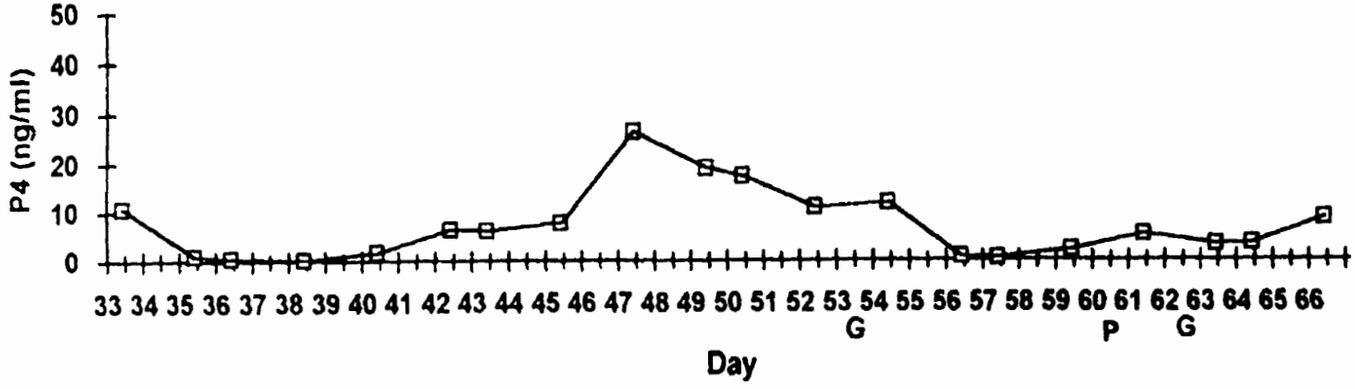
APPENDIX I

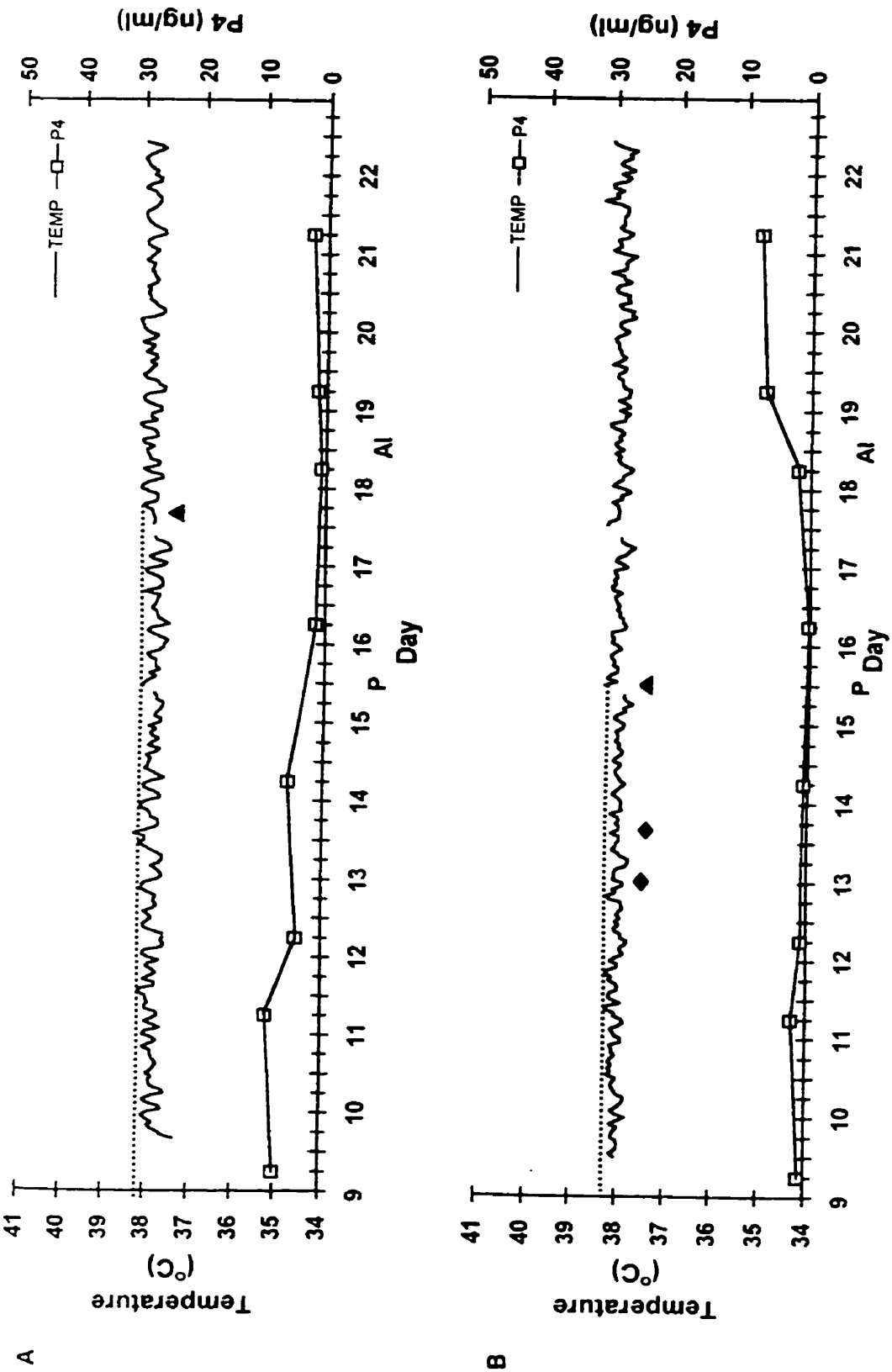
Progesterone profile of Una for days 33 to 66 of experiment 1



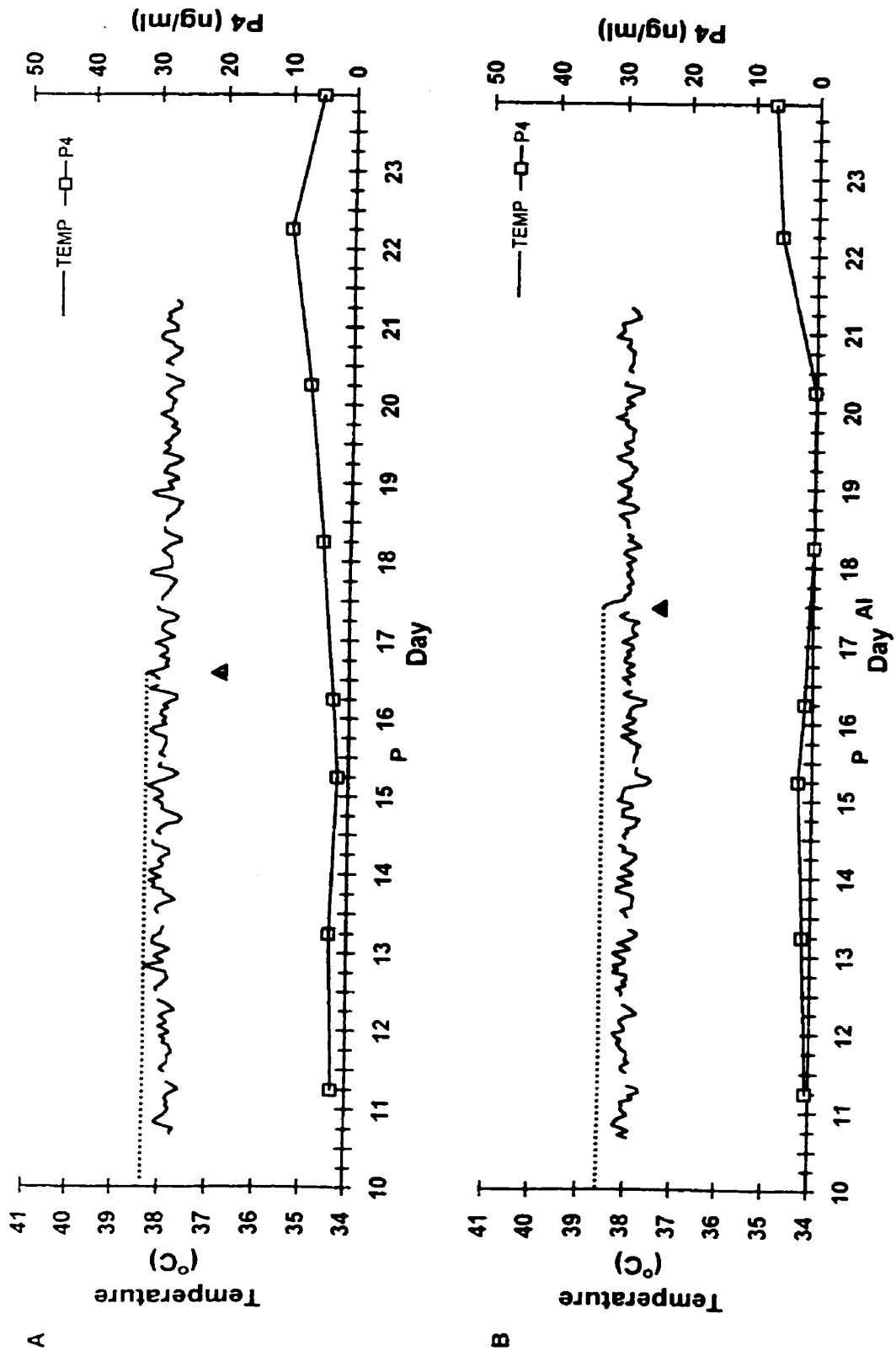
Progesterone of Una for days 33 - 66 of the trial. Progesterone samples were taken 4 times weekly. P = day of PGF injection G = day of GnRH injection

APPENDIX II

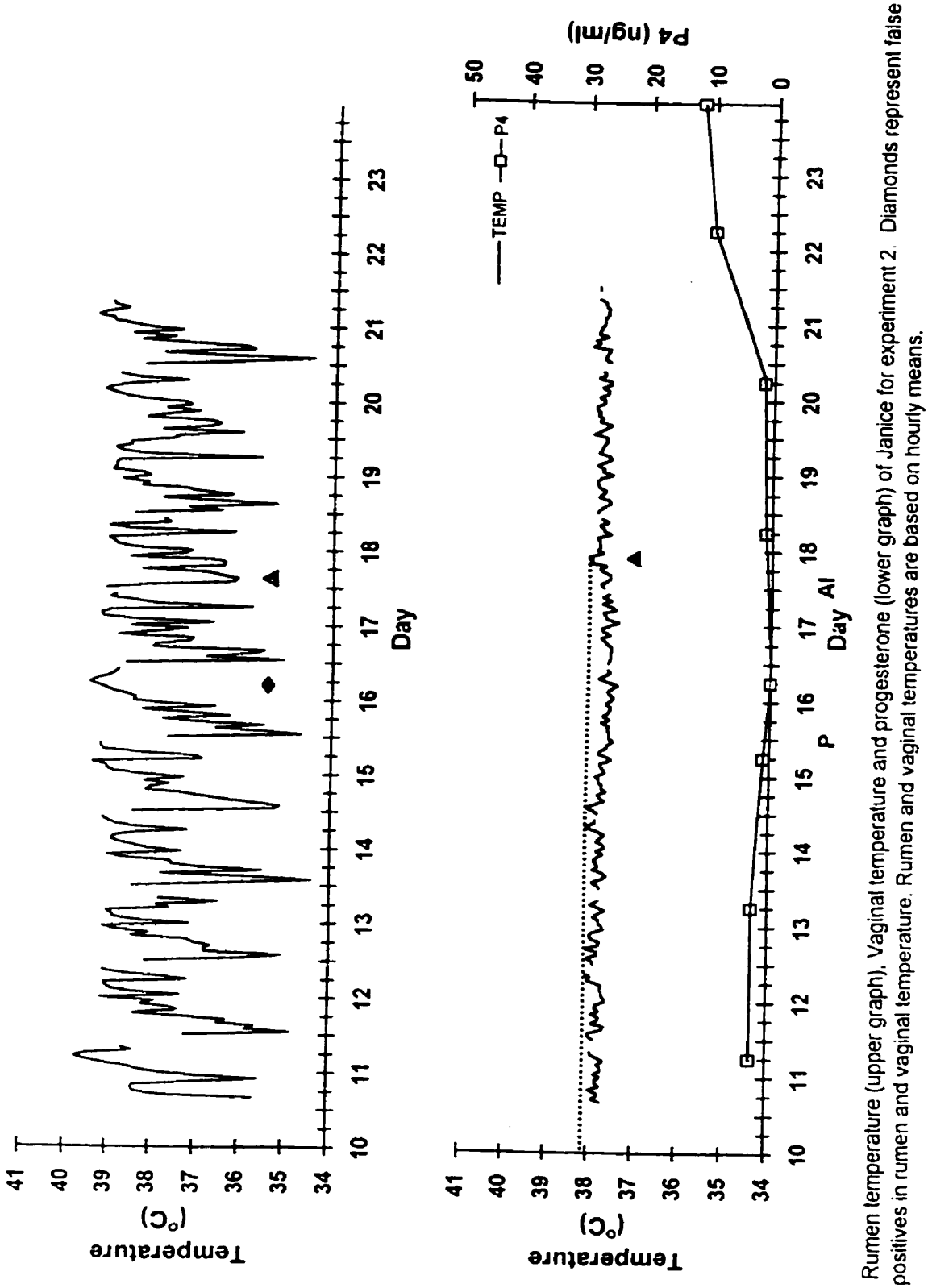
Vaginal temperature graphs for Ritz, Whitney, Ellymay and Tawny, plus vaginal and rumen temperature graphs for Janice during experiment 2. Triangles indicate commencement of an increase in temperature (true positive) according to the best criterion described in the text. Diamonds represent false positives in temperature. Temperatures are based on hourly means. Progesterone samples were taken 4 times weekly. Dashed line is a reference point representing maximum vaginal temperature on the day of a true positive. P = PGF injection AI = Day bred



Vaginal temperature and progesterone of Ritz (A) and Whitney (B) for experiment 2.



Vaginal temperature and progesterone of Ellymay (A) and Tawny (B) for experiment 2.



Rumen temperature (upper graph). Vaginal temperature and progesterone (lower graph) of Janice for experiment 2. Diamonds represent false positives in rumen and vaginal temperature. Rumen and vaginal temperatures are based on hourly means.