Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves

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Prolonged postpartum anoestrus in beef cows is due to failure of early dominant follicles to ovulate. It is hypothesized that this failure to ovulate is due to inadequate LH pulse frequency. The objective of this study was to determine whether administration of hourly LH pulses would cause the first dominant follicle to ovulate. In Expt 1, 16 cows received either saline ($n = 8$) or porcine LH (pLH; $50 \mu\text{g h}^{-1}$; $n = 8$) as hourly pulses for 3–5 days from the second day of dominance of the first dominant follicle (day 0). In Expt 2, 21 cows received either saline ($n = 7$), or $50 \mu\text{g pLH}$ ($n = 7$) or $100 \mu\text{g pLH}$ ($n = 7$) as hourly pulses for 3 days. Appropriate ovarian scanning and assays of blood samples were carried out. In Expt 1, the number of dominant follicles that underwent atresia was not affected by increasing the number of LH pulses, but the duration of dominance (days) of the first and second dominant follicles and maximum size (mm) of the second dominant follicle were increased ($P < 0.05$). Oestradiol concentrations were higher ($P < 0.05$) in cows given hourly pLH pulses ($3.1 \pm 1.2 \text{ pg ml}^{-1}$) compared with controls ($1.2 \pm 0.2 \text{ pg ml}^{-1}$). Four of eight treated cows had an anovulatory LH surge. The number of follicle waves to first ovulation was not different ($P < 0.05$) between control (4.6 ± 0.9) and pLH treated cows (3.9 ± 0.5). In Expt 2, four of seven cows given pulses of $100 \mu\text{g pLH h}^{-1}$ ovulated the first dominant follicle, and the interval from calving to first ovulation was decreased ($P < 0.05$). In the remaining three cows, the duration of dominance of the first dominant follicle was increased ($P < 0.005$), the maximum size of the first dominant follicle was greater ($P < 0.05$), and the interval (days) from the start of infusion to new wave emergence was greater ($P < 0.05$) compared with cows that failed to ovulate in either the $50 \mu\text{g pLH h}^{-1}$ or control treatments. In conclusion, hourly pulses of pLH from day 1 after dominance of the first dominant follicle in postpartum beef cows can either prolong dominance or induce it to ovulate. This finding supports the hypothesis that LH pulse frequency is a key determinant of the fate of the dominant follicle in the early postpartum period.

Introduction

The number of calves produced annually is a key component of high productivity in beef cows. However, prolonged postpartum (PP) anoestrus has an adverse effect on the size of the annual calf production of beef cows. A 12 month calving interval is biologically efficient; however, Williams (1990) estimated that only 50–70% of beef cows in the US achieve this, and the remaining cows either have an intercalving interval > 365 days, or fail to produce a calf in that year.

The emergence of the first follicle wave occurs within 10–14 days (Murphy *et al.*, 1990) of parturition and is associated with the early resumption of recurrent FSH increases (Crowe *et al.*, 1998), presumably due to the abrupt withdrawal of the negative feedback effect of oestrogens and

progesterone before parturition. Prolonged postpartum anoestrus in beef cows is due to failure of these early dominant follicles to ovulate (Murphy *et al.*, 1990; Stagg *et al.*, 1995). Dairy cows that are not under nutritional stress generally ovulate the first dominant follicle (Savio *et al.*, 1990), in contrast to a mean of 3.2 ± 0.2 dominant follicles to first ovulation in beef suckler cows with a good body condition score (Murphy *et al.*, 1990). However, beef cows in poor body condition can have 8–14 follicle waves before the first ovulation (Stagg *et al.*, 1995). Failure of the early dominant follicle to ovulate is thought to be due to inadequate LH pulse frequency (Roche *et al.*, 1992; Williams and Griffith, 1995; Stagg *et al.*, 1998), which results in low androgen production in the follicle (Fortune, 1986) and inadequate oestradiol positive feedback to induce a preovulatory gonadotrophin surge (Peters *et al.*, 1985). In support of this hypothesis, a single injection of a GnRH analogue ($20 \mu\text{g}$ Buserelin)

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administered during the growing phase of the first dominant follicle in beef cows consistently induced ovulation of that follicle (Crowe *et al.*, 1993), demonstrating that the first dominant follicle post partum is capable of ovulation if it receives an appropriate gonadotrophin surge.

Therefore, the objective of these studies was to determine whether hourly LH pulses of similar magnitude to those detected in the follicular phase of the oestrous cycle (Rahe *et al.*, 1980) would stimulate sufficient oestrogen secretion, after selection of the first dominant follicle to induce a gonadotrophin surge and ovulation.

Materials and Methods

Animals and treatments

Experiment 1. Sixteen postpartum Limousin $\times$ Friesian spring-calving cows in their first to sixth lactation were used. Cows were housed indoors until calving and fed a maintenance diet of grass silage with a mineral supplement. After calving, the cows and calves were at pasture. They were blocked on the basis of parity, body weight, body condition score and calving date, and assigned at calving to one of two treatments: (i) 0.84 ml saline h^{-1} (controls; $n = 8$); (ii) 50 μg of porcine LH (pLH; Lutropin, Vetrepharm, Canada) h^{-1} in 0.84 ml saline ($n = 8$). pLH or saline pulses were delivered once an hour through a cannula inserted into the right jugular vein using an autosyringe pulse infusion pump (Glaxo model AS6H) from 1 day (day 0) after detection of the first dominant follicle post partum by ovarian ultrasound scanning once a day.

Experiment 2. Twenty-one postpartum Limousin $\times$ Friesian spring-calving cows in their first to seventh lactation were used in year 2 and managed as described for Expt 1. They were assigned to treatments as follows: (i) 0.84 ml saline h^{-1} (controls; $n = 7$); (ii) 50 μg pLH h^{-1} in 0.84 ml saline ($n = 7$); and (iii) 100 μg pLH h^{-1} in 0.84 ml saline ($n = 7$). pLH or saline pulses were delivered once an hour for 3 days as in Expt 1.

Ultrasound examination of ovaries

In both experiments, the ovaries of each cow were scanned once a day from day 7 or 8 post partum until the first ovulation, and then once at day 4 after ovulation to verify the formation of a corpus luteum, using real-time ultrasonography (Dynamic Imaging, Livingston) with a 7.5 MHz transrectal linear probe as described by Murphy *et al.* (1990). A dominant follicle was defined as the largest follicle ≥ 10 mm in diameter that was at least 2 mm larger than all other follicles present on either ovary.

Blood sampling

In both experiments, blood samples were collected once a day for progesterone assays from day 7 post partum until either behavioural oestrus was detected or until 25 days after

the first ovulation to confirm ovulation and formation of the corpus luteum, and to determine the duration of the lifespan of the first corpus luteum post partum. Blood samples were collected twice a day to determine oestradiol concentrations from day 0 to day 5 after infusion of LH. Blood samples for LH assay were collected through a cannula inserted into the left jugular vein at 15 min intervals for 8 (day -1 relative to LH infusion) or 6 h (days 0 and 2) to determine LH pulse frequency and amplitude during the LH infusion period. Blood samples were taken every 8 h from day 0 to day 2 of pLH infusion and subsequently every 4 h until day 5 after the start of the infusion period for determination of FSH and LH concentrations. After collection, blood samples were stored at room temperature for 1 h and at 4°C for 18–24 h, and were centrifuged at 1614 g for 15 min. Serum was decanted and stored at -20°C until assayed.

Hormone assays

Progesterone concentrations were determined using the non-extraction radioimmunoassay described by Ronayne and Hynes (1990). Mean interassay coefficients of variation (CV; $n = 4$) for samples containing 0.20, 0.82 and 2.44 ng progesterone ml^{-1} were 14.0, 15.9 and 11.2%, respectively. Intra-assay CV ($n = 9$) for the same samples were 14.2, 7.4 and 8.5%, respectively. The sensitivity of the assay was 0.03 ng ml^{-1} . Oestradiol concentrations were determined using the radioimmunoassay described by Prendiville *et al.* (1995). Mean interassay CV ($n = 6$) for samples containing 0.78, 2.38 and 5.89 pg oestradiol ml^{-1} were 22.5, 15.6 and 19.1%, respectively. Intra-assay CV ($n = 3$) for the same samples were 11.8, 14.1 and 11.5%, respectively. The sensitivity of the assay was 0.2 pg ml^{-1} . LH concentrations were determined using the radioimmunoassay described by Cooke *et al.* (1997). Mean interassay CV ($n = 9$) for samples containing 2.2, 6.5 and 10.2 ng LH ml^{-1} were 11.6, 12.1 and 11.0%, respectively. Intra-assay CV ($n = 7$) for the same samples were 9.5, 9.5 and 14.2%, respectively. The sensitivity of the assay was 0.1 ng ml^{-1} . FSH concentrations were determined using a heterologous radioimmunoassay described by Crowe *et al.* (1997) using NIDDK-anti-oFSH antibody (AFP-C5288113) and the bovine FSH standard preparation (USDA bFSH B1). Mean interassay CV ($n = 4$) for samples containing 16.6, 23.3 and 51.4 ng FSH ml^{-1} were 7.2, 5.7 and 7.9%, respectively. Intra-assay CV ($n = 3$) for the same samples were 4.6, 5.3 and 9.3%, respectively. The sensitivity of the assay was 1.6 ng ml^{-1} .

Statistical analyses

Follicular data were analysed using ANOVA (Snedecor and Cochran, 1980). LH pulses were determined by PC-Pulsar pulse detection algorithm (Merriam and Wachter, 1982). The G parameters used were: G1 = 2.40, G2 = 2.00, G3 = 1.90, G4 = 1.90, G5 = 1.90. Comparisons between groups, on all hormone parameters, were made using ANOVA (Snedecor and Cochran, 1980). Proportions of cows that had an LH surge (LH concentration > 10 ng ml^{-1} for > 6 h) and that ovulated were analysed using chi-squared analysis (Swinscow, 1996).

Results

Experiment 1

Parameters before LH or saline infusion. After calving and before pLH infusion, mean body weight, body condition score (scale 0–5; 0 = emaciated, 5 = obese), and number of days post partum to the first day of infusion were not different for cows allocated to the different treatment groups (Table 1). There was no difference among treatments in the size of the first dominant follicle on the first day of infusion (Table 1).

LH pulse frequency. On day –1, there was no difference between cows assigned to treatment groups, in the mean number of LH pulses h^{-1} (0.13 ± 0.4 and 0.21 ± 0.3 for control and $50 \mu\text{g pLH h}^{-1}$ groups, respectively). However, more pulses were detected in cows treated with pLH on day 0 (0.9 ± 0.5 LH pulses h^{-1}) and day 2 (0.8 ± 0.4 LH pulses h^{-1}) compared with control cows (0.16 ± 0.5 and 0.26 ± 0.4 pulses h^{-1} on days 0 and 2, respectively). The mean concentration of LH in the treated cows ($4.1 \pm 0.7 \text{ ng ml}^{-1}$) was higher ($P < 0.05$) than in the controls ($1.4 \pm 0.3 \text{ ng ml}^{-1}$). The mean of the maximum LH concentration was higher ($P < 0.05$) in treated cows ($26.6 \pm 7.6 \text{ ng ml}^{-1}$) compared with controls ($5.1 \pm 2.0 \text{ ng ml}^{-1}$) during the infusion period. Concentrations of LH were also higher ($P < 0.05$) in cows that had a subsequent LH surge ($46.5 \pm 0.9 \text{ ng ml}^{-1}$) compared with cows in the treated group that did not have an LH surge ($6.7 \pm 2.9 \text{ ng ml}^{-1}$).

Serum oestradiol concentrations. Mean oestradiol concentrations in control cows ($0.7 \pm 0.1 \text{ pg ml}^{-1}$) during the pLH infusion period were lower ($P < 0.05$) than in cows given $50 \mu\text{g pLH h}^{-1}$ ($1.5 \pm 0.3 \text{ pg ml}^{-1}$). Peak concentrations of oestradiol in control cows ($1.2 \pm 0.2 \text{ pg ml}^{-1}$) during the pLH

infusion period were lower ($P < 0.05$) than in cows given $50 \mu\text{g pLH h}^{-1}$ ($3.1 \pm 0.7 \text{ pg ml}^{-1}$) throughout the treatment period. For cows treated with pLH, mean serum oestradiol concentrations (2.1 ± 0.4 versus $1.19 \pm 0.2 \text{ pg ml}^{-1}$) and the mean of the maximum amplitude of oestradiol attained during the pLH infusion period (4.5 ± 0.8 versus $1.7 \pm 0.2 \text{ pg ml}^{-1}$, respectively) were higher ($P < 0.05$) in cows that had an LH surge compared with those that did not have an LH surge.

Gonadotrophins. Four of eight cows given hourly $50 \mu\text{g pLH}$ pulses had a subsequent LH and FSH surge, whereas none ($P < 0.05$) of the cows infused with saline had a surge between days 0 and 5. There was no difference in mean serum FSH concentrations during the treatment period for pLH treated ($21 \pm 1.5 \text{ ng ml}^{-1}$) or control ($19 \pm 2.0 \text{ ng ml}^{-1}$) cows. There was also no difference in serum FSH concentrations between cows with ($24 \pm 2.3 \text{ ng ml}^{-1}$) or without ($18 \pm 0.4 \text{ ng ml}^{-1}$) an LH surge within the group treated with pLH.

Follicular dynamics and first ovulation. The duration of dominance of the first dominant follicle was greater ($P < 0.05$) in cows given $50 \mu\text{g pLH h}^{-1}$ compared with controls. The maximum size of the first dominant follicle was greater ($P < 0.05$) and the interval from treatment to the first day of dominance of the second dominant follicle was greater ($P < 0.05$) in the cows given pLH compared with control cows (Table 1). There was no effect of pLH infusion on the postpartum interval to first ovulation between control (38.2 ± 6.4 days) and treated cows (40.5 ± 2.9 days). There was also no effect of treatment on the number of follicle waves to first ovulation between control (4.5 ± 0.8) and treated cows (4.0 ± 0.5). After the first ovulation, all cows had a short oestrous cycle (9–12 days).

Table 1. Follicular dynamics in postpartum beef cows before, during and after hourly infusion of saline (controls) or $50 \mu\text{g}$ porcine LH (pLH) for 3 days beginning on the day after detection of the first dominant follicle (DF1) (Expt 1)

Parameters	Control	$50 \mu\text{g pLH h}^{-1}$
Pre-experimental parameters		
Body weight (kg)	510 ± 27.4	509 ± 25.2
Body condition score (scale 0–5)	2.7 ± 0.2	2.8 ± 0.2
Postpartum interval to start of experiment (days)	11.3 ± 0.41	11.5 ± 0.57
Size of DF1 at start of infusion (mm)	12.5 ± 0.80	11.8 ± 0.59
Follicular parameters during LH infusion		
Maximum size of DF1 (mm)	14.3 ± 1.42	16.6 ± 1.58
Interval from day 0 to maximum size of DF1 (days)	2.3 ± 0.3^a	6.4 ± 1.5^b
Duration of dominance of DF1 (days)	3.4 ± 0.6^a	7.6 ± 1.25^b
Duration of detection of DF1 (days)	6.5 ± 0.74^a	13.8 ± 2.40^b
Follicular parameters after LH infusion		
Day 0 to next wave emergence (days)	2.4 ± 0.35^a	8.3 ± 1.33^b
Maximum size of DF2 (mm)	14.0 ± 0.61^a	20.0 ± 1.61^b
Duration of dominance of DF2 (days)	3.6 ± 0.6^a	7.4 ± 1.3^b
Duration of detection of DF2 (days)	5.0 ± 0.45^a	10.1 ± 1.4^b

Values are mean $\pm$ SEM.

Day 0 is the first day of infusion of pLH.

^{ab}Within rows, values with different superscripts are significantly different ($P < 0.05$).

Experiment 2

Parameters before LH or saline infusion. After calving and before pLH infusion, body weight, body condition score, and the number of days post partum to the first day of infusion was not different among cows assigned to the different treatment groups. There was no difference among treatments in the size of the first dominant follicle on the first day of infusion (Table 2).

LH pulse frequency and amplitude. On day -1 relative to the start of LH infusion, there was no difference among cows assigned to treatment groups in the mean number of LH pulses h^{-1} (Table 3). Infusion of hourly pLH pulses increased ($P < 0.05$) the number of LH pulses on days 0 and 2 compared with controls. There was no difference in LH pulse amplitude among treatments on day -1 relative to the start of LH infusions (Table 3). On days 0 and 2 of LH infusions, LH pulse amplitude was between 1.9- and 2.4-fold higher ($P < 0.03$) in the cows infused with 100 μg pLH h^{-1} compared with those infused with 50 μg pLH h^{-1} . Individual LH pulse profiles are presented (Fig. 1). Mean LH concentrations (1.6 ± 0.2 , 4.09 ± 1.3 and 4.29 ± 1.6 ng ml^{-1} for cows treated with saline, 50 μg pLH h^{-1} and 100 μg pLH h^{-1} , respectively) and the mean of the maximum LH pulse amplitude (2.3 ± 0.4 , 25.8 ± 5.9 and 18.9 ± 6.2 ng ml^{-1} for cows treated with saline, 50 pLH $\mu\text{g h}^{-1}$ and 100 μg pLH h^{-1} , respectively) were higher ($P < 0.05$) in cows treated with pLH than in controls during the treatment period. Mean LH concentrations and the mean of the peak LH concentration were higher ($P < 0.05$) in the treated cows that had an LH surge compared with those that did not have a LH surge (Table 4).

Serum oestradiol concentrations. The overall mean concentrations of oestradiol were higher ($P < 0.05$) in cows treated with 100 μg pLH h^{-1} (2.1 ± 0.3 pg ml^{-1}) than in cows

Table 3. LH pulse frequency and amplitude before, during and after hourly infusion with either saline (controls) or 50 or 100 μg porcine LH (pLH) h^{-1} for 3 days beginning on the day after detection of the first dominant follicle (Expt 2).

Treatment	Control	50 μg pLH LH h^{-1}	100 μg pLH LH h^{-1}
LH pulse frequency (pulses h^{-1})			
Day -1	0.21 ± 0.026	0.21 ± 0.026	0.17 ± 0.026
Day 0	0.17 ± 0.000^a	0.81 ± 0.037^b	0.90 ± 0.025^b
Day 2	0.23 ± 0.042^a	0.92 ± 0.033^b	0.911 ± 0.028^b
LH pulse amplitude (ng ml^{-1})			
Day -1	2.1 ± 0.39	2.4 ± 0.36	3.3 ± 0.86
Day 0	2.4 ± 0.32^a	1.8 ± 0.19^b	3.4 ± 0.05^a
Day 2	2.9 ± 0.22^{ab}	1.7 ± 0.14^a	4.0 ± 0.75^b

Values are mean $\pm$ SEM.

Day 0 is the day on which infusion of pLH commenced.

^{ab}Within rows, values with different superscripts are significantly different ($P < 0.05$).

treated with 50 μg pLH h^{-1} (0.8 ± 0.2 pg ml^{-1}) or controls (0.7 ± 0.1 pg ml^{-1}). Peak oestradiol concentrations were higher ($P < 0.05$) in cows treated with 100 μg pLH h^{-1} (3.4 ± 0.3 pg ml^{-1}) than in cows treated with 50 μg pLH h^{-1} (1.4 ± 0.2 pg ml^{-1}) or controls (1.3 ± 0.3 pg ml^{-1}). Within the cows treated with pLH, mean serum oestradiol concentrations (0.8 ± 0.2 and 0.6 ± 0.1 pg ml^{-1} for cows treated with 50 μg pLH h^{-1} with and without an LH surge; and 2.15 ± 0.6 and 2.18 ± 0.5 pg ml^{-1} for cows treated with 100 μg pLH h^{-1} with or without an LH surge) were not different between those that had and those that did not have an LH surge (Fig. 2).

Table 2. Follicular dynamics in postpartum beef cows before, during and after hourly infusion with either saline (controls) or 50 or 100 μg porcine LH (pLH) for 3 days beginning on the day after detection of the first dominant follicle (Expt 2)

Treatment	Control	50 μg pLH h^{-1}	100 μg pLH h^{-1}
Pre-experimental parameters			
Body weight (kg)	564 ± 17	573 ± 20	577 ± 21
Body condition score (scale 0-5)	3.3 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
Postpartum interval to detection of DF1 (days)	10.9 ± 0.5	11.6 ± 0.6	11.1 ± 0.9
Size of DF1 at start of infusion (mm)	11.0 ± 0.6	11.7 ± 0.4	12.4 ± 0.6
Follicle parameters during LH infusion			
Maximum size of DF1 (mm)	12.9 ± 0.6^a	14.7 ± 0.7^{ab}	17.4 ± 1.3^b
Interval from day 0 to maximum size of DF1 (days)	2.1 ± 0.6	3.3 ± 0.6	4.3 ± 1.4
Duration of dominance of DF1 (days)	2.6 ± 0.6^a	4.0 ± 0.6^a	8.3 ± 2.0^b
Duration of detection of DF1 (days)	9.1 ± 1.2	9.9 ± 0.7	13.0 ± 2.5
Follicle parameters after LH infusion			
Day 0 to next wave emergence (days)	3.2 ± 0.5^a	3.9 ± 0.6^{ab}	6.3 ± 1.7^b
Maximum size of DF2 (mm)	15.4 ± 1.7	17.4 ± 0.9	20.0 ± 2.0
Duration of dominance of DF2(days)	3.0 ± 1.2	5.7 ± 0.8	6.5 ± 3.5
Duration of detection of DF2 (days)	8.6 ± 1.9	13.0 ± 1.4	13.0 ± 3.0

Values are mean $\pm$ SEM.

Day 0 is the first day of infusion of pLH.

^{ab}Within rows, values with different superscripts are significantly different ($P < 0.05$).

Fig. 1. LH profiles in representative postpartum beef suckler cows infused with either saline, 50 µg porcine LH (pLH), or 100 µg pLH every hour between days 10 and 13 post partum. Samples were collected every 15 min for 8 h on day -1 (day 0 = first infusion) and for 6 h on days 0 and 2.

Table 4. Overall mean $\pm$ SEM and mean of the maximum serum LH concentrations in beef suckler cows with or without an LH surge after infusion of porcine LH during the growing period of the first dominant follicle post partum (Expt 2)

Treatment	Mean LH (ng ml ⁻¹)	Maximum LH (ng ml ⁻¹)
Saline	1.6 $\pm$ 0.2 ^a	2.3 $\pm$ 0.4 ^a
50 µg h ⁻¹ with LH surge	7.6 $\pm$ 1.3 ^b	48.8 $\pm$ 11.9 ^b
50 µg h ⁻¹ without LH surge	1.8 $\pm$ 0.3 ^a	2.7 $\pm$ 0.5 ^a
100 µg h ⁻¹ with LH surge	5.8 $\pm$ 1.6 ^b	33.9 $\pm$ 13.4 ^b
100 µg h ⁻¹ without LH surge	2.8 $\pm$ 0.6 ^a	4.1 $\pm$ 0.4 ^a

^{ab}Within columns, values with different superscripts are significantly different ($P < 0.05$).

Gonadotrophins. Five of seven cows receiving 50 µg pLH h⁻¹ and 4 of 7 cows receiving 100 µg pLH h⁻¹ had a subsequent LH and FSH surge, whereas none ($P < 0.05$) of the cows infused with saline had a surge between days 0 and 5. There was no difference in mean serum FSH concentrations during the treatment period (15.8 ± 3.2 , 14.2 ± 2.4 and 15.2 ± 0.9 ng

ml⁻¹ for cows treated with saline, 50 µg pLH h⁻¹ and 100 µg pLH h⁻¹, respectively). There was also no difference in serum FSH concentrations within the groups treated with pLH between cows with or without an LH surge (14.7 ± 3.1 and 15.2 ± 2.7 ng ml⁻¹ for cows treated with 50 µg pLH h⁻¹ and 14.5 ± 2.4 and 13.2 ± 4.0 ng ml⁻¹ for cows treated with 100 µg pLH h⁻¹; Figs 2 and 3).

Progesterone concentrations. Progesterone concentrations increased (> 0.3 ng ml⁻¹) for 7–12 days in all cows that did not ovulate the first dominant follicle, when they ovulated a subsequent dominant follicle, and these cows were deemed to have a short cycle. In cows ovulating the first dominant follicle within 2 days of the end of treatment, progesterone concentrations increased (> 0.3 ng ml⁻¹) for > 15 days.

Follicular dynamics and first ovulation. The duration of dominance of the first dominant follicle was greater ($P < 0.005$) in cows given 100 µg pLH h⁻¹ that failed to ovulate the first dominant follicle compared with the other groups. The maximum size of the first dominant follicle was greater ($P < 0.05$) in the cows infused with 100 µg pLH h⁻¹ than in cows in the other two treatment groups. The interval from the start of pLH infusion to emergence of the second dominant follicle was

Fig. 2. LH (—) and oestradiol (—) concentrations in (a) a control cow and cows treated with (b) 50 µg porcine LH (pLH) h⁻¹ or (c) 100 µg pLH h⁻¹. (a) Cow 80 ovulated the third postpartum dominant follicle; (b) cow 70 had an LH surge but failed to ovulate until the second postpartum dominant follicle; cow 86 had no LH surge and failed to ovulate until the fourth postpartum dominant follicle; (c) cow 84 ovulated the first postpartum dominant follicle after an LH surge; cow 88 had an LH surge but ovulated the fourth dominant follicle post partum; and cow 67 had no LH surge and ovulated the third postpartum dominant follicle.

greater ($P < 0.05$) in the cows infused with 100 µg pLH h⁻¹ with anovulatory first dominant follicle compared with the other two groups (Table 2). In three cows infused with 100 µg pLH h⁻¹, the first dominant follicle ovulated within 2 days of the end of the infusion period, which was followed by an oestrous cycle of normal duration (on the basis of progesterone concentrations), whereas one cow ovulated the first dominant follicle 15 days after the start of pLH treatment and this ovulation was followed by a short oestrous cycle. Thus, the interval from calving to first ovulation was shortened in those cows that ovulated within 2 days of the end of infusion with 100 µg pLH h⁻¹ (16.5 ± 1.8 days). In the remaining cows receiving 100 µg pLH h⁻¹ the postpartum interval to first ovulation was prolonged (44.0 ± 3.8 days) compared with the saline infused cows (29.2 ± 2.1 days), and was followed by a short oestrous cycle (Fig. 4).

Discussion

This study demonstrates that increasing LH pulse frequency from one pulse every 6–8 h to one pulse every 1 h altered follicular dynamics in the early postpartum period in beef

cows nursing calves, and either caused the first dominant follicle post partum to ovulate or prolonged its duration of dominance. In cattle, LH is critical for growth of dominant follicles beyond 7 mm in diameter (Gong *et al.*, 1997). When LH pulse frequency is low, the dominant follicle grows, shows dominance for a few days and regresses, as is typical during the luteal phase of a normal oestrous cycle. Conversely, high LH pulse frequency stimulates oestradiol production from follicles, leading to a preovulatory LH surge and ovulation. When LH frequency is intermediate, the growth of dominant follicles is prolonged (Savio *et al.*, 1993). The fact that increasing LH pulse frequency to one pulse per hour in the current study either caused prolonged dominance or ovulation of the first postpartum dominant follicle shows that the fate of the dominant follicle in cows is modulated by LH pulse frequency.

Oestrogen-active dominant follicles on days 3–7 of the oestrous cycle increased LH binding to the thecal cells, and on day 7 there is increased LH but not FSH binding to the granulosa cells (Ireland and Roche, 1982). In addition, the period of dominance of the dominant follicle in heifers given sub-luteal phase concentrations of progesterone (intermediate LH; Stock and Fortune, 1993) can be significantly prolonged

Fig. 3. FSH (—) and LH (—) concentrations in (a) a control cow and cows treated with (b) 50 µg porcine LH (pLH) h⁻¹ or (c) 100 µg pLH h⁻¹. (a) Cow 80 ovulated the third postpartum dominant follicle; (b) cow 70 had an LH surge but failed to ovulate until the second postpartum dominant follicle; cow 86 had no LH surge and failed to ovulate until the fourth postpartum dominant follicle; (c) cow 84 ovulated the first postpartum dominant follicle after an LH surge; cow 88 had an LH surge but ovulated the fourth dominant follicle post partum; and cow 67 had no LH surge and ovulated the third postpartum dominant follicle.

(Savio *et al.*, 1993). Taken together, these results confirm the key role of LH in determining the growth and capacity for oestrogen production of the dominant follicle in cows. In the present study, LH and oestradiol concentrations were significantly increased in cows given pLH pulses compared with control cows, confirming the role of LH pulses in stimulating oestradiol production from prolonged dominant follicles (Bigelow and Fortune, 1998).

Monitoring ovarian follicles using ultrasonography does not give precise information on the functional state of the follicle. In this study, a dominant follicle was defined as dominant when it was at least 2 mm greater in diameter than any other follicle present on either ovary, and at least 10 mm in diameter. It is possible that once the follicle becomes dominant, it is already LH-dependent and the time required to confirm its dominance by size deviation using ultrasound scanning could have been detrimental to its subsequent developmental fate, in that apoptosis of a significant number of granulosa cells may have irreversibly started the atretic process. It may be necessary to start pLH infusion before ultrasound confirmation of the presence of the first postpartum dominant follicle to induce successful final maturation and ovulation of the first dominant follicle. Further work is necessary to correlate the size at which the

follicle becomes dominant with oestrogen activity and other biochemical markers (Mihm *et al.*, 1997) and the extent of pycnosis of granulosa cells, as it is not possible to define the state of atresia of follicles on the basis of ovarian scanning alone, to allow more precise timing of LH pulse infusions and induce more consistent responses.

In year 1, pulsing cows with 50 µg pLH h⁻¹ altered follicular dynamics, whereas in year 2 it did not. The reason for this difference is not clear since there was no difference in LH pulse frequency between controls in year 1 and 2, although body condition score was better in year 2. Treatment of cows during the early dominance period of the first dominant follicle with 50 µg pLH h⁻¹ did not induce ovulation. This may be due to inadequate LH pulse amplitude (1.8 ± 0.19 and 1.7 ± 0.14 ng ml⁻¹ on days 0 and 2 of infusion) after this treatment compared with cows in the 100 µg LH h⁻¹ group (3.4 ± 0.05 and 4.0 ± 0.75 ng ml⁻¹ on days 0 and 2 of infusion). Infusion of 100 µg pLH h⁻¹ resulted in ovulation of the first dominant follicle post partum in three of seven cows. The apparent reason for failure of the first dominant follicle to ovulate in the remaining cows in this treatment group was the inability of this LH pulse pattern to stimulate significant oestradiol production from the first postpartum dominant follicle. Inadequate oestradiol

Fig. 4. Follicular growth in (a) a control cow and cows treated with (b) 50 µg porcine LH (pLH) h⁻¹ or (c) 100 µg pLH h⁻¹. (a) Cow 80 ovulated the third postpartum dominant follicle; (b) cow 70 had an LH surge but failed to ovulate until the second dominant follicle; cow 86 had no LH surge and failed to ovulate until the fourth postpartum dominant follicle; (c) cow 84 ovulated the first postpartum dominant follicle after an LH surge; cow 88 had an LH surge but ovulated the fourth dominant follicle post partum; and cow 67 had no LH surge and ovulated the third postpartum dominant follicle. The solid bars (■) indicate period of saline or pLH infusion.

production by the first postpartum dominant follicle could have been due to inadequate pLH concentrations, use of porcine rather than bovine LH, or delayed initiation of infusion relative to onset of functional dominance. Hence, low or inadequate LH pulse frequency in cows (early postpartum period of beef cows, before puberty or the luteal phase of cyclic cows) results in decreased oestradiol concentrations (Stagg *et al.*, 1995), lack of positive feedback, and atresia rather than ovulation of the dominant follicle.

In this study, there was no detectable effect of pLH infusion on FSH concentrations. Detection of FSH increases, associated with follicle emergence in this study, is consistent with previous studies (Crowe *et al.*, 1998; Stagg *et al.*, 1998), which reported that recurrent increases in FSH concentrations occur before emergence of each wave of follicle growth during the postpartum anoestrous period of beef cows. These findings disagree with other studies in which FSH concentrations did not vary during postpartum anoestrus (Moss *et al.*, 1985; Leung *et al.*, 1986; Short *et al.*,

1990). The failure to detect FSH increases in these studies may reflect the inadequacy of the bovine FSH assays available or inadequate frequency of blood samples.

In Expt 2, normal oestrous cycles occurred in the three cows that ovulated in response to pLH infusion during the growing phase of the first dominant follicle post partum. This is in agreement with data from dairy cows that have an oestrous cycle of normal duration after ovulation of a dominant follicle on or before day 15 post partum (Savio *et al.*, 1990). The remaining cows that did not ovulate in response to LH treatment in the present study had a predominance of short oestrous cycles after the first ovulation. This finding is consistent with other studies which indicate that the first ovulation post partum after day 20 in beef cows is usually followed by a short oestrous cycle (Lamming *et al.*, 1981; Murphy *et al.*, 1990; Crowe *et al.*, 1993; Stagg *et al.*, 1995).

In conclusion, this study demonstrates that infusing cows with hourly pLH pulses in the early dominance period of the

first dominant follicle either increased the maximum size of the first dominant follicle and increased its duration of dominance or resulted in ovulation of that follicle. It also postponed the first day of dominance of the second dominant follicle by approximately 6 days. Hourly infusion with 50 µg pLH induced an LH surge in half of the cows. However, none of these cows ovulated the first dominant follicle post partum. The cows infused with pLH showed more LH pulses and higher oestradiol concentrations than the control cows. There was no difference in FSH concentrations between treatments. Hourly infusion with 100 µg pLH demonstrated that the first postpartum dominant follicle was capable of producing adequate oestradiol to induce an LH surge and ovulation if exposed to the appropriate LH pulse frequency and amplitude in the early dominant period. These results support the crucial role of LH pulses in ovulation of dominant follicles during the early postpartum period of beef cows nursing calves.

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