

## USE OF MILK PROGESTERONE PROFILES FOR THE STUDY OF FERTILITY IN THE POSTPARTUM PERIOD OF DAIRY COWS

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### Summary

The present study focused on the onset of ovarian function and occurrence of oestrus of 83 cross-bred cows (Native x Holstein-Friesian) from village farms, in Chiang Mai Province. Milk samples were collected every three or four days beginning from day 14 postpartum to analyze for progesterone content by Radioimmunoassay technique. By using progesterone profiles, cows were classified in four groups : cows with normal oestrous cycles (42.2%), short oestrous cycles followed by normal cycles (33.7%), anoestrus (20.5%) and abnormal oestrous cycles (3.6%). The period from parturition to first and second oestrus were  $29.5 \pm 12.79$  and  $50 \pm 12.47$  days, respectively. Nineteen, 39.7, 15.9, 12.7, 11.1 and 1.6 percent of cows exhibited first oestrus at under 20, 21-30, 31-40, 41-50, 51-60 and 61-70 days after parturition. These results suggest several areas for future investigation, including reduction numbers of short oestrous cycle, anoestrus and prolongation of oestrus exhibition to improve reproductive efficiency of the animals.

### Introduction

The radioimmunoassay method has been used for analyzing milk or plasma progesterone in cows. This is a significant tool to monitor reproductive performance in cattle. It had been used (Hoffmann, Gunzler, Hamburger, and Schmidt, 1976) for studying oestrous cycle and diagnosis pregnancy status in cattle (Hoffmann, *et al.* 1976). The technique had also been used (Schiavo, Matuszczak, Oltenacu and Footé, 1974; Van De Wiel, Kalis and Nasir Hussain Shah, 1979; Sharpe and King, 1981) for investigation of fertility in postpartum period in the cattle.

Postpartum ovarian activity in dairy cattle has been studied extensively by Marion and Gier (1968) who classified average days from parturition to first ovulation and first oestrus into 3 groups which were low, 13.1 and 28.4; medium,

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14.0 and 33.1; high, 15.5 and 36.9. Britt, Kitto and Harrison (1974) determined interval to first postpartum ovulation in Holstein cows as  $23.6 \pm 2.6$  days. Williams and Ray (1980) studied in 3-year-old Hereford cows on first oestrus behavior, oestrus, luteal function and conception the results were 30.6, 41.3, 32 and 63 days postpartum. Mean postpartum intervals for crossbred (Jersey x Hereford and Angus x Hereford) were prolonged as  $48 \pm 19$  days when energy intake of the cows reduced to 80 percent of NRC (1970) recommended (Humphrey, Kaltenbach, Dunn, Koritnik and Niswender, 1983). The postpartum first ovarian activity in most cows (64%) had short luteal phases (<12 d) showed longer intervals (33.4 vs 24.9 d) compare to normal luteal phases (>12 d) (Meisterling and Dailey, 1987).

The objective of this study was to study ovarian function and expression of oestrus during the postpartum period in dairy cows from smallholder farms in Chiang Mai Province.

### Materials and Methods

Eighty three cross-bred Holstein Friesian cows on smallholder farms in Chiang Mai area were used. Milk samples were collected every three or four days (twice a week) from the day 14 to the day 80 postpartum. Milk samples were analyzed for progesterone concentration by Radioimmunoassay technique. Progesterone profiles of each cow were studied and the interval from parturition to the first and second oestrus were recorded.

Progesterone in milk samples were analyzed by Radioimmunoassay technique as described by Gadsby, Heap, Henville, and Laing (1976); Holdsworth, Booth, Sharman and Rattray (1979); Dobson (1983) and Sravasi (1988). Briefly, titration to find out the optimum dilution of antiserum which was kindly donated by Prof. M. Kamonpatana. In the beginning, dilution of antiserum : phosphate-buffered saline (PBS) at 1:100, 1:200, 1:400, 1:800, 1:1 600, 1:3 200, 1:6 400 and 1:12 800 were prepared. Each of antiserum was taken 100 microlitre to incubate with 100 and 400 microlitre tritiated progesterone ( $^3\text{H-P4}$ ) and PBS respectively. The mixture were kept at  $-15^\circ\text{C}$  for 18 hours, then in an ice-bath, 200 microlitre charcoal suspension were added. After 20 minute incubation, bound and free hormone were separated by centrifugation at 3000 rpm,  $4^\circ\text{C}$ , for 15 minute. Supernatants were decanted into glass counting vials. Scintillation fluid were added 4 ml each and kept overnight before counting by 1209 RACKBETA. Radiation activities, in cpm, were calculated in percentage of the total activities (Percent Binding). For finding the optimum dilution of the antiserum, the percent binding were plotted against the various dilutions. At 50 percent binding the optimum dilution of the antisera were between 1:1 500 - 1:2 500.

The assay tube consist of standard progesterone at 2.5, 5, 10, 25, 50, 100, 250, 500 and 1 000 ng/ml and postpartum milk samples. Twenty microlitre milk

sample or 50 microlitre standard progesterone were incubated at room temperature with 100 microlitre antiserum (1:1 500) for 1 hour. Consequently, 100 microlitre  $^3\text{H}$ -P4 were added. These were then well mixed and left at 5°C overnight. Bound and free  $^3\text{H}$ -P4 were separated by charcoal suspension in the same manner as antiserum titration procedure. A standard curve of percent binding radioactivities was plotted against the logarithm of the progesterone concentration. The progesterone contents in the postpartum milk samples were determined by interpolation on the standard curve.

Table 1. Days from parturition to the first and second oestrus in normal dairy cows.

Oestrus	First	Second
No. of cows	64	63
Duration, days	$29.5 \pm 12.79$	$50 \pm 12.47$
Range	9-56	28-76

<sup>a</sup> The time interval from parturition to the first and second oestrus is mean  $\pm$  SD.

Table 2. Start of first oestrus in relation to the time after parturition of all cows with normal ovarian function.

Days postpartum	Cows starting the first oestrus	
	No.	%
<20	12	19.0
21-30	25	39.7
31-40	10	15.9
41-50	8	12.7
51-60	7	11.1
61-70	1	1.6
Total	63	100



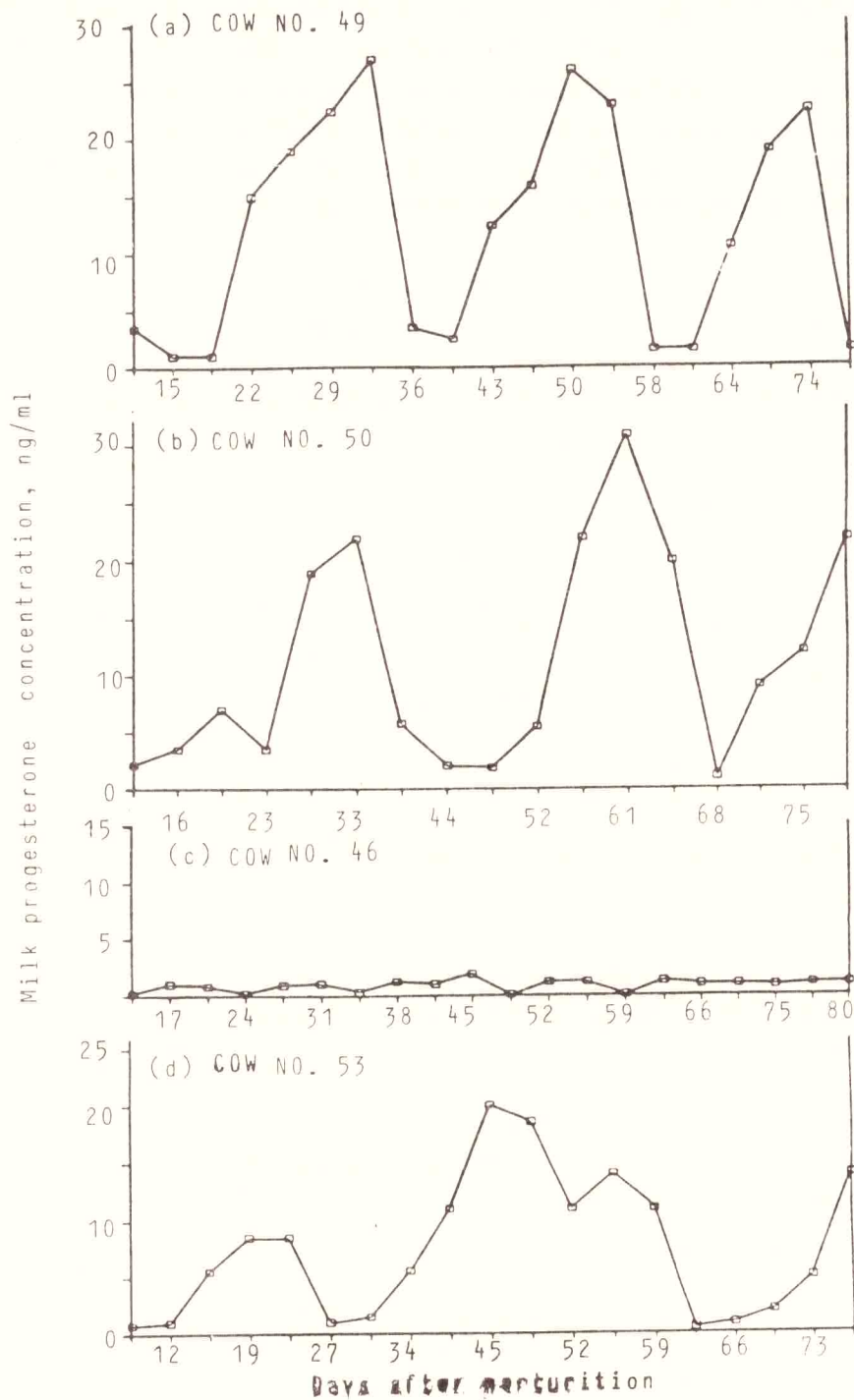


Figure 1. Profiles of postpartum milk progesterone of normal oestrous cycle (a), short oestrous cycle followed by normal cycle (b), anoestrous (c) and abnormal oestrous cycle (d).

Table 3. Start of second oestrus in relation to the time after parturition of all cows with normal ovarian function.

Day postpartum	Cows starting the first estrus	
	No.	%
<20	0	0
21-30	0	0
31-40	12	18.2
41-50	23	34.9
51-60	14	21.2
61-70	11	16.7
71-80	4	6.0
<80	2	3.0
Total	66	100

### Results and Discussion

All cow (n=83) were classified as four groups based on the progesterone profiles. The four groups were cows with normal oestrous cycles (42.2%, n=35), cows with short oestrous cycles followed by normal oestrous cycles (33.7%, n=28), cows with anoestrus that is without ovarian activity (20.5%, n=17) and cows with abnormal progesterone pattern (3.6%, n=3). These data were illustrated in figure 1. The incidence of short oestrous cycles (less than 17 days) has been reported in dairy (Macmillan and Watson, 1971; Schams, Schallenberger, Menzer, Stangl, Zottmeyer, Hoffman and Karg, 1978 and Stevenson and Britt, 1979) and beef cows (Odde, Ward, Kiracofe, McKee and Kittok, 1980). Henshelwood, Hansen and Hauser (1982) reported that short oestrous cycles were related to milk production, suckling activity, diet and calving season. The incidence of anoestrus (20.5%) was higher than the 10% reported by King (1976). On the basis of progesterone profiles, anoestrus seemed to be the most important problem in dairy production. The abnormal progesterone pattern of 3 cows was due to an especially long first oestrous cycles (more than 25 days) which was followed by a normal second cycles.

The interval from parturition to first and second oestrus, determined for all normal study, were  $29.5 \pm 12.79$  and  $50 \pm 12.47$  days, respectively (Table 1). These results are comparable to the report of Sharpe and King (1981), the interval from parturition to first and second oestrus were  $19.8 \pm 8.8$  and  $39.4 \pm 9.0$  days for Holsteins and  $28.7 \pm 15.6$  and  $46.9 \pm 13.6$  days for Jamaica Hope cows.

From Table 2 it was obvious that up to 30 days postpartum 58.7% of the cows had started the first oestrus. This affected the day postpartum of the second oestrus (Table 3). By day 50 postpartum, 53.1% of the cows were in the stage of second oestrus, this was the period that the farmers were suggested to look for sign of heat and to ask for A.I. services for their cows.

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