Use of progesterone profiling to investigate factors affecting conception rates in a large, pasture-grazed dairy herd

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Abstract

A study was conducted at the Lincoln University Dairy Farm to investigate factors influencing conception rate (CR). Relative to the planned start of mating date (Day 0), milk progesterone (MP4) concentrations were determined weekly from Day -20 to 0, twice weekly from Day 0 to 42, and then weekly to Day 70 in 666 cows. Trough-like patterns among individual profiles were identified and defined as periovulatory events. Pre-mating ovulatory status was a predictor ($P < 0.01$) of first service CR (FSCR), with anovulatory cows having a lower FSCR (37.4%) compared with 'late' (47.3%) and 'early' (56.4%) ovulating cows. In turn, interval from calving to Day 0 was a significant determinant for pre-mating ovulatory status. Oestrus detection was performed to a high standard, even though 5% of the 850 artificial inseminations were coincident with a lack of ovulation. MP4 profiles also indicated that 4% of cows that conceived during the 37-day artificial breeding period subsequently lost these pregnancies. A 2.6% error rate for diagnosing pregnancies on Day 78 was detected, mostly due to incorrectly attributing pregnancies following artificial breeding. Findings indicate the critical importance of managerial strategies that promote a high prevalence of cows having ovulated at least 21 days before mating starts.

Keywords: conception rate; progesterone; dairy cow

Introduction

Conception rate (CR) is a key driver of overall reproductive performance. The national average CR of about 52% is substantially lower than the industry target of 60% (Burke et al. 2007). The economic consequences of this 8% gap between observed and target CR is estimated from the InCalf Economic Benefits model (Burke et al. 2008) to be costing dairy farmers as much as $200 million per annum in lost operating profit.

Reports from the 1970s (Macmillan & Watson 1975) and 1980s (Macmillan et al. 1984) suggested first service CR (FSCR) of between 60 and 70% were routinely achieved. Even during the mid-1990s, a CR of 70 and 80% to the first and second inseminations was recorded in herds noted as having exceptional reproductive performance (Xu & Burton 1996). In general, however, there has been a decline in CR, most notably through the 1990s. A study that represented the industry situation during the 1998 to 2000 seasons reported average FSCR as 53% (Xu & Burton 2003) suggesting that routinely achievable CR declined about 10% during the 1990s.

The study of Xu & Burton (2003) reported that significant differences in FSCR were associated with season, region, age, breed, age x breed interactions, calving difficulties, non-cycling treatment, day of calving, and day of artificial insemination during the artificial insemination period. Body condition score at calving and loss after calving are also predictors of first service CR (Roche et al. 2007). In spite of these established risk factors it is generally difficult to pinpoint causes for consistently low CR in dairy herds. A variety of other reasons ranging from genetics, feeding levels, semen quality, insemination technique and diseases are often speculated. There is little or no accurate information on how pre-ovulatory status, oestrus detection accuracy, fetal losses and accuracy of pregnancy diagnosis influences CR. Some of these indicators are available to industry but are proxy-based estimates from data routinely collected on dairy farms.

The Lincoln University Dairy Farm has historically suffered with poor CR (Blackwell et al. 2010) with overall CR being below 45% from its establishment in 2002 to the 2008/09 season. With excellent record keeping, Lincoln University Dairy Farm was considered as an ideal case-study herd to investigate these underlying factors affecting CR in a large, pasture-grazed dairy herd. The objective was to use progesterone profiling to examine the influences of pre-mating ovulatory status, oestrus detection performance, embryonic loss and pregnancy diagnostics on CR.

Materials and methods

Animals and management

The study was conducted at Lincoln University Dairy Farm during the 2010/11 season. Objectives, management policies and animal performance measures relating to the farm are made widely available to farmers and rural professionals at *www.siddc.org.nz*. Briefly, Lincoln University Dairy Farm is an irrigated, seasonal, 160 ha (effective) pasture-based farm with 670 cows milked twice-daily through a rotary milking shed equipped with auto-drafting capability.

Milk sampling, testing and progesterone measurement

Milk samples were collected from every cow from three weeks before the planned start of mating date (PSM) (Day 0; 25 October 2010) to the end of the mating period (4 January 2011). The frequency of sampling was weekly during the pre-mating period, twice-weekly for the first six weeks of mating and weekly for the last four weeks of the 10-week mating period. A total of 13,400 milk samples were collected using Livestock Improvement Corporation (LIC) herd testing equipment. Initially, these samples were processed for routine herd test measures at the LIC TestLink facility in Christchurch. Samples were kept chilled at this facility before refrigerated transport to Gribbles Veterinary Pathology Ltd, Mosgiel, New Zealand, where progesterone was measured. A commercial radio-immune assay (RIA) double antibody kit was used in accordance with the manufacturer's instructions (ImmuChem™, ICN Biomedical Inc., Costa Mesa, CA, USA). Samples were mixed thoroughly before assay to reincorporate the cream. The inter-assay coefficient of variation for 20 assays were 18.3, 5.4 and 5.6% for standard concentrations (Trilevel Lyphochek Immunoassay Plus Control™, Bio-Rad Laboratories, Hercules, CA, USA) of 0.5, 8.2, and 20.7 ng/mL, respectively. Mean intra-assay coefficients of variation were 5.2 and 6.1% for milk samples having concentrations of 5.8 and 12.2 ng/mL with 20 replicates per pool standard, respectively.

The results were compiled in a manner allowing an individual progesterone profile to be constructed for every cow. Example profiles are shown in Fig. 1.

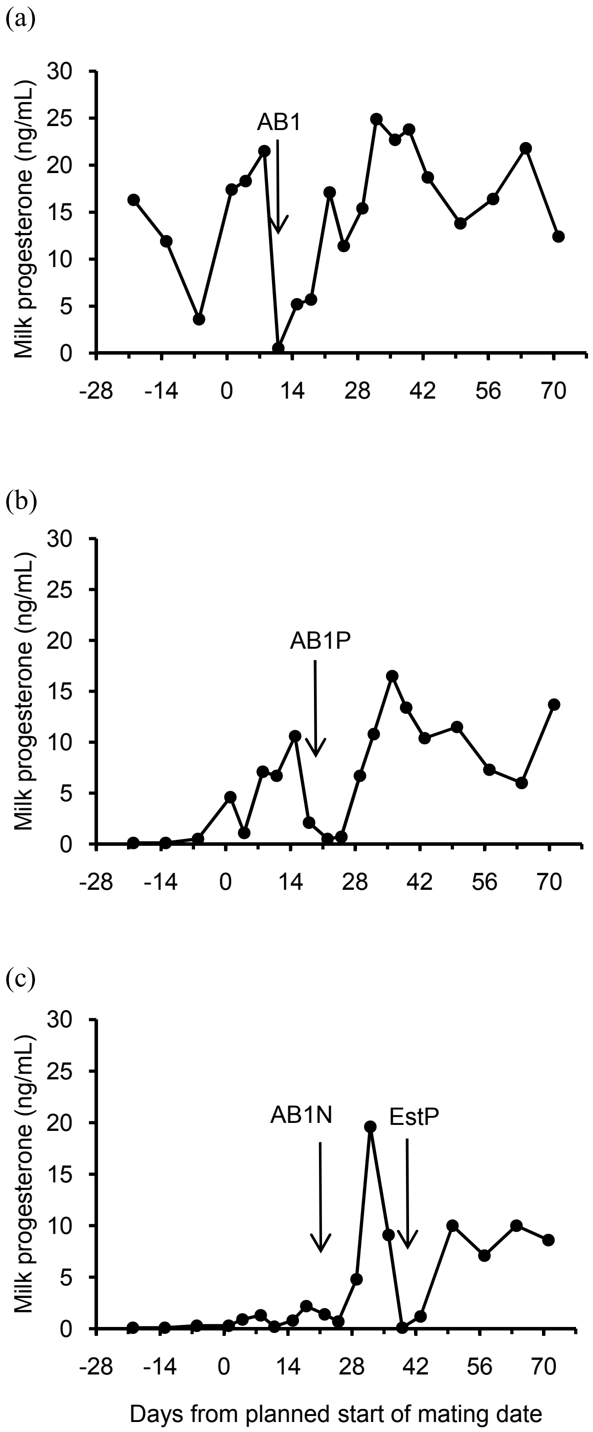
Oestrus detection and performance measure definitions

Progesterone profiling was used to assess the sensitivity and success rate of visual oestrus detection using a standard epidemiological procedure. Sensitivity was defined as the proportion of progesterone-based events that were accompanied with a recorded artificial insemination date. Success rate was defined as the proportion of artificial insemination dates that corresponded with an ovulation that was detected from progesterone profiling.

Day of oestrus was determined by visual assessment of the individual progesterone profiles overlaid with timing of artificial insemination and pregnancy diagnosis results. Initially, the trough-like curves indicating a periovulatory event such as luteolysis with subsequent development of the new functional corpus luteum, were located within the profiles. These were followed with assignment of a day of oestrus based on insemination and pregnancy diagnosis outcomes. The rules for assigning a day of oestrus were:

1. the day of oestrus was assigned as the insemination date for any inseminations that

Figure 1 (a) Example of a cow that was ovulating before the pre-mating sampling period started (Day -21 or earlier); early cycler (EC) that conceived to first service (AB1P); (b) Example of a cow that began ovulating during the pre-mating sampling period (days -20 to 0); late cycler (LC) that conceived to first service (AB1P); (c) Example of a cow that was anovulatory (AN) at the planned start of mating date (PSM; day 0), and failed to conceive to first service (AB1N), but established pregnancy to a bull after the AB period (EstP).



resulted in a positive pregnancy outcome, regardless of the progesterone profile;
2. the day of an unsuccessful insemination was also assigned as the day of oestrus when the insemination was temporarily coordinated with the trough-like curve in progesterone concentrations;
3. if there was no insemination date within a trough-like curve, then the day of oestrus was set preferentially at the nadir point of this trough such as would occur with a missed oestrus.

Progesterone profiling indicated that 116 (17.4%) cows ovulated spontaneously for the first time after the PSM date; non-cycling treatments were not used. Specific rules around assigning a date of oestrus to detected first ovulations were the same as described previously when the estimated day of first ovulation corresponded with an insemination date, but an oestrous date was not assigned when there was no supporting evidence that the first ovulation was associated with a detectable behavioural oestrus. This rule was applied to be consistent with previous reports that the majority of first ovulations are not accompanied with oestrus in pasture-grazed systems (McDougall et al. 1995; Burke et al. 1995).

Any insemination date that did not concur within this characteristic trough-like curve in progesterone concentrations was considered false, and not assigned with a date of oestrus.

Calving groups and pre-mating ovulatory status

Cows were categorised into four calving groups based on calving dates relative to the planned start of calving date (PSC) (8 August 2010). These categories were: 'Very early' - calved before the PSC date (n = 108); 'Early' - calved within the first three-week period (n = 331); 'Mid' - calved within the second three-week period (n = 127); and, 'Late' - calved after the sixth week (n = 65) from the PSC.

Progesterone profiling (Fig. 1) was used to categorise cows into those that were anovulatory (AN) at the PSM date (Day 0) as 'Late cyclers' (LC) which ovulated between Day -20 and 0, and 'Early cyclers' (EC) which had ovulated before Day -20.

Statistical analyses

The associations between FSCR and variables of interest were analysed in mixed models using REML variance components (Payne et al. 2009). Iterative models were conducted to test the inclusion or exclusion of age (first calving heifer vs. multiparous cows), pre-mating ovulatory status, calving group, and the interaction term between pre-mating cycling status and calving group. The relationship between the interval from calving to PSM and pre-mating cycling status was also analysed using this model. Results are presented as the mean ± standard error unless specified otherwise.

Table 1 Descriptive statistics of reproductive performance for cows that were anovulatory during the pre-mating period (AN; n = 117), ovulated during the 21-day period before planned start of mating (PSM) date (LC (Late cycling); n = 117) or were ovulating at the commencement of the milk sampling period 21 days before PSM (EC (Early cycling); n = 434). SE = Standard error.

Reproductive performance	Pre-mating ovulation status			Overall
	AN	LC	EC	
Calving to PSM (days), (Mean ± SE)	46.7 ± 1.4	56.3 ± 1.4	68.2 ± 0.7	62 ± 0.7
3-week submission rate (%)	65.5	90.5	94.2	88.6
First service conception rate (%)	37.4	47.3	56.4	52.2
Second service conception rate (%)	48.5	53.9	52.8	52.3
3-week in-calf rate (%)	25.7	42.6	55.9	48.4
6-week in-calf rate (%)	53.1	69.6	76.4	71.3
Empty rate (%)	23.0	12.2	10.0	12.7

Table 2 Number (and percentage) of cows classified by progesterone profiling as anovulatory (AN), Late cycler (LC) and Early cycler (EC) among the groups of cows having a recorded pre-mating oestrus or no pre-mating oestrus. Table includes a single case of a cystic cow with chronically elevated progesterone in the absence of oestrus, ovulation and pregnancy.

Pre-mating oestrus status	Pre-mating ovulation status				Total
	Cystic	AN	LC	EC	
Pre-mating oestrus	0 (-)	25 (5)	70 (14)	401 (81)	495 (74)
No pre-mating oestrus	1 (-)	91 (53)	46 (27)	33 (19)	171 (26)
Total	1 (-)	116 (17)	116 (17)	434 (65)	666 (100)

Results

Associations between pre-mating ovulatory status and FSCR

Progesterone profiling revealed that the majority (82.6 %) of the herd had ovulatory cycles before the PSM date with 65.2 % of the herd having ovulatory cycles from the commencement of milk sampling at 20 days before PSM. The remainder of the herd were equally split between those that ovulated for the first time within the 20-day period before PSM and those that had not ovulated by the PSM date (Table 1). An association for improved CR as cows initiated ovulatory cycles earlier relative to the PSM date ($P = 0.002$) was detected: EC, 56.4 ± 2.4%; LC, 47.3 ± 4.7%; AN, 37.4 ± 5.1%. Descriptive statistics for overall reproductive performance among these pre-mating categories are presented in Table 1.

Calving group was also associated with FSCR ($P = 0.003$; Very early, 54.3 ± 5.5%; Early, 57.8 ± 2.8%; Mid, 40.2 ± 4.4%; Late calvers, 42.3 ± 6.3%), but calving group did not add any predictive value when pre-mating ovulatory status was already included in the statistical model. In addition, there was no interaction ($P = 0.3$) between calving group and pre-mating cycling ovulatory status on FSCR. Calving group was a predictor ($P < 0.001$) of pre-mating ovulatory status.

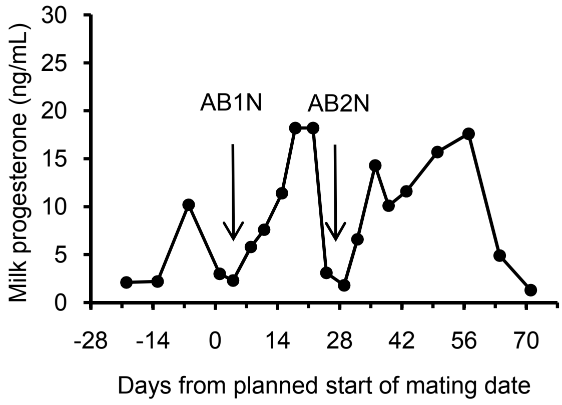
Weekly visual observations of tail paint recorded 74.3% of the herd as having had a pre-mating oestrus. There was general agreement between this indicator of pre-mating cycling status and the progesterone-based, pre-ovulatory status (Table 2).

Effects of oestrus detection performance on CR

The sensitivity of visual oestrus detection during the 37-day insemination period was 90%. That is, of all progesterone-based ovulation events, 90% were accompanied with an artificial insemination mating following detection of oestrus. The success rate was 95%, that is, of all artifical inseminations, progesterone-based ovulation events concurred with insemination dates 95% of the time.

The majority (76%) of return inseminations to first insemination were of a normal length of between 18 and 24 days. On the basis of progesterone profiles, 143 (93%) of these normal returns were classified as 'true' return intervals. That is both first and second inseminations were correct. Of the 40 (19%) returns that were shorter than 18 days, only 11 (28%) were true return intervals. Most (83%) of these 29 'false' short returns were a consequence of the first insemination being 'out of step' with the progesterone profiles. There were only eight (4%) return-to-service intervals longer than 24 days, and all were 'true' returns.

Figure 2 An example of a cow identified as having lost a pregnancy to the second insemination (AB2N); identified through an insemination at the correct time with a subsequently prolonged period of elevated progesterone, followed later with a return to basal concentrations and confirmed non-pregnant by ultrasonography.



Effect of lost pregnancies on CR for all inseminations

Inter-ovulatory cycles of 28 days or more were detected in 45 cows. These were closely examined to evaluate the likely prevalence of a lost pregnancy. Of these, 36 cow-inseminations (4% of 850 inseminations performed) had progesterone profiles characteristic of a lost pregnancy. An example is depicted in Fig. 2, where insemination was performed at the correct time and progesterone concentrations remained elevated for a prolonged time indicating pregnancy, before returning to a basal level. Pregnancy diagnosis confirmed that this cow was not pregnant. Among these 36 cows, only four (11%) had an incorrect return insemination that preceded the pregnancy loss. There were no obvious associations with age or calving date, although the nominal prevalence of lost pregnancies was 4.4% among Early cyclers compared with 9.4% of Late cyclers and 6.8% of anovulatory cows.

Errors with pregnancy diagnosis

At the first pregnancy diagnosis on 10 January 2011, results for 17 cows (2.6%) did not concur with the progesterone profiles. The most common difference was cows (n = 12) recorded as pregnant to artificial insemination where progesterone profiling indicated they later cycled during the natural bull mating period. These errors were confirmed with subsequent rechecking on 15 February 2011.

Discussion

Overall reproductive performance

Overall reproductive performance at Lincoln University Dairy Farm during this study was satisfactory relative to industry benchmarks and was consistent with an upward trend in reproductive performance since the Lincoln University Dairy Farm was established in 2002. The poor levels of CR of less than 45% that were evident in seasons before this study, have not been observed since the 2008 mating. It is not possible to attribute the observed improvement to any particular causes or management changes, of which there have been many. In spite of the Lincoln University Dairy Farm achieving an industry average CR of 52%, the progesterone profiling approach used in this study has provided novel information on the crucial influence that pre-mating ovulatory status has on CR. It has also provided some perspective on how oestrus detection performance, lost pregnancies and pregnancy diagnosis accuracy influence the CR measured in a large dairy herd.

Pre-mating ovulatory status

Pre-mating ovulatory status was a key determinant of FSCR, and hence overall reproductive performance. Although it is well documented that non-cycling cows have poorer reproductive performance (Xu & Burton 2003), the current findings indicate a ‘continuous’ advantage in FSCR with increased interval between the onset of ovulatory cycles and the PSM date. This interval was significantly influenced by calving date was also associated with FSCR. The associations between calving date and pre-mating ovulatory status are confounded, but the logical pathway, and one supported with the present data, is that FSCR is improved for earlier calving cows because they had more ovulations before the mating period started.

Oestrus detection performance

Progesterone profiling determined that 95% of artifical inseminations following visual oestrus detection were accompanied with ovulation. If the remaining 5% of artifical inseminations (n = 43) are excluded from the denominator that determines CR (i.e. 445 artificially inseminated pregnancies from 807 (850 minus 43)), then overall CR is increased from 52.3 to 55.1%. It is possible that these 5% of inseminations were triggered by visually detected signs of oestrus, but the animal failed to ovulate. Caution is therefore required when attributing such cases to mistakes with oestrus detection; particularly in this case where all indications were that this task was performed to a high standard during this study.

Among the false inter-ovulatory intervals detected, there was a nominal trend for the first artificial insemination to be incorrect with false shorter cycles. While a larger dataset would be required to test this observation statistically, it is consistent with a previous report where falsely identified short cycles are mostly generated by a false first artificial insemination (e.g. 90%, Macmillan & Watson 1975). A trade-off between sensitivity of not missing oestruses and specificity of being correct with those observed, is acknowledged within the industry. Dairy farmers will generally accept that some unnecessary inseminating will occur during the first three weeks of the period of artificial insemination as a consequence of aiming not to miss any cows in oestrus.

Lost pregnancies

The prevalence of lost pregnancies in the current study is consistent with previous reports using progesterone analysis or frequent diagnosis of pregnancy to indicate that 4 to 9% of dairy cows lose their pregnancy during the mating period (Moller et al. 1986; Nation et al. 2003; McDougall et al. 2005). It is difficult to identify causes for why cows lose pregnancies. Diseases and toxins are often implicated (*see* McDougall et al. 2005). Falsely inseminating cows that were already pregnant has been indicated as one risk factor (Burke et al. 2005), but was negligible in the current study. Among the variables of interest examined, only pre-mating cycling status had some nominal association where half (4%) the proportion of Early cyclers lost a pregnancy compared with 8% of those that had not ovulated by 21 days before the PSM. This difference would need to be validated with a larger dataset, but it is a possible contributing reason for the higher FSCR observed in Early cyclers.

Pregnancy diagnosis

The CR metric can only be quantified by ageing the fetus during pregnancy diagnosis. Incorrect results will affect the overall CR measure. The present study indicated an error rate of 2.6% over-estimation. This was mainly because a number of cows were incorrectly ‘recorded’ as being pregnant to an artificial insemination. This error rate should not be considered excessive. A recent report (J Morton, Personal communication) indicates a much larger level of inaccuracy is prevalent, at least among Australian dairy herds. The 2.6% error could have been due to incorrect fetal ageing, incorrect cow identification, or incorrect data entry. Although it was not possible to partition the error among these possibilities, the result could indicate a general overestimation of CR in the dairy industry; especially since it is common practice not to re-check cows already diagnosed pregnant at earlier examinations. Further, there is likely to be a bias toward CR being over estimated because, by default, a cow is assumed pregnant to the last recorded insemination, unless determined otherwise. This is particularly evident when pregnancy diagnosis records are incomplete, and an over-reliance on insemination information. Re-calving information may be useful to further investigate pregnancy diagnostics as a source of error in reporting CR.

Conclusions

Pre-mating ovulatory status was a key predictor of FSCR, and hence overall reproductive performance. Calving date was confounded with this association, and most likely influenced FSCR through altering the interval from first ovulation after calving to the first artificial insemination. Insemination of cows that have recently ovulated, do not ovulate coincidentally with the insemination, pregnancy losses and errors with pregnancy diagnosis, are all factors that influence CR. In combination these factors would account for the gap between the industry average of 52% and the target of 60% for levels of CR in the New Zealand dairy industry.

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