Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of Gn-RH

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Summary. Five acyclic spring-calving beef cows (20–40 days *post partum*) were bled every 15 min for 60 h and thereafter every 6 h for 5 days. Gn-RH (5 µg) was injected every 2 h for 48 h, starting 12 h after sampling began. Pulsatile patterns of LH release occurred synchronously in response to injection and 4 of the 5 treated animals subsequently ovulated and completed at least one full ovarian cycle. Four of 6 similar control cows were bled every 10 min for 8-h periods at equivalent times *post partum*. Pulses of LH were seen after approximately Day 25 *post partum* with a mean pulse frequency of 0.5 per h. There was little evidence of a discrete pulsatile mode of FSH release in any of the treated or control cows. The time to the first significant progesterone rise in the 4 treated and ovulating cows (34.5 ± 5.6 days *post partum*) was significantly shorter ($P < 0.05$) than in the 6 control animals (66.3 ± 11.4 days).

Introduction

Suckling beef cows tend to undergo longer and more variable acyclic intervals *post partum* than do milked dairy cows (Wiltbank & Cook, 1958; Casida *et al.*, 1968; Oxenreider, 1968). This may be related to intensity of the suckling stimulus, plane of nutrition, breed, age (Inskeep & Lishman, 1979) and season of calving (Peters, 1980). These factors can lead to extended individual calving intervals and extended calving seasons in beef herds, both of which are economically undesirable (Melrose, 1979).

A pulsatile or episodic pattern of LH release develops in acyclic milked dairy cows after about Day 10 *post partum*, this pattern being absent or much less apparent in intensively suckling dairy cows (Carruthers & Hafs, 1980; Peters, Lamming & Fisher, 1981b). In post-partum suckling beef cows pulsatile patterns of LH secretion did not develop until at least Day 20 *post partum* (G. M. Riley, unpublished data, see review by Lamming, Wathes & Peters, 1981). A high frequency of pulsatile LH secretion has also been observed, starting a few days before the preovulatory LH surge, during the bovine oestrous cycle (Rahe, Owens, Fleeger, Newton & Harms, 1980). It has been suggested that the development of this pulsatile pattern in the post-partum acyclic cow is a pre-requisite for initiation of ovarian activity and that the frequency of pulses may be important in determining the timing of the first ovulation *post partum* (Peters *et al.*, 1981b). Knobil (1980) has provided evidence from primate studies that both amplitude and frequency of Gn-RH release are important in controlling the pattern of release of gonadotrophins and ovarian activity. Plasma FSH concentrations rise rapidly at or

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soon after parturition in the cow (Dobson, 1978; Schams *et al.*, 1978; Webb, Lamming, Haynes & Foxcroft, 1980) but, to our knowledge, a pulsatile pattern of FSH secretion has not been described in this species.

The following experiment was carried out to induce pulsatile LH (and FSH) secretion in spring-calving post-partum beef cows which tend to be acyclic for longer than are autumn calvers (Peters, 1980) and to determine whether the establishment of this pattern would result in ovulation and the resumption of ovarian cycles.

Materials and Methods

Five spring-calving Hereford × Friesian cows (Nos 3, 5, 15, 601 and C) each suckling 2 calves were used between Days 20 and 40 *post partum*. Blood samples were taken via indwelling jugular venous catheters at 15-min intervals for 60 h and thereafter every 6 h for a further 5 days. After the first 12 h of sampling, 5 µg Gn-RH in 5 ml sterile saline (0.9% w/v NaCl) was injected i.v. at 2-h intervals for 48 h.

Four of 6 similar control cows were bled at 10-min intervals for 8-h periods at similar times *post partum* (Table 1).

Table 1. Time *post partum* at which blood samples were taken from control cows for 8-h periods at 10-min intervals

Cow		Days <i>post partum</i>			
2	20*	27	35	—	
211	17*	25	33	—	
169	17*	24*	32	40	
304	16*	23*	30	38	

* During these periods there was no evidence of pulsatile LH release.

Blood samples were assayed for LH (reference standard NIH-LH-B9) by the radioimmunoassay procedure as described by Webb, Lamming, Haynes, Hafs & Manns (1977) and for FSH (reference standard NIH-FSH-B1) by the heterologous assay of Webb *et al.* (1980). Luteal function of all 11 cows was monitored by milk progesterone measurement as described by Bulman & Lamming (1978). The assay reliability criteria did not differ significantly from those previously described (Table 2). The ovaries of all the cows were examined *per rectum* for the presence of corpora lutea 10 days after the end of Gn-RH treatment.

Table 2. Reliability criteria of radioimmunoassay procedures

	Sensitivity (ng/ml)	Coefficients of variation (%)	
		Intra-assay	Inter-assay
Plasma LH	0.6	6.5	8.0
Plasma FSH	20.3	8.3	12.0
Milk progesterone	0.4	9.1	11.0

For each treated cow mean LH and FSH concentrations were determined for the pre-treatment period. Values greater than 2 standard deviations above these means were taken as being significant elevations above the mean (Foster, Lamming & Peters, 1980). An LH peak was described as a pulse or episode if the highest point was at least 50% higher than the adjacent

baseline, there were at least two consecutive elevated points between troughs and the peak decayed at a rate no greater than that allowed by the half-life of bovine LH in plasma of approximately 35 min (Schams & Karg, 1969).

Results

The plasma LH profiles over 8 h for 2 control cows are shown in Text-fig. 1. Pulsatile LH patterns (peak height 1.5–4.5 ng/ml) occurred in the control cow profiles taken after Day 25 *post partum* (see Table 1). Mean pulse frequency in these profiles was 0.50 ± 0.07 (s.e.m.) pulses per h. LH pulses occurred in only 3 of the treated cows in the 12-h pretreatment period (peak heights 1.5–3.0 ng/ml) with a mean frequency of 0.20 ± 0.07 per h. The mean plasma LH concentration in control cow profiles taken before the appearance of pulses was 0.89 ± 0.02 ng/ml, compared to 0.77 ± 0.03 ng/ml for the pretreatment period in the injected cows.

Text-fig. 1. Plasma LH concentrations in the control Cows 211 and 169. A distinct pulsatile pattern of LH had developed at Days 25 and 33 *post partum* in Cow 211 and at Day 32 in Cow 169.

Injections of Gn-RH induced pulses of LH, although the peak heights (range 1.5–5.0 ng/ml) did not always reach statistical significance (e.g. see Text-fig. 2). The LH concentrations had invariably returned to baseline before the next injection 2 h later. The LH response sometimes decreased during the injection period and even ceased altogether in Cows C and 15 (see Text-fig. 2a).

FSH concentrations were measured in each 15-min plasma sample from 2 treated cows

Text-fig. 2. Plasma LH and FSH concentrations (15-min samples except for Cow C FSH which was hourly) in (a) Cow C and (b) Cow 3 for 12 h before, during, and for 18 h after injections of Gn-RH every 2 h. The timing of Gn-RH injections is indicated by the vertical lines. The injections caused regular pulsatile increases in LH concentrations in both cows although the response declined after about 36 h in Cow C and eventually ceased 6–8 h before the LH surge. There was no pulsatile response of FSH to the Gn-RH injections. Horizontal lines indicate 2 standard deviations above the mean pre-injection concentration and all values above these lines are considered to be significant elevations above the mean.

(Nos 3 and 5, e.g. Text-fig. 2b). There was little evidence of a discrete pulsatile mode of secretion; concentrations appeared to fluctuate in a random manner.

Injections of Gn-RH did not consistently cause elevations in plasma FSH concentrations (see Text-fig. 2b). Mean concentrations in Cows 3 and 5 immediately before each injection were 32.0 ± 3.0 and 33.8 ± 3.0 ng/ml and 5 min after injection 34.8 ± 3.0 and 38.3 ± 4.5 ng/ml, respectively (not significant, paired *t* test). Significant elevations in plasma FSH concentrations did occur but not directly after individual Gn-RH injections.

In the rest of the treated and the control cows, only hourly samples were assayed for FSH. The mean concentrations of FSH in plasma over the pretreatment period for Cow 3 were 34.7 ± 1.3 ng/ml from the 15-min samples and 33.5 ± 2.5 ng/ml from hourly samples, and for Cow 5 the values were 26.1 ± 1.3 ng/ml and 26.2 ± 2.2 ng/ml respectively. Mean FSH concentrations for the treated and control cows are shown in Table 3. There was no significant difference between the mean FSH concentrations in the control cows and for treated cows during the pretreatment period. There was a significant decrease in mean FSH concentrations over the first 12 h of treatment compared to the pretreatment period, but concentrations had then increased significantly by the fourth 12-h periods (*d* test; Bailey, 1959; see Table 3).

Four of the 5 treated cows showed full preovulatory type gonadotrophin surges after treatment and completed at least one ovarian cycle as determined by milk progesterone values (Text-fig. 3). The fifth cow (No. 601) showed pulsatile LH release in response to injection of Gn-RH but no preovulatory surge of LH or FSH. A transient rise in milk progesterone occurred in this cow between Days 7 and 12 after treatment (Text-fig. 3). No preovulatory type LH surges were seen in the 4 control cows which were bled and none of the 6 control cows ovulated

Table 3. Mean ($\pm$ s.e.m.) FSH concentrations in control and Gn-RH-treated cows

	No. of cows	FSH conc. (ng/ml)
Control cows	6	*33.8 $\pm$ 3.3
Treated cows	5	
Pre-treatment period		34.0 $\pm$ 3.3
Treatment period		
0-12 h		**28.1 $\pm$ 3.2
12-24 h		*29.9 $\pm$ 3.2
24-36 h		*39.0 $\pm$ 3.1
36-48 h		***53.0 $\pm$ 6.8

* Not significantly different from pretreatment period by *d* test (Bailey, 1959).

Significantly different from pretreatment period, ** $P < 0.01$, *** $P < 0.001$.

Text-fig. 3. Mean ($\pm$ s.e.m.) milk progesterone concentrations in the 4 treated cows (●) in which gonadotrophin surges occurred following multiple Gn-RH injections (every 2 h for 48 h starting on Day 0) and Cow 601 (○) in which no gonadotrophin surge was detected following Gn-RH injections.

around this time. The time from calving to first ovarian activity, as determined by a sustained rise (3 ng/ml) in milk progesterone concentrations, was significantly shorter ($P < 0.05$) in the 4 treated cows which ovulated (34.5 ± 5.6 days) than in the 6 control cows (66.3 ± 11.6 days). The fifth treated cow had not begun to cycle by Day 60 *post partum*. Rectal palpation of the 5 treated cows revealed a single corpus luteum in one ovary of the 4 responding cows and a lack of palpable structures in the non-responding cow (No. 601), thus confirming the milk progesterone data.

Discussion

It seems likely that pulsatile LH secretion occurs in response to pulsatile endogenous Gn-RH secretion (Rahe *et al.*, 1980), and it has been suggested that a development of pulsatile LH release in the post-partum cow is a pre-requisite for the onset of ovulation and ovarian cycles (Peters *et al.*, 1981b). Peters *et al.* (1981b) observed an LH pulse frequency of between 0.25 and 1.25 pulses per h in the post-partum dairy cow, commencing several days before the first ovulation and with a negative correlation between pulse frequency and the time to first ovulation. In the present experiment, we were attempting to mimic this situation in cows in which natural pulsatile LH secretion was likely to be infrequent. Therefore an intermediate exogenous Gn-RH pulse frequency of 0.5 per h (one per 2 h) was chosen. The dosage of

5 µg was selected on the basis of previous dose-response experiments (A. R. Peters and M. W. Fisher, unpublished data).

The incidence of natural LH pulses was similar in the pretreatment period of the treated cows to that in the control cows. Multiple injections of Gn-RH apparently induced regular synchronous pulsatile LH secretion as evidenced by the plasma profiles in Text-fig. 2. Although, in some cows, the LH response began to decrease after about 36 h of the treatment period and ceased altogether in 2, gonadotrophic surges occurred in 4 of the 5 treated cows.

In the natural cycle and in cows in which ovulation is induced by exogenous progesterone withdrawal, increases in both tonic LH secretion and LH pulse frequency have been demonstrated during the 24 h before the LH surge (Rahe *et al.*, 1980; Webb *et al.*, 1980; Peters, Kingsley & Riley, 1981a), although in the pig there is a decrease in tonic and pulsatile LH concentrations in the period immediately preceding the LH surge (Foxcroft, Pomerantz & Nalbandov, 1975; Edwards, 1980). Pulses of oestradiol-17β closely follow pulses of LH in the cyclic and seasonally anoestrous ewe (Baird, Swanson & Scaramuzzi, 1976; Scaramuzzi & Baird, 1979) and it seems likely that frequent pulses of LH during the early follicular phase of the oestrous cycle stimulate oestradiol secretion in the cow. Rising oestradiol concentrations eventually result in the preovulatory gonadotrophin surge by the positive feedback mechanism. In the present experiment the inhibition of LH secretion in some cows after approximately 36 h of treatment may have been due to negative feedback of higher concentrations of oestradiol than those produced in the presurge period of the normal cycle. Alternatively, the pulse frequency used may not have mimicked the natural neuronal discharge controlling endogenous Gn-RH release, with a resultant depression of gonadotrophin secretion, as shown in the monkey (Knobil, 1980).

Previous work in the cow has indicated that, in contrast with LH, FSH is not released as discrete pulses (see Lamming, *et al.*, 1981). Due to lack of data on the half-life in plasma of bovine FSH and the inconsistent variations in plasma concentrations in the present experiment, we were unable to identify a pulsatile pattern for this hormone. All significant rises in LH concentrations above the baseline coincided with Gn-RH pulses (although not *vice versa*) and tended to occur at regular intervals. Significant rises in FSH concentrations occurred at times unrelated to Gn-RH injections, albeit more frequently as the baseline value increased before the preovulatory surge. It was therefore considered that FSH did not behave in a pulsatile manner. Gn-RH can stimulate immediate FSH release but the dose of Gn-RH used was much larger than in the present study (Foster *et al.*, 1980).

Mean FSH concentrations measured in samples taken hourly were in good agreement with those taken at 15-min intervals and therefore only hourly samples were measured for FSH in some cows. Mean FSH concentrations decreased over the first 12 h of the treatment period and then began to rise after approximately 24 h of treatment. Changes in FSH concentrations in treated cows could not be directly related to Gn-RH injection and the reason for the initial decrease is not clear. However, preovulatory type surges of both FSH and LH occurred in 4 of the 5 treated cows and these were followed by at least one full ovarian cycle, as indicated by a normal milk progesterone profile. It is possible that the short-lived rise in progesterone concentrations of Cow 601 may have been due to the presence of a luteinized follicle, growth of which was stimulated by the Gn-RH injections but which failed to ovulate.

Ovulation and ovarian cycles have also been induced by frequent Gn-RH injections in prepubertal monkeys (Wildt, Marshall & Knobil, 1980) and in seasonally anoestrous ewes (B. Mcleod & W. Haresign, personal communication). In the present experiment, Gn-RH treatment caused the 4 responding cows to ovulate and exhibit an ovarian cycle significantly earlier than did the control cows. It remains to be determined whether the pulse mode of Gn-RH administration is important to induce ovulation and luteal function or whether a continuous administration, e.g. by infusion or implant, would achieve the same result. If the latter were so then this would offer a convenient form of treatment for the post-partum anoestrous beef cow.

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