Evaluation of oestrus observation and conception rates in suckling beef cows using whole milk progesterone concentration

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ABSTRACT

A 2-sample regime was used to measure whole milk progesterone concentration on the day of oestrus and insemination (Day 0) and 6 days later (Day 6) in a sample of 50 primiparous and 100 multiparous suckling beef cows. Exposure to teaser bulls and observation by cattlemen identified the occurrence of oestrus. Three sets of criteria used to define ovulatory oestrus were compared: a) milk progesterone concentration less than 6 nmol/ℓ on Day 0; b) milk progesterone less than 6 nmol/ℓ on Day 0 and rising to greater than 6 nmol/ℓ on Day 6; c) milk progesterone less than 6 nmol/ℓ on Day 0 and rising to greater than 6 nmol/ℓ on Day 6, or cow diagnosed pregnant to 1st insemination. Using only a single milk sample on Day 0 (criterion a) would have resulted in the positive predictive value of heat detection being estimated at 98.7 %. Using a paired measurement (criterion b) resulted in a significantly lower estimate of 84.7 %. The inclusion of cows that conceived despite not showing a marked rise in milk progesterone concentration (criterion c) resulted in a more accurate estimate of 89.3 %. Use of a 2-sample regime also allowed calculation of conception rates while eliminating the effect of heat detection errors. In the cows sampled, of those in ovulatory oestrus that were inseminated, 73.1 % conceived to the 1st insemination. These results demonstrate that artificial insemination within a limited breeding season can be successful if nutrition is optimal and management is intensive. The use of a 2-sample milk progesterone test may be a valuable tool in investigating heat detection and conception problems in beef herds in which artificial insemination is used.

Key words: milk progesterone, oestrus observation, suckling beef cows.

INTRODUCTION

Reproductive performance in beef herds has a marked effect on profitability. The average inter-calving period for the herd should be as close as possible to 365 days. The key period of the reproductive cycle that determines overall reproductive efficiency is the calving to conception interval. The length of this interval is influenced by the *post partum* onset of oestrous cycles and oestrus detection efficiency[5,7,14,15,17,21,24].

Accuracy of oestrus detection is commonly defined as the percentage of oestrus events observed that are true oestrus. However, this is more correctly termed the positive predictive value (PPV) of oestrus detection. Accurate detection of oestrus and the correct timing of insemination are critical for the successful use of artificial insemination in beef herds and even more so if a restricted breeding season is used. Cows must conceive within 1–3 oestrous cycles. Since the average calving rate to a given insemination is usually only 0.5 to 0.6, the earlier a cow is correctly observed in oestrus and inseminated the easier it is to achieve the target 365-day inter-calving period[21]. Major difficulties may exist in oestrus detection, with at least 10 % and possibly up to 36 % of cows in problem herds failing to conceive because of insemination at the wrong time due to faulty observation[3,10,13,14,16,19].

Artificial insemination is widely practised in dairy cattle. For many reasons, however, its use in beef cattle is limited. Compared to natural breeding, low success rates have been achieved, with the extensive nature of beef herd management complicating oestrus observation[8,14,23]. Also, the period of standing oestrus is short and poorly defined, with cows often showing oestrus for only a few hours, very often only at night[8,14,16,23]. Oestrus detection has often been cited as a major factor limiting the widespread use of artificial breeding in beef herds. Even with innovative management of breeding herds, the use of artificial breeding in beef herds has not increased significantly over the years[8,23].

Changes in plasma progesterone concentrations (PPC) during the bovine oestrous cycle are well documented. Progesterone concentrations are low (<1.5 nmol/ℓ or <0.5 ng/mℓ) at oestrus and for several days thereafter, *i.e.* during the follicular phase. PPC begins to rise on Day 3 or 4 and peaks at about Day 11 of the cycle. Concentrations fluctuate over the next few days but remain in excess of 6 nmol/ℓ in plasma, until about 48 hours before the next oestrus. A precipitous decrease to the typical oestrus level then occurs[14,19,20].

There is a high correlation between blood and milk progesterone concentrations (MPC) throughout the bovine oestrous cycle, with the milk concentration being consistently higher than in plasma[10,19,27]. Measurement of either blood, skim milk, whole milk or milk fat progesterone concentrations can be used to confirm or monitor *post partum* ovarian activity and oestrus, as well as pregnancy diagnosis, early embryonic mortality and ovarian pathology[3,4,9,11,13–16,19–21,23,25,27].

In cattle, MPC has been used in various ways to evaluate oestrus detection. By monitoring the MPC of a group of animals once to 3 times per week, post-ovulation rises in milk progesterone can be detected[15,21,25]. Single samples taken at the time of insemination may be used to confirm that progesterone concentrations are low; the finding of cows with high MPC at the time of insemination would indicate a lack of specificity for detection of oestrus[4,6,13,14,16].

Oestrus observation may also be evaluated by a double sampling regime on the day of heat detection and 6–7 days later[6,10,11,20]. A cow in true ovulatory oestrus would be expected to have a very low progesterone concentration in the 1st sample, followed by a high concentration

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in the 2nd sample, indicating that ovulation has taken place. Low progesterone concentrations in both samples indicate either a non-cyclic cow (*i.e.* an error in heat detection) or an anovulatory oestrus (cystic ovaries). High concentrations in the 1st sample usually indicate an error in oestrus detection[14] but have also been shown to occur in repeat breeders[27].

In beef herds, however, there is little information on the evaluation of oestrus observation in artificial breeding programmes using progesterone concentrations. The objective of this study was to investigate the use of a 2-sample (day of insemination and 6 days later) milk progesterone analysis to evaluate the positive predictive value of heat detection as well as conception rates in suckling beef cows.

MATERIALS AND METHODS

The cows used in the study were from a semi-intensive, pasture-based beef cattle system in the Gauteng province of South Africa. The herd size was 245 primiparous and 827 multiparous cows. The farm is 2397 ha in size and lies at an elevation of 1380 m, with an average annual rainfall of 604 mm. The pasture is a ryegrass-kikuyu mixture, with ryegrass predominating in autumn and spring and kikuyu in summer. The pasture is irrigated with purified sewage effluent from the Johannesburg Metropolitan area.

A limited breeding season is practised, using artificial insemination with frozen semen exclusively. The heifer breeding season commences 2 weeks ahead of the rest of the cows, thus allowing the primiparous cows a longer *post partum* rest period of 14 days before falling in with the breeding season of the multiparous cows. All other cows are bred for 60 days, starting in early October. The calving season commences in late June and continues until the end of August. Primiparous and multiparous cows are managed in separate groups.

In order to stimulate maximum cyclic activity, breeding females receive an energy supplement, starting 10 days before and continuing for 14 days into the breeding season. Teaser bulls are introduced at a ratio of 2:100 cows. Introduction to the primiparous cows takes place 3 weeks before the breeding season and to the multiparous cows at the start of the breeding season. Temporary weaning of calves at foot is practised for 48 hours at the start of the breeding season.

Heat spotting is performed during the day by the section supervisor and his cattlemen and at night by the night watchmen. Cows on heat are marked with a water-based paint on the hair over

the sacrum. Cows seen on heat from midday through to midnight are inseminated the following morning. Those on heat from midnight to midday are inseminated that afternoon. All insemination dates, pregnancy diagnoses and calving dates are recorded.

Whole milk samples were collected from each of the first 50 primiparous and first 100 multiparous cows bred. Cows were sampled on the day of insemination (Day 0) and again 6 days later (Day 6). Each milk sample was collected in a 20 mℓ plastic tube containing a 40-mg potassium dichromate preservative tablet. The tubes were labelled with the cow identification number and date of collection, and stored at 4 °C. The samples were assayed as a batch to minimise variation between assays.

Whole milk progesterone concentration was determined using a solid-phase radio-immunoassay, in which I^{125}-labelled progesterone competes with progesterone in the sample for antibody sites (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA). This kit has been validated for use on milk samples[26]. Milk or reference standard was added to tubes coated with specific progesterone antibody. Buffered I^{125}-labelled progesterone (1 mℓ) was then added to all tubes, which were then mixed on a multi-shaker and left to incubate for 3 hours at 37 °C. Tubes were then decanted and cleaned with a cotton swab above the 1-mℓ mark to remove excess fluid. The tubes were counted for radioactivity on a gamma counter (Packard Auto-Gamma 500, Packard instruments, Downers Grove, USA). The results were calculated against a standard spline-smoothed curve (Cobra Model 5003 Automatic gamma-counter, with RIA-SMART RIA data-reduction software). The sensitivity of the assay was 0.15 nmol/ℓ.

Pregnancy diagnoses were performed by trans-rectal palpation of all cows 3–5 months after insemination. All reproductive data, including oestrus and insemination date, pregnancy diagnosis and calving date, as well as MPC on Days 0 and 6, were recorded. General herd fertility parameters such as conception rate of cows served, 1st insemination conception

rate and average number of inseminations per conception were calculated.

MPC on Days 0 and 6 were compared between parities using Student's *t*-test. Logarithmic transformation was used for highly skewed data. First insemination conception rates of cows sampled, calculated for those cows inseminated and for those showing ovulatory oestrus (using criterion (c) below), were compared between parities using the Fisher exact test. Data analysis was performed using a commercial statistical analysis software package (NCSS 2001, NCSS, Kaysville, USA).

Using the MPC and pregnancy diagnosis data, alone or in combination, various criteria were used to determine whether each cow had been in ovulatory oestrus at the time of insemination:
(a) MPC less than 6 nmol/ℓ on Day 0
(b) MPC less than 6 nmol/ℓ on Day 0 and rising to greater than 6 nmol/ℓ on Day 6
(c) MPC less than 6 nmol/ℓ on Day 0 and rising to greater than 6 nmol/ℓ on Day 6, or cow diagnosed pregnant to 1st insemination

By calculating the number of cows fulfilling each of the above criteria as a proportion of the total number of cows observed to be on heat, positive predictive values for heat detection accuracy were obtained. Positive predictive values determined using the different criteria were compared using the Fisher exact test. Positive predictive values were also compared between parities using the Fisher exact test.

RESULTS

The results of the MPC determinations are summarised in Table 1. Except for 1 primiparous and 1 multiparous cow, all had low MPC (<6 nmol/ℓ) on Day 0. The difference in MPC between primiparous and multiparous cows was statistically significant at Day 0 ($P < 0.05$), but not at Day 6. Of the 148 cows with low progesterone on Day 0, 127 (85.5 %) showed a rise to above 6 nmol/ℓ on Day 6. However, of the 21 cows that did not show a rise in progesterone to above 6 nmol/ℓ, 7 were diagnosed pregnant to their 1st insemination. The number of cows fulfilling

Table 1: Mean whole milk progesterone concentrations of primiparous and multiparous cows on day of insemination and 6 days later.

Parity	Day 0 (nmol/ℓ)	Day 6 (nmol/ℓ)	n
Primiparous	1.84[a]	14.77[a]	50
Multiparous	2.14[b]	13.88[a]	100
Total	2.04	14.17	150

Values within a column with different superscripts differ significantly ($P < 0.05$).

Table 2: **Positive predictive value of heat detection** (%) **using different criteria to define ovulatory oestrus.**

Parity	Criteria used to define ovulatory oestrus			n
	Day 0 low*	Day 0 low *and* Day 6 high**	(Day 0 low *and* Day 6 high) *or* PD+***	
Primiparous	98	84	86	50
Multiparous	99	85	91	100
Total	98.7[a]	84.7[b]	89.3[b]	150

*Milk progesterone concentration on Day 0 less than 6 nmol/ℓ.
**Milk progesterone concentration on Day 6 greater than 6 nmol/ℓ.
***Diagnosed pregnant to 1st insemination.
Values with different superscripts differ significantly ($P < 0.05$).

Table 3: **First insemination conception rates.**

Parity	Including all cows observed on heat		Including only cows showing ovulatory oestrus	
	Conception rate (%)	n	Conception rate (%)	n
Primiparous	70.0[a]	50	81.4[a]	43
Multiparous	63.0[a]	100	69.2[a]	91
Total	65.3	150	73.1	134

Values with different superscripts differ significantly ($P < 0.05$).

criterion (c) for being in ovulatory oestrus was therefore 134.

The positive predictive values of heat detection are summarised in Table 2. Estimates differed significantly depending on whether a single or a paired progesterone measurement was used to define ovulatory oestrus. No significant differences were observed between primiparous and multiparous cows.

The 1st insemination conception rates are summarised in Table 3. The observed differences in conception rate between primiparous and multiparous cows were not statistically significant ($P > 0.05$). The difference between the estimates of conception rate using the 2 methods was also not statistically significant ($P > 0.05$).

For the entire herd, the conception rate of cows served, 1st insemination conception rate and average number of inseminations per conception were 83 %, 53 %, and 1.45 for primiparous cows and 88 %, 50 % and 1.83 for multiparous cows.

DISCUSSION

The proportion of cows observed in oestrus that are truly in oestrus is often referred to as oestrus detection 'accuracy'. This parameter, however, is correctly termed the positive predictive value (PPV) of oestrus detection. Correct assessment of oestrus observation accuracy would also require determination of the true status of all cows not observed to be in oestrus in order to determine sensitivity and specificity. This was not done in this study.

Accurate detection of oestrus and the correct timing of insemination are 2 important prerequisites for the successful use on artificial insemination in cattle. In the absence of progesterone data it is impossible to know whether a cow detected on heat is truly in ovulatory oestrus. A single milk sample taken on the day of insemination was originally considered to be useful in evaluating the 'accuracy' of oestrus observation, high levels being attributed to faulty observation[4,10,13,16,19]. If MPC at the time of insemination is high in less than 10 % of the samples, heat detection accuracy was considered to be good. There is a very low probability of conception if insemination occurs while progesterone levels are elevated[4].

Low progesterone concentrations on the day of insemination, however, do not necessarily indicate ovulatory oestrus, as they also occur with certain gynaecological disorders such as follicular cysts and inactive ovaries. Taking a 2nd sample 6 or 7 days later may avoid such misinterpretations, when in a normally cycling animal progesterone concentrations will be elevated, indicating that ovulation has taken place. A 2-sample assay thus allows distinction between ovulatory and anovulatory oestrus[6,10,11,14].

In this study, 3 sets of criteria used to define ovulatory oestrus were compared. Using only a single measurement of milk progesterone on the day of insemination (Day 0), the PPV of heat detection would have been estimated at 98.7 %, as only 2 cows showed high progesterone levels at heat detection. Using a paired measurement, *i.e.* defining an ovulatory oestrus

when milk progesterone increased from less than 6 nmol/ℓ on Day 0 to greater than 6 nmol/ℓ on Day 6, resulted in a significantly lower estimate of 84.7 %. However, the fact that several additional cows conceived despite not showing a rise in progesterone to above 6 nmol/ℓ, indicates that the use of a single cut-off value does not necessarily lead to the best estimate of PPV heat detection. The retrospective inclusion of these additional cows should result in a better estimate of the parameter. In this case, their inclusion resulted in an estimate of 89.3 %; still significantly lower than the estimate obtained from a single measurement on Day 0.

Studies in dairy herds have shown that on average 10 % of cows, and possibly up to 36 % in problem herds, fail to conceive due to faulty observation resulting in insemination at the wrong time[3,6,10,13,14,16,19]. This is consistent with the results of the present study. Using paired MPC and subsequent pregnancy diagnosis, it was estimated that 10.7 % of cows failed to conceive due to inaccurate observation. It is worth noting, however, that the progesterone trough, beginning approximately 48 hours before the onset of oestrus and lasting on average 5 days, is much longer than the average duration of standing heat, which is the optimal time of insemination. Hence, false timing of insemination may be even more frequent than indicated by the progesterone concentrations[22].

The choice of the cut-off value for MPC is to a certain extent arbitrary, and will influence the results obtained. In this study it was found that using a cut-off of 5 nmol/ℓ rather than 6 nmol/ℓ (1.88 ng/mℓ) made a negligible difference to the results obtained. However, reducing the cut-off to 4 nmol/ℓ or less, or raising the cut-off to 7 nmol/ℓ or more, resulted in an increase in the misclassification of cows, *i.e.* cows not fulfilling the criteria for ovulatory oestrus and yet conceiving to the 1st insemination.

In this study progesterone was measured in whole milk and the results were consistent with concentrations reported in other publications[1,4,9,13,20,25]. However, although plasma and milk progesterone concentrations are highly correlated[13,16], there are several additional factors that may affect the milk progesterone concentration, including handling procedures, type of sample (whole milk, fat free milk or milk fat), time of sampling, suckling and laboratory technique[18]. Because of its liposolubility, progesterone concentration is higher in the lipoid fraction of milk. Progesterone concentration in milk is thus correlated with butter-fat content and tends to be lowest in first milk and

highest in last milk obtained during the usual milking sequence in dairy cows[9,13,16,18]. In beef cows with calves, the timing of sampling relative to suckling will therefore influence milk progesterone concentration. It has been shown that the progesterone and fat levels in milk after suckling were significantly higher than the levels before suckling[1]. In this study, milk samples were collected from beef cows whose calves had continual access to them.

In order to minimise this source of variation, consistent timing of sampling relative to suckling should be attempted. However, this may be highly unpractical under field conditions and certainly was not possible in the current trial. It is unclear why the mean milk progesterone concentration of primiparous cows on Day 0 was lower than that of multiparous cows, although the observed difference (1.84 vs 2.14 nmol/ℓ) is presumably physiologically and possibly clinically insignificant. A more likely explanation is that the milk fat content of multiparous cows may be higher than that of primiparous cows.

In the calculation of conception rate, the total number of cows inseminated is usually used as the denominator. The insemination of cows that are not in ovulatory oestrus will therefore lead to a reduction in the conception rate. The use of paired progesterone measurements to determine whether a cow is truly in ovulatory oestrus allows one to calculate a conception rate using as denominator only those cows shown to be in ovulatory oestrus at the time of insemination. This therefore eliminates the effect of heat detection errors on the conception rate and may be of value in assessing the effect of other factors on the conception rate. In the animals sampled in this study, of the cows in ovulatory oestrus that were inseminated, 73.1 % conceived to the 1st insemination. This differs markedly from the conception rate of 65.3 % based on all cows inseminated and indicates that oestrus detection errors accounted for the failure of some cows to conceive.

Because only the first 50 primiparous and first 100 multiparous cows were sampled, the sample was not representative of all cows coming into oestrus during the breeding season. This is evident when comparing the 1st insemination conception rate of the 150 cows sampled (65 %) with that of the entire herd (51 %). It order to investigate the entire herd, it would have been preferable to have taken a random sample of cows detected in oestrus. However, this does not detract from the usefulness of paired MPC measurements in investigating oestrus detection and conception problems in defined groups of animals.

The overall fertility performance of this herd compares favourably with accepted standards. The good results may be ascribed to several factors, including the more intensive nature of the system[23], optimal nutrition and condition[7,14,17,24], supplementary feeding[7], temporary weaning[5,7,12,24], intensive 24-hour heat observation[8,23], the use of teaser bulls[2,14] and the strict quality control applied to frozen semen before it is used for insemination. These results demonstrate that artificial breeding of beef cattle within a limited breeding season can be applied successfully with intensive management.

The testing regime described here provides a more accurate estimate of the PPV of oestrus detection than the use of a single milk progesterone sample. It may therefore be used in the investigation of suspected oestrus observation problems and the evaluation of differences between observers and inseminators. It also eliminates the effect of heat detection errors in calculation of the conception rate, thus facilitating further investigation of conception problems. Although its routine use may not be practical or cost-effective in many situations, it may be a valuable tool for problem-solving in certain herds.

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