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The Use of An Elisa Milk Progesterone Test As an Aid to Oestrus Prediction in Dairy Cows

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Introduction

Oestrus detection in dairy cows has been the subject of many studies and various methods of improving oestrus detection have been investigated and reviewed by Boyd (1984). Poor oestrus detection will extend the calving to conception interval by prolonging both the calving to first service and first service to conception intervals (Eddy 1980). It is known that each one day extension of the herd calving interval beyond 365 days represents a potential loss of £3.00 per cow (see HMSO publication: Dairy Herd Fertility). The availability of the enzyme immunoassay (EIA) for progesterone in milk presents the possibility of a useful aid to oestrus detection. Foulkes, Cookson and Sauer (1982) measuring progesterone in milk samples taken daily from 42 days post calving demonstrated a significant reduction in calving to first service interval. Here fixed time insemination was employed on the third day of low progesterone. Stimpson (1984) using the same test before and after first service demonstrated both the feasibility of performing the test in a practice laboratory and significant improvements in oestrus detection rates and calving to conception intervals in two herds.

The availability of a commercially produced EIA test kit (Ovucheck; Cambridge Veterinary Sciences) stimulated the setting up of trials to study oestrus detection rates and detection efficiency after first service. This paper reports the results of these trials.

Materials and Methods

Trial 1A

Herd T.W.C. consisted of 250 cows calving September to June. Foremilk samples were taken from all cows 16 to 24 days post service. All the sample was taken from one teat with the first 5 teatfuls of milk discarded before sampling. The trial took place in August and September 1984.

Trial 1B

Herd 229 consisted of 104 cows mainly summer calving. Samples were taken daily on days 17 to 23 post service during the period August to December 1984.

Samples were transported to the practice laboratory six days a week for progesterone assay using EIA and the progesterone values plotted for each cow. If a level less than 5ng/ml was recorded the farmer was notified by telephone of the cows identity and instructed to apply a heat mount detector (HMD) (Kramer Inc.). Cows were inseminated immediately following an observed oestrus or a HMD colour change. Pregnancy was confirmed by rectal palpation 7-8 weeks post service. Cows with low progesterone and not detected in oestrus were presented at the first veterinary herd health visit after 25 days post service and where possible treated with Prostaglandin to induce oestrus.

Trial 1C

Using the data gained from trials 1A and 1B the day post service on which Progesterone fails and the time lapse from Progesterone fall to service will be observed in order to determine the minimum number of samples that could be considered practical.

Results

The distribution of inter-service intervals before and after the introduction of the EIA regime can be seen in Figs. 1 and 2. Fig. 1 (herd TWC) demonstrates considerable improvement in oestrus detection as the percentage of intervals occurring 18-24 days improved from 38 to 67. All other intervals were reduced in number. Fig. 2 (herd 229) shows an improvement of the 18-24 day intervals from 21 to 45 percent.

FIGURE 1. Interservice Intervals For Herd TWC Before (a) and After (b) Using Ovucheck.

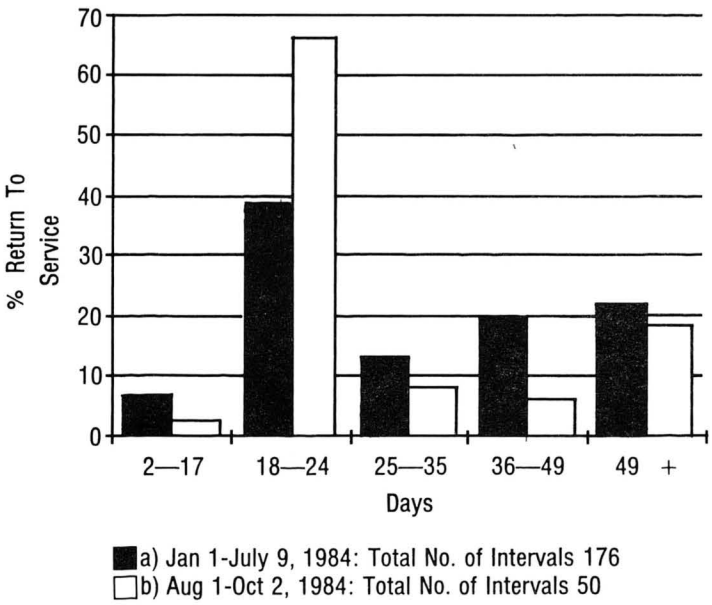
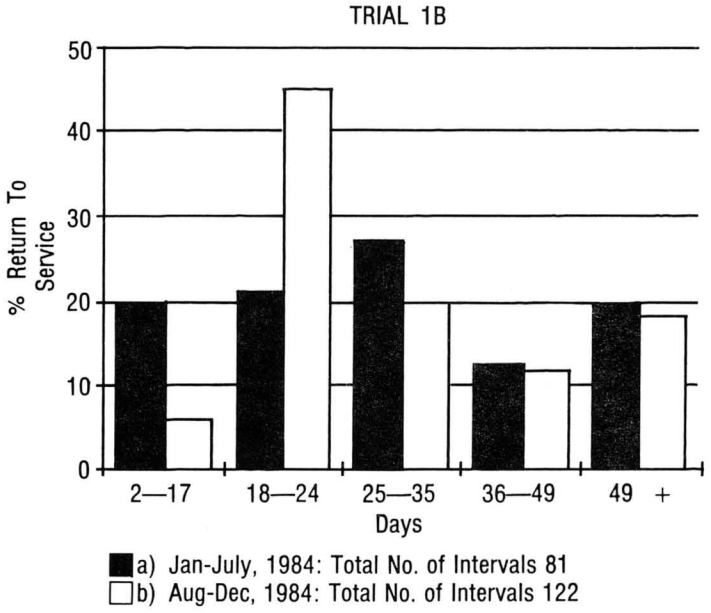


FIGURE 2. Interservice Intervals For Herd 229 Before (a) and After (b) Using Ovucheck.



The improvement here came from more accurate detection as the 2-17 day and 25-35 day intervals were reduced from 20 to 5.5 percent and from 28 to 20 percent respectively.

The day number on which the first low progesterone was determined is seen in table 1. 66.4 percent of cows with low progesterone on day 24 were low on day 19 and only 94.0 percent low on day 22. The day number of low progesterone on which service occurred is shown in table 2. 82 percent of cows were low progesterone for more than 48 hours before service occurred. As only 66 percent were served on days 3 and 4 fixed time insemination was not considered to be advisable.

These results indicate that sampling on days 18, 20, 22 and 24 would appear to be satisfactory but day 19, 21 and 23 sampling could be a practical alternative and should be considered in an effort to reduce sampling to a minimum.

Trial 2

The objectives of trial 2 were to evaluate the effect of alternate day milk testing for progesterone on oestrus detection post service and also to make an economic assessment of the effect of the programme on herd fertility. The opportunity was also presented to examine whether the colour change of the reaction in the test wells could be read by eye rather than using a plate reader thus determining whether a qualitative assessment would be possible.

Materials and Methods

The trial took place on two commerical dairy herds (herds 200 and 220) from December 1984 to July 1985. On alternate

TABLE 1. Day Number Following Service On Which Low Progesterone Was First Determined.

	Day of First Low Progesterone									
Herd	16	17	18	19	20	21	22	23	24	Total
TWC	7	2	11	15	5	6	4	3	1	
229	1	17	12	12	6	7	3	2	1	
Total	8	19	23	27	11	13	8	5	2	116
Cumulative %	6.9	23.2	43.1	66.4	75.9	87.1	94.0	98.3	100	

TABLE 2. Day Number of Low Progesterone on Which Service Occurred.

	Day of Low Progesterone						
Herd	1	2	3	4	5	6	Total
TWC	0	5	19	12	4	1	
229	3	8	18	10	7	3	
	3	15	42	24	12	4	100
			82%				

TABLE 2. Fertility Indices For Three Years Herd 200. Cows Calving July 1 to January 31 Each Year.

	82-83	83-84	84-85
Number Calved	317	256	249
Number Served	284	229	217
Percent Served	89	89	87
Calving To 1st Service (days)	83	72	61
Number Conceived	248	217	200
% of Served	87	94	92
Calv-Conception Days	119	115	84
All Service Pregnancy Rate %	38	41	48
% of Re-Services at 16-28 Days	31	33	68
1st Service-Conception Days	36	43	23

days samples were taken from all cows 18 to 24 days post service on the day of sampling. The samples were assayed for progesterone in the practice laboratory.

From time to time during the trial period the colour change was assessed visually in daylight and the results compared with those obtained using the plate reader.

Results

Figs. 3 and 4 show the interservice intervals obtained in both herds compared with cows calving in the same period the previous year.

In herd 200 (Fig. 3) the 18-24 day returns improved from 25.5 to 53 percent as a result of both improved accuracy (2-17 day intervals) and improved detection rates (36-48 day and 49 + intervals).

In herd 220 (Fig. 4) the 18-24 days intervals improved from 38 to 60 percent as a result of fewer returns occurring 36 days or more.

FIGURE 3. Interservice Intervals For Herd 200 Before (a) and After (b) Using Ovucheck.

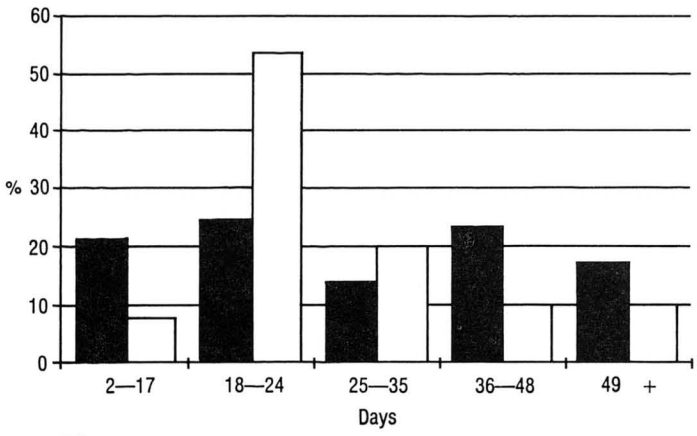
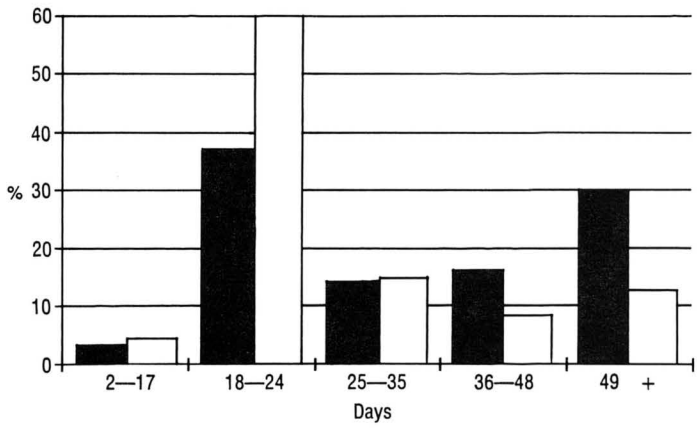


FIGURE 4. Interservice Intervals For Herd 220 Before (a) and After (b) Introduction of Ovucheck.



The fertility indices of herd 200 for the 1984-85 calving season are shown in table 2 and can be compared with the indices for the previous two years. Compared with 1983-84 the calving to conception improved 31 days from 115 to 84. 23 days of this improvement were the result of reducing first service to conception and could be attributable to the progesterone testing regime. It is noticeable that the all service pregnancy rate improved during 1984-85, presumably as a result of improved detection accuracy.

The cost of the testing and the potential benefits in herd 200 accruing from the improved fertility are shown in table 3. The potential benefits exceed the costs of the programme by 7.5 : 1.

Visual Assessment

357 test reactions were assessed visually in daylight before using the plate reader to measure optical density of the colour change. The colour change in each well was compared with the reaction in the well containing 5ng standard. Reactions darker than the standard were considered low progesterone and if lighter were considered high progesterone. Colour reactions indistinguishable from the 5ng standard were considered inconclusive. The results are shown in table 3, from which it can be seen that visual assessment was highly accurate in determing both high and low progesterone.

TABLE 3. The Costs and Potential Benefits of the Progesterone Testing Regime in Herd 200.

Costs	
Laboratory Fees @ £1.20 Per Test 1160 Tests	£1392
Extra Labour Costs For Sampling @ 20p Per Sample	£ 232
	£1624
(IE. £8 Per Cow Conceived)	
Potential Benefits	
20 Days Improvement in Calving to Conception Attributable to E.I.A. Programme at £3.00 Per Day for 200 Cows Conceived	£12000
(IE. £60 Per Cow Conceived)	

TABLE 3. A Comparison of Visual Assessment of the Colour Reactions With the Use of Plate Reader.

Visual Assessment	Number	Plate Reader
High Progesterone	265	All Over 10 NG (5NG (74)
Low Progresterone	77	(5NG (2) (7.1NG (1)
Inconclusive	15	Ranged Form 4.3 to 7.8NG
Total	357	

Discussion

Trial 1 demonstrated that the E.I.A. for progesterone could be readily performed in the practice laboratory and

when used to test milk samples 18-24 days post service could be used to predict the onset of oestrus. Both oestrus detection rates and accuracy showed considerable improvements. Trial 2 demonstrated that similar improvements in accuracy and detection rates could be achieved by alternate day sampling. Oestrus detection remains a problem in many large dairy herds using artificial insemination and these trials demonstrate a place for oestrus prediction using E.I.A. It was encouraging to see the improvements occurring in these herds in which oestrus detection was probably worse than average thus demonstrating that the technique could be used in herds of below average management ability. The accurancy of the visual assessment leads the way to the development of an on farm test using a qualitative assessment.

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Titers for BVD, IBR, PI_3, and BRSV in Dairy Cattle. What Do They Tell Us?

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Blood samples from 160 dairy animals in 15 client herds were tested for titers to Bovine Virus Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR), Parainfluenza 3 (PI_3), and Bovine Respiratory Syncytial Virus (BRSV). Sampling occurred from September through December, 1984. The results are summarized by disease and by vaccination status. Data from other surveys in the same geographical area are included in the 9-18 month age group summary.

Client Herds:

The management and vaccination history was known by the author in all client herds. The client herds included open and closed herds of 40-150 cows housed in stanchion, comfort stall and free stall barns.

Two herds with negative titers for IBR and BVD and no history of vaccination for these diseases were vaccinated for IBR, PI_3, and BVD after the initial sampling and then retested. They are included in both the non-vaccinate and vaccinate summaries. (Table 5)

Survey Design:

A minimum of ten animals in each herd were sampled including one 3 month calf, one yearling, two 1st calf heifers, two 2nd calf heifers, two 5-6 year old cows, and two aged cows.

Sampling:

10 cc of venus blood was drawn into a red top vaccutainer tube. The blood was allowed to clot and 2 cc of serum was transferred to another tube. The serum was chilled or frozen and sent to the Diagnostic Laboratory at the University of Minnesota.

Serological Testing:

Microtiter serum neutralization (SN) tests were performed for IBR, BVD, and BRSV. PI_3 titers were measured by a hemaglutination inhibition (HI) test. Testing was performed using serial dilutions from 2-2048.

BVD Titers:

Closed vs open herds: 30-40% of the animals in 5 closed herds had titers ranging from 2-512. All animals (22) in two open herds had titers ranging from 8-2048 with a titer of 256, the most common finding. Clinical evidence of BVD infection was not present in any of these herds.

Killed vaccine: Table 1 summarizes titers from 5 herds where killed BVD vaccine was used. The titers following initial vaccinations were lower than the literature suggests.[1] The yearly booster injection appears to raise the titer significantly.

MLV vaccine: In one herd, MLV BVD vaccine (Resbo[8a]) was given yearly to all animals 2-6 weeks post-calving. This is a closed herd and no other viral vaccines are given at any age. Titers (19) ranged from 16-256 with titers of 128 and 256 the most common finding.

These titers may reflect natural exposure to BVD instead of vaccination titers when serological results from non-vaccinated animals in the herd are considered (Table 2).

Serological testing was funded by Norden Laboratories, Lincoln, Nebraska. The author thanks Dr. Douglas Armstrong for technical assistance and many colleagues for conducting some of the surveys.

(a) MLV IBR, PI_3, BVD plus Leptoferm 5, Norden Laboratories, Lincoln, NE.