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Milk progesterone concentrations at selected times following oestrus and insemination in relation to the success of pregnancy establishment

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ABSTRACT

Positive relationships between concentrations of progesterone in milk and subsequent pregnancy rates suggest that measurement of milk progesterone concentration at selected times following insemination may facilitate identification of cows for strategic progesterone supplementation to enhance the probability of pregnancy. This study investigated some factors associated with variation in milk progesterone concentration.

Following a standard oestrus synchronisation treatment with CIDR-B™ devices (InterAg, NZ), cloprostenol (Estroplan injection, Parnell Laboratories NZ Ltd, NZ) and oestradiol benzoate (CIDIROL Injection, InterAg, NZ) every cow artificially inseminated (AI) at 72 h after CIDR-B™ withdrawal (Day 0) was enrolled in the trial. Milk samples (fore-milk: Herd 1, n = 153, Holstein-Friesian; HF; composite: Herd 2, n = 50 HF and 76 Jersey; J) were taken 4, 8 and 22 days after AI to measure milk progesterone concentration. Pregnancy to the insemination was confirmed by rectal palpation 60-70 days later.

Lower milk progesterone concentrations were found in fore-milk (Herd 1) than composite samples (Herd 2), but followed a similar pattern of change throughout Days 4, 8 and 22. Neither return to service, nor pregnancy status was associated with altered milk progesterone concentration on Days 4 or 8, but levels on Day 22 were higher ($P < 0.001$) in pregnant cows. Mean milk progesterone concentrations were higher ($P < 0.001$) in J than HF in Herd 2 on Day 8.

Absolute milk progesterone concentration on Day 4 or 8 following AI did not predict the probability of pregnancy establishment, but the difference between milk progesterone concentration in the two samples was of predictive value on a herd, but not an individual cow, basis. Milk collection methods and breed effects need to be considered when developing milk progesterone concentration as a predictor of pregnancy establishment. Although the non-parametric analyses showed that non-pregnant animals tended to have lower milk progesterone concentration, the requirement for analysis of at least two samples will limit its use as a practical means to identify the subset of cows for which strategic progesterone supplementation might be considered.

Keywords: milk progesterone; pregnancy; cattle.

INTRODUCTION

The relationships between progesterone concentration in milk following artificial insemination (AI) and the probability of subsequent pregnancy have been extensively described (Erb *et al.,* 1976, Bulman and Lamming, 1978, Lamming *et al.,* 1989). Low progesterone concentrations in early dioestrus may alter responsiveness to oxytocin later in the oestrous cycle in such a manner that luteolytic signals then initiate a stronger response (Mann and Lamming, 1995), while high progesterone concentrations have also been suggested to enhance embryo development (Garrett *et al.,* 1988). Thus have arisen suggestions that progesterone supplementation during dioestrus may enhance the probability of pregnancy establishment (Macmillan and Peterson, 1993). Israeli research has shown that milk progesterone concentration 21 days after AI is highly correlated to the success of pregnancy. Furthermore, when cows received strategic progesterone supplementation for 7 days from 23 days after AI, the pregnancy rate was significantly greater in treated cows than in contemporary cows (Francus, 1997).

Cost constraints limit the effectiveness of progesterone supplementation as a practical tool for the New Zealand dairy farmer. One would expect that such supplementation would be of benefit to only about 30% of cows, since 55% of inseminations result in successful pregnancy establishment without intervention (Lamming *et al.,* 1989). Cost-effective utilisation of such technology requires a method to identify which proportion of the herd may benefit. To be effective, it must be applied soon after AI. Measurement of progesterone concentrations in milk samples taken at selected times following insemination may facilitate identification of the sub-group of cows for which supplementation could be beneficial. Recent studies in the United Kingdom have shown a positive relationship between progesterone supplementation based on milk progesterone concentration in samples taken 3 or 4 days following AI and subsequent pregnancy rates (Lamming, *pers. comm.*).

The study described here investigated some factors which influence progesterone concentrations in cows' milk following breeding and examined for predictive relationships. This was done to determine whether an index based on milk progesterone concentration could be established which would identify that sub-population for which strategic progesterone supplementation might enhance the probability of pregnancy establishment.

MATERIALS AND METHODS

Following diagnosis by rectal palpation as anoestrus (An) or cycling (Cyc) in spring 1997, the oestrous cycles of

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lactating cows from two dairy herds were synchronised using a progesterone-containing CIDR-B™ device (InterAg, NZ) for seven days. Cycling cows received 2 mg oestradiol benzoate (CIDIROL™ Injection, InterAg, NZ) by intramuscular injection (im) at CIDR-B™ insertion and 500 mg cloprostenol im (Estroplan injection, Parnell Laboratories NZ Ltd, NZ) on the day of CIDR-B™ removal. Cows which did not show oestrus by 48 h after CIDR-B™ withdrawal were injected with 1 mg oestradiol benzoate im at that time. Cows which were inseminated 72 h after CIDR-B™ withdrawal were enrolled in the trial (n = 281).

Milk samples of approximately 30 ml were collected from each cow at the morning milking on Days 4, 8 and 22 following AI (day of AI = Day 0). For Herd 1, comprising 155 Holstein-Friesian (HF) cows, fore-milk samples (removed from the udder by hand prior to machine milking) were collected. Composite samples from Jersey (J; n = 76) and HF (n = 50) cows in Herd 2 were gathered using a proportioning milk meter (Tru Test Co, NZ).

Oestrous activity in the herds was monitored twice daily, and any cows which were observed to return to oestrus were noted. Each cow was pregnancy tested by rectal palpation between 60 and 70 days following insemination to confirm conception dates.

Each milk sample was mixed with 33 mg potassium dichromate as a preservative, and chilled to 4°C within 1 h of collection. Milk samples were stored at 4°C and analysed within 3 days of collection. Determination of progesterone concentration was performed in duplicate using a commercial RIA kit (Count-a-Coat™, Diagnostic Products Corp., California) which had been previously validated for use with fresh bovine milk (McDougall, 1994). The sensitivity of the assay was 0.08 ng/ml. The intra-assay coefficients of variation (CVs) were 7.3%, 6.1% and 15.7% for samples with mean concentrations of 4.3, 3.0 and 0.4 ng/ml of progesterone, respectively. The inter-assay CVs were 6.1%, 3.1% and 9.5% respectively, for the reference samples as above.

Results are reported as mean ± SEM. The variables examined were the milk progesterone concentration on each day and the difference between concentrations on Days 4 and 8. Data were examined using a GLM-ANOVA (Minitab v.10; Minitab Statistical Software, Pennsylvania) for effects of herd, pre-synchrony status (An/Cyc), return to oestrus and pregnant to AI. Because of highly significant herd effects, analyses were repeated within herd and within breed for Herd 2, to determine effects of breed, An/Cyc status, ± return to oestrus status and ± pregnancy to insemination. Variables were further examined by non-parametric analyses including Wilcoxon Rank Sums and the Kolmogorov-Smirnov Test (SAS; SAS Institute Ltd, North Carolina). Data were ranked and the proportions of non-pregnant cows below the median and in the first quartile of each group were compared with the probability of non-pregnancy using a Chi-square analysis.

RESULTS

Results were analysed from a total of 273 animals after exclusion of 8 cows (2.8%) which showed evidence of false heats by either returning to oestrus within 10 days of AI or having milk progesterone concentration of < 1 ng/ml in the sample collected in Day 8. Pregnancy rates to AI were 63% for Herd 1, and 55% for both J and HF groups in Herd 2.

Mean milk progesterone concentration for pregnant and non-pregnant cows are given in Table 1. Levels were lower ($P < 0.001$) for Herd 1, from which foremilk samples had been collected, than for Herd 2, from which composite samples were collected; but generally followed a similar pattern of change between Days 4, 8 and 22. Mean milk progesterone concentration for the J cows in Herd 2 was greater than for the HF cows on Days 8 and 22 ($P < 0.01$).

TABLE 1: Mean (± SEM) milk progesterone concentrations (ng/ml) for samples collected on Days 4, 8 and 22 from cows which were subsequently diagnosed pregnant or not pregnant to an insemination on Day 0.

	Status	n	Day 4	Day 8	Day 22
Herd 1					
	Pregnant	96	0.7 (0.1)	3.1 (1.3)	6.1 (3.3)
	Not pregnant	57	0.7 (0.1)	2.9 (0.9)	1.4 (2.5)
Herd 2					
Jersey	Pregnant	40	2.5 (0.1)	9.8 (0.5)	16.7 (0.8)
	Not pregnant	33	2.5 (0.2)	9.1 (0.6)	4.3 (1.1)
Holstein Friesian	Pregnant	26	2.5 (0.2)	7.9 (0.6)	13.6 (0.9)
	Not pregnant	21	2.1 (0.2)	6.5 (0.5)	2.9 (1.1)

Neither the occurrence of return to oestrus nor pregnancy status altered milk progesterone concentration on Days 4 or 8; but levels were higher ($P < 0.001$) on Day 22 in cows which either did not return to oestrus or were subsequently diagnosed pregnant. There was no effect of Cyc *vs*. An status on subsequent milk progesterone concentration in either herd on any day.

Non-parametric analyses demonstrated that absolute milk progesterone concentrations on Days 4 and 8 had no predictive value. However, when the difference between milk progesterone concentrations on Days 4 and 8 was examined, there was a tendency for more cows that failed to become pregnant to be in the lower quartile of the range (Table 2). Within each group, the proportions of non-pregnant cows below the median and in the lowest quartile were similar to the proportions of non-pregnant cows in the total group, but when data from all cows were combined, the proportion of non-pregnant cows in the lowest quartile were greater than would have been predicted by pregnancy rate ($P < 0.05$).

DISCUSSION

Results showed that progesterone concentrations in milk samples collected from two herds on Days 4, 8 and 22 following synchronisation and AI at a detected oestrus differed between herds and breeds within herds. These differences may be due to the manner in which progesterone is partitioned within milk as it associates with the milk fat fraction (Schiavo *et al.*, 1975). In Herd 1, the samples were obtained by hand prior to machine milking, while in Herd 2 composite samples were obtained during the entire

TABLE 2: Ranges of the difference in milk progesterone concentrations between Days 4 and 8 (ÐMPC, ng/ml) for cows in the bottom quartile and below the median, proportions (n) of non-pregnant cows in each group, and the overall non-pregnancy rate (n) in each group for cows in each herd.

	Range of ÐMPC		Proportion of non-pregnant cows in each category (n)		Overall non-pregnant (n)
	Bottom quartile	Below median	Bottom quartile	Below median	
Herd 1	0.3-1.7	0.3-2.3	47% (18)	43% (33)	37% (57)
Herd 2					
Jersey	2.6-5.1	2.6-6.3	65% (12)	50% (19)	45% (33)
Holstein-Friesian	1.5-3.4	1.5-4.5	66% (8)	54% (13)	45% (21)

period of milk let-down using a proportioning milk meter. Differences in milk fat content may account for the variation between fore-milk and composite milk samples (Atwood and Hartmann, 1992; Stelwagen *et al.,* 1996). Greater milk progesterone concentrations in Jersey compared to Holstein-Friesian cows may also be due to varying milk fat content.

Analysis of the data for evidence of predictive indicators of pregnancy using absolute milk progesterone concentrations was unsuccessful. Although the overall trend in the data was towards cows which failed to become pregnant having lower milk progesterone concentrations both at Days 4 and 8, these were all shown to be non-significant effects with both analyses of variance and non-parametric analyses. This is consistent with previous studies which showed that differences in progesterone concentrations in milk between pregnant and non-pregnant cows only became apparent during Days 10 to 16 of the oestrous cycle following insemination (Lamming *et al.*, 1989).

Only when data for the increase in milk progesterone concentration between Day 4 and Day 8 were examined non-parametrically did we find any association with success of pregnancy. Experimental observations by Lamming and Mann (1995) that low or oscillating progesterone concentrations during metoestrus and early dioestrus can alter development of oxytocin receptors and thus modulate responsiveness to the luteolytic oxytocin signal later in dioestrus conform with this finding. On this basis, it may be possible to derive an index using milk progesterone concentration which could identify a sub-population for which strategic progesterone supplementation might enhance the probability of pregnancy establishment. Such an index would need to consider the variability of milk progesterone concentrations caused by milk sampling methods and differences in milk fat content. Nevertheless, such an index would require analysis of two milk samples to establish the increase in milk progesterone concentration, and is unlikely to be cost-effective for the New Zealand dairy farmer.

CONCLUSION

Absolute milk progesterone concentrations in samples taken on Days 4 or 8 following oestrus and insemination did not provide a reliable prediction of pregnancy, but the difference between these concentrations may be of some use. Any further development of using milk progesterone measurements to determine a sub-population of cows for which strategic progesterone supplementation might be efficacious will need to account for differences produced by methods of milk collection and breed.

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