A comparison of plasma LH concentrations in milked and suckling post-partum cows

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Summary. Plasma samples were taken every 10 min for periods of 8 h on 3 occasions during the post-partum period from 8 cows milked twice daily (Groups M₁ and M₂) and from 4 cows each suckling 4 calves (Group S). All samples were assayed for LH, and ovarian activity was monitored by measurement of milk progesterone. Three of the Group M₁ cows had a delayed return to ovarian activity after calving (> 70 days) whereas all 4 Group M₂ cows resumed ovarian cycles by 25.25 ± 3.35 days *post partum*. Three Group S cows resumed cycles by a mean of 47.7 days *post partum* while the fourth had not done so by Day 100. Mean plasma LH concentrations were low *pre partum* (0.67 ± 0.05 ng/ml) and up to Day 5 *post partum* (0.80 ± 0.03 ng/ml, Group S; 0.90 ± 0.12 ng/ml, Group M₁; 0.80 ± 0.25 ng/ml, Group M₂). Concentrations then increased up to Day 12 (1.23 ± 0.20 , 1.66 ± 0.16 and 1.67 ± 0.21 ng/ml in Groups S, M₁ and M₂ respectively). Between Days 13 and 20 a distinct pulsatile pattern was seen in the profiles of milked but not of suckling cows. The pulse frequency was lower (approximately 2/8 h) in those milked cows in which ovarian activity was delayed and varied from 4 to 10 episodes/8 h in milked cows that commenced ovarian activity early in the post-partum period. It is suggested that the pulsatile LH pattern is a prerequisite for the onset of ovarian cycles.

Introduction

A detailed knowledge of the pattern of gonadotrophin release is important in understanding the factors controlling the return of ovarian activity in the post-partum cow. There is evidence that both ovulation and overt oestrous behaviour occur earlier after calving in milked cows than in suckling cows (Wiltbank & Cook, 1958; Casida *et al.*, 1968; Oxenreider, 1968) and that an increased intensity of suckling increases the anoestrous interval (Wettemann, Turman, Wyatt & Totusek, 1978).

It has been suggested that suckling might inhibit ovarian activity via an effect on gonadotrophin release (Carruthers & Hafs, 1980). Previous studies in this laboratory and elsewhere have suggested that plasma FSH levels are unlikely to exert a limitation on the onset of ovarian activity in the post-partum cow (Dobson, 1978; Schams *et al.*, 1979; Webb, Lamming, Haynes & Foxcroft, 1980), but changes in LH concentrations over this period appear to be of more significance (Lamming, 1978; Schams *et al.*, 1979; Webb *et al.*, 1980). Most previous studies of hormone changes during the post-partum period have involved blood sampling at intervals of 24 h or longer (Schams, Hoffman, Fischer, Marz & Karg, 1972;

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Edgerton & Hafs, 1973; Arije, Wiltbank & Hopwood, 1974), although gonadotrophin data based on 6-hourly sampling schedules have been published (Schams *et al.*, 1979; Webb *et al.*, 1980). However, in the cow, as in other species, LH is released as discrete short pulses or episodes, each lasting approximately 1 h (Carruthers & Hafs, 1980; Forrest, Fleegeer, Long, Sorenson & Harms, 1980), and therefore a sampling regimen more frequent than every 6 h is required to define such changes.

The following experiments were undertaken to compare short-term changes in plasma LH concentrations in milked and suckling cows during the early post-partum period to determine whether the patterns could explain differences in ovarian activity between the two groups. The experiment was first completed in the winter of 1977–78, but it was found necessary to obtain more data in 1979 due to the unpredicted behaviour of the original group of milked cows.

Materials and Methods

Animals

Three groups of Friesian cows in their second or third pregnancy/lactation were used as follows. In Group S, 4 cows (A, B, C and D) calving between 12 and 25 September 1977 were each allowed to suckle 4 calves from immediately after parturition. In Group M₁, 4 cows (G, H, I and J) calving between 11 November and 7 December 1977 were milked twice daily. In Group M₂, 4 cows (L, M, N and O) calving between 13 October and 11 November 1979 were milked twice daily.

Group S cows were fed hay and a standard dairy ration sufficient for the production of 25 kg milk/day. Cows in Groups M₁ and M₂ were fed a similar diet, calculated according to individual weekly milk yields.

Sampling procedures

Milk progesterone concentrations of each cow were monitored three times weekly from calving until the resumption of normal ovarian cycles. On three occasions during the first 20 days *post partum*, as shown in Table 1, blood samples were obtained from each cow via indwelling jugular venous catheters, at 10-min intervals for 8-h periods. In addition, most cows in Groups S and M₁ were sampled by this regimen once during the last 14 days of gestation (see Table 1).

Table 1. Days relative to calving on which blood samples were taken at 10-min intervals for 8 h

Reproductive stage (days)	Group S cows (suckling)				Group M ₁ cows (milked)				Group M ₂ cows (milked)			
	A	B	C	D	G	H	I	J	L	M	N	O
<i>Pre partum</i>	—3	—11	—10	—1	—3	—	—4	—4	—	—	—	—
<i>Post partum</i>												
1–5	3	1	2	2	2	2	2	4	1	3	4	5
6–12	9	10	11	6	9	7	6	8	8	10	11	12
13–19	15	16	17	15	17	14	13	16	15	17	18	19

Blood samples (10 ml) were collected into heparinized tubes, centrifuged at 1500 g for 15 min and the plasma stored at –20°C until assayed for LH.

Hormone assays

Milk progesterone concentrations were measured as described by Bulman & Lamming (1978). The assay sensitivity, intra-assay coefficient of variation (CV) and interassay CV were

0.40 ng/ml, 9.25% and 11.4% respectively. Plasma LH concentrations were measured as described by Webb, Lamming, Haynes, Hafs & Manns (1977), and the sensitivity, intra-assay CV and interassay CV values were 0.25 ng/ml, 9.2% and 10.5% respectively. Other reliability criteria of these assays did not differ significantly from those previously described.

Analysis of data

An elevation in the concentration of LH was defined as a pulse or episode if the highest point was at least 50% higher than the baseline, there were at least two consecutive elevated points between troughs and the decay phase of the episode was similar to the half-life of LH in bovine plasma, i.e. approximately 35 min (Schams & Karg, 1969).

Mean LH values were calculated for each 8-h sampling period ($n = 49$) to produce a representative LH concentration for a particular day. These values were then meaned together within groups for various time periods (see 'Results'). Statistical analysis of changes in mean LH concentrations relative to time was undertaken by the calculation and comparison of regression coefficients using the Genstat V computer package (Rothamsted Experimental Station, Harpenden, Herts, U.K.).

Results

Resumption of ovarian cycles

The time at which ovarian cycles began was defined as the first rise in milk progesterone concentration to ≥ 3 ng/ml followed immediately by a normal cyclic pattern (Bulman & Lamming, 1978).

In Group S, Cows A, B and D resumed cycles 49, 42 and 52 days after calving respectively (47.7 ± 2.96 days). Cow C had not resumed cycles by Day 100 *post partum*. In Group M₁, Cows G, H and I resumed cycles after 71, 36 and 74 days. Cow J had not begun to cycle by Day 100 *post partum*. In Group M₂, Cows L, M, N and O resumed cycles after 35, 24, 20 and 22 days respectively (25.25 ± 3.25 (s.e.m.) days).

Plasma LH concentrations

Examples of individual 8-h patterns for the three groups are shown in Text-figs 1 and 2 and the mean LH values for the four sampling periods are shown in Table 2. Plasma LH concentrations were low before parturition and during the first 5 days *post partum* in all cows. Values increased significantly in 11/12 of the cows (not in Cow I) between sampling periods 1 and 2 ($P < 0.01$) but there were no significant differences in the mean slopes for the different

Table 2. Mean ($\pm$ s.e.m.) plasma concentrations of LH (ng/ml) in milked and suckling cows at various stages after calving

Reproductive stage (days)	Sampling period	Group S (suckling)	Group M ₁ (milked)	Group M ₂ (milked)
<i>Pre partum</i> *		0.67 ± 0.05		—
<i>Post partum</i>				
1–5	1	0.80 ± 0.03	0.90 ± 0.12	0.80 ± 0.25
6–12	2	1.23 ± 0.20	1.66 ± 0.16	1.67 ± 0.21
13–19	3	0.98 ± 0.16	2.06 ± 0.57	1.83 ± 0.13

* All pre-partum values (taken within 14 days of parturition) were combined because cows had not been allocated to Groups S or M₁ at this time.

Text-fig. 1. Individual 8-h profiles of LH concentrations in representative cows from Group M₁ (milked) and Group S (suckling) *pre* and *post partum*. Note the low levels up to Day 9 *post partum* and the development of a pulsatile pattern in Cows G, H and J of Group M₁ between Days 14 and 17. Cow H exhibits a greater frequency of LH pulses than do Cows G and J, while Cows D, B and C have few pulses.

Text-fig. 2. Individual 8-h profiles of LH concentrations in 2 milked cows from Group M₂. A pulsatile LH pattern had developed by Days 15 and 17.

groups. Between sampling periods 2 and 3, LH concentrations in Groups M₁ and M₂ continued to rise, whereas those of Group S did not, although the differences were not significant (see Table 2). If the data for Cow I are excluded then the slopes of the mean LH concentrations over the three sampling periods are significantly steeper for Groups M₁ and M₂ than for Group S ($P < 0.05$), mean LH concentration between Days 13 and 19 being significantly higher in Groups M₁ and M₂ than in Group S ($P < 0.05$). During sampling period 3 in 3 Group M₁ cows and all 4 Group M₂ cows, a distinct pulsatile pattern had developed (Text-figs 1 and 2). Pulse frequency varied between cows from 2/8 h to 10/8 h. Excluding Cow I for which there was no evidence of a pulsatile pattern, there was a correlation between pulse frequency and the time to the first progesterone rise *post partum* (Kendall's Rank Correlation $T = 0.7857$; $P < 0.01$). There was no correlation between maximum peak height and the time to first progesterone rise.

LH pulses occurring between Days 13 and 19 in Groups M₁ and M₂ were associated with a possible ovulation only in Cow M (Group M₂; see Text-fig. 2) which exhibited 4 pulses in the 8-h sampling period on Day 17 *post partum*. The first progesterone rise was on Day 24 in this cow and so ovulation may have occurred on approximately Day 21.

Although isolated pulses did occur in Group S cows during sampling period 3, they were far less apparent visually and less frequent, and both the mean and peak LH concentrations over this period were lower than in Groups M₁ and M₂ (see Text-fig. 1). Within Group S cows there was no correlation between pulse frequency and time to the first progesterone rise.

Discussion

From previous studies in this and other laboratories, it was anticipated that the cows in Group M₁ would recommence ovarian cycles between Days 20 and 50 *post partum*. For example, in the study of Bulman & Lamming (1978), only 5.2% of 535 milked dairy cows had not started to cycle again by Day 50 *post partum*. However, in Group M₁ of the present study, only Cow H started to cycle within this time (first progesterone rise on Day 36). Cows G and I each had a retained placenta after parturition, a condition predisposing to delay in onset of ovarian cycles (Roberts, 1971). The remaining cow of this group (Cow J) exhibited an extended post-partum anoestrus of unknown cause. It was therefore considered necessary to repeat the experiment with another group of milked cows (Group M₂) and this was performed at the same time of year, i.e. autumn/winter 1979 under similar management conditions. These cows recommenced ovarian cycles at an earlier and more uniform time (25.25 ± 3.25 days *post partum*), and are therefore considered representative of milked cows.

However, in spite of the above reservations concerning cows in Group M₁, the overall patterns of change in LH concentrations were similar in Groups M₁ and M₂ (see Table 2). The higher mean LH concentrations in all 3 groups between Days 6 and 12 compared with that at Days 1 to 5 are equivalent to the greater release of LH after exogenous GnRH injection at 7–10 days than at 1–5 days in post-partum milked dairy cows (Foster, Lamming & Peters, 1980). LH concentrations before Day 5 were similar to those *pre partum* and it therefore seems likely that low pituitary output at this time may be due to a continuation of the inhibitory effects of pregnancy.

The increase in LH concentrations in both groups of milked cows between Days 13 and 20 was due to an increase in the tonic circulating levels of LH and the development of a distinct pulsatile pattern superimposed on these rising tonic levels (see Text-figs 1 and 2). Pulses were fewer and less distinct in Group S cows which also had lower mean LH levels (Table 2). This suggests that intensive suckling (or the presence of calves) inhibits this component of LH secretion and similar findings have been reported by Carruthers & Hafs (1980).

The appearance of LH episodes does not necessarily reflect the imminent resumption of ovarian cycles, since in most cows episodes were observed at least 10 days before the first progesterone rise. However, it may be that this period is a potential initiator of the first

post-partum follicular phase, but that ovulation does not occur because the follicular steroidogenic response is insufficient to induce LH release.

Studies of cyclic and seasonally anoestrous ewes have demonstrated a close relationship between pulses of LH and oestradiol-17 β (Baird, Swanston & Scaramuzzi, 1976; Scaramuzzi & Baird, 1979). This relationship has not been demonstrated in the cow, although pulsatile patterns of LH secretion have previously been reported for post-partum, cyclic and ovariectomized cows (Carruthers & Hafs, 1980; Rahe, Owens, Fleeger, Newton & Harms, 1980; Forrest *et al.*, 1980), and a positive relationship between LH and progesterone peaks has been demonstrated in the cyclic cow (Foster *et al.*, 1980). Scaramuzzi & Baird (1979) found that LH pulses were less frequent in anoestrous Welsh Mountain ewes than in Merino ewes of similar status and suggested that this might be related to the 'depth' of anoestrus. In the milked cows in the present study, there was a correlation between pulse frequency and time to the initiation of ovarian activity *post partum*. In fact, the 5 milked cows which ovulated within the expected time showed a more rapid pulse frequency (4–10/8-h period) than did the other 3 in which ovulation was delayed (0–2 pulses/8-h period).

Within the limited number of profiles available taken at intervals *post partum* there was no correlation between mean LH concentrations and the time to onset of ovarian activity. Mean LH values in Cows C and J (which had had no significant rise in progesterone by Day 100 *post partum*) were not significantly different from those of the other cows in their respective groups. In conclusion it is apparent that (1) the development of a frequent pulsatile pattern of LH secretion is a prerequisite for early return to ovarian activity, and (2) suckling, or the presence of calves, suppresses this development.

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