ELSEVIER

MODIFICATION OF FOLLICULAR DYNAMICS BY EXOGENOUS FSH AND PROGESTERONE, AND THE INDUCTION OF OVULATION USING hCG IN POSTPARTUM BEEF COWS

Y. Yavas,[1a] W.H. Johnson[2] and J.S. Walton[1b]

[1]Department of Animal and Poultry Science and [2]Department of Population Medicine
University of Guelph, Guelph, Ontario N1G 2W1 Canada

Received for publication : March 2, 1999
Accepted : May 17, 1999

ABSTRACT

Follicular growth and ovulation in response to FSH, progesterone and hCG were evaluated in postpartum beef cows. In Experiment 1, on Day 21 post partum, cows received an injection of either saline (control; n=6), FSH (200 mg; n=6), or a PRID (n=5) for 10 d. Both FSH and PRID prolonged maintenance of a dominant follicle (15.5±1.16 and 14.4±1.29 d, respectively, vs 8.4±1.22 d in control; P<0.01), and increased the maximum diameter of the dominant follicle (14.0±0.91 and 16.4±1.01 mm, respectively, vs 10.9±0.95 mm in control; P<0.05). The PRID-maintained dominant follicle ovulated in 60% of cows, followed by normal estrous cycles (vs 0% in control; P=0.01), whereas the dominant follicle ovulated in 33% of FSH-treated cows (P=0.08). The PRID regimen shortened the interval to first ovulation preceding a normal cycle and continued cyclicity (44±4.1 vs 60±4.4 d in control; P=0.02). In Experiment 2, on Day 21 post partum, cows received either saline (control), saline+PRID, or FSH+PRID (n=16/group). Sixty hours after PRID withdrawal, cows received either saline or hCG (1,500 IU, n=8/treatment). The FSH+PRID regimen increased the number of large (>10 mm in diameter) follicles (3.6±0.43 vs 1.9±0.39 in control; P=0.005). Both PRID and FSH+PRID prolonged maintenance of the largest follicle (11.0±0.82 and 11.2±0.91 d, respectively, vs 8.7±0.81 d in control; P<0.05). The PRID-maintained dominant follicle ovulated in 50% of cows, followed by normal estrous cycles. The FSH+PRID-maintained largest follicle had become atretic at PRID withdrawal and was anovulatory. The FSH+PRID+hCG regimen increased the incidence of ovulation preceding a cycle of normal duration and continued cyclicity (100 vs 50% in PRID; P=0.03), and reduced the interval to first ovulation preceding a cycle of normal duration and continued cyclicity (38±6.5 vs 58±6.3 d in control; P=0.04). The area under the progesterone curve during the induced cycle was reduced after (PRID±FSH)+hCG than after PRID±FSH (P=0.002). These results indicate that PRID alone or with FSH/hCG has the potential to modify the dominant follicle and initiate cyclicity in postpartum beef cows.

Key words: cow, postpartum, follicle, ovulation, PRID

Acknowledgments
The authors thank the staff of the Elora Beef Cattle Research Centre; Sanofi Animal Health, Inc. for donation of PRIDs; and the Ontario Ministry of Agriculture, Food and Rural Affairs, Natural Sciences and Engineering Research Council of Canada, and the Ontario Cattlemen's Association for funding this research.

[a]Current address: Toronto Fertility Sterility Institute, 66 Avenue Road, Toronto, Ontario, M5R 3N8, Canada.

[b]Correspondence and reprint requests: Phone: (519) 824-4120, ext. 3706;
Fax: (519) 767-0573; E-mail: jwalton@aps.uoguelph.ca.

Theriogenology 52:949–963, 1999

0093-691X/99/$–see front matter
PII S0093-691X(99)00185-5

INTRODUCTION

In suckled beef cows, failure to resume ovulatory cyclicity and the associated delay in rebreeding reduces the calf crop and causes economic loss to beef cattle producers. Numerous follicular waves precede the first postpartum ovulation (23,44) that occurs between Days 30 and 65 post partum (18,20,23). Inguinal perception of a calf by the cow during suckling (45,52) is believed to increase the sensitivity of the hypothalamus to the negative feedback of ovarian estradiol-17ß (1,14), which results in delayed resumption of LH pulsatility (21), and anovulation. Postpartum follicles have the potential to ovulate in response to exogenous GnRH (54), hCG (19), PMSG (56), estrogens (28) and anti-estrogens (39). Such induced ovulation is usually followed by a short cycle and a return to acyclicity. Postpartum follicles also ovulate after weaning of calves; however, the induced ovulation is usually followed by a short cycle (8). Furthermore, weaning is costly, requires intensive labor, and reduces growth rate of weaned calves (9), making it impractical for beef cattle producers. Therefore, an efficient procedure for establishing cyclicity in suckled beef cows is needed.

The ovulatory follicle of cyclic cows undergoes FSH-dependent recruitment and growth (12,22), followed by LH pulse frequency-dependent terminal maturation (36,55). Maintaining circulating progestin at intermediate concentrations increases LH pulse frequency (5,33,48) and extends the life span of the dominant follicle (35,43,47,49). The hypothesis of this study was that the life span of dominant follicles in early postpartum beef cows would be maintained by subluteal progesterone and would ovulate after withdrawal, as in cyclic cows. As a positive control, treatment with FSH was included to determine the responsiveness of the follicles to FSH stimulation. The objectives were to evaluate follicular growth and ovulation in response to 1) a single injection of exogenous FSH on Day 21 post partum, 2) maintenance of circulating progesterone at subluteal concentrations beginning on Day 21 post partum using a PRID, and 3) FSH plus PRID. In addition, whether hCG was required to cause ovulation of the follicles was determined.

MATERIALS AND METHODS

Animals and Diet

The experiments were conducted from April to August over two consecutive breeding seasons. Cross-bred beef cows were housed with their calves in straw-bedded pens except during ultrasonographic examination of ovaries and blood sampling. Cows were given access to haylage (39% dry matter [DM], 20% crude protein [CP]) supplemented with dicalcium phosphate:trace mineralized salt (50:50) at 0.3% of the diet DM ad libitum. Cows were uniform and in good body condition (scores 3 to 3.5 on a scale of 1 to 5; 11).

Treatments

In Experiment 1, cows were randomly assigned at parturition, in approximately equal numbers within parity (primi- or multiparous) and frame size (large or small), to 1 of 3 treatments: 1) control (n=6); 2) FSH (n=6); and 3) PRID (n=5). At 0900 h on Day 21 post partum, cows received an injection of either 10 mL, sc physiological saline (control), FSH equivalent to 200 mg NIH-FSH-P1 in 10 mL, sc saline (Folltropin-V[c]), or received a PRID[d] (1.4 g progesterone) for 10 d. In Experiment 2, cows were randomly assigned at parturition, in approximately equal numbers within parity and frame

---

[c]Vetrepharm, London, Ontario, Canada.

[d]Sanofi Animal Health, Victoriaville, Quebec, Canada.

size, to 1 of 3 treatments (n=16/treatment): 1) control, 2) PRID, and 3) FSH+PRID. At 0900 h on Day 21 post partum, cows received an injection of either 10 mL, sc physiological saline or FSH equivalent to 200 mg NIH-FSH-P1 in 10 mL, sc saline (Folltropin-V[c]). At the time of injection, cows in treatments 2 and 3 received a PRID[d]. Sixty hours after PRID withdrawal, equal numbers of cows across treatments received an injection of 1,500 IU, im hCG in 1.5 mL saline (Progon 10,000[d]), or an injection of 1.5 mL, im physiological saline. The above 3 treatments were subdivided into 6 (n=8/treatment): 1) control, 2) hCG, 3) PRID, 4) PRID+hCG, 5) FSH+PRID, and 6) FSH+PRID+hCG.

Ovarian Ultrasonography

In Experiment 1, ovarian structures were monitored daily from Day 18 to 50 post partum, using a real-time B-mode transrectal ultrasound scanner[e] with a 5-MHz transducer.[f] In Experiment 2, ovarian structures were monitored daily from Day 18 until detection of ovulation and the resulting corpus luteum (CL). Cows were restrained in a metal chute, and ultrasonographic examinations of both ovaries were performed by the same operator as described previously (17).

Blood Sampling and Radioimmunoassay

Blood samples were collected daily by jugular venipuncture into 10-mL heparinized tubes[g] before each ultrasonographic examination from Day 18 to 50 in Experiment 1, and from Day 18 until ovulation and thereafter every 3 d for 30 d in Experiment 2. To monitor the preovulatory surge of LH in Experiment 2, additional blood samples were collected via jugular catheters every 4 h from 48 to 80 h after PRID withdrawal. A similar sampling schedule was followed at the corresponding times in cows that did not receive a PRID. Plasma was separated by centrifugation at 2,000 x g for 20 min at the site of the experiment, and plasma samples were stored at -20°C. Concentrations of plasma progesterone were measured using a solid-phase radioimmunoassay kit[h] as described previously (32). In Experiment 1, all samples were assayed for progesterone in a single assay. Sensitivity of the assay was 0.125 ng/mL, and the intra-assay coefficient of variation was 6.3%. In Experiment 2, the intra- and inter-assay coefficients of variation over 4 assays for progesterone were 11.6 and 13.7%, respectively. Concentrations of plasma LH were measured by a double-antibody method in a single assay as described previously (53). Sensitivity was 0.05 ng/mL, and the intra-assay coefficient of variation was 7.8%.

Statistical Analyses

The test of normality using the Univariate Procedure of SAS (34) failed to reject the hypothesis that the data were normally distributed. Data were analyzed by least squares analysis of variance using the General Linear Models Procedure of SAS (34). Parity and frame size were included in the model. In Experiment 2, the follicular data from the day of initiation of treatments (Day 21) until the day of saline/hCG injection (Day 33) were pooled for control, PRID and FSH+PRID treatments. An LH surge was presumed to have occurred if the concentration of LH exceeded 15 ng/mL in 2 consecutive plasma samples. Area under the progesterone curve for each cow was determined by the trapezoidal summation method (50) using SAS (34).

---

[e]Aloka SSD-500, Tokyo, Japan.

[f]Aloka UST-588U-5, Tokyo, Japan.

[g]Sherwood Medical, St. Louis, MO.

[h]Coat-A-Count, Diagnostic Products, Los Angeles, CA.

RESULTS

Experiment 1

Follicular dynamics. In controls, 3.9±0.47 anovulatory dominant follicles were detected from Day 21 until first ovulation post partum (Figure 1). Treatment with FSH or PRID prolonged the duration of dominance (P=0.001 and P=0.005, respectively) and increased the maximum diameter of the first dominant follicle (P=0.03 and P=0.002, respectively; Table 1, Figure 1). The first dominant follicle attained a maximum diameter later in PRID than in control cows (P=0.05; Table 1).

Ovulation and postpartum interval. Treatment with PRID but not with FSH increased the incidence of ovulation of the first dominant follicle within 5 d after withdrawal (P=0.01; Table 1, Figure 1), followed by a normal cycle and continued cyclicity. Treatment with PRID shortened the intervals to first ovulation preceding cyclicity (P=0.05) and to first ovulation preceding a normal cycle and continued cyclicity (P=0.02; Table 1).

Experiment 2

Follicular dynamics. In controls, 4.1±0.68 anovulatory dominant follicles were detected from Day 21 until first ovulation post partum. The first dominant follicle remained dominant longer (P=0.05), and attained a maximum diameter later (P=0.03) in PRID than in control cows (Table 2). One of the cows in FSH+PRID treatment was removed from the study because the PRID was lost during the treatment period. Injection of FSH plus PRID insertion increased the number of large (>10 mm in diameter) follicles (P=0.005; Table 2, Figure 2). In the FSH+PRID-treated cows, the first largest follicle was maintained longer than in controls (P=0.04), and it attained its maximum diameter earlier than in PRID cows (P=0.002; Table 2).

Ovulation and postpartum interval. The first ovulation on Day 50 was followed by a short cycle in 7/8 (87.5%) control cows. Two of eight (25%) hCG cows had already ovulated before hCG injection. In another 2/8 (25%) cows, hCG injection was followed by ovulation, a short cycle, and continued cyclicity (Figure 2). In the remaining 4 cows (50%), the hCG injection was followed by ovulation, a short cycle, and a return to acyclicity (Figure 2). The hCG treatment did not affect postpartum interval (Table 3).

In the PRID and PRID+hCG treatments, ovulation occurred within 5 d after PRID withdrawal in 4/8 (50%) and 6/8 (75%) cows, respectively. Ovulation was followed by a cycle of normal duration and continued cyclicity (Table 3, Figure 2). In the remaining 4/8 (50%) and 2/8 (25%) cows, respectively, the PRID-maintained dominant follicle regressed following PRID withdrawal (Figure 2). Regression was followed by continued acyclicity in PRID cows, whereas it was followed by ovulation of a new follicle, a short cycle and a return to acyclicity in PRID+hCG cows. The PRID or PRID+hCG treatment did not affect postpartum interval (Table 3).

In the FSH+PRID and FSH+PRID+hCG treatments, ovulation occurred within 5 d after PRID withdrawal in 2/7 (29%) and 8/8 (100%) cows, respectively. Ovulation was followed by a cycle of normal duration and continued cyclicity (Table 3, Figure 2). In the remaining 5/7 (71%) of FSH+PRID cows, the maintained follicles regressed following PRID withdrawal (Figure 2). These cows exhibited continued acyclicity.

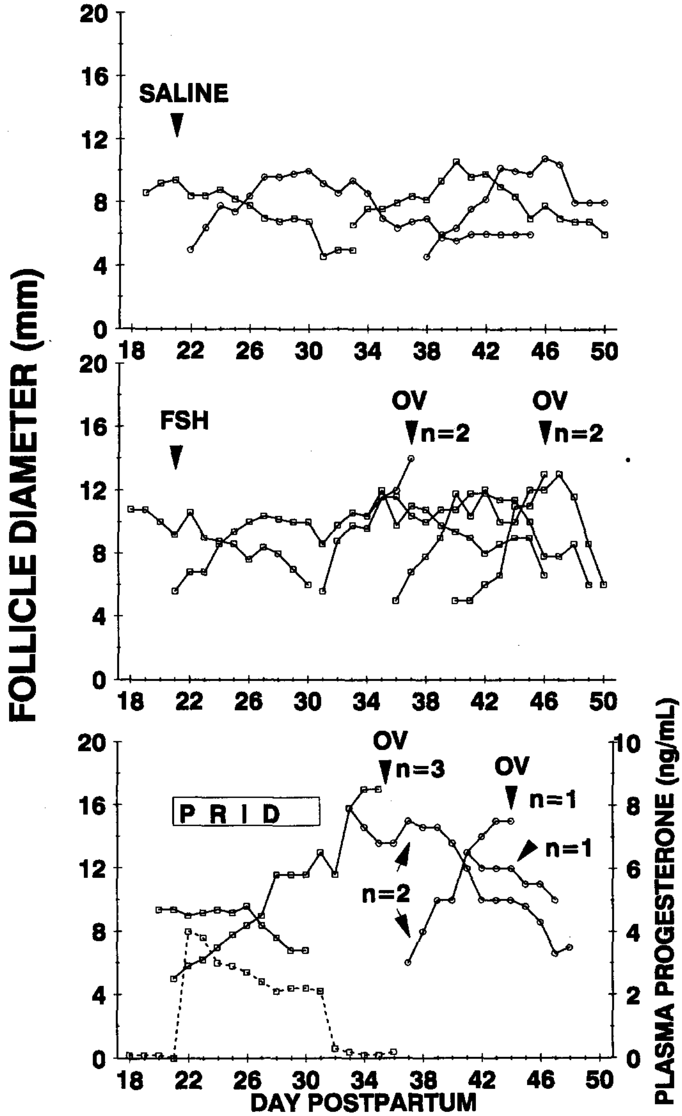

Figure 1. Diameters of dominant follicles (solid lines) in cows receiving saline (controls; n=6, top), FSH (n=6, middle) or PRID (n=5, bottom), and mean plasma progesterone concentrations (broken line) during PRID treatment in Experiment 1. (OV=ovulation)

Table 1. Effect of FSH (200 mg, sc) on Day 21 post partum or of a 10-day PRID treatment, beginning on Day 21 post partum, on ultrasonographic characteristics of the first dominant follicle after initiation of treatments, on the number of large follicles at Day 21 through Day 31 post partum, and on resumption of cyclicity post partum. (Experiment 1)

	Treatment			
Variable	Control	FSH	PRID	Pooled SEM
n	6	6	5	-
Duration of dominance (days)	8.4[a]	15.5[b]	14.4[b]	1.22
Maximum diameter (mm)	10.9[a]	14.0[b]	16.4[b]	0.96
Growth rate (mm/day)	0.9[a]	0.8[a]	1.1[a]	0.12
Day of maximum diameter	28[a]	31[ab]	34[b]	1.8
Ovulation of dominant follicle	0%[a] (0/6)	33%[ab] (2/6)	60%[b] (3/5)	-
No. of large follicles / cow	0.8[a]	1.3[a]	1.3[a]	0.20
Days to first ovulation	51[a]	48[ab]	40[b]	3.5
Days to first ovulation preceding a normal cycle	60[a]	57[a]	44[b]	4.2
Duration of first cycle (days)	9.8[a]	8.9[a]	16.4[b]	1.59

[a,b]Within a row, means lacking a common superscript differ ($P<0.05$). Numbers in parentheses represent the proportions of cows.

Table 2. Effect of PRID and FSH+PRID on ultrasonographic characteristics of the first dominant/largest follicle after initiation of treatments and on numbers of follicles at Day 21 through Day 33 post partum. The data prior to saline/hCG injection were pooled. (Experiment 2)

	Treatment			
Variable	Control	PRID	FSH+PRID	Pooled SEM
n	16	16	15	-
Duration of maintenance (days)	8.7[a]	11.0[b]	11.2[b]	0.85
Maximum diameter (mm)	12.7[a]	14.1[a]	14.4[a]	0.63
Growth rate (mm/day)	1.1[a]	1.1[a]	1.1[a]	0.10
Day of maximum diameter	30[a]	33[b]	29[a]	0.9
No. of medium follicles/cow	1.4[a]	1.2[a]	2.7[a]	0.68
No. of large follicles/cow	1.9[a]	1.6[a]	3.6[b]	0.40

[a,b]Within a row, means lacking a common superscript differ ($P<0.05$).

Of the PRID-, PRID+hCG-, and FSH+PRID-treated cows in which the maintained follicle ovulated, 3/4, 1/6 and 1/2 cows, respectively, exhibited a preovulatory LH surge at 62 ± 3.2 h after PRID withdrawal ($P=0.02$; PRID vs control and hCG; Table 3). The FSH+PRID treatment did not affect postpartum interval (Table 3). The FSH+PRID+hCG treatment reduced days to first ovulation preceding a cycle of normal duration and continued cyclicity ($P=0.04$ vs control; $P=0.05$ vs PRID; Table 3).

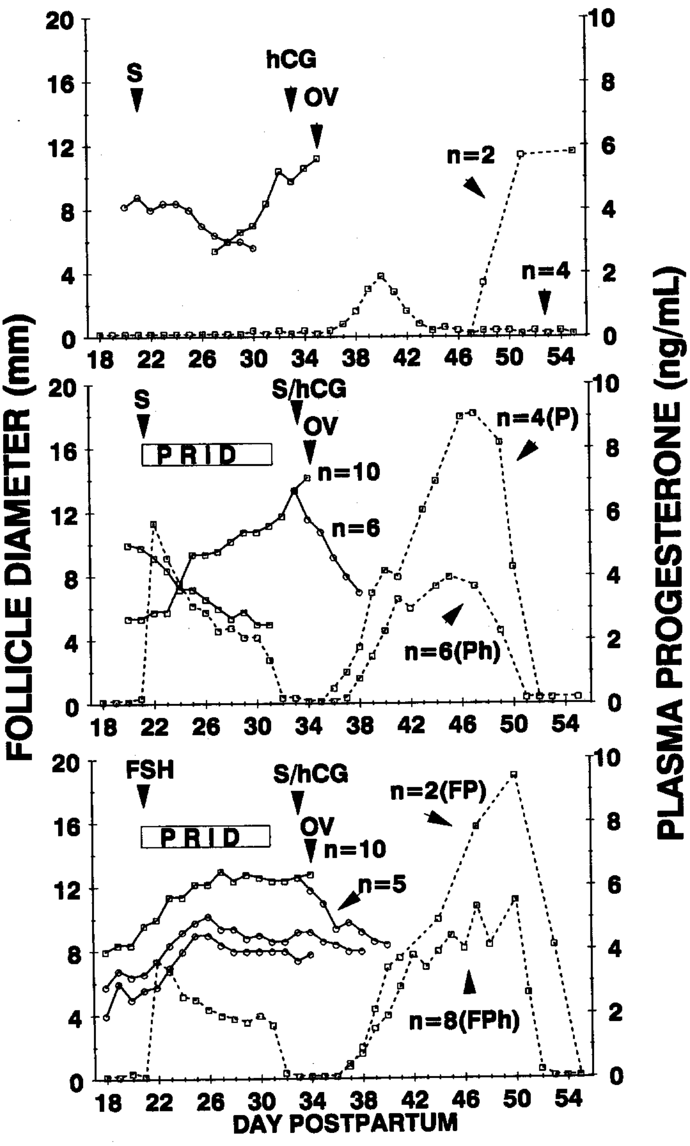

Figure 2. Diameters of dominant follicles (solid lines) and mean plasma progesterone concentrations (broken lines) in cows receiving hCG (n=6, top), PRID±hCG (n=16, middle), and (FSH+PRID)±hCG (n=15, bottom) in Experiment 2. (S=saline, OV=ovulation, P=PRID, Ph=PRID+hCG, FP=FSH+PRID, FPh=FSH+PRID+hCG)

Table 3. Effect of PRID with or without FSH and hCG on occurrence of the LH surge and resumption of cyclicity post partum. (Experiment 2)

Variable	Treatment						
	Control	hCG	PRID	PRID hCG	FSH PRID	FSH PRID hCG	Pooled SEM
n	8	8	8	8	7	8	-
LH surge	0%^{a}	0%^{a}	38%^{b}	13%^{ab}	14%^{ab}	0%^{a}	-
	(0/8)	(0/8)	(3/8)	(1/8)	(1/7)	(0/8)	
Ovulation of largest follicle preceding cyclicity	25%^{a}	13%^{a}	50%^{ab}	75%^{bc}	29%^{a}	100%^{c}	-
	(2/8)	(1/8)	(4/8)	(6/8)	(2/7)	(8/8)	
Days to first ovulation preceding cyclicity	50^{ab}	55^{a}	52^{ab}	44^{ab}	54^{ab}	38^{b}	5.9
Days to first ovulation preceding a cycle of normal duration and continued cyclicity	58^{ab}	66^{a}	57^{ab}	47^{bc}	61^{ab}	38^{c}	6.5

^{a,b,c}Within a row, means lacking a common superscript differ ($P<0.05$). Numbers in parentheses represent the proportions of cows.

Ultrasonographic characteristics of ovulatory and anovulatory follicles. The diameters of the ovulatory follicles in control cows at ovulation were greater than the maximum diameter of the postpartum anovulatory dominant follicle ($P \leq 0.03$; Table 4). Maximum diameter of the PRID-maintained ovulatory follicle was greater ($P=0.02$) and occurred later ($P=0.01$) than that of contemporary anovulatory control follicles (Table 5). Maximum diameter of the FSH+PRID-maintained largest anovulatory follicle was greater than that of contemporary anovulatory control follicles ($P=0.04$; Table 5). In PRID treatment, the diameter of the maintained anovulatory follicle 2 d after PRID withdrawal (Day 33) was not different from its maximum diameter at PRID withdrawal (Day 31; $P=0.22$) but was smaller than the PRID-maintained ovulatory follicle 2 d after PRID withdrawal (Day 33; $P=0.03$; Table 5). In the FSH+PRID treatment, diameter of the maintained anovulatory largest follicle 2 d after PRID withdrawal (Day 33) was smaller than its maximum diameter during PRID treatment (Day 27; $P=0.004$; Table 5).

The induced CL. Area under the progesterone curve during the cycle induced by (PRID±FSH)+hCG was smaller than in PRID±FSH ($P=0.003$; Table 6, Figure 2). Maximum diameter of the induced CL in (PRID±FSH)+hCG was smaller than the spontaneously-formed CL during the first normal cycle in controls ($P=0.04$; Table 6). The diameter of the ovulatory follicle which gave rise to the induced CL in (PRID±FSH)+hCG was smaller than in PRID±FSH ($P=0.03$), and was smaller than that which gave rise to the spontaneously-formed CL during the first normal cycle in controls ($P=0.003$; Table 6).

DISCUSSION

Control cows exhibited approximately 4 follicular waves from Day 21 through the first ovulation post partum. This indicates that dominant follicles develop and turn over within 3 wk post partum in suckled beef cows (23,44). However, the anovulatory dominant follicle attained a maximum diameter smaller than that of the ovulatory follicle preceding both the short and the normal cycles, suggesting that postpartum anovulatory dominant follicles in suckled beef cows undergo atresia before they attain the optimal size for ovulation.

Administration of FSH on Day 21 post partum prolonged dominance and increased maximum diameter of the dominant follicle. Previous studies indicated that dominant follicles of postpartum beef cows have receptors for FSH (7), and that endogenous FSH is not a limiting factor to postpartum ovulation (29,57). The dose of FSH administered in the present study is half the total dose usually given twice daily over 4 d to induce superovulation (4). A single subcutaneous injection of 330 mg (37) or 400 mg (6) FSH induced superovulation similar to that obtained from intermittent intramuscular injections of the same total doses. In the present study, 1 injection of FSH (200 mg, sc) enhanced the growth of the dominant follicle in postpartum cows but did not increase the number of large follicles unless accompanied by PRID as in Experiment 2.

Table 4. Ultrasonographic characteristics of the first anovulatory dominant follicle after Day 21 post partum, and of the ovulatory dominant follicles preceding the short and the normal cycle in control cows. (Experiment 2)

	Type of follicle			
Variable	Anovulatory	Preceding short cycle	Preceding normal cycle	Pooled SEM
n	6^a	7^b	5^c	-
Duration of dominance (days)	8.3^e	11.0^e	8.8^e	1.20
Maximum diameter (mm)^d	12.4^e	15.6^f	17.0^f	0.98
Growth rate (mm/day)	1.1^e	1.2^e	1.6^e	0.19

^{a}Data unavailable for 2 cows in which the first ovulation occurred shortly after Day 21.
^{b}Data unavailable for 1 cow in which the first cycle was normal.
^{c}Data unavailable for 3 cows due to discontinuation of ultrasonography after first ovulation.
^{d}Maximum diameter for ovulatory follicles refers to diameter at ovulation.
^{e,f}Within a row, means lacking a common superscript differ ($P<0.05$).

Table 5. Ultrasonographic characteristics of the PRID- and FSH+PRID-maintained dominant/largest follicle which did or did not ovulate after a 10-day PRID treatment beginning on Day 21 post partum compared with anovulatory follicles in control cows. (Experiment 2)

	Treatment					
	Control	PRID		FSH+PRID		Pooled
Maintained follicle ovulated:	No	No	Yes	No	Yes	SEM
n	6	4	4	5	2	-
Duration of maintenance (days)	8.5^a	11.3^a	10.2^a	9.6^a	11.4^a	1.75
Growth rate (mm/day)	1.1^a	1.2^a	1.3^a	1.1^a	0.9^a	0.21
Day of maximum diameter	29^{ab}	31^{bc}	34^c	27^a	34^{bc}	1.6
Maximum diameter (mm)	12.4^a	14.5^{abd}	16.3^{bd}	15.3^{bd}	13.9^{abd}	1.18
Diameter on Day 33 (mm)	-	12.5^{ad}	16.3^{bd}	10.6^{ae}	12.5^{abd}	1.26

^{a,b,c}Within a row, means lacking a common superscript differ ($P<0.05$).
^{d,e}Within a column, means for diameters lacking a common superscript differ ($P<0.05$).

Table 6. Function of the induced CL with normal life span in cows that did or did not receive hCG after PRID±FSH compared with the spontaneously-formed CL in control cows during the first normal cycle post partum and diameter of the preceding ovulatory follicle. (Experiment 2)

Variable	Treatment			Pooled SEM
	Control	PRID±FSH	(PRID±FSH) +hCG	
n	7	6	14	-
Duration of cycle (days)	20^{a}	22^{a}	21^{a}	1.3
Area under progesterone curve (arbitrary units)	23^{ab}	30^{a}	17^{b}	2.9
Maximum diameter of CL (mm)	23.0^{a}(4)	21.1^{ab}(5)	18.5^{b}	1.44
Diameter of ovulatory follicle at preceding ovulation (mm)	16.7^{a}(5)	14.9^{a}	12.3^{b}	0.90

[a,b]Within a row, means lacking a common superscript differ ($P<0.05$). Numbers in parentheses represent the numbers of cows; numbers vary due to discontinuation of ultrasonography after first ovulation in some cows.

Subluteal amounts of progesterone from the PRID extended the life span and increased maximum diameter of the dominant follicle. This finding is similar to that of previous reports that norgestomet treatment of postpartum beef cows increased diameter of the dominant follicle (16), and that norgestomet or PRID treatment of postpartum dairy cows maintained the dominant follicle (46). The maximum diameter of the PRID-maintained ovulatory follicle after PRID withdrawal was greater than contemporary anovulatory follicles in the controls but similar to the ovulatory follicle at ovulation preceding the short or the normal cycle in controls, indicating that the PRID-maintained follicle escapes atresia and is capable of terminal maturation, at least in some cows. In cyclic cows, maintaining circulating progestin at intermediate concentrations with norgestomet (31,48,49) or CIDR (35,43,47) maintained the dominant follicle until withdrawal of progestin. Thus, despite the physiological differences between postpartum dominant follicles and those of normal cycles, subluteal amounts of progesterone from PRID maintained the dominant follicle in postpartum cows in a pattern similar to that in cyclic cows. Failure of postpartum dominant follicles to ovulate in suckled beef cows results from a delay in reestablishment of an appropriate pulsatile pattern of LH release (18,25,27), and maintaining circulating progestin at intermediate concentrations increases LH pulse frequency in cyclic cows (5,10,33), and in beef cows 21 to 35 d post partum (15). Treatment with PRID may, therefore, have mimicked the short cycle, increased the frequency of LH pulses, which, in turn, maintained the dominant follicle.

An injection of 200 mg FSH at the time of PRID insertion increased the number of large follicles. The fact that multiple large follicles were observed in FSH+PRID treatment but not in FSH or PRID alone indicates a potential synergism between FSH and subluteal concentrations of circulating progesterone. As in the PRID treatment, life span of the FSH+PRID-maintained largest follicle was prolonged. However, unlike the PRID treatment, the FSH+PRID-maintained largest follicle attained its maximum diameter earlier than the PRID-maintained dominant follicle, which may have been due to initial stimulation by the large dose of FSH. The FSH+PRID-induced multiple large follicles had already begun to regress by the time of PRID withdrawal. The PRID was, therefore, unable to maintain these multiple large follicles. In cyclic cows, subluteal progesterone concentrations (CIDR) were also unable to maintain multiple follicles induced by FSH (3).

In control cows, the interval to first ovulation was 50 to 51 d, which is similar to that in previous reports (18,20,23). Ovarian stimulation with FSH did not affect the incidence of ovulation, although it prolonged dominance and increased maximum diameter of the dominant follicle.

The PRID-maintained dominant follicle ovulated within 5 d after PRID withdrawal in 60 and 50% of cows in Experiments 1 and 2, respectively. This differs from that in cyclic cows, in which the dominant follicle ovulated in 75 to 100% of cows after withdrawal of progestin (31,35,47,49). Failure of the PRID-maintained dominant follicle to ovulate in some cows could not be ascribed to parity, frame size or body condition score. Parity and frame size were included in the statistical model, and all cows were in good body condition. Failure to ovulate may be due to variation among cows in the sensitivity of the positive feedback system to the calf effect. Peters (26) reported that 80% of beef cows receiving a 10-day PRID treatment were bred within 5 d after PRID withdrawal. However, the cows in that study received PRID beginning between Days 20 and 40 post partum, whereas in the present study, all cows received a PRID beginning on Day 21 post partum. Between Days 21 and 31 post partum, the suppressive effect of suckling on the GnRH pulse-generator is more profound than at later stages (14), which may have prevented the PRID-maintained dominant follicle from ovulating after PRID withdrawal in some cows during the present study.

Failure of the FSH+PRID-maintained multiple follicles to ovulate resulted in no difference in postpartum intervals among the control, PRID, and FSH+PRID treatments. The FSH+PRID-maintained largest anovulatory follicle grew to a size similar to that of the PRID-maintained follicle. However, this large anovulatory follicle attained its maximum diameter sooner (Day 27) after PRID insertion than did FSH+PRID-maintained ovulatory follicles (Day 34) and PRID-maintained follicles (Day 33), and underwent atresia thereafter through Day 33. It is unlikely that high concentrations of circulating progesterone during the first 2 to 3 d after PRID insertion caused atresia of the FSH+PRID-maintained largest follicle, as the follicle attained its maximum diameter 6 d after PRID insertion. The multiple large follicles in FSH+PRID-treated cows may, however, resulted in increased circulating concentrations of estradiol-17ß, and the increased negative feedback tone would suppress LH pulse frequency, followed by premature atresia.

An injection of hCG on Day 33 post partum was followed by ovulation and a short cycle in all cows that were still acyclic, which is consistent with previous reports (15,19,40). Since an LH surge was not detected in these cows, ovulation was likely caused by hCG. Injection of hCG after PRID withdrawal increased the incidence of ovulation preceding cyclicity. After FSH+PRID+hCG, the incidence of ovulation preceding cyclicity was 100% even though these follicles were undergoing atresia. The increased incidence of ovulation in PRID+hCG and FSH+PRID+hCG was not associated with an increased incidence of LH surge. This indicates that without hCG fewer follicles would have ovulated, or that the hCG pre-empted the positive feedback signal.

The first postpartum ovulation was usually followed by a short cycle, associated with a single follicular wave and subluteal concentrations of circulating progesterone, similar to previous reports (23,24,44). Resumption of cyclicity in FSH-treated cows followed a pattern similar to that in controls. Ovulation after PRID withdrawal was followed by a normal cycle and continued cyclicity. In cows where ovulation did not occur within 5 d after PRID withdrawal, resumption of cyclicity occurred later in a pattern similar to that of the controls. Treatment with hCG alone was followed by ovulation of the largest follicle and resumption of cyclicity in 25% of cows in a pattern similar to that in control cows. In 50% of cows that received hCG alone, and in 25% of cows that received hCG after PRID the induced ovulation was followed by a short cycle and a return to acyclicity. Thus, hCG stimulation alone was sufficient to induce ovulation, but insufficient to continue cyclicity.

In PRID+hCG and FSH+PRID+hCG treatments, ovulation was followed by a cycle of normal duration and continued cyclicity in 75 and 100% of the cows, respectively. Thus, pretreatment with PRID±FSH before hCG prolonged the life span of the hCG-induced CL, as did norgestomet pretreatment before hCG (15,30,40). However, hCG injection after PRID±FSH treatment reduced the area under the progesterone curve during the induced cycle. A luteal phase of normal duration with reduced circulating progesterone concentrations has been termed a luteal phase with reduced level of function of the CL (40) or an inadequate luteal phase (51). The reduced level of function of the induced CL in those cows receiving hCG after PRID±FSH was associated with the reduced size of the induced CL in comparison with controls. In cyclic cows, an injection of hCG on Days 6 to 7 of the estrous cycle also induced ovulation of the dominant follicle, and formation of an accessory CL (13,38,41,42) of a smaller size and a shorter life span (41,42) which secreted less progesterone in vivo (41,42) and in vitro (13) in comparison to the spontaneously-formed CL of similar age. The diameter of the ovulatory follicle at ovulation preceding the induced cycle in those cows receiving hCG after PRID±FSH was also smaller than in those cows not receiving hCG after PRID±FSH, and smaller than that at ovulation preceding the first normal cycle in controls. This indicates that the occurrence of a subfunctional CL in (PRID±FSH)+hCG was a result of ovulation of the follicle before it reached the optimal size for formation of a normal CL. The premature follicles in these cows may have received hCG stimulation early in their development that resulted in a premature luteinization and formation of a smaller CL with fewer large luteal cells (LLC). Because secretion of progesterone is highly dependent on the number of LLC (2), this may have resulted in decreased secretion of progesterone during induced cycles in (PRID±FSH)+hCG cows.

In conclusion, postpartum anovulatory dominant follicles exhibit waves of growth and atresia, but are smaller than the ovulatory follicles at the resumption of cyclicity. Exogenous FSH and hCG stimulated follicular growth and induced ovulation, respectively, but did not initiate cyclicity. This study demonstrated for the first time that treatment with a PRID early postpartum maintained the postpartum dominant follicle in a pattern similar to that in cyclic cows, and allowed the dominant follicle to undergo terminal maturation, followed by an LH surge, ovulation, normal life span and function of the CL, and continued cyclicity in 50 to 60% of the cows. Exogenous FSH at the time of PRID insertion disrupted follicular dominance and stimulated the growth of multiple large follicles, most of which underwent atresia before PRID withdrawal. Exogenous FSH at the time of PRID insertion plus hCG after PRID withdrawal maximized the incidence of ovulation of the PRID-maintained largest follicle. The present study demonstrates that use of PRID alone or in conjunction with FSH and hCG has the potential to establish ovulations and normal cycles at 3 to 4 wk post partum in up to 100% of suckled beef cows. The induced CL has a normal life span and produces less progesterone.

REFERENCES

1. Acosta B, Tarnavsky GK, Platt TE, Hamernik DL, Brown JL, Schoenemann HM, Reeves JJ. Nursing enhances the negative effect of estrogen on LH release in the cow. J Anim Sci 1983;57:1530-1536.
2. Alila HW, Dowd JP, Corradino RA, Harris WV, Hansel W. Control of progesterone production in small and large bovine luteal cells separated by flow cytometry. J Reprod Fertil 1988;82:645-655.
3. Ambrose JD, Rajamahendran R. Folltropin-V-stimulated multiple follicles cannot be maintained by low progesterone in cows. Theriogenology 1995;43:158 abstr.
4. Armstrong DT. Recent advances in superovulation of cattle. Theriogenology 1993;39:7-24.
5. Bergfeld EGM, Kojima FN, Cupp AS, Wehrman ME, Peters KE, Mariscal V, Sanchez T, Kinder JE. Changing dose of progesterone results in sudden changes in frequency of luteinizing hormone pulses and secretion of 17ß-estradiol in bovine females. Biol Reprod 1996;54:546-553.

6. Bo GA, Adams GP, Pierson RA, Mapletoft RJ. Effect of progestogen plus estradiol-17ß treatment on superovulatory response in beef cattle. Theriogenology 1996;45:897-910.
7. Braden TD, Manns JG, Cermak DL, Nett TM, Niswender GD. Follicular development following parturition and during the estrous cycle in beef cows. Theriogenology 1986;25:833-843.
8. Breuel KF, Lewis PE, Inskeep EK, Butcher RL. Endocrine profiles and follicular development in early-weaned postpartum beef cows. J Reprod Fertil 1993;97:205-212.
9. Comerford JW, Cash EH, Harpster HW, Wilson LL. Effects of early weaning and return to pasture on health and performance of beef calves. J Anim Sci 1995;73(Suppl.1):238 abstr.
10. Duchens M, Forsberg M, Edqvist LE, Gustafsson H, Rodriguez-Martinez H. Effect of induced suprabasal progesterone levels around estrus on plasma concentrations of progesterone, estradiol-17ß and LH in heifers. Theriogenology 1994;42:1159-1169.
11. Field J. Body condition scoring of beef cows. Ontario Ministry of Agriculture and Food. Factsheet. 1985. No. 85-099.
12. Fortune JE. Ovarian follicular growth and development in mammals. Biol Reprod 1994;50:225-232.
13. Fricke PM, Reynolds LP, Redmer DA. Effect of human chorionic gonadotropin administered early in the estrous cycle on ovulation and subsequent luteal function in cows. J Anim Sci 1993;71:1242-1246.
14. Garcia-Winder M, Imakawa K, Day ML, Zalesky DD, Kittok RJ, Kinder JE. Effect of suckling and ovariectomy on the control of luteinizing hormone secretion during the postpartum period in beef cows. Biol Reprod 1984;31:771-778.
15. Garcia-Winder M, Lewis PE, Deaver DR, Smith VG, Lewis GS, Inskeep EK. Endocrine profiles associated with life span of induced corpora lutea in postpartum beef cows. J Anim Sci 1986;62:1353-1362.
16. Garcia-Winder M, Lewis PE, Townsend EC, Inskeep EK. Effects of norgestomet on follicular development in postpartum beef cows. J Anim Sci 1987;64:1099-1109.
17. Griffin PG, Ginther OJ. Research applications of ultrasonic imaging in reproductive biology. J Anim Sci 1992;70:953-972.
18. Humphrey WD, Kaltenbach CC, Dunn TG, Koritnik DR, Niswender GD. Characterization of hormonal patterns in the beef cow during postpartum anestrus. J Anim Sci 1983;56:445-453.
19. Johnson SK, Del Vecchio RP, Townsend EC, Inskeep EK. Role of prostaglandin $F_{2\alpha}$ in follicular development and subsequent luteal life span in early postpartum beef cows. Dom Anim Endocrinol 1992;9:49-56.
20. King GJ, Macleod GK. Reproductive function in beef cows calving in the spring or fall. Anim Reprod Sci 1984;6:255-266.
21. Lamming GE, Peters AR, Riley GM, Fisher MW. Endocrine regulation of post-partum function. In: Karg H, Schallenberger E (eds), Factors Influencing Fertility in the Postpartum Cow. Current Topics in Veterinary Medicine and Animal Science, vol. 20. The Hague: Martinus Nijhoff, 1982;148-172.
22. Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 1992;70:3615-3626.
23. Murphy MG, Boland MP, Roche JF. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J Reprod Fertil 1990;90:523-533.
24. Perry RC, Corah LR, Kiracofe GH, Stevenson JS, Beal WE. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. J Anim Sci 1991;69:2548-2555.
25. Peters AR, Lamming GE, Fisher MW. A comparison of plasma LH concentrations in milked and suckling post-partum cows. J Reprod Fertil 1981;62:567-573.
26. Peters AR. Calving intervals of beef cows treated with either gonadotrophin releasing hormone or a progesterone releasing intravaginal device. Vet Rec 1982;110:515-517.

27. Peters AR, Riley GM. Pulsatile LH secretion and its induction in post-partum beef cows. In: Karg H, Schallenberger E (eds), Factors Influencing Fertility in the Postpartum Cow. Current Topics in Veterinary Medicine and Animal Science, vol. 20. The Hague: Martinus Nijhoff, 1982;225-228.
28. Peters, AR. Effect of exogenous oestradiol-17ß on gonadotrophin secretion in post-partum beef cows. J Reprod Fertil 1984;72:473-478.
29. Peters AR, Lamming GE. Reproductive activity of the cow in the post-partum period. II. Endocrine patterns and induction of ovulation. Br Vet J 1984;140:269-280.
30. Pratt BR, Berardinelli JG, Stevens LP, Inskeep EK. Induced corpora lutea in the postpartum beef cow. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestogen and estrogen. J Anim Sci 1982;54:822-829.
31. Rajamahendran R, Taylor C. Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. J Reprod Fertil 1991;92:461-467.
32. Robinson NA, Leslie KE, Walton JS. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J Dairy Sci 1989;72:202-207.
33. Sanchez T, Wehrman ME, Kojima FN, Cupp AS, Bergfeld EG, Peters KE, Mariscal V, Kittok RJ, Kinder JE. Dosage of the synthetic progestin, norgestomet, influences luteinizing hormone pulse frequency and endogenous secretion of 17ß-estradiol in heifers. Biol Reprod 1995;52:464-469.
34. SAS. SAS/STAT User's Guide (Release 6.03). Cary NC: SAS Institute. Inc, 1988.
35. Savio JD, Thatcher WW, Morris GR, Entwistle K, Drost M, Mattiacci MR. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. J Reprod Fertil 1993;98:77-84.
36. Schallenberger E, Schams D, Bullermann B, Walters DL. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J Reprod Fertil 1984;71:493-501.
37. Schallenberger E, Ulrich P, Mostl E, Fuchs S, Tenhumberg H. Induction of superovulation in cattle comparing single subcutaneous and repeated epidural with standard intramuscular administration of FSH. Theriogenology 1994;41:290 abstr.
38. Schmitt EJ, Barros CM, Fields PA, Fields MJ, Diaz T, Kluge JM, Thatcher WW. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on Day five of the estrous cycle. J Anim Sci 1996;74:1915-1929.
39. Schramm RD, Roberge S, Reeves JJ. Enclomiphene does not alter the postpartum interval of suckled beef cows. J Anim Sci 1991;69:4112-4116.
40. Sheffel CE, Pratt BR, Ferrell WL, Inskeep EK. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J Anim Sci 1982;54:830-836.
41. Sianangama PC, Rajamahendran R. Effect of hCG administration on Day 7 of the estrous cycle on follicular dynamics and cycle length in cows. Theriogenology 1996;45:583-592.
42. Sianangama PC, Rajamahendran R. Characteristics of corpus luteum formed from the first wave dominant follicle following hCG in cattle. Theriogenology 1996;45:977-990.
43. Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990;127:916-925.
44. Stagg K, Diskin MG, Sreenan JM, Roche JF. Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. Anim Reprod Sci 1995;38:49-61.
45. Stevenson JS, Knoppel EL, Minton JE, Salfen BE, Garverick HA. Estrus, ovulation, luteinizing hormone, and suckling-induced hormones in mastectomized cows with and without unrestricted presence of the calf. J Anim Sci 1994;72:690-699.
46. Stevenson JS, Pursley JR. Resumption of follicular activity and interval to postpartum ovulation after exogenous progestins. J Dairy Sci 1994;77:725-734.

47. Stock AE, Fortune JE. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 1993;132:1108-1114.
48. Taylor C, Rajamahendran R, Walton JS. Ovarian follicular dynamics and plasma luteinizing hormone concentrations in norgestomet-treated heifers. Anim Reprod Sci 1993;32:173-184.
49. Taylor C, Manikkam M, Rajamahendran R. Changes in ovarian follicular dynamics and luteinizing hormone profiles following different progestagen treatments in cattle. Can J Anim Sci 1994;74:273-279.
50. Thomas GB Jr. Calculus. Cambridge MA: Addison-Wesley, 1953.
51. Troxel TR, Kesler DJ. The effect of progestin and GnRH treatments on ovarian function and reproductive hormone secretions of anestrous postpartum suckled beef cows. Theriogenology 1984;21:699-711.
52. Viker SD, Larson RL, Kiracofe GH, Stewart RE, Stevenson JS. Prolonged postpartum anovulation in mastectomized cows requires tactile stimulation by the calf. J Anim Sci 1993;71:999-1003.
53. Vorstermans JPM, Walton JS. Effect of intermittent injections of gonadotrophin releasing hormone at various frequencies on the release of luteinizing hormone and ovulation in dairy cows. Anim Reprod Sci 1985;8:335-347.
54. Walters DL, Short RE, Convey EM, Staigmiller RB, Dunn TG, Kaltenbach CC. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small dose injections of GnRH. Biol Reprod 1982;26:655-662.
55. Walters DL, Schallenberger E. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the periovulatory phase of the oestrous cycle in the cow. J Reprod Fertil 1984;71:503-512.
56. Wettemann RP, Beck TW, Turman EJ, Hintz RL. Endocrine response of postpartum anestrous beef cows to GnRH or PMSG. Theriogenology 1982;18:599-613.
57. Williams GL. Suckling as a regulator of postpartum rebreeding in cattle: a review. J Anim Sci 1990;68:831-852.