Diagnosis of pregnancy in dairy cows based on the progesterone content of milk. Part 2. Effect of day of sampling and number of samples on accuracy of diagnosis

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Two of the many factors which may affect the accuracy of pregnancy diagnosis using milk progesterone levels are day of sampling and number of samples taken per cow. These two aspects were analysed using information obtained from progesterone profiles encompassing 359 pregnancy tests. Where a single sample was obtained between 20 and 24 days after insemination the accuracy of pregnancy diagnosis was less than for two samples taken on the 21st and 22nd day (89,1 *vs* 97,4% accuracy). The best time to take a single sample was 24 days after insemination when only 1,5% incorrect classifications were made.

Twee van die faktore wat die akkuraatheid van dragtigheidsdiagnose deur middel van melkprogesteroonvlakke mag beïnvloed, is die dag van monsterneming en getal monsters per koei. Hierdie twee aspekte is ondersoek met behulp van inligting verkry vanaf die progesteroonprofiele wat 359 dragtigheidstoetse behels het. Waar net een monster tussen 20 en 24 dae na inseminasie geneem is, was die korrektheid laer as vir twee monsters verkry op die 21ste en 22ste dag (89,1 *vs* 91,4% korrektheid). Die beste tyd om 'n enkele monster te neem was 24 dae na inseminasie wanneer slegs 1,5% van die koeie foutiewelik dragtig verklaar is.

Keywords: Accuracy, day of sampling, milk progesterone, number of samples, pregnancy diagnosis

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In the dairy cow, the secretory function of the corpus luteum has been used as an indicator of reproductive status (Gadsby, Heap, Henville & Laing, 1974). In the non-pregnant cow the corpus luteum regresses on about day 17 or 18 (day 0 = oestrus), while in the pregnant cow the luteolytic property of the uterus is neutralized, allowing the corpus luteum to survive (Heap, Holdsworth, Gadsby, Laing & Walters, 1976). Progesterone concentrations in blood and milk reflect this ovarian activity, and have been used to diagnose pregnancy (Bulman & Lamming, 1976).

It was originally suggested (Dobson & Fitzpatrick, 1976; Heap *et al.*, 1976) that a milk sample taken 21 days after insemination would provide the most reliable result, since this sample was most likely to reflect peak oestrus. However, one of the major aims of early pregnancy diagnosis in the cow is to enable the timeous recognition of the non-pregnant animal. For this reason, it may be advantageous to sample earlier than day 21, provided that this does not reduce the accuracy of the test. In this way, the loss of an entire oestrous cycle may be avoided.

By taking milk samples on more than one day, initial progesterone concentrations may be confirmed and any variation with time exposed. Interpretation of these results may lead to a more accurate diagnosis of pregnancy/non-pregnancy than examination of a single sample datum. The effect of day of sampling and number of samples on the accuracy of pregnancy diagnosis was thus analysed in order to determine the extent of this effect.

Whole milk samples for pregnancy diagnosis were collected during afternoon milking from cows in four herds. In three of the herds a single milk sample was drawn from cows (n = 98) which had been inseminated 20—24 days previously. Two samples, taken on days 21 and 22 after insemination, were from 193 cows in the fourth herd. In addition, daily milk samples were obtained from 38 cows in two of the herds. Daily sampling commenced approximately 10 days post-partum and ended when the cow was confirmed to be pregnant by rectal palpation 60 days post-insemination. In these 38 cows, 68 post-insemination periods were monitored.

Milk samples were preserved with 0,25% potassium dichromate and either refrigerated or frozen. The concentration of progesterone in the milk was determined using the radioimmunoassay method of Holdsworth, Chaplin & Booth (1979) as modified by Butterfield (1986). The assay was performed without ether extraction of whole milk samples. The antibody produced for the study showed negligible cross-reactivity with the important progesterone derivatives (Butterfield, 1986). The within- and between-assay CV's were 6,7% and 13,8% respectively.

To determine on which day after insemination a single milk sample should be taken so as to yield the most accurate pregnancy test in the first analysis of the results, the progesterone levels of milk samples taken 17—28 days after insemination were examined and thereby the normal variation in oestrous-cycle length (17—25 days) was included. Putative pregnancy was then diagnosed on the basis of the progesterone levels measured, and the accuracy of the diagnosis was determined (Table 1) following rectal palpation. Secondly, the accuracy of diagnosis based on two samples (day 21 and 22 post-insemination) was compared (Table 2) to that obtained from a single sample (obtained day 20—24 post-insemination).

Table 1 Effect of day of sampling after insemination on accuracy of pregnancy test where diagnosis is based on a single sample only

Sampling day[a]	Incorrectly classified (%)
17	25,0
18	16,0
19	8,8
20	8,8
21	8,8
22	2,9
23	2,9
24	1,5
25	2,9
26	2,9
27	7,4

[a] Day 0 = day of AI.

Table 2 Relative accuracy of the pregnancy test based on a single sample taken 20—24 days after insemination *vs* two consecutive samples on days 21 and 22

	Sample error rates (%)		
Sampling routine	Incorrect pregnant diagnosis	Incorrect non-pregnant diagnosis	Total mean error rate
Single	7,5	3,4	10,9
Double	4,8	3,8	8,6

Samples taken on days 22—26 allowed cows to be accurately classified as pregnant or non-pregnant, with the 24-day sample providing the most accurate estimate (Table 1). It was notable that estimates obtained from samples taken on days 17—21 were less accurate than those taken on days 26 and 27. The relatively large number of cows incorrectly classified from samples taken before day 19 or after day 27 was expected, but erroneous classification from samples taken on days 19—21 was surprising.

The advantage of examining data obtained from two samples rather than one was more pronounced in the diagnosis of pregnancy rather than non-pregnancy (Table 2). The second sample may be used to determine whether an initial progesterone concentration of 5 ng/ml on day 21 will be sustained on day 22, in which case the cow is pregnant, or will decline in which case the cow is non-pregnant. In non-pregnant cows the error rates were virtually identical for both sampling procedures (Table 2).

The results of this analysis confirm the importance of day of sampling on accuracy of classification of pregnancy status in cows. For the pregnancy test a milk sample is usually taken 20—25 days after insemination as this range normally covers the known variation in oestrous cycle length (Inaudi, Bacigalupo, Monittola, Lugaro & Genazzani, 1982). The results presented in Table 1 indicate that samples taken during the latter half of this period were more accurate than those taken earlier. Although this finding was contradictory to those of other researchers who claimed that maximum accuracy was achieved 21—23 days after insemination (Pennington, Spahr & Lodge, 1976; Pope, Majzlik, Ball & Leaver, 1976; Inaudi *et al.*, 1982), it is in agreement with results obtained by Heap *et al.*, (1976).

The results of the first analysis (Table 1) and the high total mean error rates in the second analysis (Table 2) suggest that the timing of sampling in the second study may have been incorrect. This could have affected the outcome of the analysis. Nevertheless, the advantage of the double sampling routine is that a decline toward basal progesterone levels in non-pregnant cows in which oestrous cycles were relatively long, or when sampling occurred too early after insemination, can be detected.

Butterfield (1986) found that oestrous cycles occurring after insemination were significantly longer than those where no insemination had been performed. This probably explains the more accurate classification of pregnancy from milk samples taken on day 24 after insemination. Where the oestrous cycle was extended, samples obtained on day 21 would provide an inaccurate diagnosis of pregnancy status. A milk sample taken on day 24 after insemination rather than day 21 provides an added advantage in that it enables the farmer to exclude from the analysis any cows which have returned to oestrus before this time.

The results of these two analyses suggest that where a single milk sample is to be used to test for pregnancy the sample should be taken 24 days after insemination. Two consecutive samples representing days 21 and 22 post-insemination can be expected to be more accurate than a single sample obtained on any day between 20 and 24 days post-insemination, but not as accurate as a single sample taken exactly on day 24.

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