Pituitary and ovarian responses of post-partum acyclic beef cows to continuous long-term GnRH and GnRH agonist treatment

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Summary. Post-partum acyclic beef cows received continuous long-term treatment with GnRH (200 or 400 ng/kg body wt/h) or the GnRH agonist buserelin (5.5 or 11 ng/kg body wt/h) using s.c. osmotic minipumps which were designed to remain active for 28 days. All treatments increased circulating LH concentrations whereas FSH remained unchanged. Ovulation and corpus luteum (CL) formation as judged by progesterone concentrations ≥ 1 ng/ml occurred in 0/5 control, 4/5 200 ng GnRH, 4/4 400 ng GnRH, 4/5 5.5 ng buserelin and 3/5 11 ng buserelin cows. The outstanding features of the progesterone profiles were the synchrony, both within and across groups, in values ≥ 1 ng/ml around Day 6, and the fact that most CL were short-lived (4–6 days). Only 3 cows, one each from the 400 ng GnRH, 5.5 ng buserelin and 11 ng buserelin groups, showed evidence of extended CL function. Cows failed to show a second ovulation which was anticipated around Day 10 and this could have been due to insufficient FSH to stimulate early follicular development, or the absence of an endogenously driven LH surge. The highest LH concentrations for the respective groups were observed on Days 2 and 6 and by Day 10 LH was declining, although concentrations did remain higher than in controls up to Day 20. The fact that LH remained elevated in treated cows, but CL were nevertheless short-lived, suggested that endogenous LH secretion in post-partum cows following a GnRH-induced ovulation is not limiting to CL function, and that other factors cause early demise of the first CL. The doses of GnRH used were not directly limiting to CL function since in a second trial extended CL were observed when GnRH was preceded by progesterone.

Keywords: cow; post partum; continuous GnRH; ovary

Introduction

Exogenous gonadotrophin-releasing hormone (GnRH) can be used to promote ovarian activity and ovulation in acyclic post-partum cows. In initial studies GnRH was administered in pulsatile regimens which were designed to mimic endogenous patterns of LH secretion (Riley *et al.*, 1981; Walters *et al.*, 1982). However, more recent work has shown that ovulation can also be induced in post-partum cows by continuous low-dose infusion of GnRH over 2–5 days (Capel *et al.*, 1984; Jagger *et al.*, 1987; Peters *et al.*, 1987) or bolus injection (Smith *et al.*, 1987). Corpora lutea (CL) arising from GnRH-induced ovulations *post partum* tend to have a short life-span which can be

extended if GnRH is preceded by a period of progesterone treatment (Troxel & Kesler, 1984; Rutter *et al.*, 1985; Peters *et al.*, 1987; Smith *et al.*, 1987). The first ovulation *post partum* under normal conditions also usually produces a short-lived CL (Manns *et al.*, 1983) and the suggestion has been made, although the mechanism remains unclear, that progesterone produced by the first CL *post partum* is a prerequisite for a normal CL to develop in the ensuing cycle (Rutter *et al.*, 1985).

The requirement of progesterone pretreatment detracts from practical development of GnRH therapies in post-partum beef cows. An alternative approach was suggested by the study of Webb *et al.* (1977) in which GnRH was given as two injections around 10 days apart. Cows which responded to the first injection with a short-lived CL showed normal CL function after the second injection. This GnRH schedule essentially achieved the same objective as progesterone pretreatment by utilizing endogenous progesterone priming.

GnRH therapies would become particularly attractive if GnRH could be administered as a single slow-release formulation. The present study therefore examined whether sustained CL function can be achieved with continuous long-term GnRH therapy. It was considered that chronic GnRH treatment was unlikely to extend the life-span of the first induced CL, but that it might induce a second ovulation with a normal CL. Post-partum acyclic cows received continuous GnRH from subcutaneous osmotic minipumps designed to remain active for 28 days and observations were made on pituitary and ovarian responses.

Materials and Methods

Animals. Shorthorn cows ranging from 5 to 8 years of age (400–450 kg body weight) and which were in at least their third post-partum period were used in the first trial. Cows calved during early winter and were maintained under field conditions with their calves in a temperate environment. A group of 2½-year-old post-partum Hereford heifers that were similarly managed were used in a second trial to examine the effects of progesterone pretreatment on responses to continuous long-term GnRH.

Experimental procedures. The treatment groups and days *post partum* (mean $\pm$ s.e.m.) for cows in the first trial were as follows: control (N = 5), 16.6 $\pm$ 1.7; 200 ng GnRH/kg body wt/h (N = 5), 17.6 $\pm$ 1.8; 400 ng GnRH (N = 4), 18.0 $\pm$ 1.6; 5.5 ng buserelin (N = 5); 17.0 $\pm$ 1.7; 11 ng buserelin (N = 5), 16.8 $\pm$ 1.9. Native GnRH and the GnRH agonist, buserelin ([D-Ser(tBu)⁶,Pro⁹NEt]¹GnRH), were delivered continuously using osmotic minipumps (2ML4, Alza Corporation, Palo Alto, CA, USA) which were placed subcutaneously in the lower neck under aseptic conditions and local lignocaine anaesthesia. Cows received one or two pumps depending on whether they were in the low- or high-dose treatment groups, both for GnRH and buserelin. Dose rates were calculated for a body weight of 450 kg. Cows were bled at 30 min intervals for 4 h by jugular venepuncture on the day before treatment (Day -1) and again on Days 2, 6, 10 and 20 of treatment. Additional single blood samples were taken on Days 4, 8, 12, 16 and 28 at which time the pumps were removed. Heparinized blood samples were kept on ice and the plasmas obtained after centrifugation were stored at -20°C until required for hormone assay.

To determine whether the doses of GnRH used in the first trial might be limiting to CL function primiparous heifers received 200 or 400 ng GnRH, or 5 ng of the GnRH agonist, D-Trp⁶GnRH, with or without 7 days pretreatment with progesterone using intravaginal devices (PRID, Bomac Laboratories, Sydney, Australia). Days *post partum* at the start of GnRH treatment were (N = 5/group): control, 41.3 $\pm$ 3.8; 200 ng GnRH, 41.1 $\pm$ 3.8; progesterone + 200 ng GnRH, 41.8 $\pm$ 3.2; 400 ng GnRH, 42.1 $\pm$ 3.5; progesterone + 400 ng GnRH, 41.3 $\pm$ 2.6; 5 ng D-Trp⁶, 39.7 $\pm$ 3.5; progesterone + 5 ng D-Trp⁶, 42.2 $\pm$ 3.7. Osmotic minipumps were used as described above and blood samples were taken on Days -7, -1, 0, 6, 11, 14, 18, 22 and 29 relative to the start of GnRH treatment.

Hormone assays. Plasma concentrations of LH and FSH were determined by double-antibody radioimmunoassays (D'Occhio *et al.*, 1986) only in serial samples collected in the first trial. An extraction single-antibody radioimmunoassay (D'Occhio *et al.*, 1988) was used to measure plasma progesterone concentrations in both trials. Progesterone concentrations $\geq$ 1 ng/ml were taken as being indicative of ovulation and formation of active CL. Intra- and inter-assay coefficients of variations for all assays were < 10% based on duplicate samples. Sensitivities were 4 ng FSH/ml, 0.2 ng LH/ml and 0.1 ng progesterone/ml.

Statistical analyses. Data for LH and FSH in the first trial were transformed to log₁₀ before testing for treatment effects using analysis of variance procedures. The 5 days were analysed both separately and together. Using Day -1 concentrations as a covariate (log scale) in analysis of Days 2, 6, 10 and 20 did not influence the analyses. The data (log scale) were also orthogonally transformed to give a mean, linear, quadratic and cubic effect for each cow and these were analysed for treatment differences, firstly with all 5 days for the shape of the response over time, and then

with the 4 treatment days. Increases in LH were determined to be a pulse if the rise was >40% above basal concentrations and was followed by a series of points that showed a progressive decline. Basal LH concentrations were calculated from points not associated with the apex or descending portions of pulses.

Results

Luteinizing hormone

Plasma LH concentrations for cows in the first trial were uniformly low on Day -1 and there were no differences ($P > 0.05$) between groups; however, pulses of LH were observed in some cows (Table 1). LH values in control cows remained constant during the study whereas for all treated groups LH increased sharply up to Days 2-6 and then decreased, still being slightly, but significantly ($P < 0.01$), above pretreatment concentrations on Day 20. Treated cows consequently had higher ($P < 0.01$) plasma LH concentrations than did controls on Days 2, 6, 10 and 20. Increased LH concentrations during treatment resulted primarily from elevated basal concentrations rather than increased pulsatile secretion. However, pulses of LH were observed at various times during treatment, particularly in cows receiving 200 ng GnRH (Table 1). Differences in LH between treated groups were observed only on Day 6 when concentrations were higher ($P < 0.01$) for cows receiving 200 ng GnRH. However, when Days -1 to 20 were analysed together, the treated groups were not different in the mean, linear, or quadratic or cubic effects. On the $\log_{10}$ scale the 4 treatments had the same significant quadratic and cubic components over the 20-day period but the controls did not change.

Table 1. Plasma LH concentrations* in post-partum cows receiving continuous GnRH or buserelin

Treatment (/kg body wt/h)	No. of cows	LH conc. (ng/ml)				
		Day -1	Day 2	Day 6	Day 10	Day 20
Control	5	0.33 $\pm$ 0.03 (1)	0.31 $\pm$ 0.04 (0)	0.32 $\pm$ 0.04 (0)	0.32 $\pm$ 0.03 (0)	0.38 $\pm$ 0.04 (1)
200 ng GnRH	5	0.43 $\pm$ 0.03 (2)	0.80 $\pm$ 0.13 (2)	0.98 $\pm$ 0.14 (4)	0.69 $\pm$ 0.07 (4)	0.63 $\pm$ 0.10 (5)
400 ng GnRH	4	0.35 $\pm$ 0.06 (1)	0.72 $\pm$ 0.09 (1)	0.59 $\pm$ 0.09 (1)	0.63 $\pm$ 0.08 (1)	0.48 $\pm$ 0.10 (2)
5.5 ng buserelin	5	0.33 $\pm$ 0.04 (0)	0.61 $\pm$ 0.08 (1)	0.57 $\pm$ 0.06 (0)	0.56 $\pm$ 0.08 (1)	0.45 $\pm$ 0.03 (0)
11 ng buserelin	5	0.34 $\pm$ 0.02 (0)	0.67 $\pm$ 0.06 (0)	0.54 $\pm$ 0.03 (0)	0.53 $\pm$ 0.03 (0)	0.50 $\pm$ 0.04 (0)

Values are mean $\pm$ s.e.m. of individual 4-h means; numbers in parentheses indicate no. of cows with pulsatile LH secretion.

*At each sampling period cows were bled at 30-min intervals for 4 h.

Follicle-stimulating hormone

In contrast to LH, circulating FSH was not increased during treatment with GnRH or buserelin (Table 2). FSH concentrations therefore remained constant during the study in control and treated cows. There was no evidence of pulsatile FSH secretion, before or during treatment.

Progesterone

None of the control cows in the first trial showed progesterone concentrations ≥ 1 ng/ml whereas 15/19 treated cows had elevated progesterone values. The outstanding features of the

Table 2. Plasma FSH concentrations in post-partum cows receiving continuous GnRH or buserelin

Treatment (/kg body wt/h)	No. of cows	FSH conc. (ng/ml)				
		Day -1	Day 2	Day 6	Day 10	Day 20
Control	5	12.7 ± 1.7	11.7 ± 1.8	11.6 ± 1.4	12.2 ± 1.1	11.3 ± 1.1
200 ng GnRH	5	15.4 ± 3.6	16.7 ± 3.4	13.3 ± 2.6	14.8 ± 3.5	16.1 ± 2.9
400 ng GnRH	4	13.5 ± 2.2	14.6 ± 1.5	13.4 ± 2.4	13.7 ± 1.8	14.4 ± 2.7
5.5 ng buserelin	5	12.3 ± 1.5	14.1 ± 1.6	13.9 ± 2.1	13.1 ± 1.3	14.4 ± 1.0
11 ng buserelin	5	14.8 ± 2.6	14.1 ± 1.7	13.1 ± 2.1	12.7 ± 2.0	14.8 ± 2.0

At each sampling period cows were bled at 30-min intervals for 4 h and the results are presented as the mean ± s.e.m. of individual 4-h means.

progesterone profiles in cows receiving GnRH or buserelin were the synchrony, both within and across groups, in appearance of elevated progesterone around Day 6, and the fact that most CL appeared to be short-lived (Table 3). Only 3 cows, one from each of the 400 ng GnRH, 5.5 ng buserelin, and 11.0 ng buserelin groups, showed evidence of extended CL function. Two of these cows continued to have elevated progesterone on Day 28 but it was not possible to conclude whether this was due to persistence of the first CL, or the result of a second ovulation between Days 20 and 28.

Table 3. Ovarian responses in post-partum cows receiving continuous GnRH or buserelin

Treatment (/kg body wt/h)	No. of cows	Progesterone ≥ 1 ng/ml	Corpus luteum	
			Short-lived	Extended
Control	5	0	0	0
200 ng GnRH	5	4	4	0
400 ng GnRH	4	4	3	1
5.5 ng buserelin	5	4	3	1
11 ng buserelin	5	3	2	1

In the second trial pretreatment with progesterone extended the life-span of CL induced by GnRH and D-Trp⁶ (Fig. 1). This indicated that the doses of GnRH used in the first trial were not directly limiting to CL function. The short life-span CL in heifers that received GnRH or D-Trp⁶ alone were similar to those observed in cows in the first trial.

Discussion

The present study sought to determine whether continuous long-term GnRH treatment could be used to induce ovulation with normal CL function in the post-partum cow. In the first trial the majority of cows receiving GnRH or buserelin ovulated, as judged by increases in plasma progesterone, but CL were short-lived. Plasma LH concentrations were increased for at least 20 days yet only 5/14 cows with elevated progesterone on Days 6 and 8 continued to have high progesterone on Day 10. Chronically increased LH secretion therefore did not sustain the activity of CL associated with the first induced ovulation. It has been concluded from previous studies that short-lived CL do not have reduced LH receptor concentrations (Rutter *et al.*, 1985); however, it remained

Fig. 1. Plasma progesterone profiles in (a) control post-partum heifers and post-partum heifers that received (b) 200 ng GnRH/kg body wt/h or progesterone + 200 ng GnRH/kg body wt/h, (c) 400 ng GnRH/kg body wt/h or progesterone + 400 ng GnRH/kg body wt/h, (d) 5 ng D-Trp⁶/kg body wt/h or progesterone + 5 ng D-Trp⁶/kg body wt/h. Heifers received progesterone from Day -7 to Day -1 and continuous GnRH or D-Trp⁶ treatment was started on Day 0.

unclear whether endogenous LH secretion was sufficient to sustain CL function. In the first trial the highest LH concentrations were observed in cows receiving 200 ng GnRH and yet none of these cows had an extended luteal phase. Also, the 3 cows that showed evidence of continued progesterone secretion did not necessarily have the highest LH for their respective groups. It would appear, therefore, that endogenous LH secretion in post-partum cows following a GnRH-induced pre-ovulatory LH surge is not limiting to CL function, and that other factors cause early demise of the first GnRH-induced CL *post partum*.

Short life-span CL were reported to have increased numbers of large luteal cells (Manns *et al.*, 1983; Rutter *et al.*, 1985). These cells contain prostaglandin receptors and it is thought that under normal conditions an increase in large cells sensitizes the CL to prostaglandin (Fitz *et al.*, 1982). Small and large luteal cells are initially derived from theca and granulosa cells, respectively, and it has been suggested that a proportion of small cells may later differentiate into large cells (Niswender *et al.*, 1985; Hansel *et al.*, 1987; O'Shea, 1987). This secondary differentiation could occur prematurely in short-lived CL leading to earlier sensitization to prostaglandin (Rutter *et al.*, 1985). The hormonal requirements for normal differentiation of luteal tissue are unknown but a balance of LH and FSH might be necessary. FSH remained unchanged in the present study and both increased (Walters *et al.*, 1982; Jagger *et al.*, 1987) and decreased (Peters *et al.*, 1985, 1987) FSH secretion in response to GnRH has been reported. Williams *et al.* (1984) suggested that progesterone acts on the hypothalamus and pituitary to normalize LH and FSH secretion ensuring proper follicular maturation. If this proves to be the mechanism whereby pre-exposure to progesterone facilitates normal CL development, then FSH responses to GnRH might be of particular significance. A role for FSH in the final stages of follicular development was suggested by the failure to ovulate of GnRH-treated seasonally anoestrous ewes in which FSH was suppressed with bovine follicular fluid (McLeod & McNeilly, 1987). However, it was concluded from another study in ewes that facilitation of normal CL development by progesterone pretreatment was not related to FSH secretion (McLeod & Haresign, 1987). Rather, progesterone priming appeared to influence gonadotrophin binding and steroidogenesis in follicles (Hunter *et al.*, 1986).

In the first trial only 3/15 cows that had elevated progesterone also showed evidence of sustained CL function. It was not possible to conclude whether increased progesterone in 2 cows on Day 28 reflected continued activity of the first CL, or resulted from a second ovulation between Days 20 and 28. Webb *et al.* (1977) reported that normal CL function was induced by the second of two injections of GnRH given ~10 days apart. On Day 10 in the first trial progesterone had declined in 9/14 cows which showed increased progesterone on Days 6 and 8, but none of these cows showed evidence of a second ovulation. Similarly, no heifers receiving GnRH or D-Trp⁶ alone in the second trial showed a second ovulation. Progesterone inhibits follicular maturation by suppressing LH secretion rather than by direct actions on the ovary. Thus, pulsatile exogenous GnRH over 3–5 days can promote follicular growth and development in the presence of elevated progesterone (Glencross, 1987). If progesterone declines, the follicles can proceed to ovulation (Glencross, 1987). This sequence of events apparently failed to occur in the present study, even though LH remained elevated at Day 10 in the first trial. However, FSH was unchanged and it is therefore possible that FSH concentrations were inadequate to stimulate early follicles to a point where they could respond to LH. Oestradiol-17 β secretion would consequently have been insufficient to trigger an endogenous LH surge. Additional studies detailing patterns of secretion of oestradiol-17 β , LH and FSH in post-partum cows receiving continuous long-term GnRH are required to elucidate the factors which in the present study caused failure of a second ovulation. In some cows progesterone remained elevated for only 2–4 days which could be considered insufficient time to achieve progesterone priming. However, in ewes, 2 days' exposure to progesterone was adequate for normal development of subsequent CL (McLeod & Haresign, 1984). In the second trial heifers that showed extended CL function after progesterone pretreatment also apparently failed to undergo a second ovulation.

Continuous GnRH has been used to induce ovulation in seasonally (Wright *et al.*, 1983; McLeod *et al.*, 1983; Williams *et al.*, 1984) and post-partum (Williams *et al.*, 1984) acyclic ewes. Normal CL function in ewes requires progesterone pretreatment (McLeod *et al.*, 1982; Hunter *et al.*, 1986) and the same requirement was confirmed for cows in the present study. In contrast, fertile ovulations and normal CL have been induced in seasonally (Hyland *et al.*, 1985; Allen *et al.*, 1987) and post-partum (Allen *et al.*, 1987) acyclic mares with continuous GnRH in the absence of progesterone priming. Similar responses can be produced in post-partum acyclic sows with pulsatile GnRH (Cox & Britt, 1982; Armstrong & Britt, 1985). These species differences are consistent with

results of other studies which indicate that normal CL function requires progesterone priming in cows and ewes, but not in sows and mares.

In conclusion, continuous long-term therapy with GnRH or GnRH agonists induced ovulation in acyclic post-partum beef cows and heifers but CL were short-lived and a second ovulation with normal CL anticipated around Day 10 of treatment did not occur. Failure of a second ovulation could have been due to insufficient FSH to stimulate early follicular development; this would have precluded events leading to an endogenous LH surge. The similarity in gonadotrophin and ovarian responses observed with GnRH agonists at doses 20–80 times lower than those of GnRH illustrate the potential of agonists as practical substitutes in GnRH therapies.

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