Short-term relationships between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of Gn-RH injection

J. P. Foster, G. E. Lamming and A. R. Peters

Department of Physiology and Environmental Studies, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, U.K.

Summary. Jugular venous blood samples were obtained from 7 dairy cows every 10 min for 10–19 h during the early- or mid-luteal phase of the oestrous cycle, and each cow was given 1 or 2 i.v. injections of 100 µg synthetic Gn-RH. Four of these cows were also sampled in a different cycle with no treatment being administered. Peaks of plasma LH, FSH and progesterone were detected in each animal in the absence of treatment; those of LH and progesterone often occurred in parallel. Injection of Gn-RH was always followed by a significant increase in plasma LH and progesterone concentrations and in most cases by a significant FSH increase. There was a significant temporal relationship between the peaks of all 3 hormones.

A further 8 cows were sampled during the first 10 days *post partum* when the mean plasma progesterone concentration was low. An i.v. injection of 200 µg synthetic Gn-RH was given to each animal and this resulted in a significant increase in plasma LH and FSH concentrations, but in only one cow was the Gn-RH injection followed by a significant increase in plasma progesterone concentration. The LH response to Gn-RH injection was significantly less in cows injected on or before Day 5 *post partum* than in cows injected on Days 7–10 *post partum*.

Introduction

Study of the relationship between plasma concentrations of reproductive hormones during the post-partum period of the dairy cow provides information about the control of ovarian activity and the transition from anoestrus to resumption of ovarian cycles.

Plasma luteinizing hormone (LH) and progesterone concentrations have usually been measured in samples taken at daily or longer intervals during the post-partum period of cows (Schams, Hoffmann, Fischer, Marz & Karg, 1972; Edgerton & Hafs, 1973; Gaverick, Brady, Mather, Bierschwal & Erb, 1973; Smith, Edgerton, Hafs & Convey, 1973; Arije, Wiltbank & Hopwood, 1974; Corah, Quealy, Dunn & Kaltenbach, 1974; Dickey, Henricks & Bierly, 1975), but we have reported studies on animals sampled at 6-h intervals from calving until 35 days *post partum* (Lamming, 1978; Webb, Lamming, Haynes & Foxcroft, 1980), a technique also used by Schams *et al.* (1978). Measurement of follicle-stimulating hormone (FSH) has also been carried out (Akbar, Reichert, Dunn, Kaltenbach & Niswender, 1974; Dobson, 1978; Webb *et al.*, 1980). To the best of our knowledge no studies have yet been reported in which short-term variations in the plasma levels of these 3 hormones have been measured concurrently in cattle, although Baird, Swanson & Scaramuzzi (1976) and Scaramuzzi & Baird (1979) have measured plasma LH and oestradiol-17β respectively in cyclic and seasonally anoestrous sheep at 10-min

intervals and found that each peak of LH release was followed by a rise in oestradiol concentrations.

The present study was undertaken (1) to examine the short-term relationship between LH, FSH and progesterone in cyclic dairy cows when luteal tissue was known to be present, and (2) to compare these hormone patterns in normal cyclic animals with those in anoestrous cows during the early post-partum period. Gonadotrophin-releasing hormone (Gn-RH) causes LH and FSH release in anoestrous and cyclic cows (see Akbar *et al.*, 1974) and has been reported to cause progesterone release in cows injected during the luteal phase of the oestrous cycle (Kittock, Britt & Convey, 1973; Zolman, Convey & Britt, 1974). We therefore investigated the effect of Gn-RH on the 3 hormones being studied.

Materials and Methods

Friesian dairy cows in their second or third lactation were used. They were machine milked twice daily and fed a high quality dairy ration according to yield. Milk samples were collected from each cow 3 times weekly and assayed for progesterone concentration to monitor ovarian cycles. All blood samples were taken via an indwelling jugular catheter.

Experimental design

Cyclic cows. Seven (7) autumn-calving cows were used between 22 and 105 days *post partum*. Cows 1–3 were sampled during the mid-luteal phase of the oestrous cycle (7–14 days after the beginning of the milk progesterone rise, i.e. assumed to be Days 11–18 of cycle) as follows. Blood samples were taken every 10 min for 8 h, 100 µg synthetic Gn-RH (Lutal: Hoechst, Frankfurt, West Germany) were then injected i.v. and the animals were sampled every 10 min for a further 11 h with a second Gn-RH injection administered 6 h after the first. Cows 4–7 were sampled every 10 min in the luteal phase for periods of 4–6 h, then injected i.v. with 100 µg Gn-RH and sampled at 10-min intervals for a further 6 h. Cows 4 and 5 were in the early luteal phase (1–6 days after the start of the progesterone rise) and Cows 6 and 7 were mid-luteal.

Cows 4–7 were also sampled for 5–8 h during a different cycle when they were in the early luteal (Cows 4 and 5) or mid-luteal (Cows 6 and 7) phase but no treatment was administered.

Post-partum anoestrous cows. Eight (8) autumn-calving cows (Nos 8–15) were sampled every 10 min for 4 h before and for 10 h after a single i.v. injection of 200 µg synthetic Gn-RH (Hoechst) during the first 10 days *post partum*. Cows 8–11 were sampled on Days 4 or 5 *post partum* and Cows 12–15 on Days 7–10. Blood samples (5 ml) were collected in heparinized tubes and centrifuged at 1500 *g* for 15 min. The plasma was removed and stored at –20°C until assayed.

Hormone assays

All hormones were measured by radioimmunoassay procedures in routine use in this laboratory. Plasma LH (reference standard NIH-LH-B9) and progesterone levels were assayed as described by Webb, Lamming, Haynes, Hafs & Manns (1977). The intra- and inter-assay coefficients of variation (CVs) for the LH assay were 6.5 and 8.0% respectively and the sensitivity was 0.15 ng/ml. For the progesterone assay, the mean recovery was $64.1 \pm 2.3\%$ ($n = 14$) and values were corrected accordingly. The intra- and inter-assay CVs were 4.8 and 11.3% respectively and the sensitivity was 25 pg/tube. Plasma FSH was measured using the heterologous system described by Webb *et al.* (1980) with reference standard NIH-FSH-B1. The intra- and inter-assay CVs were 8.3 and 12.0% respectively, and the sensitivity was 9.0 ng/ml. Milk progesterone was assayed by the method of Lamming & Bulman (1976). The intra- and inter-assay CVs were 8.9 and 10.3% respectively and the sensitivity was 0.4 ng/ml.

For each animal the mean LH, FSH and progesterone concentrations were determined for the entire sampling period for the untreated animals and for the period before injection of Gn-RH for the treated animals. Values greater than 2 standard deviations above these means were taken as being significantly greater than the mean.

Results

Cyclic cows

The changes in Cows 1 and 2 before and after injection of synthetic Gn-RH in the mid-luteal phase are shown in Text-fig. 1. Significant elevations of all 3 hormones occurred in all 7 cows in the absence of any treatment and injection of synthetic Gn-RH was always followed by a significant increase in LH and progesterone concentrations. Plasma FSH concentrations sometimes increased significantly after injection. There was no difference between the early and mid-luteal phases of the oestrous cycle in the responses obtained.

Progesterone peaks were often associated with LH peaks in the absence of treatment and sometimes also with FSH peaks. In only one instance (Cow 6) was a progesterone peak associated with an FSH peak but not an LH peak. Each progesterone value in the control and treated periods was categorized according to whether it was significantly greater than the mean progesterone concentration in that animal and to its relationship with significant LH and FSH increases (Table 1). A χ^2 analysis of the pooled LH and progesterone data indicated that there was a temporal relationship between significant increases in plasma LH and progesterone concentrations ($\chi^2 = 77.6$, $P < 0.001$). There was a greater correspondence of progesterone increases with the LH increases than would be expected if there were no relationship between the two. There was also a significant relationship between significant increases in plasma FSH and progesterone concentration ($\chi^2 = 30.2$, $P < 0.01$).

Table 1. Occurrence of plasma samples with increased progesterone concentrations/total number of samples in relation to LH and FSH peaks

Time when progesterone conc. significantly > mean value	Significant peaks of:	
	LH	FSH
During	64/163 (62.7%)*	18/43 (17.7%)
After		
0-1 h	12/92 (11.8%)	29/108 (28.4%)
1-3 h	17/99 (16.7%)	16/122 (15.7%)
Unrelated	9/246 (8.8%)	39/327 (38.2%)
Total	102/600	102/600

* Calculated as 64/102, i.e. 62.7% of significant progesterone increases occurred during significant LH increases which occupied 163/600 (27%) of the sampling time.

Additionally, there was a relationship between significant increases in plasma LH and FSH concentration since 79% of all samples with significantly elevated FSH were taken during or within 1 h of a significant increase in plasma LH. The plasma concentrations of both gonadotrophins usually increased significantly after synthetic Gn-RH injection (although in 2 cyclic cows FSH did not increase). At other times, peaks of the 2 hormones were unrelated.

Anoestrous cows

In 7 of the 8 animals injected during the first 10 days *post partum* the plasma progesterone concentration was low (<0.05 ng/ml) and although Gn-RH injection was always followed by a significant increase in plasma LH and FSH concentration there were no significant changes in

Text-fig. 1. Plasma hormone concentrations in (a) Cow 1 and (b) Cow 2, sampled during the mid-luteal phase of the oestrous cycle. The arrows indicate the time of injection of synthetic Gn-RH. Horizontal lines indicate 2 standard deviations above the mean pre-injection value, and all values above these lines are considered to be significant peaks.

progesterone concentration (Text-fig. 2a). However, in Cow 15 (Text-fig. 2b) there was a significant increase, lasting 2.3 h, in plasma progesterone concentration 4.5 h after Gn-RH injection. The mean plasma LH (1.7 ng/ml) and progesterone (0.56 ng/ml) concentrations in

this cow before Gn-RH injection were greater than those of the other animals in this group (LH 0.2–0.89 ng/ml; progesterone <0.1–0.38 ng/ml) and significant fluctuations in concentration were observed only in this animal. When the response to Gn-RH injection was assessed in terms of area under the induced LH and FSH peaks, the LH response in Cow 15 (12 793 mm²) was greater than that of the other animals (1665–8047 mm²) and the FSH response (5424 mm²) was the second highest of the group (1140–6156 mm²). Cow 15 began to cycle within 6 days of treatment (as indicated by an increase in milk progesterone concentration) whereas none of the other cows ovulated within 16 days of treatment.

Text-fig. 2. Plasma hormone concentrations in (a) Cow 14 and (b) Cow 15 sampled on Day 10 *post partum*. The arrow indicates the time of injection of synthetic Gn-RH. Cow 14 was typical of 7/8 of the cows studied but Cow 15 was the only one in which a significant increase in plasma progesterone was obtained in response to the Gn-RH. Horizontal lines indicate 2 standard deviations above the mean pre-injection value, and all values above these lines are considered to be significant peaks.

The 4 cows injected on or before Day 5 *post partum* had a significantly lower LH response to Gn-RH (mean $\pm$ s.e.m. area of induced peak 3091 ± 499 mm²) than the 4 cows injected on Days 7–10 *post partum* (8841 ± 2090 mm²). There was, however, no difference between these times in the FSH response (Day 5 mean area 3891 ± 958 mm²; Day 7–10 mean area 2715 ± 992 mm²).

Discussion

The short duration of the hormone peaks measured in these animals (often less than 30 min) emphasizes the importance of frequent sampling for studies of this type. Although in dairy cows with a functional corpus luteum there was a significant temporal relationship between plasma peaks of FSH and progesterone as well as of LH and progesterone, the latter correspondence was more marked. The relationship between FSH and progesterone may be indirect, occurring because the release of LH is related to that of FSH, and the immediate increase in progesterone following Gn-RH injection in cows with a functional corpus luteum is probably due to stimulation by LH rather than FSH. A positive relationship between LH and progesterone

release has been reported *in vitro* (Armstrong & Black, 1966; Seifart & Hansel, 1968) and passive immunization studies have suggested that LH is the main luteotrophic factor in the cow (Hoffmann *et al.*, 1974).

The lack of progesterone response to injection of Gn-RH in all but one of the anoestrous cows suggests that luteal tissue has to be present for such a response to be elicited. The one animal that did respond to Gn-RH had a higher pre-treatment plasma level of progesterone and LH and a higher pituitary responsiveness to Gn-RH than did the others in this group. In many animals there is an elevated plasma progesterone concentration before the first post-partum ovulation (see Donaldson, Bassett & Thorburn, 1970; Robertson, 1972; Humphrey, Koritnik, Kaltenbach, Dunn & Niswender, 1976; Lamming & Bulman, 1976; Lavoie & Moody, 1976; Lamming, 1978) which may be associated with changes in gonadotrophin secretion. This was probably the situation in this particular cow since it began to show ovarian cycles within 6 days of treatment. If this is so, then it is tempting to speculate that there was luteal tissue present in the ovaries of this animal at the time of treatment, i.e. before the first ovulation. Rectal examination of other anoestrous cows after parturition has revealed the presence of small palpable ovarian structures concurrent with small increases in milk progesterone concentration before the first post-partum ovulation (A. R. Peters & J. P. Foster, unpublished observation). These structures were no longer palpable after the milk progesterone concentration had decreased to basal values. It may be possible to demonstrate the presence of luteal tissue in bovine ovaries by a positive progesterone response to Gn-RH injection.

The lack of an ovulatory response to Gn-RH in most of the anoestrous dairy cows is probably due to the early time *post partum* at which they were injected. The pituitary LH response to Gn-RH is reported to be low in the first few days *post partum* and to increase thereafter (Lamming, 1978; Schallenberger, Schams & Zoltmeier, 1978) and this was confirmed in these animals. This increase in LH responsiveness to Gn-RH during the first 10 days *post partum* may be responsible for the increase in endogenous LH release which occurs at this time (unpublished data).

The relationship between peaks of LH and FSH is not as clear-cut as might perhaps be expected if these two hormones are released by the same releasing hormone. Separate mechanisms may therefore operate for at least the tonic release of LH and FSH. In the mare, the LH/FSH response ratio to exogenous Gn-RH changes significantly during the oestrous cycle (Foster, Evans & Irvine, 1979) and a different LH and FSH release pattern could therefore be explained by the different way in which pituitary gonadotrophs respond to a common releasing hormone.

The relationship between LH and progesterone is obviously important in the control of cow ovarian cycles; the stimulation of progesterone secretion by LH in animals with a corpus luteum provides an important luteotrophic influence. The results from early post-partum anoestrous cows suggest that LH is not capable of stimulating progesterone secretion at this stage but that it may be capable of doing so a few days before the first ovulation when responsive ovarian tissue is apparently present.

Studies of cyclic and seasonally anoestrous ewes have shown a close relationship between episodic release of LH and oestradiol-17 β (Baird *et al.*, 1976; Scaramuzzi & Baird, 1979), but a possible relationship between LH and progesterone in sheep during the luteal phase was not demonstrated. Since both prolactin and LH are necessary luteotrophins in sheep (Schroff, Klindt, Kaltenbach, Graber & Niswender, 1971; Denamur, Martinet & Short, 1973), it is possible that such a distinct relationship between progesterone and LH may not exist.

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