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Induced ovulation of the first *postpartum* dominant follicle in beef suckler cows using a GnRH analogue

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There is a low incidence of ovulation of the first dominant follicle that develops in the early *postpartum* period of beef suckler cows, which prolongs the interval from calving to first ovulation. The objective of this study was to determine whether a single injection of a GnRH analogue would ovulate the first *postpartum* dominant follicle. Limousin × Friesian beef suckler cows were assigned at parturition, over two years (16 cows in year 1; 19 cows in year 2), to one of three treatments: (1) untreated (control; $n = 12$), (2) GnRH analogue (20 μg buserelin i.m.) administered in the growing–plateau phase of the first *postpartum* dominant follicle (GnRH-G; $n = 12$) and (3) GnRH analogue administered in the declining phase of the first *postpartum* dominant follicle (GnRH-D; $n = 11$). From day 8 or 9 *post partum*, the ovaries of each cow were examined daily by ultrasound to determine the time of GnRH injection and ovulation. Blood samples were collected daily for progesterone measurement to confirm ovulation and in year 2 to determine the duration of the first oestrous cycle. The mean (±SEM) number of days from parturition to development of the first dominant follicle was 11.0 ± 0.3, 10.3 ± 0.5 and 10.1 ± 0.7 for cows assigned to treatments 1–3, respectively ($P > 0.05$). The proportion of cows ovulating the first dominant follicle was higher ($P < 0.05$) following GnRH treatment (12 of 12 and 7 of 10; GnRH-G and GnRH-D, respectively) than with controls (2 of 12). The mean interval from parturition to first ovulation in all cows in the GnRH-G treatment was reduced (16.1 ± 0.8 days; $P < 0.01$) compared with the interval for cows in the GnRH-D treatment (24.5 ± 3.6 days) or controls (27.1 ± 2.5 days). There was no difference in the duration of the first luteal phase *post partum* in control (9.3 ± 3.0 days) and GnRH-treated cows (8.0 ± 1.0 and 9.6 ± 3.7 days; GnRH-G and GnRH-D, respectively) or in the proportion of cows with short cycles (4 of 7, 5 of 6 and 4 of 5; control, GnRH-G and GnRH-D, respectively). In conclusion, a single injection of GnRH analogue during the growing–plateau or declining phase of the first *postpartum* dominant follicle of beef suckler cows induced ovulation in most cows but did not alter the proportion of cows with short cycles compared with controls.

Introduction

Beef suckler cows have a longer *postpartum* anoestrous period than do dairy cows. Follicular growth resumes early after calving with the formation of the first dominant follicle, detected morphologically by ultrasound, within 10.2 days (Murphy *et al.*, 1990). There is a low (11%) incidence of ovulation of the first dominant follicle *post partum* (Murphy *et al.*, 1990). This is in contrast to dairy cows, in which the first dominant follicle ovulated in more than 70% of cows (Savio *et al.*, 1990). In beef suckler cows, there was recurrent growth and regression of dominant follicles with a mean of 3.2 ± 0.2 dominant follicles until first ovulation (Murphy *et al.*, 1990). Thus, the prolonged anoestrous period in beef cows is due to a failure of ovulation of dominant follicles rather than a delay in development of dominant follicles.

This early resumption of follicular development in both beef and dairy cows is due to the rise in FSH concentrations after parturition (Schallenberger, 1985). Lack of ovulation of the early dominant follicle is hypothesized to be due to inadequate LH pulse frequency, which results in low androgen production in the follicle (Fortune, 1986) and inadequate oestradiol positive feedback to induce the LH surge (Peters *et al.*, 1985). Factors affecting LH pulse frequency, such as suckling stimulus (Lamming *et al.*, 1982; Myers *et al.*, 1989), presence of the cow's own or an alien calf (Silveira and Williams, 1991), energy balance (Canfield and Butler, 1990) and body condition score of the cow (Wright *et al.*, 1992), will therefore ultimately affect the time of first ovulation and the duration of the *postpartum* interval.

The administration of LH or GnRH will induce ovulation in a variety of species, including cattle, depending on the follicular

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status at the time of treatment. In dairy cows, Britt *et al.* (1974) showed that GnRH (100 μg in an ear implant) during the early *postpartum* period could advance the time of first ovulation. A single injection of GnRH to *postpartum* anoestrous beef cows results in a significant rise in serum LH within 2 h of treatment (Wettemann *et al.*, 1982). GnRH administration will therefore overcome the inadequate secretion of pituitary LH in the early *postpartum* period. However, in previous studies with beef cows, in which the follicular status at the time of GnRH administration was unknown, the ovulatory response was variable. Single injections of GnRH or an analogue induced ovulation in 18–93% of cows (Fonseca *et al.*, 1979; Mawhinney *et al.*, 1979). Similar studies involving multiple injections of GnRH induced ovulation in 73–80% (Riley *et al.*, 1981; Walters *et al.*, 1982) and 10–42% of cows (Edwards *et al.*, 1983).

Because there is sequential development and atresia of dominant follicles in the majority of beef suckler cows before first ovulation, failure of some cows to ovulate following GnRH administration could be due to the state of development of the dominant follicle at the time of treatment. Our objective was therefore to determine whether the first *postpartum* dominant follicle, identified by ultrasound examination, could be ovulated using a single injection of a GnRH analogue. Specifically, we looked at the effect of administration of the GnRH analogue during either the growing–plateau phase of the first *postpartum* dominant follicle or the declining phase of the first *postpartum* dominant follicle. The growing–plateau phase, before emergence of a new wave of follicular growth, was selected to represent a physiologically active dominant follicle and the declining phase a dominant follicle undergoing atresia (Badinga *et al.*, 1992).

Materials and Methods

Animals and treatments

The experiment was carried out over 2 years using 16 Limousin × Friesian cows in year 1 and 19 Limousin × Friesian cows in year 2. All cows calved in spring (April–May) and were in their second to fifth lactations. Cows were housed indoors up to calving and fed a maintenance diet of grass silage with a mineral supplement in year 1 and grass silage with barley straw *ad libitum* in year 2; after calving, the cows and calves were run at pasture. They were randomly assigned at calving each year to one of three treatments: (i) controls ($n = 12$); (ii) 20 μg buserelin (Hoechst, Ireland, Ltd) administered (i.m.) during the growing–plateau phase of the first *postpartum* dominant follicle (GnRH-G; $n = 12$) and (iii) 20 μg buserelin administered (i.m.) during the declining phase of the first *postpartum* dominant follicle (GnRH-D; $n = 11$).

Ovaries of cows were scanned daily from days 8 or 9 *post partum* to the time of first ovulation and formation of the corpus luteum was confirmed, using an ultrasound scanner (Dynamic Imaging, Livingston, Scotland) fitted with a 7.5 MHz transrectal linear probe, as described previously (Murphy *et al.*, 1990). A dominant follicle was defined as the largest follicle, ≥ 10 mm in diameter and at least 2 mm larger than other follicles present on either ovary, that suppressed the growth of other follicles. The growing–plateau phase of the first dominant follicle was defined as the time when the diameter of the dominant follicle increased in size or remained at the same value for two consecutive days and the declining phase was defined as the time when the diameter decreased by ≥ 3 mm over ≥ 2 days. The experiment was terminated 4–5 days after first ovulation in year 1 and either when first oestrus was detected or 25 days after first ovulation in year 2. Blood samples were collected once a day throughout the experiment. Serum samples were obtained after centrifugation at 700 *g* for 20 min, following storage at room temperature for 1 h and at 4°C for 18–24 h; they were frozen at −20°C until assayed for progesterone concentrations using the non-extraction radioimmunoassay described by Ronayne and Hynes (1990). The intra-assay coefficients of variation ($n = 4$) for samples containing 0.85 and 3.13 ng progesterone ml^{-1} were 6.6 and 5.6%, respectively. The interassay coefficients of variation ($n = 9$) for the same samples were 7.6 and 8.3%, respectively. The sensitivity of the assay was 0.03 ng progesterone ml^{-1}. Progesterone concentrations were used to confirm the ultrasound results on timing of ovulation and corpus luteum formation. In year 2, they were also used to determine the lifespan of the corpus luteum (defined as the interval between the first day serum progesterone was ≥ 0.3 ng ml^{-1} until the day serum progesterone decreased below 0.3 ng ml^{-1}) after the first *postpartum* ovulation.

Statistical analyses

Data relating to intervals *post partum* and duration of the luteal phase were analysed using ANOVA (Snedecor and Cochran, 1980). Where significant differences occurred, specific differences between treatments were determined using Dunnett's *t* test with a priori comparisons. Proportions of cows ovulating the first dominant follicle and numbers with short cycles after first ovulation were analysed using χ^2 analysis.

Results

One of the 11 cows in the GnRH-D-treated group developed a luteinized follicle, after GnRH analogue treatment, which persisted for at least 29 days. At this stage, she was no longer monitored and was excluded from the analysis.

The mean (± SEM) number of days from parturition to development of the first dominant follicle was 11.0 ± 0.3, 10.3 ± 0.5 and 10.1 ± 0.7 for cows assigned to treatments 1–3, respectively (Table 1). The proportion of cows ovulating the first *postpartum* dominant follicle was significantly higher ($P < 0.05$) following injection of the GnRH analogue (12 of 12 and 7 of 10 for GnRH-G-treated and GnRH-D-treated cows, respectively) than in controls (2 of 12; Table 1). In control cows, the mean (± SEM) number of dominant follicles until first ovulation was 2.7 ± 0.3. The mean interval from parturition to first ovulation (Table 1) in GnRH-G-treated cows was significantly shorter (16.1 ± 0.8 days; $P < 0.01$) than the interval for GnRH-D-treated (24.5 ± 3.6 days) or control cows (27.1 ± 2.5). However, the mean interval to first ovulation for GnRH-D-treated cows was extended by three cows which failed to ovulate the first dominant follicle. In cows given the GnRH analogue (growing or declining phase), the mean interval to first ovulation for those that ovulated the first dominant follicle was

Table 1. The effect of administration of GnRH (buserelin) to *postpartum* beef suckler cows. The ovaries were scanned to identify the first dominant follicle *postpartum*, ovulation of the first dominant follicle, *postpartum* interval to first ovulation and duration of the first *postpartum* luteal phase. GnRH was administered either during the growing–plateau (GnRH-G) or declining (GnRH-D) phases of the first *postpartum* dominant follicle

Parameter	Control	GnRH-G	GnRH-D
Number of cows	12	12	10
PPI to first dominant follicle (days)	11.0 ± 0.3[a]	10.3 ± 0.5[a]	10.1 ± 0.7[a]
PPI to buserelin	—	13.9 ± 0.8	15.6 ± 0.7
Number ovulating first dominant follicle	2[a]	12[b]	7[b]
PPI to first ovulation	27.1 ± 2.5[a]	16.1 ± 0.8[b]	24.5 ± 3.6[a]
Number of cows in year 2	7	6	5
Number with short cycles	4[a]	5[a]	4[a]
Duration of luteal phase*	9.3 ± 3.0[a]	8.0 ± 1.0[a]	9.6 ± 3.7[a]

[ab]Values in a row with differing superscripts are significantly different ($P < 0.05$).
*Defined as the interval between the first day on which serum progesterone was ≥ 0.3 ng ml^{-1} until the day on which serum progesterone decreased below 0.3 ng ml^{-1}.
PPI: *postpartum* interval.

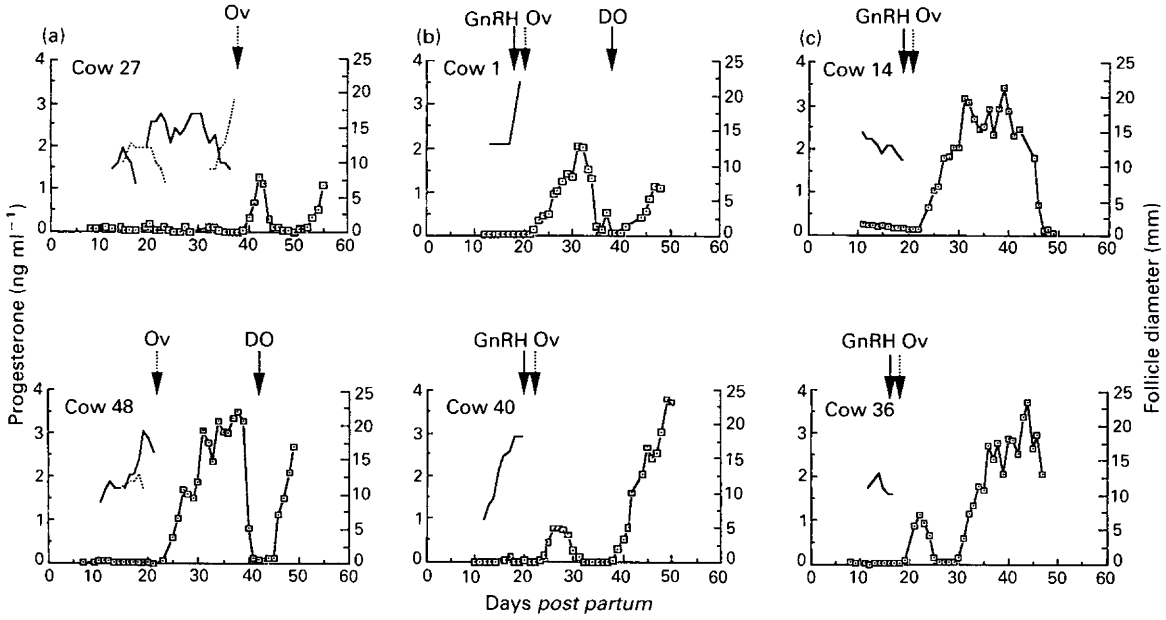

Fig. 1. Follicular development (——; · · · ·), times of GnRH treatment (GnRH), ovulation (Ov) and detected oestrus (DO), and progesterone profiles (—⊡—) for two representative cows (year 2) from each treatment group: (a) controls, (b) 20 μg GnRH analogue administered in the growing phase of growth of the first *postpartum* dominant follicle and (c) 20 μg GnRH analogue administered in the declining phase of growth of the first *postpartum* dominant follicle.

16.8 ± 0.6 days ($n = 19$) compared with 39.7 ± 4.9 days ($n = 3$) for those cows in which the first dominant follicle did not ovulate. Cows ovulating in response to the GnRH analogue were not detected in oestrus before ovulation.

The mean interval from ovulation to the first day on which serum progesterone concentrations increased above 0.3 ng ml^{-1} was not different between treatments (2.0 ± 0.3, 2.0 ± 0.3 and 2.0 ± 0.2 days; control, GnRH-G and GnRH-D, respectively; Fig. 1). In year 2, there was no difference in the duration of the first luteal phase (all cows) *post partum* (Table 1) between control (9.3 ± 3.0 days) and GnRH-treated cows (8.0 ± 1.0 and 9.6 ± 3.7 days; GnRH-G and GnRH-D, respectively) or in the proportion of cows with short cycles (4 of 7, 5 of 6 and 4 of 5; control, GnRH-G and GnRH-D, respectively).

Discussion

The present data confirm the original finding that a dominant follicle develops within 10–15 days *post partum* in beef suckler cows, but the majority undergo atresia rather than ovulate (Murphy *et al.*, 1990). However, the current results clearly show

that the first dominant follicle identified can ovulate following a single injection of the GnRH analogue. Previous work showed that GnRH treatment of *postpartum* beef cows results in a variable ovulatory response with 10–80% of treated cows ovulating after GnRH administration by single, multiple or pulsatile injections (Fonseca *et al.*, 1979; Mawhinney *et al.*, 1979; Riley *et al.*, 1981; Walters *et al.*, 1982; Edwards *et al.*, 1983). In those studies, however, the follicular status at the time of GnRH administration was not known and this, together with various nutritional states, probably accounted for the poor and variable ovulatory responses obtained. Thus, the data reported here may help to explain the reported variable ovulatory response by showing that it is the presence of a growing or mature dominant follicle that is the key factor determining the success of GnRH treatment in inducing ovulation early in the *postpartum* period.

The formation of a dominant follicle early in the *postpartum* period suggests that FSH secretion is not deficient, but the failure of ovulation of the majority of the first dominant follicles implicates LH deficiency as the probable cause of anovulation. Gonadotrophin release is primarily controlled by binding of GnRH to specific receptors on the gonadotroph cells of the pituitary (Braden and Conn, 1991). GnRH evokes other cellular responses including the biosynthesis of LH, as it stimulates the production of mRNA for the β subunit (Braden and Conn, 1991). Ovulation of the first dominant follicle in response to the GnRH analogue suggests that there is an adequate pituitary store of LH that can induce ovulation. Furthermore, Wise (1990) demonstrated, in *postpartum* ewes, that resumption of pulsatile LH release paralleled changes in pulsatile GnRH secretion. Thus the negative effect of suckling on reproductive function appears to act to a greater extent at the hypothalamic rather than at the pituitary level, by decreasing pulsatile secretion of GnRH. However, since the precise role of GnRH in stimulating LH synthesis is unclear, definitive conclusions cannot be drawn. In cows, the content of LH in the anterior pituitary gland decreases during gestation (Nalbandov and Casida, 1940), remains low during the early *postpartum* period (days 1 and 15 *post partum*; Nett *et al.*, 1988) and gradually increases to values similar to those present in cyclic animals by day 30 *post partum* (Moss *et al.*, 1985; Nett *et al.*, 1988). Our results indicate that there is sufficient releasable LH to induce ovulation 14–16 days *post partum*. Wettemann *et al.* (1982) demonstrated that GnRH can induce an LH surge in *postpartum* beef cows, which supports our evidence that there is in fact adequate LH available for release provided that the appropriate GnRH signal occurs.

A number of factors affect LH secretion and ovarian function in *postpartum* cows, including body condition score and plane of nutrition (Wright *et al.*, 1992), energy balance (Canfield and Butler, 1990) and suckling frequency (Lamming *et al.*, 1982). In beef heifers, Murphy *et al.* (1991) demonstrated that the plane of nutrition affected the diameter of the dominant follicle: heifers on a high feed intake had significantly larger dominant follicles than did heifers on maintenance or below maintenance diets. In addition, the proportion of beef cows in low body condition at 9 weeks *post partum* with large oestrogenic follicles was significantly decreased compared with controls (Prado *et al.*, 1990). The oestrogen content of follicles classed as oestrogen active in beef heifers that were rapidly losing weight was significantly decreased compared with the oestrogen content of similar follicles in heifers gaining weight (Spicer *et al.*, 1991), suggesting that nutritionally induced changes in gonadotrophins also affect the physiological function of dominant follicles. Thus, body condition of the cows and energy balance are likely to affect the resumption of follicular development and the ovulatory response to GnRH.

Monitoring of ovarian follicles using ultrasound does not give precise information on the oestrogenic activity or functional state of the follicle. In this study, we arbitrarily defined a decrease in size of ≥ 3.0 mm as an indicator of atresia of a dominant follicle. There was no difference in the ability of the first dominant follicle to ovulate or form a normal corpus luteum following GnRH injection in the declining phase of development compared with the growing–plateau phase of development, suggesting that either a dominant follicle which decreased in size by ≥ 3 mm is not atretic or a dominant follicle in the early stages of atresia can still ovulate and form a corpus luteum. It is unclear which of these options is correct but, since atresia is a gradual process resulting in slow degeneration of granulosa cells, the follicle may ovulate, even after degeneration has commenced. However, there was a variable response in the GnRH-D group in which four cows failed to ovulate (in one cow the dominant follicle luteinized and in the other three the dominant follicle regressed normally). This variation may be attributable to the dominant follicles being at different stages of atresia, which are undetectable by ultrasound scanning, at the time of GnRH treatment. Further work is necessary to correlate the size of the declining dominant follicle with oestrogen activity of the follicle and rate of development of pycnotic granulosa cells and thus define the state of atresia of follicles based on ultrasound examination.

The occurrence of short cycles after first ovulation in this study was not surprising, as short cycles occur spontaneously before oestrus in prepubertal heifers (Berardinelli *et al.*, 1979) and ewe lambs (Keisler *et al.*, 1983), and following parturition in cows and ewes (Lamming *et al.*, 1981; Sharpe *et al.*, 1986). Follicular development during short cycles is associated with the development of a single dominant follicle (Savio *et al.*, 1990). *Postpartum* dairy cows ovulating before day 10 had a low incidence of short cycles; those ovulating between days 10 and 20 had short, normal and long cycles and those ovulating 20–40 days *post partum* had a high incidence of short cycles (Savio *et al.*, 1990). This finding suggests that beef cows ovulating in response to GnRH treatments on either 16.1 ± 0.8 (GnRH-G) or 18.0 ± 1.1 (GnRH-D; responders) days *post partum* might have had a lower incidence of short cycles compared with the controls ovulating 27.1 ± 2.5 days *post partum*. However, there was no difference in the incidence of short cycles between treatments, suggesting that short cycles are associated with first ovulation at about day 16–30 in *postpartum* beef cows. Further work is necessary to overcome the occurrence of short cycles and lack of oestrus in response to GnRH-induced ovulation. A potential approach would be to incorporate progesterone treatment in conjunction with GnRH administration (when a dominant follicle is present).

In conclusion, this study demonstrates that in beef suckler cows exogenous GnRH will consistently induce ovulation of the first dominant follicle that develops within 10–15 days *post partum*. In addition, this study lends support to the hypothesis that anovulation in the early *postpartum* period is due to inadequate LH secretion (Roche *et al.*, 1992). Use of GnRH requires

ultrasound scanning of dominant follicles, but the problems of lack of expression of behavioural oestrus and high incidence of short cycles need to be overcome.

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