

## Endocrine patterns of the post-partum cow

G. E. Lamming, D. Claire Wathes\* and A. R. Peters†

Department of Physiology & Environmental Studies, University of Nottingham School of Agriculture, Sutton Bonington, Nr Loughborough, Leics LE12 5RD, U.K.

**Summary.** Milked dairy cows generally have a shorter post-partum interval to ovarian cyclicity than suckling dairy or beef cows. In milked and suckling cows, there is a strong seasonal influence with spring-calving cows remaining anoestrous longer. Increasing the suckling intensity further delays the onset of ovarian cyclicity, probably by increasing the frequency or strength of its inhibitory influence on hypothalamic activity. Plasma FSH levels rise in most cows 5-10 days after calving and thereafter the random changes observed have little relationship to the onset of cycles. Recovery of FSH release therefore occurs earlier *post partum* than recovery of LH release. Hyperprolactinaemia is not a cause of reproductive failure in milked or suckling cows because there is no correlation between plasma prolactin levels and the onset of ovarian cycles. Plasma LH concentrations undergo significant changes directly related to the initiation of ovarian cycles, with low plasma levels immediately *post partum*, followed by an increase in basal secretion and the development of clear LH episodes. This pulsatile pattern appears earlier in dairy than in beef cows and is further delayed by suckling compared to milking. Before the first ovulation there is an increased frequency and peak height of LH episodes leading to a rise in plasma LH levels and eventually to a preovulatory-type LH surge which results in the first ovulation. These changes in the pattern of LH release appear definitive in the initiation of ovarian activity in post-partum cows.

### Introduction

In contrast to the situation in many eutherian and marsupial mammals, lactation in cattle is not an absolute block to ovarian cyclicity, conception or pregnancy. However, there is clear evidence that the frequency of milking, milk yield, nutritional status, the frequency and intensity of suckling and environmental factors influence the oestrous cycle and conception (Lamming, 1978). Furthermore, the production of dairy cattle capable of higher individual milk yields has increased the economic importance of maintaining high levels of fertility (Esslemont, 1974). In view of the lack of detailed information on plasma hormone levels in lactating and non-lactating cows it is important to define gonadotrophin and ovarian steroid hormone profiles in post-partum cows and to elucidate the nature of endocrine changes associated with subfertility.

Concentration on endocrine problems is justified because improved techniques of veterinary control of cattle diseases and the application of artificial insemination have dramatically decreased cow and calf losses due to venereal infections.

This paper covers two areas of study. The first section defines the incidence and nature of

\* Present address: University of Bristol, Department of Anatomy, The Medical School, Bristol BS8 1TD, U.K.

† Present address: Meat & Livestock Commission, P.O. Box 44, Queensway, Bletchley, Milton Keynes MK2 2EF, U.K.

subfertility of animals in their natural environment. During the past decade milk progesterone radioimmunoassays have been developed and widely used for this purpose as well as for detecting pregnancy in normal cows (Gadsby, Heap, Henville & Laing, 1974; Lamming & Bulman, 1976; Bulman & Lamming, 1979; Foote *et al.*, 1979; Gunzler *et al.*, 1979; Van de Weil, Kalis & Nisir Husain Shah, 1979). The second section gives information on changes in plasma hormone levels during early cycles after parturition, when there is peak milk yield or an intense suckling stimulus, because this is the time at which the next conception is required. Since steroid hormones and gonadotrophins are released in episodes, frequent plasma samples are required to define more precisely the patterns of hormone release.

### Field data from dairy and beef cows

#### *The interval to first oestrus and ovulation in post-partum cows*

In most milked cows there is suppression of follicular development immediately after parturition but changes occur rapidly between 7 and 20 days *post partum* (Wagner & Hansel, 1969; Morrow, Roberts & McEntee, 1969a, b). Full uterine involution requires about 30 days but suckling enhances this change (Reisen, Saiduddin, Tyler & Casida, 1968; Wagner & Hansel, 1969). Before data from hormone profiles were available the average interval to the first post-partum oestrus in both dairy and beef cows was reported as 30–104 days (Casida *et al.*, 1968; Marion & Gier, 1968; Callahan, Erb, Surve & Randel, 1971; Thatcher & Wilcox, 1973; King, Hurnik & Robertson, 1976). Others claimed that the first oestrus occurred earlier in dairy cows (30–72 days) than in beef cows (46–104 days) (Morrow *et al.*, 1969a, b; Casida, 1971; Bulman, 1978). Suckling extended the post-partum anoestrus in both beef and dairy cows (Wiltbank & Cook, 1958; Saiduddin, Reisen, Tyler & Casida, 1968; Casida *et al.*, 1968; Wagner & Hansel, 1969; Wagner & Oxenreider, 1971; Short, Bellows, Moody & Howland, 1972; England, Hauser & Casida, 1973; Lavoie & Moody, 1976; Wettemann, Turman, Wyatt & Totusek, 1976). Similarly, ovarian activity was initiated earlier in non-suckling than in suckling beef cows (Tribble, 1973; Connor, Tribble, Woodward, Beverly, Sorensen & Fleegeer, 1974) and the early weaning of calves reduced the interval to first oestrus.

Many cows do not exhibit oestrus in association with first or, sometimes, the second or subsequent ovulations and accurate detection of oestrus is a major problem. Estimates of 'silent' ovulations range from 43 to 70% (Menge, Mares, Tyler & Casida, 1962; Callahan *et al.*, 1971; Casida, 1971; Erb, Surve, Callahan, Randel & Garverick, 1971; Whitmore, Tyler & Casida, 1974). Since King *et al.* (1976) found that 50% of cows under continual survey showed oestrus at first ovulation compared with only 20% of those observed in the normal way, the validity of data on oestrus unaccompanied by data on endocrine activity is open to question. Therefore, although beef cows have longer post-partum 'anoestrous' intervals than dairy cows and suckling extends anoestrus in both, data collected solely by discontinuous observation cannot be used to define precisely the onset of ovarian cyclicity, and measurements of ovarian steroids such as progesterone are a valuable aid.

#### *Progesterone profiles of post-partum dairy cows*

Using milk progesterone profiles to monitor ovarian cyclicity we have shown that the average dairy cow, i.e. not suckling, resumes ovarian cyclicity  $24.1 \pm 0.6$  days ( $N = 505$ ) after calving (Lamming & Bulman, 1976; Bulman & Lamming, 1978). For brevity the validity criteria for all the radioimmunoassays quoted from our own work in this paper are given in Table 1. The data in Table 2 for the distribution of the post-partum interval in milked dairy cows indicate that 95% had resumed ovarian cycles within 50 days *post partum*. A short period of elevated progesterone ( $>3$  ng/ml milk and  $<10$  days' duration) precedes the first full-length oestrous cycle in

approximately 50% of milked cows and oestrus is frequently not observed until the end of the first complete cycle. When data collected by the herdsmen and milk progesterone profiles were compared there was a gradual increase in the proportion of cows observed in oestrus with succeeding ovulations (see Text-fig. 1). A full analysis of factors influencing the post-partum interval of dairy cows and its effects on fertility has been published elsewhere (Bulman & Lamming, 1978; Bulman & Wood, 1980). Briefly this showed a significant variation ( $P < 0.01$ ) in the interval to ovarian cyclicity according to the month of calving, with animals calving in the spring (March–May in Britain) taking longer than those calving during the rest of the year. The extreme averages were  $52 \pm 15$  days in April and  $20 \pm 1$  days in October (mean  $\pm$  s.e.m.  $N = 5$

**Table 1.** Validation of hormone assays cited in text

Assay	Sensitivity* (ng/ml)	Coefficient of variation (%)		Standard	Reference
		Intra-assay	Inter-assay		
Milk progesterone	0.40	8.9	10.3	—	Bulman & Lamming (1978)
Plasma progesterone†	0.025	6.0	12.3	—	Haresign <i>et al.</i> (1975)
Plasma LH	0.15–0.25	9.4	8.0	NIH-LH-B9	Webb <i>et al.</i> (1977)
Plasma FSH	10.00	8.7	16.9	NIH-FSH-B1	Webb <i>et al.</i> (1980)
Plasma prolactin	1.70	3.7	7.8	NIH-P-B3	Webb <i>et al.</i> (1980)

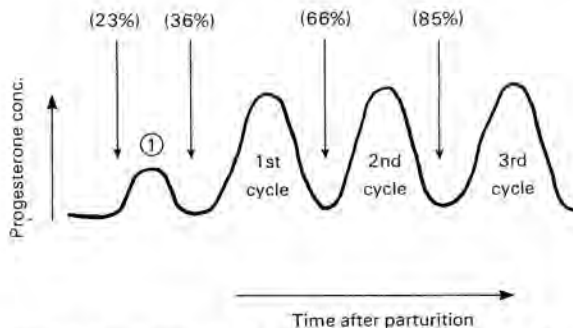
\* The value corresponding to twice the standard deviation of the blank values.

† Extraction recovery of progesterone (15 assays) =  $74.1 \pm 3.7\%$ .

**Table 2.** Interval to ovarian activity in milked dairy cows *post partum*

Days from parturition	No. of cows resuming ovarian activity within this period*	Cumulative %
1–10	13	2.6
11–20	240	50.4
21–30	157	81.7
31–40	54	92.4
41–50	16	95.6
51–>160	22	100.0

\* Milk progesterone levels  $>3$  ng/ml in two consecutive samples taken 2 or 3 days apart.



**Text-fig. 1.** Diagrammatic representation of milk progesterone levels of a normal post-partum cow showing a short period of elevated progesterone (1,  $>3$  ng/ml for  $<10$  days) preceding regular ovarian cycles. The percentage values indicate the proportion of cows in oestrus at each stage of ovarian activity.

and 120 cows respectively). There appeared to be a trend towards longer post-partum intervals in older animals, especially those of more than 5 lactations, but the effect just failed to achieve significance at  $P = 0.05$ . There was no correlation between yield per lactation and the post-partum interval to the start of ovarian cycles.

If a 'normal' cow is defined as one in which regular ovarian cycles resume within 50 days of calving and oestrus is observed after the second full-length cycle, then about 23% of the milked dairy cows that we have studied were 'abnormal'. In addition to delayed cycles (5%), there were problems of temporary cessation of ovarian cycles (5%), prolonged luteal activity (2%) and silent oestrus (11%). This last category included cows in which two consecutive ovulations were unaccompanied by observed oestrus. In all these groups the intervals from calving to conception tended to be longer than for the normal cows, although only the cessation of ovarian cycles gave a significant difference. A further analysis of profiles of 600 post-partum milked cows has revealed a similar incidence of subfertility (Lamming, 1980).

#### *Milk progesterone profiles in post-partum beef cows*

Ovarian activity was monitored by radioimmunoassay of milk progesterone in samples collected 3 times weekly from suckling beef cows of 3 herds. Table 3 indicates the times to resumption of ovarian activity as indicated by consecutive milk progesterone levels of  $>3$  ng/ml. Initial short cycles ( $>3$  ng/ml for  $<10$  days) were present in 46 (52.9%) of the cows.

**Table 3.** Post-partum interval to resumption of ovarian activity† in suckling beef cows

Herd	No. of cows	Breed	Time to ovarian cycles (days)
1	20	H × F	37.6 ± 4.4
2	26	H × F	91.5 ± 2.4*
	14	Blue Grey	81.1 ± 2.8*
3	27	H × F	24.5 ± 1.6

Values are mean ± s.e.m.

H = Hereford; F = Friesian; Blue Grey = female derived from Galloway ♀ × Shorthorn ♂.

† Milk progesterone levels  $>3$  ng/ml in two consecutive samples taken 2 or 3 days apart.

\* Significantly different,  $P < 0.001$ .

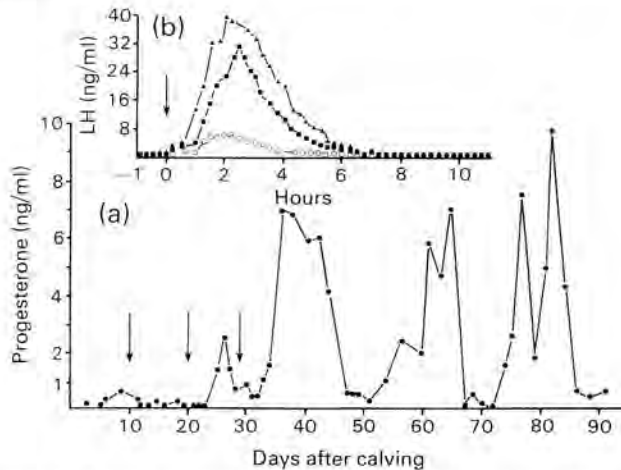
Because there were large differences between herds (cows in Herd 1 calving at various times of the year, those in Herd 2 from February to March and those in Herd 3 from September to October) the data were re-analysed on the basis of month of calving. Cows calving between January and June took significantly longer ( $P < 0.001$ ) to resume ovarian cycles ( $85.8 \pm 2.3$  days,  $N = 44$ ) than did those calving between July and December ( $26.9 \pm 1.6$  days,  $N = 43$ ), as found for dairy cows (see above). Deficiencies of nutrient intake could be implicated but the effect was highly significant even after removal of any effect of bodyweight at calving, thus suggesting that the season of calving can influence the time of return of ovarian cyclicity and that seasonal differences in photoperiod may be important.

#### **Detailed investigations of gonadotrophin and steroid secretion patterns in post-partum cows**

##### *Pituitary gonadotrophin content and response to LH-RH*

Pituitary LH content increases from low levels *pre partum* and immediately *post partum* to higher levels at 10 and 20 days *post partum*. Pituitary FSH content decreases from Days 1 to

20 (Labhsetwar, Collins, Tyler & Casida, 1964; Saiduddin *et al.*, 1968; Wagner, Saatman & Hansel, 1969) and is accompanied by increased plasma FSH levels (Dobson, 1978). This evidence supports the concept that early post-partum follicular development may be initiated by FSH secretion, whereas the time of first ovulation is more closely related to the level of LH. These changes probably reflect an increased pituitary response to LH-RH. There is increased responsiveness to LH-RH in the milked dairy cow at 10 days *post partum* (Kesler, Garverick, Younquist, Elmore & Bierschwal, 1977; Fernandes, Thatcher, Wilcox & Call, 1978; Lamming, 1978; Schallenberger, Schams & Zottmeier, 1978; Foster, Lamming & Peters, 1980; see also Table 4), while the suckling beef cow requires 20 days (autumn calving) or 30 days (spring calving) to achieve full pituitary response to synthetic LH-RH (Text-fig. 2; Webb, Lamming, Haynes, Hafs & Manns, 1977).



**Text-fig. 2.** Peripheral plasma progesterone (a) and LH (b) concentrations in a suckling beef cow given three injections of LH-RH (arrows) at 10 (○), 20 (■) and 29 (▲) days *post partum*. Note the significantly decreased LH response at 10 days *post partum*. (From Webb *et al.*, 1977.)

Work on rats (Aiyer, Chiappa & Fink, 1974) and sheep (Crighton, Foster, Haresign & Scott, 1975; Crighton & Foster, 1977) as well as that on cows (Kittok, Britt & Convey, 1973; Zolman, Convey & Britt, 1974; Foster, 1978a, b) suggests that pituitary responses to LH-RH are influenced both by steroid hormones and LH-RH. In cows, 2 injections of LH-RH (100 µg) given 90 min apart during the luteal phase of the cycle induced a much greater total LH release than did a single injection of 200 µg LH-RH (Table 4; for full details see Foster, 1978a, b). It is possible that changes in pituitary responsiveness with time *post partum* and differences between cows at a given time is controlled by the pattern of endogenous LH-RH release and that this situation may partly explain variations in response between cows to injections of synthetic LH-RH.

The transition from relatively low pituitary responsiveness to LH-RH during the pre-partum and immediate post-partum period to higher responsiveness between 10 and 20 days *post partum* appears a natural sequence of events following removal of the suppressive effects of high levels of steroid hormones during pregnancy. Our studies on plasma LH patterns provide circumstantial evidence that an increased frequency of LH-RH release with time *post partum* influences changes in plasma LH profiles and lack of LH-RH release is probably one major factor responsible for lack of reproductive activity in post-partum cows. Once ovarian cyclicity is established, pituitary response to injected LH-RH is influenced by ovarian hormone levels, with both LH and FSH release declining during the luteal phase (see Table 4).

A single injection of synthetic LH-RH administered after pituitary responsiveness has been

**Table 4.** Gonadotrophin response to injection of synthetic LH-RH in post-partum milked dairy cows (adapted from Foster, 1978a, b)

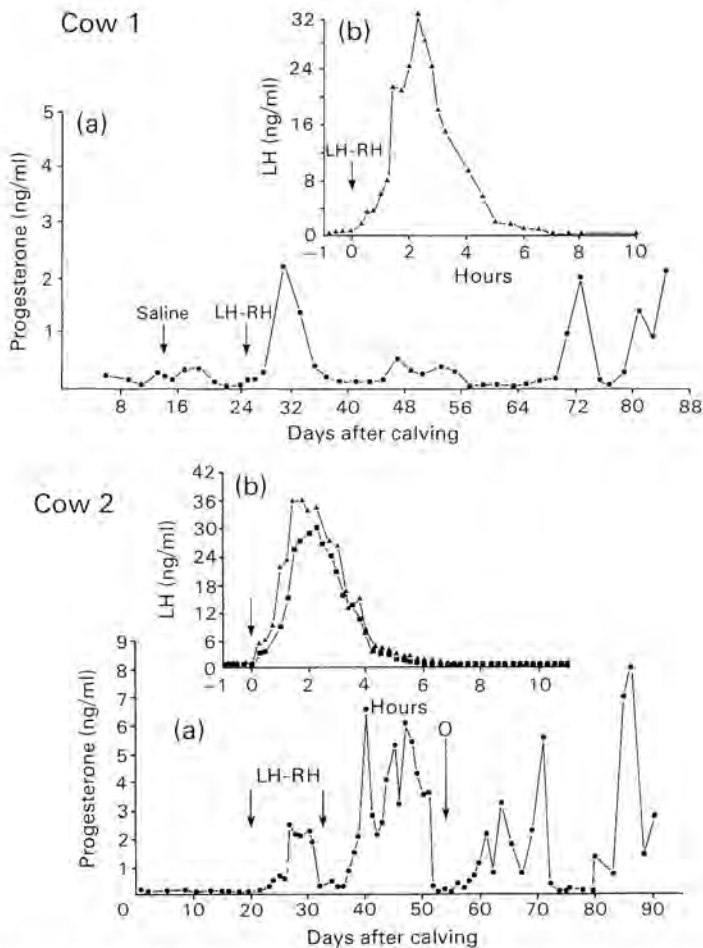
Reproductive stage	Dose ( $\mu\text{g}$ )	No. of animals	Day <i>post partum</i>	LH ( $\text{mm}^2$ )*	FSH ( $\text{mm}^2$ )*	Progesterone conc.
Post-partum anoestrus	200	4	4-5	3091 $\pm$ 499	3891 $\pm$ 958	Low
	200	4	7-10	8841 $\pm$ 2090	2715 $\pm$ 992	Low
Luteal phase of cycle	100	3	36-50	3492 $\pm$ 639	618 $\pm$ 263	High
	200	4	71-85	3998 $\pm$ 989	—	High
	(1 $\times$ 200) 100	3	102-138	3215 $\pm$ 732	603 $\pm$ 166	High
At end of short plasma progesterone rise	200	1	10	12793	5424	Falling

\* Calculated using 1 cm = 1 ng/ml : 3 cm/h for LH and 1 cm = 20 ng/ml : 3 cm/h for FSH.

restored will induce ovulation in most anoestrous dairy cows (Schams, Hofer, Hoffmann, Ender & Karg, 1973; Britt, Kittok & Harrison, 1974; Bulman & Lamming, 1978; Bulman, McKibbin, Appleyard & Lamming, 1978), but in beef cows suckling 2 calves a single injection may cause ovulation and a short period of elevated plasma progesterone followed by a return to anoestrus (see Text-fig. 3, Cow 1). Two spaced injections of LH-RH given at 10-day intervals, the first designed to cause ovulation and mimic the first short progesterone rise, the second given as the plasma progesterone declined to basal levels (see Text-fig. 3, Cow 2), induced normal oestrous cycles (Webb *et al.*, 1977). However, during a study of short-term changes in LH, FSH and progesterone concentrations, Foster, *et al.* (1980) measured very large LH and FSH responses to an injection of synthetic LH-RH in a 10-day-post partum milked cow which had already naturally exhibited the transient plasma progesterone rise (see Table 4; Text-fig. 4). The cow subsequently ovulated on Day 16 *post partum*.

#### *Plasma FSH and LH concentrations*

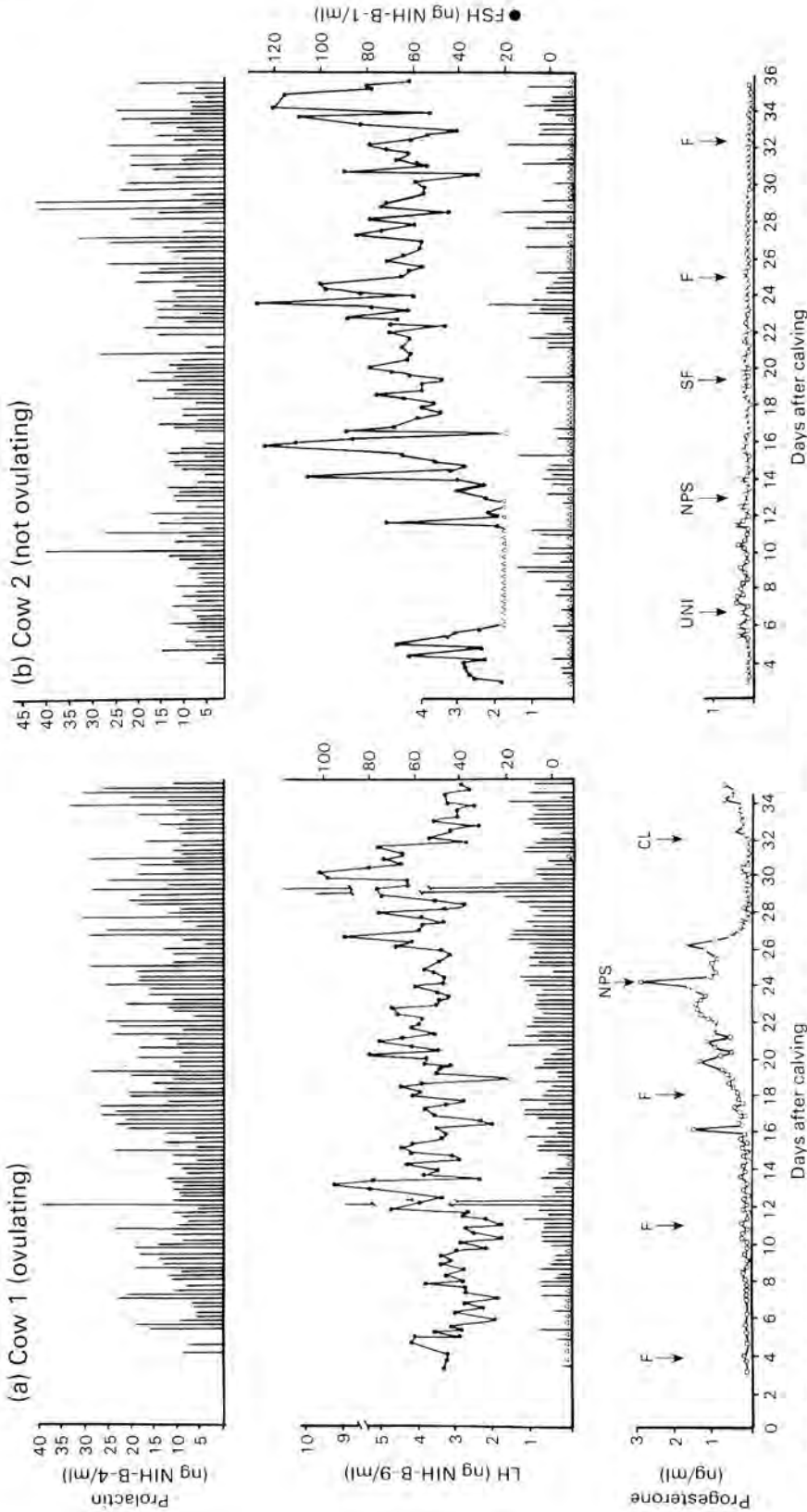
The availability of sensitive radioimmunoassay procedures has led to more detailed study of the endocrinology of cows *post partum*. The results of our studies of plasma gonadotrophin and prolactin in milked and suckling dairy cows to 30 days *post partum* are summarized in Table 5. Before parturition, plasma LH and FSH concentrations were low and FSH values remained low until 1-5 days *post partum*. There are no clearly defined episodes of release of FSH or LH during this early post-partum period. In the milked cow mean tonic plasma LH levels increase by 10-20 days *post partum* but mean plasma FSH levels remain relatively constant with marked, but largely random, temporal fluctuations. Typical examples of changes in plasma gonadotrophins and plasma prolactin in ovulating and non-ovulating milked dairy cows *post partum* are given in Text-fig. 4. In both animals the mean FSH values increased initially and then remained constant; there was an apparent rise of FSH concomitant with the preovulatory type LH surges at 12 and 29 days in Cow 1 which ovulated at the normal and expected time (30 days) *post partum* (Text-fig. 4a). The main feature in the non-ovulating cow is the lower basal values of plasma LH. Equivalent profiles have been published for milked cows by Schams *et al.* (1978) who observed similar mean FSH values, with strong FSH oscillations, and irregular spikes and widely differing FSH peak values, all independent of the onset of cyclicality and first oestrus after parturition. From these and studies described later, once an increased level of plasma FSH has been established about 5-10 days *post partum*, differences in plasma FSH are not considered a limiting factor in influencing the onset of ovarian cycles *post partum*. After the previous inhibitory influences of late pregnancy, FSH levels recover earlier than LH levels, reaffirming our



**Text-fig. 3.** Peripheral plasma progesterone (a) and LH (b) concentrations in suckling beef cows given a single injection of synthetic LH-RH (Cow 1) or two injections 10 days apart (Cow 2). Note the adequate LH response in both cows, with Cow 1 becoming acyclic after 33 days *post partum*, whereas Cow 2 continued to cycle and was representative of a group with a post-partum interval to resumed ovarian cyclicity of 36 days compared with 72 days for untreated controls. O = Oestrus. (From Webb *et al.*, 1977.)

previous evidence of a different mechanism of control in the cow for these two gonadotrophins (Foster *et al.*, 1980).

However, in all the ovulating cows that we have studied, we have observed a rise in the basal level of plasma LH and the development of an episodic pattern of LH release before ovarian cyclicity recommenced. Furthermore, in the ovulating animals (of which Cow 1 is typical), an LH surge of the magnitude seen at ovulation preceded the transient rise in plasma (or milk) progesterone and, after a decline of plasma progesterone concentration <1 ng/ml, a similar LH surge again occurred and was followed by ovulation and an oestrous cycle of normal length (Webb, Lamming, Haynes & Foxcroft, 1980). Schams *et al.* (1978) have also recorded a significant preovulatory-type peak of plasma LH before the first full cycle. Immediately before the first LH peak we have noted an increase in the frequency and peak height of LH episodes (see Text-fig. 5), indicating a possible increase in pituitary sensitivity to, and more frequent release of, endogenous LH-RH. This pattern of LH release develops as stages in a defined sequence, each



**Text-fig. 4.** Changes in hormone concentrations in an ovulating (a) and non-ovulating (b) milked dairy cow. Concentrations indicated by  $\Delta$  were below the sensitivity of the particular assay. UNI, uterus not involuted; NPS, no palpable ovarian structures; SF, small follicle; F, large follicle; CL, corpus luteum. Note the difference in basal plasma LH concentrations and the lack of preovulatory-type LH peaks in Cow 2. From Day 12 onwards plasma FSH concentrations in this cow appeared adequate. The mean plasma prolactin level of Cow 1 was higher than for Cow 2. (From Webb *et al.*, 1980.)



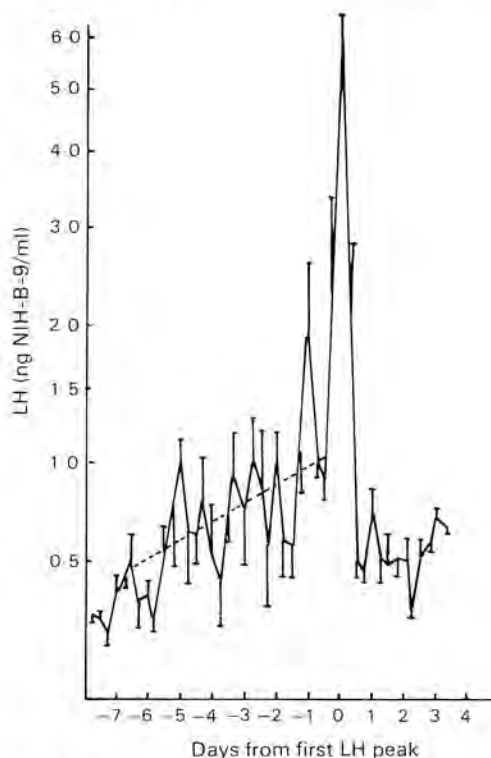
**Table 5.** Mean ( $\pm$  s.e.m.) plasma concentrations of LH, FSH and prolactin in suckling and milked Friesian cows

	LH (ng/ml)		FSH (ng/ml)		Prolactin (ng/ml)	
	Suckling cows	Milked cows	Suckling cows	Milked cows	Suckling cows	Milked cows
<i>Pre partum*</i>	0.67 $\pm$ 0.05		18.6 $\pm$ 2.6		16.0 $\pm$ 2.5	
<i>Days post partum</i>						
1-5	0.08 $\pm$ 0.03	0.90 $\pm$ 0.12	50.9 $\pm$ 15.3	45.3 $\pm$ 15.8	58.9 $\pm$ 15.9	29.1 $\pm$ 19.5
6-10	1.39 $\pm$ 0.20	1.66 $\pm$ 0.16	60.7 $\pm$ 11.7	41.5 $\pm$ 9.3	20.5 $\pm$ 7.8	16.4 $\pm$ 1.0
11-20	0.94 $\pm$ 0.16	2.06 $\pm$ 0.57	46.4 $\pm$ 10.1	36.9 $\pm$ 8.8	16.1 $\pm$ 3.1	21.8 $\pm$ 5.0
21-30	0.81 $\pm$ 0.09	1.17 $\pm$ 0.19	47.7 $\pm$ 9.6	39.2 $\pm$ 5.4	10.2 $\pm$ 2.2†	22.6 $\pm$ 3.6†

Blood plasma samples were taken at 10-min intervals for 8 h approximately weekly during the period 14 days *pre partum* to 30 days *post partum*. A total of 9 cows were used of which 5 suckled 4 calves each and the remaining 4 were machine-milked twice per day ( $n \geq 196$ ).

\* All the pre-partum values were combined because the cows had not been allocated to a group at this time.

† Significantly different,  $P < 0.05$  (Student's *t* test).



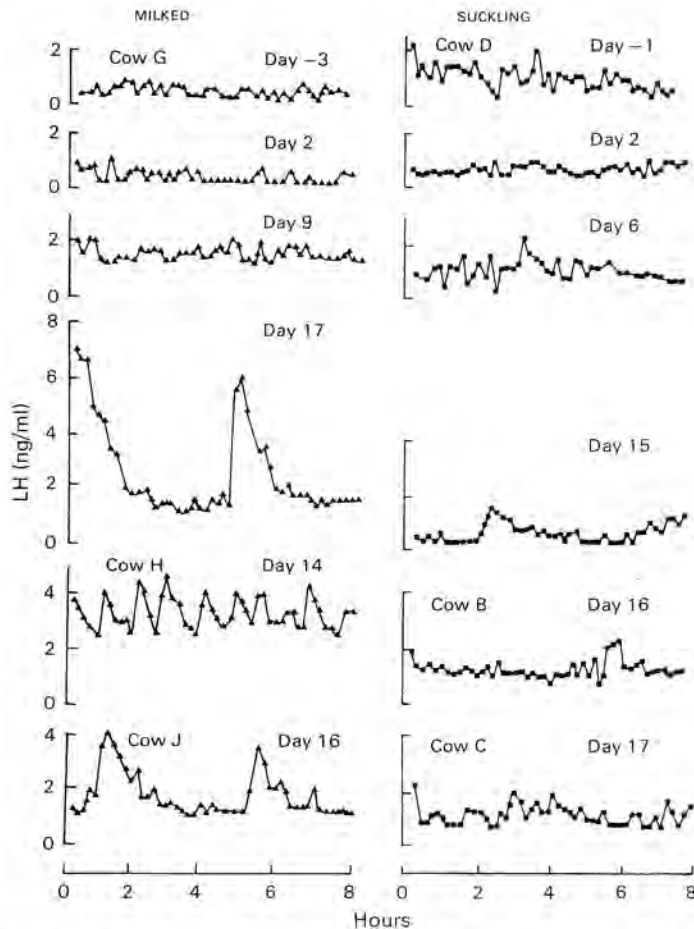
**Text-fig. 5.** Mean plasma LH concentrations ( $\pm$  s.e.m.) normalized to the first LH peak of ovulatory magnitude from 3 milked dairy cows 20-40 days *post partum*. The regression equation for the change marked by the broken line is  $y = 1.15 - 0.11x$ ,  $r = 0.46$  ( $N = 90$ ;  $P < 0.001$ ). (From Webb *et al.*, 1980.)

necessary for ovulation and for normal luteal function, as indicated by milk progesterone measurements.

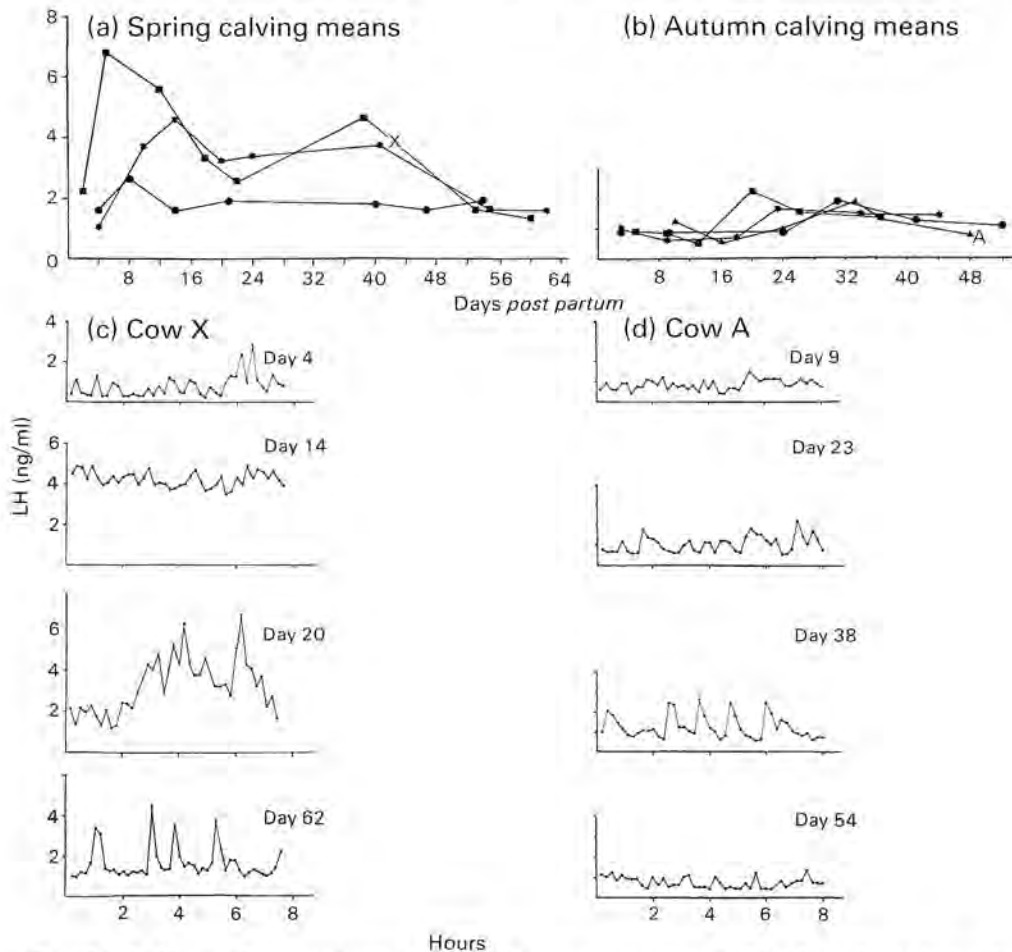
Although milked and suckling dairy cows have different patterns of reproductive activity, their plasma FSH levels were not markedly different. There were, however, significant effects of

suckling on mean plasma LH levels and particularly on the time of appearance of the episodic pattern of LH release. In milked Friesian cows episodic patterns of plasma LH developed between Days 10 and 20 *post partum* while few episodes were seen in Friesian cows suckling several calves (see Text-fig. 6). We have also observed a marked seasonal effect on LH profiles in suckling beef cows. In autumn-calving cows, which recommenced ovarian cycles at about 40 days *post partum*, there was a delay in the increase in mean plasma LH until about 25–30 days and a marked delay (>30 days) in the appearance of significant episodes of LH release (see Text-fig. 7). In spring-calving cows which did not cycle until at least 60 days *post partum*, there were surprisingly high basal levels of LH in most cows until Day 12 ( $2.67 \pm 0.4$ – $6.82 \pm 0.2$  ng/ml) but then a decline, perhaps related to higher circulating plasma oestradiol from developing ovarian follicles (see Text-fig. 7). Following the decline from high levels of LH at Day 12, an irregular pattern of LH release developed at about Day 20 *post partum*, with a more consistent typical pulsatile pattern of release by Day 40. During this period pulses occurred at approximately 2–4-h intervals and were similar in magnitude to those seen in milked cows at 18–20 days *post partum* (Text-fig. 7).

These results suggest that although a pulsatile pattern of LH secretion may be observed early



**Text-fig. 6.** LH profiles of plasma samples collected at 10-min intervals from 09:00 to 17:00 h for milked and suckling Friesian cows. Cows G and D were studied before and after parturition. Note the clear episodic pattern in the milked animals. (From Peters *et al.*, 1981.)



**Text-fig. 7.** Mean LH concentrations ( $n = 48$ ) in plasma samples collected at 10-min intervals from 09:00 to 17:00 h from 3 spring (a) and 4 autumn-calving (b) suckling cows. Plasma LH profiles (c, d) are shown for representative cows (X = spring, A = autumn) at various intervals *post partum*. Note the high mean plasma LH levels in the spring-calving cows (a) until after Day 40. In Cow A an irregular episodic pattern developed by Day 23 with a more distinct pattern by Day 38, with 7 LH episodes in 8 h. This cow ovulated on Day 40 and was in the late luteal phase on Day 54, when there was again a lack of clear LH episodes in the profile. The 3 spring-calving cows are typical of 6 out of 8 animals sampled at this time. The high mean LH levels (see Cow X, Day 14) occurred without clear episodes of release. Note the irregular pattern on Day 20 and the 4 clear episodes above the lower basal LH level on Day 62. This animal was anovulatory for more than 100 days (see text), and the hormone pattern was typical of that in other anoestrous cows. Such animals do, however, develop the regular episodic LH pattern before the first ovulation.

in the post-partum period, especially in milked cows, this is not necessarily followed immediately by a preovulatory-type LH surge and in such animals ovulation occurs later. In milked cows a significant relationship has been measured between frequency of LH episodes and the occurrence of the first progesterone rise (Peters, Lamming & Fisher, 1981). In suckling cows there is a marked delay in the presence of these LH episodes. The identity of the immediate trigger to the first preovulatory-type LH surge is not yet known. Working on the 28-day cycle of the rhesus monkey, Knobil (1980) has postulated that the arcuate nucleus is the centre for a control system that signals, at approximately once per hour, the release of a bolus of LH-RH to which the

pituitary responds by releasing pulses of FSH and LH. Monkeys with lesions in the arcuate nucleus were given infusions of 1 µg LH-RH/min for 6 min every hour. An increase or decrease in the dose of LH-RH or a change in the frequency of pulses resulted in a reduced pituitary response. Knobil (1980) further suggests that suckling inhibits the neural oscillator present in the arcuate nucleus. Our work with the cow confirms the importance of using small repeated pulses of LH-RH in inducing ovarian cyclicity *post partum*.

As indicated in Text-fig. 6, there was an increase in the basal concentration of LH several days before the first preovulatory-type LH surge which preceded the first progesterone rise, a phenomenon also noted by Chenault, Thatcher, Kalra, Abrams & Wilcox (1975). This rise may initiate the development of ovarian follicles and the resulting positive feedback due to oestrogen, because Shemesh & Hansel (1975) observed increased oestradiol production *in vitro* by follicles collected from cows on Day 16–17 of the cycle after stimulation with LH. A significant positive relationship between increasing LH and oestradiol before the preovulatory LH surge is known to occur in sheep (Hauger, Karsch & Foster, 1977).

#### *Plasma prolactin concentrations and the effect of hyperprolactinaemia*

The mean prolactin levels from daily samples and serial samples taken frequently over an 8-h period from the last 2 weeks of pregnancy to 90 days *post partum* have been determined for autumn-calving milked and autumn-calving suckling cows, and the data for the first 30 days *post partum* are shown in Table 5. Concentrations were low *pre partum* ( $16.0 \pm 2.5$  ng/ml), increased around parturition ( $139.9 \pm 3.2$  ng/ml) and then declined linearly up to Day 25 ( $r = 0.7$ ,  $P < 0.01$ ) in the suckling animals, while those in the milked group showed a gradual increase over the same period ( $r = 0.64$ ,  $P < 0.001$ ). Thereafter, up to Day 90, concentrations remained significantly ( $P < 0.01$ ) higher in the milked cows ( $27.1 \pm 2.1$  ng/ml) than in those suckling calves ( $10.1 \pm 1.0$  ng/ml). We have found no correlation between prolactin levels in individual cows of either type and the time to resumption of ovarian cycles or milk yield. These results were unexpected because previous workers have reported higher prolactin concentrations in suckling beef cows than in milked cows (Hart, Bines, Balch & Cowie, 1975; Hart, Bines, Cowie & Balch, 1975). However, Webb (1977) and Webb & Lamming (1981) have shown that suckling beef cows that calved in the spring have plasma prolactin patterns similar to those of the autumn-calving suckling dairy cows cited above, with prolactin levels in agreement with those reported by Ingalls, Convey & Hafs (1973). It is possible that, if suckling beef animals are infrequently handled and venepuncture is used, stress effects may be implicated as a causal factor of the higher mean plasma prolactin levels sometimes recorded (Webb & Lamming, 1981).

The linear decline in mean plasma prolactin concentrations in suckling cows from parturition to 80 days *post partum* (Webb & Lamming, 1981) and the significantly higher plasma prolactin levels in milked than in suckling cows suggest that higher prolactin levels in cows cannot be implicated in explaining differences between breeds and the effects of suckling on the return of post-partum cyclicity, a view contrary to suggestions for other species (human female: Rolland, Lequin, Schellekens & de Jong, 1975; sheep: Kann, Martinet & Shirar, 1978). Our view is supported by the results of Schallenberger *et al.* (1978) who found no effect of the dopamine agonist, bromocriptine (Sandoz, Basle), on FSH and LH basal levels or release rates in cows, although plasma prolactin values were significantly depressed. We conclude that hyperprolactinaemia cannot be a cause of the longer post-partum interval to resumption of ovarian cycles in suckling cows. However, suckling clearly has a marked inhibitory effect on reproductive behaviour. This inhibition is possibly mediated via a neural pathway from the mammary gland.

#### *Significance of the early progesterone increase*

In the post-partum period approximately 50% of suckling beef and milked dairy cows exhibit a plasma progesterone increase of short duration before full cyclicity, and this incidence is

similar to that observed by Corah, Quealy, Dunn & Kaltenbach (1974) and Schams *et al.* (1978). Tribble (1973) thought that this progesterone probably came from luteinized follicles; we have shown that short progesterone cycles occur more frequently in animals which exhibit ovarian activity soon after parturition and believe that this requires further study especially in relation to the level of follicular development before ovulation. Schams *et al.* (1978) suggested that this short early cycle was caused by inadequate levels of LH or its receptor. However, our results from examination of basal plasma LH levels at this time could not demonstrate a lack of luteotrophic support to account for the lower plasma progesterone or earlier luteolysis during this first cycle. A more likely explanation would be defects of follicular development leading to a lack of steroidogenic ability of the luteal structure, which might be related to a lack of LH receptor activity. For example, corpora lutea occurring in anoestrous sheep following intramuscular injection of synthetic LH-RH did not secrete progesterone (Haresign, Foster, Haynes, Crighton & Lamming, 1975), but if PMSG was administered before the LH-RH normal progesterone release occurred (Haresign & Lamming, 1978), suggesting that follicular maturation before ovulation may be important.

The first short cycle in cows is normally followed by ovulation and a corpus luteum of normal life-span and secretory activity although oestrus is often not observed until the end of the second cycle. There is no evidence from our work, or that of Schams *et al.* (1978) that plasma LH concentration changed substantially during the transient progesterone rise, so it must be assumed that the second LH surge and subsequent normal luteal activity are influenced more by the stimulatory effect of removing a negative feedback influence of progesterone, rather than a possible positive or modulating effect of low levels of progesterone on the hypothalamus and/or pituitary to produce a preovulatory-type LH release, as previously suggested by Lamming (1978). However, the 'organizational' role of the progesterone produced by the first ovarian cycle on physiological and behavioural changes at the second ovulation is probably important. The fact that an LH-RH challenge given as the plasma progesterone level declines produces a massive release of LH and FSH (see Table 4) supports the view that, once the pituitary response to LH-RH is restored in the early post-partum period, inadequate pituitary sensitivity or availability of LH cannot be the reason for the lower progesterone release during the first short cycle.

### Conclusions

Lactation in the cow does not provide a complete block to pregnancy, but milking and suckling can inhibit ovulation at the hypothalamic, pituitary or ovarian levels. The lack of ovarian follicular activity generally observed at the end of pregnancy is continued during early lactation.

There is good evidence of suppressed pituitary sensitivity to synthetic gonadotrophin releasing hormone (LH-RH) during the early post-partum period extending to 10 days in milked dairy cows and 20–30 days in suckling beef animals.

Plasma FSH levels in cows are low immediately *post partum* but generally increase within 5 days with levels fluctuating widely. There are no consistent changes associated with the first preovulatory LH surge and ovulation.

Plasma prolactin levels rise in milked cows from parturition to approximately 30 days *post partum* (peak lactation) and then steadily decline. In suckling cows prolactin levels decline linearly from parturition, and are usually lower than in milked cows. There was no significant relationship in individual animals between plasma prolactin values and the onset of ovarian activity and hyperprolactinaemia cannot be implicated as the causal factors in the maintenance of anoestrus.

During early lactation in the milked cow and later in the suckling cow, the low plasma LH concentrations are generally followed by increased pulsatile LH release, leading to a

preovulatory-type LH surge. This probably occurs due to (i) a change in pituitary sensitivity to hypothalamic stimulation by an increased frequency of releasing factor release and/or (ii) a positive feedback effect of oestradiol from the developing follicles. The first preovulatory-type LH surge is preceded by episodes of LH release of increasing frequency and peak height and this increases basal LH levels. It can be induced by injections of synthetic LH-RH.

In approximately 50% of cows the first preovulatory-type LH surge is followed by a small transitory rise in plasma progesterone lasting for 5–10 days. This precedes the second preovulatory LH surge, ovulation and the first full ovarian cycle. In other animals the first preovulatory-type LH surge is followed by ovulation, with or without oestrus and an oestrous cycle of normal length. The physiological effects of progesterone from the first ovarian cycle are probably important in affecting (1) a possible neural oscillator co-ordinating hypothalamic activity and particularly releasing factor release, (2) pituitary and ovarian activity, and (3) oestrous behaviour.

The mechanism responsible for the increase in frequency and peak height of LH episodes and the first LH preovulatory-type surge before the low transitory rise in plasma progesterone in post-partum cows is, as yet, undisclosed but appears to hold the key to the resumption of cyclic ovarian activity in the cow *post partum*.

This work was supported by grants from the Agricultural Research Council, the Meat & Livestock Commission, the Milk Marketing Board and the Royal Society.

### References

- Aiyer, M.S., Chiappa, S.A. & Fink, G. (1974) The priming effect of luteinising hormone releasing factor on the anterior pituitary gland in the female rat. *J. Endocr.* **62**, 573–588.
- Britt, J.H., Kittok, R.J. & Harrison, D.S. (1974) Ovulation, estrus and endocrine response after Gn-RH in early *post partum* cows. *J. Anim. Sci.* **39**, 915–919.
- Bulman, D.C. (1978) *Progesterone levels and fertility of lactating cows*. Ph.D. thesis, University of Nottingham.
- Bulman, D.C. & Lamming, G.E. (1978) Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *J. Reprod. Fert.* **54**, 447–458.
- Bulman, D.C. & Lamming, G.E. (1979) The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryo mortality in dairy cows. *Br. vet. J.* **135**, 559–567.
- Bulman, D.C. & Wood, P.D.P. (1980) Abnormal patterns of ovarian activity in dairy cows and their relationship with reproductive performance. *Anim. Prod.* **30**, 177–188.
- Bulman, D.C., McKibbin, P.E., Appleyard, W.T. & Lamming, G.E. (1978) Effect of a progesterone-releasing intravaginal device on the milk progesterone levels, vaginal flora, milk yield and fertility of cyclic and non-cyclic dairy cows. *J. Reprod. Fert.* **53**, 289–296.
- Callahan, C.J., Erb, R.E., Surve, A.H. & Randel, R.D. (1971) Variables influencing ovarian cycles in *post partum* dairy cows. *J. Anim. Sci.* **33**, 1053–1059.
- Casida, L.E. (1971) The *post partum* interval and its relation to fertility in the cow, sow and ewe. *J. Anim. Sci.* **32**, Suppl. 1, 66–72.
- Casida, L.E., Graves, W.E., Hauser, E.R., Lauderdale, J.W., Reisen, J.W., Saiduddin, S. & Tyler, W.J. (1968) Studies on the *post partum* cow. *Res. Bull. agric. Exp. Stn Univ. Wis.* No. 270, 1–54.
- Chenault, J.R., Thatcher, W.W., Kalra, P.S., Abrams, R.M. & Wilcox, C.J. (1975) Transitory changes in plasma progesterone, estradiol and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci.* **58**, 709–717.
- Connor, J.S., Tribble, R.L., Woodward, T.L., Beverly, J.R., Sorensen, A.M., Jr & Fleeger, J.L. (1974) Serum estrogens in suckled and non-suckled heifers. *J. Anim. Sci.* **39**, 203, Abstr.
- Corah, L.R., Quealy, A.P., Dunn, T.G. & Kaltenbach, C.C. (1974) *Pre-partum* and *post-partum* levels of progesterone and estradiol in beef heifers fed two levels of energy. *J. Anim. Sci.* **39**, 380–385.
- Crighton, D.B. & Foster, J.P. (1977) Luteinizing hormone release after two injections of synthetic luteinizing hormone releasing hormone in the ewe. *J. Endocr.* **72**, 59–67.
- Crighton, D.B., Foster, J.P., Haresign, W. & Scott, S.A. (1975) Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle. *J. Reprod. Fert.* **44**, 121–124.
- Dobson, H. (1978) Radioimmunoassay of FSH in the plasma of *post-partum* dairy cows. *J. Reprod. Fert.* **52**, 45–49.
- England, B.G., Hauser, E.R. & Casida, L.E. (1973) Some effects of unilateral ovariectomy in the *post partum* beef cow. *J. Anim. Sci.* **36**, 45–50.
- Erb, R.E., Surve, A.H., Callahan, C.J., Randel, R.D. & Garverick, H.A. (1971) Reproductive steroids in the bovine. VII. Changes *post partum*. *J. Anim. Sci.* **33**, 1060–1071.

- Esslemont, R.J. (1974) Economic and husbandry aspects of the manifestation and detection of oestrus in cows. *ADAS Q. Rev.* **12**, 175-184.
- Fernandes, L.C., Thatcher, W.W., Wilcox, C.J. & Call, E.P. (1978) LH release in response to GnRH during the postpartum period of dairy cows. *J. Anim. Sci.* **46**, 443-448.
- Foote, R.H., Oltenuacu, E.A.B., Kummerfeld, H.L., Smith, R.D., Rick, P.M. & Braun, R.K. (1979) Milk progesterone as a diagnostic aid. *Br. vet. J.* **135**, 550-558.
- Foster, J.P. (1978a) Plasma LH concentrations after single or double injections of synthetic LH-RH in dairy cows. *J. Reprod. Fert.* **54**, 119-121.
- Foster, J.P. (1978b) The response of the pituitary gland to hypothalamic stimulation. In *Control of Ovulation*, pp. 101-116. Eds D. B. Crighton, N. B. Haynes, G.R. Foxcroft & G. E. Lamming. Butterworths, London.
- Foster, J.P., Lamming, G.E. & Peters, A.R. (1980) Short-term relationship between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of Gn-RH injection. *J. Reprod. Fert.* **59**, 321-327.
- Gadsby, J.E., Heap, R.B., Henville, A. & Laing, J.A. (1974) A semi automated technique for the estimation of progesterone in cows milk and its application in pregnancy diagnosis. *J. Physiol., Lond.* **242**, 3P-5P.
- Gunzler, O., Rattenberger, E., Gorchl, A., Hahn, R., Hocke, P., Klause, R. & Karg, H. (1979) Milk progesterone determination as applied to the confirmation of oestrus, the detection of cycling and as an aid to veterinarian and biotechnical measures in cows. *Br. vet. J.* **135**, 541-549.
- Haresign, W. & Lamming, G.E. (1978) Comparison of LH release and luteal function in cyclic and LH-RH-treated anoestrous ewes pretreated with PMMSG or oestrogen. *J. Reprod. Fert.* **52**, 349-353.
- Haresign, W., Foster, J.P., Haynes, N.B., Crighton, D.B. & Lamming, G.E. (1975) Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod. Fert.* **43**, 269-279.
- Hart, I.C., Bines, J.A., Balch, C.C. & Cowie, A.T. (1975) Hormone and metabolite differences between lactating beef and dairy cattle. *Life Sci.* **16**, 1285-1292.
- Hart, I.C., Bines, J.A., Cowie, A.T. & Balch, C.C. (1975) A comparison of hormones and metabolites in the circulation of beef and dairy cattle. *J. Endocr.* **65**, 3P-4P. Abstr.
- Hauger, R.L., Karsch, S.T. & Foster, D.L. (1977) A new concept for control of the estrus cycle of the ewe based on the temporal relationship between luteal hormone oestradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. *Endocrinology* **101**, 807-817.
- Ingalls, W.G., Convey, E.M. & Hafs, H.D. (1973) Estrus and related behaviour in post-partum Holstein cows. *Appl. Anim. Ethol.* **2**, 55-62.
- Kann, G., Martinet, J. & Shirar, A. (1978) Hypothalamic pituitary control during lactation. In *Control of Ovulation*, pp. 319-344. Eds D. B. Crighton, G. R. Foxcroft, N. B. Haynes & G. E. Lamming. Butterworths, London.
- Kesler, D.J., Garverick, H.A., Youngquist, R.S., Elmore, R.G. & Bierschwal, C.J. (1977) Effects of days post-partum and endogenous reproductive hormones on Gn-RH release in dairy cows. *J. Anim. Sci.* **46**, 797-803.
- King, G.J., Hurnik, J.P. & Robertson, H.A. (1976) Ovarian function and estrus in dairy cows during early lactation. *J. Anim. Sci.* **42**, 688-692.
- Kittok, R.J., Britt, J.H. & Convey, E.M. (1973) Endocrine response after Gn-RH in luteal phase cows and cows with ovarian follicular cysts. *J. Anim. Sci.* **37**, 985-989.
- Knobil, E. (1980) Neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.* **36**, 53-88.
- Labhsetwar, A.P., Collins, W.E., Tyler, W.J. & Casida, L.E. (1964) Some pituitary-ovarian relationships in the periparturient cow. *J. Reprod. Fert.* **8**, 85-90.
- Lamming, G.E. (1978) Reproduction during lactation. In *Control of Ovulation*, pp. 335-353. Eds D. B. Crighton, G. R. Foxcroft, N. B. Haynes & G. E. Lamming. Butterworths, London.
- Lamming, G.E. (1980) Milk progesterone for assessing response to treatment of sub-fertile cattle. *Proc. 9th Int. Congr. Anim. Reprod. & A.I., Madrid* **2**, 143-152.
- Lamming, G.E. & Bulman, D.C. (1976) The use of milk progesterone radioimmunoassay in the diagnosis and treatment of sub-fertility in dairy cows. *Br. vet. J.* **132**, 507-517.
- Lavoie, V.L. & Moody, E.L. (1976) Suckling effect on steroids in postpartum cows. *J. Anim. Sci.* **43**, 292-293.
- Marion, C.B. & Gier, H.T. (1968) Factors affecting bovine ovarian activity after parturition. *J. Anim. Sci.* **27**, 1620-1626.
- Menge, A.C., Mares, S.E., Tyler, W.J. & Casida, L.E. (1962) Variation and association among post-partum reproduction and production characteristics in Holsten Friesian cattle. *J. Dairy Sci.* **45**, 233-241.
- Morrow, D.A., Roberts, S.J. & McEntee, K. (1969a) Post-partum ovarian activity and involution of the uterus and cervix in dairy cattle. 1. Ovarian activity; 2. Involution of uterus and cervix; 3. Days non-gravid and services per conception. *Cornell Vet.* **59**, 173-190.
- Morrow, D.A., Roberts, S.J. & McEntee, K. (1969b) A review of post-partum ovarian activity and involution of the uterus and cervix in cattle. *Cornell Vet.* **59**, 134-154.
- Peters, A.R., Lamming, G.E. & Fisher, M.W. (1981) A comparison of plasma LH concentrations in milked and suckling post-partum cows. *J. Reprod. Fert.* **62**, 567-573.
- Reisen, J.W., Saiduddin, S., Tyler, W.J. & Casida, L.E. (1968) Relation of post-partum interval to corpus luteum development, pituitary prolactin activity and uterine involution in dairy cows. *Res. Bull. agric. Exp. Stn Univ. Wis.* No. 270, pp. 27-40.
- Rolland, R., Lequin, R.M., Schellekens, L.A. & de Jong, F.H. (1975) Role of prolactin in the restoration of ovarian function during the early post-partum period in the human female. 1. Study during physiological lactation. *Clin. Endocr.* **4**, 15-25.

- Saiduddin, S., Reisen, J.W., Tyler, W.J. & Casida, L.E.** (1968) Pituitary LH activity in the postpartum dairy cow. *J. Anim. Sci.* **25**, 930 Abstr.
- Schallenger, E., Schams, D. & Zottmeier, K.** (1978) Response of lutropin (LH) and follitropin (FSH) to the administration of gonadoliberin (Gn-RH) in pregnant and post-partum cattle including experiments with prolactin suppression. *Theriogenology* **10**, 35-53.
- Schams, D., Hofer, F., Hoffmann, B., Ender, M.L. & Karg, H.** (1973) Effects of synthetic LH-RH treatment on bovine ovarian function during oestrus cycle and post-partum period. *Acta endocr., Copenh., Suppl.* **177**, 296, Abstr.
- Schams, D., Schallenger, E., Menzer, C.H., Stangl, J., Zottmeier, K., Hoffmann, B. & Karg, H.** (1978) Profiles of LH, FSH and progesterone in post-partum dairy cows and their relationship to the commencement of cycling functions. *Theriogenology* **10**, 453-468.
- Shemesh, M. & Hansel, W.** (1975) Stimulation of follicular steroidogenesis by PGF<sub>2α</sub> and LH. *J. Anim. Sci.* **41**, 379, Abstr.
- Short, R.E., Bellows, R.A., Moody, E.L. & Howland, B.E.** (1972) Effects of suckling and mastectomy on bovine postpartum reproduction. *J. Anim. Sci.* **34**, 70-74.
- Thatcher, W.W. & Wilcox, C.J.** (1973) Post-partum estrus as an indicator of reproductive status in the dairy cow. *J. Dairy Sci.* **56**, 608-610.
- Tribble, R.L.** (1973) Peripheral serum progesterone, 17-hydroxy progesterone and 20β-hydroxy progesterone levels in suckled and non-suckled primiparous Hereford heifers. *Diss. Abstr.* **35B**, 1251.
- Van de Weil, D.F.M., Kalis, C.H.J. & Nisir Husain Shah, S.** (1979) Combined use of milk progesterone profiles, clinical examination and oestrus observation for the study of fertility in the post-partum period of dairy cows. *Br. vet. J.* **135**, 568-577.
- Wagner, W.C. & Hansel, W.** (1969) Reproductive physiology of the post partum cow. I. Clinical and histological findings. *J. Reprod. Fert.* **18**, 493-500.
- Wagner, W.C. & Oxenreider, J.L.** (1971) Endocrine physiology following parturition. *J. Anim. Sci.* **32**, Suppl. 1, 1-16.
- Wagner, W.C., Saatman, R. & Hansel, W.** (1969) Reproductive physiology of the post-partum cow. II. Pituitary, adrenal and thyroid function. *J. Reprod. Fert.* **18**, 501-508.
- Webb, R.** (1977) *Reproductive physiology of the post-partum cow*. Ph.D. thesis, University of Nottingham.
- Webb, R. & Lamming, G.E.** (1981) Patterns of plasma prolactin in post-partum suckled cows. *J. Endocr.* **90** (in press).
- Webb, R., Lamming, G.E., Haynes, N.B., Hafs, H.D. & Manns, J.G.** (1977) Response of cyclic and post-partum suckled cows to injections of synthetic LH-RH. *J. Reprod. Fert.* **50**, 203-210.
- Webb, R., Lamming, G.E., Haynes, N.B. & Foxcroft, G.R.** (1980) Plasma progesterone and gonadotrophin concentrations and ovarian activity in post-partum dairy cows. *J. Reprod. Fert.* **59**, 133-143.
- Wettemann, R.P., Turman, E.J., Wyatt, R.D. & Totusek, R.** (1976) Suckling intensity and reproduction in range cows. *J. Anim. Sci.* **42**, 267-268.
- Whitmore, H.L., Tyler, W.J. & Casida, L.E.** (1974) Effects of early postpartum breeding in dairy cattle. *J. Anim. Sci.* **38**, 339-346.
- Wiltbank, J.N. & Cook, A.C.** (1958) The comparative reproductive performance of nursed and milked cows. *J. Anim. Sci.* **17**, 640-648.
- Zolman, J., Convey, E.M. & Britt, J.H.** (1974) Relationships between the luteinising hormone response to gonadotrophin releasing hormone and endogenous steroids. *J. Anim. Sci.* **39**, 355-359.