
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

An evaluation of a progesterone-based diagnostic as an aid to re-insemination decisions in a seasonal, pasture-grazed dairy cow herd

CR Burke[a]*, C Kamphuis[a], S Meier[a] and R Pellow[b]

[a]*DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand;* [b]*South Island Dairy Development Centre, PO Box 160, Lincoln University, Christchurch 7647, New Zealand*
**Corresponding author. Email: chris.burke@dairynz.co.nz*

Abstract

Progesterone-based diagnostic tests are commercially available to predict pregnancy status 19 to 23 days following an insemination, thus providing decision-making support for artificial breeding. The study objective was to evaluate the likely performance of such a test in a pasture-grazed, seasonal dairy farm. Progesterone was determined in milk samples collected twice weekly from 553 cows at the Lincoln University Dairy Farm. Receiver operating characteristic analysis was applied to these data to determine sensitivity, false positive rate and the positive predictive value using a range of progesterone threshold values for detecting non-pregnant cows. The gold standard was non-pregnancy as measured by ultrasonography. At an optimised threshold of 4.1 ng/mL, the sensitivity, false positive rate and positive prediction rate were 78%, 2.6% and 96% for detecting non-pregnant cows on Days 19 to 23. A major source of inaccuracy was the 28 cows with elevated levels of progesterone in milk on Days 19 to 23 after first insemination that were subsequently diagnosed non-pregnant. Another source of error was the inherent variance in progesterone concentration profiles among individuals. At a current cost of about $5 per test, this technology would have limited appeal for detecting non-pregnant cows in dairy systems where a satisfactory level of oestrus detection performance can be achieved.

Keywords: pregnancy diagnosis; progesterone; dairy cow

Introduction

Submission of dairy cows for artificial breeding (AB) based on visual observations for signs of behavioural oestrus can be a difficult task and prone to a high error rate. Inexperienced staff, poor use of aids and weak displays of oestrus, all contribute to reducing oestrus detection performance. This problem is particularly evident in high-yielding, confinement-style dairy systems, where the chances of correctly detecting cows in oestrus can be less than 50%. To alleviate this inefficiency, a test that quickly measures the concentration of progesterone in the cow's milk so as to provide an immediate diagnosis, that is a 'cow-side' test, has been developed and commercialised (Eddy & Clark 1987; Nebel 1988). Several companies are currently marketing kits kits to determine pregnancy status at 19 to 23 days following an insemination. A positive test is meant to indicate pregnancy whilst a negative test indicates that the cow is not pregnant, and should be inseminated.

The cow-side tests are accurate in differentiating between low and high concentrations of progesterone (Nebel et al. 1987; Nebel et al. 1989) and, therefore, are accurate in diagnosing between non-pregnant cows due to re-ovulate, with a low level of circulating progesterone, and pregnant cows with a high level of circulating progesterone, that will not re-ovulate. These diagnoses are time specific to 19 to 23 days after an animal has been inseminated at the correct time. This technology is 85% to 100% accurate for diagnosing non-pregnancy 19 to 23 days after an insemination (Nebel et al. 1987; Romagnolo & Nebel, 1993). Diagnosing pregnancy by this method is less accurate, but still able to make a correct prediction in 68 to 84% of cases (Nebel et al. 1987; Romagnolo & Nebel, 1993).

The purpose of this study was to evaluate the predictive performance of using progesterone in milk as a diagnostic tool for detecting non-pregnant cows 19 to 23 days after first insemination in a large, pasture grazed dairy herd. A receiver operating characteristic (ROC) analysis was employed to consider a range of threshold levels for optimising the performance of this diagnostic test.

Materials and methods

A subset of the data collected during a study at the Lincoln University Dairy Farm was used. A detailed methodology of the Lincoln University Dairy Farm study has been described elsewhere (Burke et al. 2012). Briefly, milk samples were collected weekly from all cows between Day -20 to 70, relative to the herd's planned start of mating date (25 October 2010; Day 0), and twice weekly during the first six weeks of mating (Day 0 to 42). Progesterone was measured in these milk samples using a commercial assay kit. To test the accuracy of progesterone concentration in milk to diagnose non-pregnant cows at 19 to 23 days after insemination, the test was only applied after the first insemination, cows with a return-to-service date prior to a test being applied were excluded, and the sampling date nearest to 21 days, and within 19 to 23 days, from first insemination was used as the test result. From these criteria, 553 cows were tested using ROC analysis (Detilleux et al. 1999; Fawcett

2006). Essentially, ROC analysis allows sensitivity and specificity indices to be calculated on a continuous basis for any given value for the predictor variable, in this case the concentration of progesterone in milk. The ‘gold standard’ (GS) was pregnancy state confirmed by presence or absence of a fetus using transrectal ultrasonography at the ‘early’ pregnancy test for this herd (Day 88). The absence of a fetus, indicating non-pregnancy, was considered a GS-positive, while pregnancy was classified as GS-negative in this evaluation. A progesterone-based alert for GS-positive was deemed as a true-positive (TP) whereas an alert for a GS-negative case was considered a false-positive (FP). Similarly, a false-negative (FN) occurred when a GS-positive was not alerted, and true-negative (TN) when a GS-negative was not alerted. Predictive performance indices of interest were sensitivity (SN) where SN =100 x TP / (TP + FN); false positive rate (FPR) where FPR = 100 – (100 x TN / (TN + FP)); and positive predictive value (PPV) where PPV = 100 x TP / (TP + FP).

The SAS (2009) package was used for these analyses.

Results

A low (Table 1) and high (Table 2) milk progesterone threshold level were arbitrarily selected to demonstrate the diagnostic performance indicators, and to describe the extreme outcomes that are produced with this approach to detecting non-pregnant cows 19 to 23 days after first insemination. A progesterone threshold level of 2.1 ng/mL (Table 1) was required to avoid false alerts completely. That is, FPR = 0% and PPV = 100%, but the test is not sensitive as SN = 65%. In contrast, an SN of 90% could be achieved with a 9.7 ng/mL threshold, but only 60% of the alerts for non-pregnancy would then be correct (Table 2). The trade-offs between SN and PPV when considering any of the progesterone levels as a threshold setting for creating an alert for non-pregnancy are presented in Fig. 1. In assessing these data, a threshold of 4.1 ng/mL, that is values less than 4.1 ng/mL 19 to 23 days after first AB to generate an alert for non-pregnancy, provided a SN of 78%, a FPR of 2.6% and a PPV of 96%. Of all the GS-positive, or non-pregnant, cows, this test would find 78% of them. Only 2.6% of GS-negative, or pregnant, cows would be identified falsely, and 96% of all alerts for non-pregnancy would be correct.

Among the 553 progesterone profiles considered in this study, 28 indicated that a cow may have conceived to first insemination but failed to maintain the pregnancy to the time of pregnancy confirmation. This indication is based on a prolonged elevation of progesterone in milk followed by a decline to basal levels any time beginning 28 days after first insemination. In addition to these aberrant patterns, variance in the levels of progesterone within individual cow profiles was also observed. Representative examples of cows with low and high levels of progesterone in milk are depicted in Fig. 2. The timing of the premating ovulation and day of conception (12 Nov 2010) were identical. The cow with naturally low levels of progesterone would be falsely identified 19 to 23 days after first insemination as non-pregnant at any progesterone threshold below 2.3 ng/mL.

Table 1 Incidence of true-positive (TP), true-negative (TN), false-positive (FP) and false-negative (FN) outcomes for detecting non-pregnancy in 553 cows at 19 to 23 days after first insemination using a low progesterone level threshold value in milk of 2.1 ng/mL.

Action	Gold standard (GS)		Number of cows
	Non-pregnant (Above GS)	Pregnant (Below GS)	
Alert	160 (TP)	0 (FP)	160
No alert	86 (FN)	307 (TN)	393
Total	246	307	553

Table 2 Incidence of true-positive (TP), true-negative (TN), false-positive (FP) and false-negative (FN) outcomes for detecting non-pregnancy in 553 cows at 19 to 23 days after first insemination using a low progesterone level threshold value in milk of 9.7 ng/mL.

Action	Gold standard (GS)		Number of cows
	Non-pregnant (Above GS)	Pregnant (Below GS)	
Alert	222 (TP)	148 (FP)	370
No alert	24 (FN)	159 (TN)	183
Total	246	307	553

Discussion

Performance of applying progesterone-based test to insemination decisions

The ROC approach allows user-specific priorities to be considered because the progesterone threshold value used to provide alerts can be varied to best suit the specific query for the diagnosis and the amount of tolerance for errors. For example, if the goal is to detect as many as possible of the cows that fail to conceive to an insemination and are therefore non-pregnant, but to falsely re-inseminate no more than 5% of those already pregnant, then the current results indicate that a threshold progesterone value of less than 4.1 ng/mL would provide this degree of accuracy while detecting 78% of non-pregnant cows. On the other hand, if the aim is to detect at least 90% of non-pregnant cows, a greater progesterone

Figure 1 Effect of progesterone threshold level in milk truncated to a maximum of 14 ng/mL, on sensitivity (SN) and positive predictive value (PPV) 19 to 23 days after first insemination, as a predictor of non-pregnancy. Diagnostic alerts for non-pregnancy are generated by progesterone values below the threshold level.

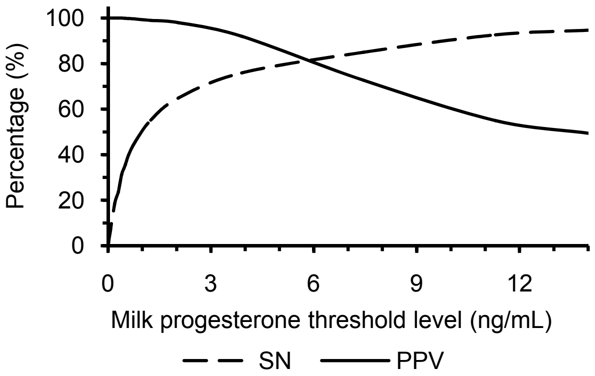
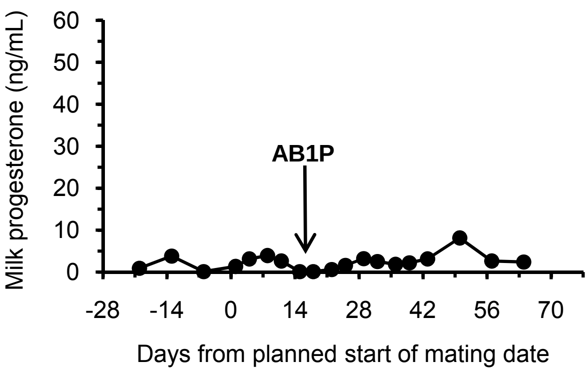
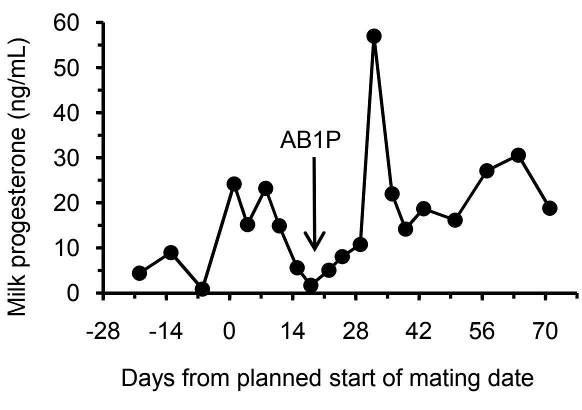


Figure 2 Representative examples of cows with (a) low and (b) high levels of progesterone in their milk. The cycling pattern and day of conception (12 Nov 2010) are identical in these extreme cases.

(a)



(b)



threshold is required of 9.7 ng/mL. In this case 40% of cows already pregnant would be re-inseminated to achieve this level of SN.

Conception rate to first AB was 52%. The reciprocal, non-pregnancy was therefore 48%. Thus the usefulness of the test diminishes as PPV declines towards this inherent level of non-pregnancy (Fig. 1), such that a PPV of 48% provides no more value than considering the whole population as being non-pregnant. If the SN of this technology is emphasised then there will be pregnant cows that are falsely alerted. The level of false alerts considered tolerable will depend on the farmer's ability to apply secondary decision-making criteria such as tailpainting, as well as their attitude to the risk of inseminating cows that are already pregnant.

The main limitation to SN was non-pregnant cows with elevated levels of progesterone on Days 19 to 23. The 28 cases detected may have been pregnant at this stage, but if they were, the pregnancy failed well before the ultrasonography diagnosis that was performed 88 days after the planned start of mating date (Burke et al. 2012). It is unlikely that progesterone-based diagnostics could be improved to overcome this limitation, unless new technologies that predict pregnancy failure are developed and incorporated into the decision-making criteria. Between-cow variations in the nature of the progesterone profile was another source of inaccuracy. Such variations have been previously reported (Friggens et al. 2008), even among animals which achieve comparable reproductive success. The source of such variation is not well known. It may reflect variation in the circulating levels of progesterone in the cow or it may be a consequence of different milk characteristics influencing the progesterone assay. The variation provides an additional challenge for establishing a single threshold level of progesterone to indicate pregnancy status accurately.

Cost-benefit considerations

Progesterone test kits are currently available for about \$5 per test. The policy for using such tests will obviously influence the cost of using this technology in the dairy herd. If a test is applied routinely to all cows at 19 to 23 days after first insemination, then in the Lincoln University Dairy Farm example, there would be a requirement for 550 kits at a total cost of \$2,750. This cost excludes labour and the consequences of the inaccuracies described in this paper. In agreement with Ruiz et al. (1989), routine use of such a test to prevent insemination errors is not likely to be cost-effective, especially when oestrus detection is performed to a high standard and standard semen costs apply. The test, however, may have beneficial application in situations where there is some evidence that a cow is in oestrus, but the signs of oestrus are not strong enough for the herd manager to be confident that the cow has a chance of conceiving without a confirmatory test. If the test could be successfully applied in these circumstances, then cows that are truly in oestrus would have a 53% chance of conceiving 21 days earlier than if the decision not to inseminate was made. This may provide a positive economic return, but routine use would not be financially justifiable under normal circumstances.

Conclusions

Assessment of progesterone levels in milk 19 to 23 days after an insemination to detect non-pregnant cows was reasonably efficient and accurate. Performance was subject to a trade-off between maximizing the success of detecting non-pregnant cows versus incorrectly classifying pregnant cows as being non-pregnant. Cows that have prolonged inter-ovulatory intervals indicative of lost pregnancies were the greatest source of inaccuracy. A diligent and effective oestrus detection programme would provide a greater level of accuracy and profitability in pasture-based systems where cows express oestrus more intensely.

Acknowledgements

The authors acknowledge the staff of the Lincoln University Dairy Farm for routine management and animal handling assistance, Bill Templeton for milk sampling, and Neil Cox and Barbara Dow for data handling advice. This study was supported by funds from DairyNZ (AN1004), Ministry of Agriculture and Forestry (now Ministry of Primary Industry), Sustainable Farming Fund (10/024) and South Island Dairy Event. In-kind support from South Island Dairy Development Centre and Livestock Improvement Corporation was also appreciated.

References

Burke CR, Meier S, Pellow R 2012. Use of progesterone profiling to investigate factors affecting conception rates in a large, pasture-grazed dairy herd. Proceedings of the New Zealand Society of Animal Production 72: 45–50.

Detilleux J, Arendt J, Lomba F, Leroy P 1999. Methods for estimating areas under receiver operating characteristic curves: illustration with somatic-cell scores in subclinical intramammary infections. Preventative Veterinary Medicine 41: 75–88.

Eddy RG, Clark PJ 1987. Oestrus prediction in dairy cows using an ELISA progesterone test. Veterinary Record 120: 31–34.

Fawcett T 2006. An introduction to ROC analysis. Pattern Recognition Letters 27: 861–874.

Friggins NC, Bjerring M, Ridder C, Højsgaard S, Larsen T 2008. Improved detection of reproductive status in dairy cows using milk progesterone measurements. Reproduction in Domestic Animals 43: 113–121.

Nebel RL 1988. On-farm milk progesterone tests. Journal of Dairy Science 71: 1682–1690.

Nebel RL, Altemose DL, Munkittrick TW, Sprecher DJ, McGilliard ML 1989. Comparisons of eight commercial on-farm milk progesterone tests. Theriogenology 31: 753–764.

Nebel RL, Whittier WD, Cassell BG, Britt JH 1987. Comparison of on-farm laboratory progesterone assays for identifying errors in detection of estrus and diagnosis of pregnancy. Journal of Dairy Science 70: 1471–1476.

Romagnolo D, Nebel RL 1993. The accuracy of enzyme-linked immunosorbent assay and latex agglutination progesterone test for the validation of estrus and early pregnancy diagnosis in dairy cattle. Theriogenology 39: 1121–1128.

Ruiz FJ, Oltenacu PA, Smith RD 1989. Evaluation of on-farm milk progesterone tests to determine nonpregnant cows and to prevent insemination errors. Journal of Dairy Science 72: 2718–2727.

SAS 2009. SAS/STAT 9.2. User's guide. SAS Institute Inc., Cary, NC, USA.