STIMULATION OF ESTROGEN AND LUTEINIZING HORMONE SECRETION IN POSTPARTUM BEEF COWS[1,2]

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SUMMARY

Three experiments were conducted to determine whether and how weaning, quantity of milk produced, suckling intensity, age of dam, or a combination of these factors influence ovarian and/or pituitary response to pregnant mare serum gonadotropin (PMS), synthetic gonadotropin-releasing hormone (Gn-RH), or ovariectomy in postpartum beef cows. Ovarian and pituitary responses, as determined by plasma estrogen, progesterone, and luteinizing hormone (LH) concentrations, were evoked in both 3-year-old and mature anestrous, suckled cows (experiment 1) at 42 days postpartum with either PMS, Gn-RH, or cessation of lactation. All three dosages of PMS (750, 1,500, or 2,250 IU) stimulated estrogen secretion and a subsequent LH surge in anestrous suckled and nonsuckled cows; whereas, PMS only stimulated estrogen secretion in the cows already cyclic at 42 days postpartum. Cows given Gn-RH (75, 150, or 300 μg) had a dose related, biphasic increase in peripheral plasma LH concentration. A comparison between 2-year-old suckled Brown Swiss and Angus heifers at 42 days postpartum (experiment 2) indicated that Brown Swiss produced more milk, and had a longer interval from administration of 2,250 IU PMS to occurrence of the preovulatory LH surge; both breeds had increased ovarian and pituitary stimulation with 2,250 IU PMS as compared with 750 IU. Effect of suckling intensity on LH secretion was evaluated in nonsuckled, restricted nursed (twice daily), and unrestricted nursed 2-year-old ovariectomized heifers (experiment 3) from which blood samples were collected at 3, 9, 16 and 30 days postpartum. Mean LH was not affected by treatment, but did increase linearly between 9 and 30 days postpartum. However, nonsuckled heifers had an increased incidence of spontaneous LH releases and increased minimal LH concentration at 30 days postpartum when compared to the nursed groups. These results suggest that lactation suppresses gonadotropin secretion in early postpartum cows.

(**Key Words**: Postpartum Cows, Luteinizing Hormone, Estrogen, Progesterone, Pregnant Mare Serum Gonadotropin.)

INTRODUCTION

Reducing the interval from parturition to conception in beef cows is advantageous to efficient beef production. Duration of postpartum anestrous can be influenced by lactation, age, breed, nutrition, or a combination of these factors (Wiltbank and Cook, 1958; Wagner and Hansel, 1969; Dunn *et al.*, 1969; Oxenreider and Wagner, 1971). Laster *et al.* (1973) reported that early weaning of calves from beef cows, especially 2- and 3-year-olds, increased the percentage of cows exhibiting estrus during the subsequent breeding period. Because early weaning is not feasible in most beef production systems, identification of lactational effects on the reproductive system of postpartum cows is warranted. Cessation of nursing removes both the endocrine events associated with the neural stimulus required for milk ejection and the

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metabolic demands of milk production; hence, their effects on postpartum anestrus should be evaluated independently. The present study was conducted to determine whether and how such factors as weaning, quantity of milk produced, suckling intensity, age of dam, or a combination of these factors influence ovarian and/or pituitary response to pregnant mare serum gonadotropin (PMS), synthetic gonadotropin-releasing hormone (Gn-RH), or ovariectomy in postpartum beef cows.

MATERIALS AND METHODS

Three experiments were conducted to evaluate ovarian and pituitary responses in postpartum cows. Ovarian responsiveness, sensitivity, and status were evaluated by quantitation of plasma estradiol (E_2), estrone (E_1), and progesterone after PMS. Pituitary responsiveness and sensitivity were evaluated on the basis of plasma LH concentration after Gn-RH (experiment 1) and PMS (experiments 1 and 2) and after ovariectomy (experiment 3). Cows were fed a ration that provided 100% NRC requirements for lactating cows producing 5 kg of milk except in experiment 3 when nonsuckled cows were fed 100% NRC for nonlactating cows. Estrus was monitored twice daily for 10 days prior to treatment in both experiment 1 and 2 but was not detected in any of the cows prior to treatment; therefore, pretreatment ovarian status for cows given PMS or Gn-RH was determined by palpation of the ovaries one day before treatment and by plasma progesterone concentration. A portion of the PMS-treated cows in experiment 1 was classified as cyclic at 42 days postpartum because a corpus luteum was palpable, and/or a preovulatory LH surge was not detected but plasma progesterone was >1 ng/ml at the time of or within 2 days after PMS treatment and remained elevated for at least 6 days.

Estradiol, E_1, and progesterone were extracted from plasma, separated on Sephadex LH-20 columns, and assayed as described by Echternkamp and Hansel (1973). Assays were validated by quantitation of known amounts of steroids added to distilled water or charcoal absorbed plasma from an ovariectomized cow. Interassay coefficients of variation for a plasma pool included in each assay were 7.2% for progesterone, 12.7% for E_1, and 9.4% for E_2.

Plasma LH concentrations were determined by the double antibody radioimmunoassay for bovine LH described by Niswender *et al.* (1969), except the pH of the .1M sodium phosphate buffer was 7.5. Purified LH (LER-1056-C2) was labeled with ^{125}I as described by Greenwood *et al.* (1963), placed on a 1- × 30-cm Sephadex G-100 column and eluted with .05 M sodium phosphate buffer, pH 7.5. The eluate corresponding to the descending part of the LH-^{125}I peak was diluted with gelatin-PBS (.1% gelatin in .14 M NaCl, .01M sodium phosphate buffer, pH 7.5) to 50,000 cpm/.1 ml. More than 90% of the LH-^{125}I was bound by the anti-LH serum, DJB 3-12/11, at the dilution of 1:300, 45 to 50% was bound by the serum at the working dilution of 1:40,000. The ovine anti-rabbit γ-globulin (DJB 5×3) was used at a dilution of 1:80. An assay was composed of 16 concentrations of bovine LH, NIH-LH-B8, ranging from 25 pg to 50 ng/tube, 75 unknown plasma samples in duplicate, and aliquots of two plasma pools. Each plasma sample was assayed at .1 and .2 ml, and the coefficient of variation of the mean was less than 10%. Mean LH concentrations and interassay coefficients of variation for the two plasma pools were .6 and 8.2 ng/ml, and 8.7 and 5.3%, respectively. Inhibition curves obtained with National Institutes of Health (NIH) hormone preparations of prolactin (PRL-B2), follicle stimulating hormone (FSH-B1), growth hormone (GH-B14), and thyroid stimulating hormone (TSH-B3) were parallel to the LH standard, and the inhibition was equal to the bioassayable LH contamination of the preparation. Minimum and maximum detectable concentrations of LH were defined as amounts of LH standard yielding 95 and 5%, respectively, of the counts per minute obtained for the control buffer tubes; these concentrations were .2 and 175 ng LH/ml of plasma, respectively. Recovery of added amounts of NIH-LH-B8 from .2 ml of plasma ranged from 97 to 100%. Plasma LH concentrations are expressed as ng of NIH-LH-B8/ml of peripheral plasma.

Assays were evaluated and concentrations determined as described by Robard *et al.* (1968). Data were analyzed by analysis of variance and Duncan's multiple-range test (Steel and Torrie, 1960). A split-plit analysis of variance was used for data involving repeated measurements within the same animal (Steel and Torrie, 1960). Response to Gn-RH was quantitated both by plasma LH concentration and area under the LH response curve. Relationships between quantitated parameters were established by simple correlations.

Experiment 1. Effects of suckling (suckled *vs* nonsuckled) and age [3-year-old (second calf) *vs* mature] on ovarian and pituitary sensitivity and function were evaluated in 48 Hereford cows at 42 days postpartum. Ovarian and pituitary sensitivity and function were monitored by determination either of plasma estradiol and LH concentrations for 24 cows after three dosages of PMS (750, 1500, or 2,250 IU) or of LH in another 24 cows after three dosages of Gn-RH (75, 150, or 300 μg). Two cows were assigned per lactational status, age, and dosage of hormone in a 2 × 2 × 3 factorial arrangement of treatments. Zero dosages of PMS and Gn-RH were not included because objectives of this study were to determine 1) whether gonadotropin and steroid secretion could be stimulated in the postpartum cow and 2) the influence of age and lactational variables on these responses. Exogenous hormones were administered intramuscularly in saline at 42 days postpartum. Calves were weaned from the nonsuckled groups at 35 days postpartum; thus 7 days were allowed before treatment for the nonsuckled cows to acclimate to the stress of weaning.

Jugular vein blood (50 ml) of cows treated with PMS was collected by venipuncture at 6-hr intervals from 1 day before (for establishment of hormone concentration baseline) through 5 days after treatment. Blood collections were continued every 6 hr for another 6 days in nonsuckled 3-year-old and suckled mature cows to assure a complete response as estrus was not detected after PMS in these two groups. Jugular blood (10 ml) of cows treated with Gn-RH was sampled by venipuncture at 10-min intervals beginning 1 hr before and continuing for 4 hr after Gn-RH treatment. Blood was collected into heparinized synringes, refrigerated, and centrifuged, and plasma stored at −10 C until assayed.

Experiment 2. Two-year-old lactating Angus and Brown Swiss heifers, 12 of each breed, were treated with 750 or 2,250 IU of PMS at 42 days postpartum as in experiment 1 to determine 1) cause(s) of previously observed breed differences in duration of the postpartum anestrous period and 2) the relationship between postpartum anestrous and milk production. Ovarian responsiveness and status were evaluated after measurement of E_2 and progesterone in peripheral plasma samples collected at 12-hr intervals from 2 days before through 10 days after the PMS injection and at 2-day intervals for another 6 days. Plasma LH concentration was determined for blood samples collected at 6-hr intervals for 6 days after PMS. Blood samples were collected by venipuncture and handled as in experiment 1.

Daily milk production for each cow was estimated at 34, 40 and 60 days postpartum from amounts of milk consumed by its calf in two consecutive feedings; each of which followed 12 hr of separation between the calf and dam. Calves were weighed just before and after feeding and weight increase associated with nursing was attributed to milk consumed.

Experiment 3. Influence of suckling intensity on LH secretion was determined with 15 2-year-old Charolais heifers; they were ovariectomized at 3 days postpartum and assigned to one of three groups: 1) calf weaned; 2) calf present for 30 min at 8 am and 5 pm; 3) calf present continuously. Jugular vein blood for LH determination were collected via indwelling catheters at 15-min intervals for 4 hr at −1, 6, 13, and 27 days after surgery and handled as in experiment 1.

RESULTS

Experiment 1

PMS. Treatment with PMS increased plasma estrogen concentrations within 12 hr (figure 1)

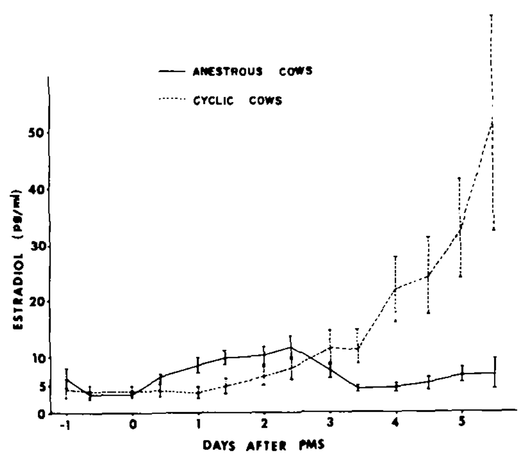

Figure 1. Combined plasma estradiol concentrations (experiment 1) for 10 anestrous and 14 cyclic cows treated with pregnant mare serum gonadotropin (PMS), regardless of the PMS dosage, age of cow or lactational status (Day 0 = day of treatment). Criteria for classification of cows as anestrus or cyclic are described in Materials and Methods.

and caused a surge (>10 ng/ml) in LH secretion within 3 days after injection (table 1) in five of six and two of six suckled and nonsuckled 3-year-old cows, and two of six and one of six suckled and nonsuckled mature cows, respectively. The LH surge in these anestrous cows coincided with or preceded a precipitous decrease in E_2 (table 2). Estradiol rather than E_1 was the predominant estrogen secreted before the LH surge (table 2). Maximal plasma E_1 concentrations were about 10% of maximal E_2 concentrations when compared within an animal. Increases in plasma E_2 >5 pg/ml were parallel with increases in E_1 (r = .92); however, when E_2 concentrations were lower, concentrations of the two steroids fluctuated independently (r = .19). Progesterone began to increase about 6 days after treatment, or 4 days after the LH surge (table 2), and was increased for the duration of the blood sampling period. Dosage of PMS, age, and lactational status did not affect plasma E_2 concentrations before the LH surge or the interval from PMS to the LH surge (table 1).

In the other 14 cows treated with PMS, PMS stimulated estrogen secretion (cyclic, figure 1), so that E_2 concentration increased 5 or 6 days but did not cause an increase in LH ≥4 ng/ml. Rate of E_2 reduction was gradual (2 to 3 days) in cyclic cows; whereas, the E_2 reduction associated with the LH surge was precipitous in anestrous cows. Again, more E_2 was secreted than E_1. The 14 cows without an LH surge had plasma progesterone ≥1 ng/ml at the time of or within 2 days after PMS that remained elevated for at least 6 days.

Gn-RH. Treatment with 75, 150, or 300 μg of Gn-RH increased LH concentrations (table 3) in plasma samples collected at 10 min intervals. Initially, all three dosages of Gn-RH induced an LH peak within 20 to 30 min (mean = 28.7 ± 2.4 min) (figure 2). An additional LH peak was detected at 110.4 ± 3.2 min within cows treated with 300 μg Gn-RH and to a lesser extent within those treated with 75 or 150 μg. These results suggest a biphasic LH response to Gn-RH (figure 2). Also, magnitude of the total LH response (area) varied directly (P<.01) with Gn-RH dosage (table 3). Unlike cows receiving PMS, palpable corpora lutea and plasma proges-

TABLE 1. OVARIAN AND PITUITARY RESPONSE TO PMS IN ANESTROUS COWS

Treatment	n[a]	Interval from PMS to LH surge	Maximum E_2 concentration first peak	Maximum E_2 concentration second peak
		(hr)	(pg/ml)	(pg/ml)
Experiment 1				
Suckled				
3-year-old	5	61.6 ± 6.7	11.2 ± 1.7	
Mature	2	63.0 ± .0	18.2 ± 1.0	
Mean		62.0 ± 4.7	13.2 ± 1.7	
Nonsuckled				
3-year-old	2	68.5 ± 3.5	18.6 ± 6.0	
Mature	1	39.0	9.1	
Mean		58.7 ± 10.0	15.5 ± 4.7	
Overall mean	10	61.0 ± 4.1	13.9 ± 1.7	
Experiment 2				
750 IU of PMS				
Angus	3	82.0 ± 12.2[b]	8.7 ± 1.3[bc]	2.5 ± .4[d]
Brown Swiss	5	69.6 ± 7.0[b]	8.3 ± 1.5[bc]	3.5 ± .5[d]
Mean		74.3 ± 6.2[b]	8.4 ± 1.0[bc]	3.1 ± .4[d]
2,250 IU of PMS				
Angus	6	55.0 ± 3.9[c]	8.6 ± .7[b]	18.4 ± 3.6[e]
Brown Swiss	6	68.0 ± 4.0[b]	12.1 ± 1.3[c]	26.6 ± 6.7[e]
Mean		61.5 ± 3.3[bc]	10.4 ± .9[bc]	22.5 ± 3.8[e]

[a]Each treatment group contained six cows, but data are reported for only cows with both increased estradiol (E_2) concentrations and a luteinizing hormone (LH) surge.

[b,c,d,e]Superscripts b and c denote significance within a column for experiment 2 at P<.05; d and e at P<.01.

TABLE 2. MEAN DAILY PLASMA LH, ESTRADIOL, ESTRONE, AND PROGESTERONE CONCENTRATIONS FOR ANESTROUS COWS TREATED WITH PMS (Experiment 1)[a]

Hours[b]	LH concentration	Estradiol concentration	Estrone concentration	Progesterone concentration
	(ng/ml)	(pg/ml)	(pg/ml)	(ng/ml)
-96	1.25 ± .22	2.78 ± .50	2.13 ± .34	.08 ± .03
-72	1.83 ± .18	3.85 ± .66	2.09 ± .37	.74 ± .44
-48	2.45 ± .43	6.47 ± 1.05	1.92 ± .25	.37 ± .15
-24	2.66 ± .33	9.43 ± 1.16	2.35 ± .29	.32 ± .10
0	32.22 ± 3.04	13.65 ± 1.96	2.48 ± .34	.22 ± .05
24	2.45 ± .40	4.75 ± .92	1.35 ± .16	.17 ± .05
48	2.02 ± .18	5.22 ± .95	1.38 ± .18	.19 ± .04
72	1.91 ± .27	7.54 ± 1.91	1.51 ± .22	.35 ± .11
96	1.96 ± .51	11.76 ± 3.03	1.47 ± .24	2.15 ± 1.32

[a]Mean daily hormone concentrations for 10 anestrous postpartum cows treated with 750, 1,500 or 2,250 IU of PMS.

[b]Hours before and after the LH surge (Day 0). The LH surge occurred 61.0 ± 4.1 hr after the cows were treated with PMS.

terone >1 ng/ml were not detected at the time Gn-RH was administered to these 24 cows. If any of these cows were cyclic at the time of Gn-RH was given, it did not affect incidence or timing of the LH response, but may have increased among animal variation in magnitude of LH release. The LH response to Gn-RH at 42 days postpartum was not affected (P>.05) by age or lactational status of the 24 beef cows used in this arrangement of treatments.

Experiment 2

Plasma E_2 concentrations increased within 12 hr after the injection of 750 IU of PMS into 12 2-year-old suckled Angus and Brown Swiss heifers at 42 days postpartum (figure 3). A subsequent LH surge (table 1) occurred in three of six Angus, and five of six Brown Swiss heifers; after the surge, E_2 concentration decreased precipitously. Maximal E_2 concentration in all but one of the heifers without an LH surge was lower than that in cows with the surge (5.0 ± 1.2 *vs* 8.4 ± 1.0 pg/ml). The excepted cow had a maximum E_2 concentration of 8.0 pg/ml at 60 hr after injection. Plasma E_2 concentrations were not different (P>.05) between breeds. A small increase in plasma E_2 concentration was detected between 6 and 10 days after injection of PMS (figure 3) for heifers with an LH surge.

Administration of 2,250 IU of PMS resulted in two successive E_2 peaks (figure 3) separated by an LH surge in all 12 heifers. Maximal plasma E_2 concentrations for the first peak (table 1) were higher (P<.05) for Brown Swiss (12.1 ± 1.3 pg/ml) than for Angus heifers (8.6 ± .7). Interval from PMS injection to occurrence of the LH surge was longer (P<.05) for Brown Swiss (68 ± 4 hr) than for Angus (55 ± 4 hr). The second E_2 peak (figure 3) occurred 202 ± 6.4 hr after the PMS injection, had an average magnitude of 22.5 ± 3.8 pg/ml, was higher (P<.01) than the first E_2 peak, and was not affected by breed.

The difference in magnitude of the first E_2 peak (table 1) between heifers treated with 750 and 2,250 IU of PMS approached significance (P<.10) for Brown Swiss (8.3 ± 1.5 and 12.1 ± 1.3 pg/ml, respectively) but not for Angus heifers. Magnitude of the second E_2 peak (table 1) was greater (P<.01) for heifers treated with 2,250 IU of PMS than for those treated with 750 IU.

An increase >.5 ng/ml in plasma progesterone was observed 5 to 6 days after PMS in the 20 animals exhibiting an LH surge and lasted from 3 to 13 days (mean = 6.7 ± .8 days). Maximum increase ranged from 1.8 to 9.5 ng/ml (mean = 4.4 ± .6 ng/ml). Figure 4 shows plasma progesterone concentrations for Brown Swiss heifers treated with 2,250 IU of PMS. The high degree of variability in both magnitude and duration of progesterone secretion typifies all treatment groups. Thus, progesterone secretion was not influenced significantly

TABLE 3. LH RESPONSE FOR SUCKLED AND NONSUCKLED 3-YEAR-OLD AND MATURE BEEF COWS TREATED WITH 75, 150, OR 300 μg OF Gn-RH[a]

Age and lactational status	n[b]	75 μg Gn-RH			150 μg Gn-RH			300 μg Gn-RH		
		LH (first peak)	LH (second peak)	Area[c]	LH (first peak)	LH (second peak)	Area[c]	LH (first peak)	LH (second peak)	Area[c]
		(ng/ml)	(ng/ml)	(cm^2)	(ng/ml)	(ng/ml)	(cm^2)	(ng/ml)	(ng/ml)	(cm^2)
3-year-old										
Suckled	2	6.9	4.7	3.94	14.1	7.4	7.91	30.7	70.0	62.91
Nonsuckled	2	7.9	5.8	6.10	13.4	14.8	6.29	32.0	139.4	91.33
Mean		7.4 ± 1.9	5.2 ± 2.5	5.02 ± 2.29	13.7 ± 5.3	11.1 ± 6.1	7.10 ± 3.10	31.3 ± 5.4	104.7 ± 25.6	77.12 ± 12.92
Mature										
Suckled	2	15.9	4.1	4.71	14.6	19.4	13.84	50.5	94.2	65.52
Nonsuckled	2	12.5	11.0	8.07	12.3	16.7	13.81	13.0	61.8	28.78
Mean		14.2 ± 5.6	7.6 ± 3.4	6.39 ± 2.17	13.4 ± 3.7	18.0 ± 5.9	13.82 ± 4.44	31.7 ± 11.3	78.0 ± 23.2	47.15 ± 15.58
Overall mean[d]	8	**10.8 ± 3.0**	**6.4 ± 2.0**	**5.70 ± 1.48**	**13.6 ± 3.0**	**14.5 ± 4.2**	**10.46 ± 2.81**	31.5 ± 5.8	91.3 ± 16.8	62.13 ± 10.95

[a]The LH response to Gn-RH consisted of two LH peaks at 28.7 ± 2.4 and 110.4 ± 3.2 min after Gn-RH injection. Means of maximal LH concentrations.

[b]n = number of animals per dosage group.

[c]Total area under the LH response curve.

[d]Boldface means are not significantly different (P>.05).

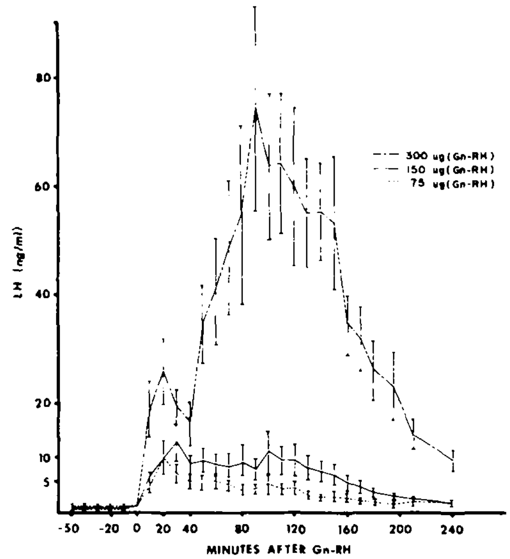

Figure 2. Plasma LH concentrations before and after treatment with 75, 150 or 300 µg of gonadotrophin releasing hormone (Gn-RH) on Day 42 postpartum. The LH concentrations for each dosage of Gn-RH are the mean of eight cows.

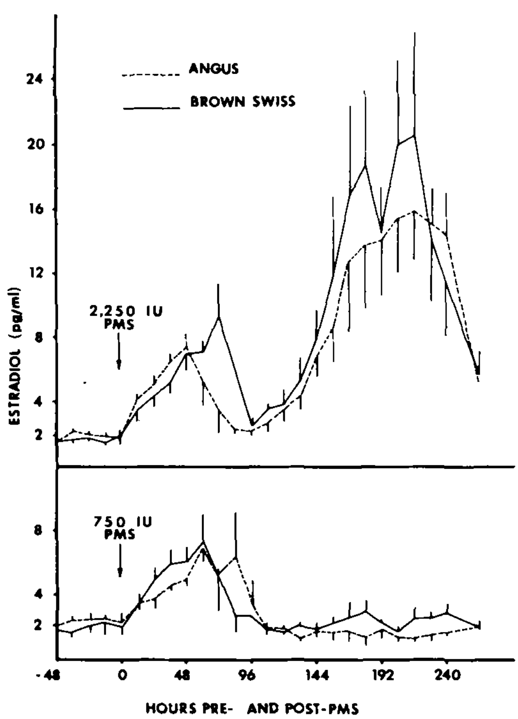

Figure 3. Plasma estradiol concentrations, before and after treatment with 750 or 2,250 IU of pregnant mare serum gonadotropin (PMS) on Day 42 postpartum, for 2-year-old Angus and Brown Swiss heifers (six heifers/PMS dosage/breed).

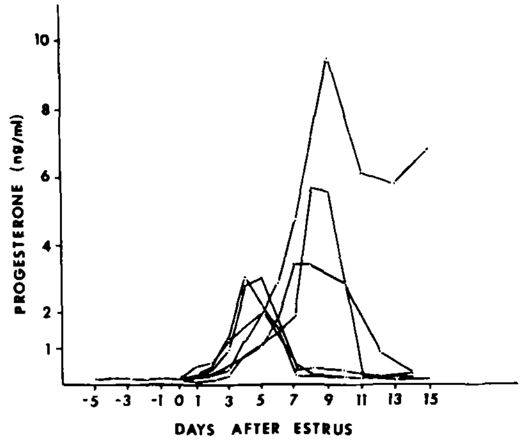

Figure 4. Plasma progesterone concentrations for six Brown Swiss heifers treated with 2,250 IU of pregnant mare serum gonadotropin. Day 0 is the day of the LH surge.

by PMS dosage or breed of cattle.

Table 4 contains means for daily milk consumption, body weight, and simple correlations between milk consumption and body weight for Angus and Brown Swiss calves at about 34, 40 and 60 days of age. Brown Swiss calves were heavier ($P<.01$) and consumed more ($P<.01$) milk than Angus calves at all three ages (overall mean difference, milk consumption, 2.2 kg; body weight 17.7 kg). The overall within-breed correlation between daily milk consumption and empty body weight (table 4) was significant ($P<.05$) for the Angus ($r = .39$) but not for the Brown Swiss calves ($r = .24$). The overall between-breed correlation for these two parameters ($r = .21$) was also significant ($P<.01$).

Experiment 3

For each cow at each sampling date, a mean plasma LH concentration was calculated from LH concentrations of the 17 blood samples collected over the 4-hr sampling period; the means of these means were then calculated according to sampling date and treatment group. Similarly for each cow and sampling date, the minimal and maximal plasma LH concentrations were recorded; and their respective means were calculated for each sampling period by treatment (table 5). The means in table 5 then allowed comparison of the effects of suckling and time after parturition on LH secretion.

TABLE 4. MEAN DAILY MILK CONSUMPTION AND BODY WEIGHT FOR ANGUS AND BROWN SWISS CALVES AT 34, 40 AND 60 DAYS OF AGE

Item	Angus	Brown Swiss	Diff.
No. of cows[a]	12	11	
Period I			
Age (days)	33.6 ± .8	34.6 ± .9	
Milk consumption (kg)	4.5 ± .3	6.9 ± .4	2.4**
Body weight (kg)[b]	42.4 ± 1.4	58.1 ± 1.9	15.7**
r^2 (milk consumption *vs* body wt)[c]	.03 (.17)	.02 (.14)	
Period II			
Age (days)	40.1 ± .8	41.1 ± .9	
Milk consumption (kg)	4.8 ± .3	6.2 ± .4	1.4**
Body weight (kg)	45.5 ± 1.4	61.9 ± 1.8	16.4**
r^2 (milk consumption *vs* body wt)[c]	.45 (.67)*	.12 (.35)	
Period III			
Age (days)	59.6 ± .8	60.6 ± .9	
Milk consumption (kg)	5.0 ± .4	7.8 ± .7	2.8**
Body weight (kg)	54.0 ± 1.7	75.2 ± 2.6	21.2**
r^2 (milk consumption *vs* body wt)[c]	.13 (.36)	.02 (.14)	
Overall			
Milk consumption (kg)	4.8 ± .2	7.0 ± .3	2.2**
Body weight (kg)	47.4 ± 1.1	65.1 ± 1.8	17.7**
r^2 (milk consumption *vs* body wt)[c]	.15 (.39)*	.06 (.24)	

[a]Data for one Brown Swiss cow that stopped lactating were not included in the statistical analysis.

[b]Body weight is prenursing am weight.

[c]r values are in parentheses.

*P<.05; **P<.01.

Minimal, maximal, and mean plasma LH concentrations increased (P<.01) linearly over the 28-day experimental period. Basal secretion of LH fluctuated over the 4-hr blood sampling period. Spontaneous LH surges ≥5 ng/ml were detected in all cows at 27 days after surgery; whereas, less than one-half of the cows within a treatment group had LH surges before 27 days. Both frequency of spontaneous LH surges and minimal LH concentrations were greater at 27 days after surgery for nonsuckled cows than for the restricted and unrestricted nursing groups. Also, without inclusion of LH concentrations greater than two standard deviations above the animal mean, mean LH concentration was higher for the nonsuckled cows than for those in the other groups at 27 days after surgery. In contrast, suckling intensity had no effect (P>.05) on maximal and mean LH concentrations (table 5).

Discussion

Ovarian and pituitary responses, as determined by estrogen and LH concentrations in peripheral blood, were evoked in anestrous 42-day postpartum cows treated with PMS or Gn-RH. Hormonal and palpation data indicated that PMS dosages of 750, 1,500, or 2,250 IU stimulated estrogen secretion, which resulted in a subsequent LH surge and ovulation in both suckled and nonsuckled anestrous cows. Preovulatory plasma E_2 and LH concentrations and relationships resulting from PMS treatment were similar to those reported for proestrous cows (Echternkamp and Hansel, 1973; Glencross *et al.*, 1973). Because five of six nonsuckled 3-year-old cows but only one of six suckled cows were cyclic at 42 days postpartum, weaning of calves at 35 days postpartum appeared to reduce duration of the postpartum anestrous interval.

Gn-RH at 42 days postpartum in experiment 1 caused dose-related increases in plasma LH concentrations (figure 2), regardless of lactational status. The biphasic LH secretion pattern suggests that the initial response to Gn-RH was

TABLE 5. EFFECT OF SUCKLING ON LH SECRETION (ng/ml) IN OVARIECTOMIZED COWS

Treatment	Days after surgery[a]													
	−1 Day			6 Days			13 Days			27 Days				
	Group mean[b]	Minimum[c]	Maximum[d]	Group mean[b]	Minimum[c]	Maximum[d]	Group mean[b]	Minimum[c]	Maximum[d]	Group mean[b]	Minimum[c]	Maximum[d]	No. of LH surges[e]
Nonsuckled	.5 ± .1	.3 ± .1	.7 ± .1	.7 ± .2	.5 ± .1	.8 ± .1	1.1 ± .2	.7 ± .2	2.0 ± .5	3.7 ± .6	2.5 ± .5[f]	7.5 ± .4	2.3 ± .3[h]
Restricted nursing	.9 ± .2	.7 ± .1	1.3 ± .3	1.3 ± .2	.8 ± .1	1.9 ± .5	2.4 ± .9	1.4 ± .4	4.8 ± 2.1	2.7 ± .4	1.8 ± .3[fg]	4.8 ± 1.7	1.0 ± .4[i]
Unrestricted nursing	.9 ± .2	.6 ± .1	1.5 ± .6	.6 ± .1	.3 ± .1	.9 ± .2	1.0 ± .2	.6 ± .1	1.9 ± .5	2.7 ± .6	1.3 ± .3[g]	7.2 ± 2.4	1.2 ± .4[i]
Overall mean[j]	.8 ± .1	.5 ± .1	1.1 ± .2	.8 ± .1	.5 ± .1	1.2 ± .2	1.5 ± .3	.9 ± .2	2.9 ± .8	3.0 ± .3	1.9 ± .2	6.4 ± 1.0	1.5 ± .2

[a]All cows were ovariectomized on day 3 postpartum.

[b]Overall mean of plasma LH concentrations for the five cows in the treatment group.

[c]Mean of minimum plasma LH concentration for each cow.

[d]Mean of maximum plasma LH concentration for each cow.

[e]Number of spontaneous LH releases occurring within the 4-hr sampling period on day 27 after surgery. Average time interval between LH releases at 27 days was 80.0 ± 5.0 min for nonsuckled, 162.0 ± 21.9 min for restricted nursing, and 180.0 ± 25.5 min for unrestricted nursing.

[f,g,h,i]Different superscripts within a column denote significance at P<.05.

[j]The increase in LH concentrations with time was significant at P<.01.

the release of LH; then, both LH synthesis and release resulted in a second dose-dependent increase in plasma LH concentration. Biphasic responses to Gn-RH have also been reported for cows with ovarian follicular cysts (100 μg of Gn-RH; Kittok *et al.*, 1973), mature bulls (500 μg of Gn-RH; Schanbacher and Echternkamp, *unpublished data*), cyclic ewes (25 μg of LRF; Pelletier and Thimonier, 1975), and anestrous ewes (200 μg of LH-RH/FSH-LH; Reeves *et al.*, 1972). The biphasic response to Gn-RH was not observed in cows treated during the luteal phase (100 μg of Gn-RH; Kittok *et al.*, 1973) or after removal of a progesterone implant (240 μg of Gn-RH; Kaltenbach *et al.*, 1974). Stage of an estrous cycle (Reeves *et al.*, 1970; Pelletier and Thimonier, 1975) and concentrations of exogenous estrogen (Reeves *et al.*, 1971) and progesterone (Hooley *et al.*, 1974) influence the timing and magnitude of the pituitary response to Gn-RH in ewes. Therefore, the presence of the initial release of LH to Gn-RH in the biphasic response may depend upon the reproductive status and pituitary LH content of the animal, regardless of species.

Treatment of 2-year-old heifers (experiment 2) with PMS induced LH surges in eight of 12 heifers treated with 750 IU of PMS and in 12 of 12 heifers treated with 2,250 IU. Three of the four heifers without an LH surge had little or no increase in plasma E_2 concentration. Subminimal steroidogenic responses suggest that the gonadotropic stimulus was not of sufficient magnitude to completely initiate steroidogenesis or that level of ovarian sensitivity to PMS was lower in these animals; therefore, induction of an ovarian response with 750 IU of PMS may depend upon the degree of follicular development, or quantity of endogenous gonadotropin secretion, or both. The higher incidence of E_2 secretion after treatment with 2,250 IU of PMS than with 750 IU suggests that ovarian function and duration of the postpartum interval in lactating cows may be related to endogenous FSH or LH secretion rates, or both.

The interval from the administration of 2,250 IU of PMS to the appearance of an LH surge (table 1) was longer, and the preovulatory E_2 concentrations after 2,250 IU of PMS were higher for Brown Swiss than for Angus heifers. The longer interval for Brown Swiss heifers may indicate that their pituitaries were less responsive to, or had a higher threshold for, estrogen stimulation. Because the Brown Swiss pituitary required a longer period of estrogen stimulation to induce an LH surge, their preovulatory E_2 concentrations were higher as a result of additional time for ovarian follicular development and E_2 secretion. The higher E_2 concentrations did not appear to be essential for release of LH because lower concentrations of E_2 mediated an LH surge within the same time interval in Brown Swiss heifers treated with 750 IU of PMS. In experiment 3, the basal mode of LH secretion and the frequency of episodic LH releases were greater for nonsuckled than for suckled cows at 30 days postpartum. These differences indicate that gonadotropin secretion is influenced by the suckling stimulus, or production of milk, or both. Because Brown Swiss calves consumed more milk than Angus calves (table 4) – and presumably the amount consumed reflected the quantity of milk produced by the dams – the delayed LH response in the Brown Swiss heifers after treatment with 2,250 IU of PMS may have been associated with the increased milk production.

Results in experiment 1 and 2 showed that plasma E_2 concentrations (tables 1 and 2) for the initial E_2 peak in postpartum anestrous cows treated with PMS were similar to those reported for proestrous cows (Echternkamp and Hansel, 1973) but were considerably less than those reported for superovulated cows (Booth *et al.*, 1975). The initial E_2 peak was not influenced significantly by PMS dosage; but the second E_2 peak, which was observed in experiment 2 (table 1) about 5 days after the first, was related to dosage of PMS. The presence of two E_2 peaks for heifers treated with 2,250 IU of PMS is interpreted to mean that the initial E_2 peak was due to stimulation of an existing graafian follicle and subsequent ovulation as occurs during proestrus, and that the second E_2 peak resulted from the stimulation of secondary or atretic follicles, which required 3 to 6 more days to grow, develop and mature. The bovine estrous cycle is characterized by follicular growth (Bane and Rajakoski, 1961) and increased plasma E_2 concentrations (Hansel and Echternkamp, 1972; Glencross *et al.*, 1973) between Days 2 and 5 of the cycle (estrus = Day 0); hence the second E_2 peak could also have resulted from enhanced stimulation of these follicles by the 2,250 IU of PMS.

Only five of the 20 PMS-treated heifers with an LH surge in experiment 2 had progesterone concentrations characteristic of the luteal phase of the bovine estrous cycle (Hansel and Echternkamp, 1972). The other 15 heifers had progesterone patterns below average in quantity, or duration, or both, as typified in figure 4; these progesterone patterns suggest either inadequate luteal development and support or follicular luteinization without ovulation. Possibly, the short luteal phases after PMS treatment were analogous to the short luteal phases (<10 days) with reduced plasma progesterone concentrations detected before the first postpartum estrus in spontaneously ovulating cows (Corah *et al.*, 1974; Castenson *et al.*, 1976). Castenson *et al.* (1976) concluded that the increase in peripheral progesterone which preceded the first postpartum estrus resulted from corpus luteum formation preceded by ovulation without estrus. Even though ovulation can be induced, apparently the status of the reproductive system of some postpartum cows is not conducive to normal luteal function.

In experiment 3, plasma LH concentrations increased with length of the postpartum interval in both suckled and nonsuckled ovariectomized cows as has been reported for intact lactating dairy (Echternkamp and Hansel, 1973; Edgerton and Hafs, 1973) and beef cows (Arije *et al.*, 1974). Fernandes *et al.* (1976) reported that the LH response to Gn-RH (100 μg) in lactating dairy cows increased in magnitude with time when compared at 3, 10 and 20 days postpartum. Presumably, the increase in LH secretion reflected an increase in LH content of the bovine pituitary, and such an increase in the pituitary during the same postpartum period has been observed (Saiduddin *et al.*, 1966).

The higher tonic secretion (baseline) and greater frequency of LH surges (table 5) for nonsuckled than for unrestricted nursing cows at 30 days postpartum indicate that lactation may suppress LH secretion. Randel *et al.* (1976) noted higher plasma LH concentrations in nonsuckled than in suckled cows within 7 days postpartum. Short *et al.* (1974) reported that suckling, in comparison with nonsuckling, tended to decrease the proportion of cows with an acute LH release in response to 10 mg of exogenous estradiol-17β at 2 weeks postpartum, but not at 4, 6, or 8 weeks. Results of experiment 1 in the present study showed that the effect of suckling on LH secretion had apparently disappeared by 42 days postpartum because suckling had no effect on the pituitary LH response to 75, 150, or 300 μg of exogenous Gn-RH. Because plasma LH concentration and the LH response to its mediators appear to be related to pituitary LH content, suckling may suppress LH secretion by retarding the transition of the bovine pituitary from a state of low LH content during gestation to a state of higher LH content in the cyclic cow. The greater ovarian response to 2,250 IU than to 750 IU of PMS in the 2-year-old heifers suggests that FSH may also have a role in postpartum reproduction, but the suggestion cannot be confirmed because of the lack of information regarding FSH secretion in the cow.

LITERATURE CITED

Arije, G. R., J. N. Wiltbank and M. L. Hopwood. 1974. Hormone levels in pre- and post-parturient beef cows. J. Anim. Sci. 39:338.

Bane, A. and E. Rajakoski. 1961. The bovine estrous cycle. Cornell Vet. 50:3.

Booth, W. D., R. Newcomb, H. Strange, L. E. A. Rowson and H. B. Sacher. 1975. Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. Vet. Rec. 97:366.

Castenson, P. E., A. M. Sorensen, Jr., C. R. Cobos and J. L. Fleeger. 1976. Source of postpartum P and 20β-OHP preceding estrus in heifers. J. Anim. Sci. 43:277. (Abstr.)

Corah, L. R., A. P. Quealy, T. G. Dunn and C. C. Kaltenbach. 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. J. Anim. Sci. 39:380.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman and J. N. Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci. 29:719.

Echternkamp, S. E. and W. Hansel. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Anim. Sci. 37:1362.

Edgerton, L. A. and H. D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

Fernandes, L. C., W. W. Thatcher and C. J. Wilcox. 1976. LH responses to GnRH in postpartum dairy cows. J. Anim. Sci. 42:269. (Abstr.)

Glencross, R. G., I. B. Munro, B. E. Senior and G. S. Pope. 1973. Concentrations of oestradiol-17β, oestrone and progesterone in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta. Endocrinol. 73:374.

Greenwood, F. D., W. M. Hunter and J. S. Glover. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. 90:114.

Hansel, W. and S. E. Echternkamp. 1972. Control of ovarian function in domestic animals. Amer. Zool. 12:225.

Hooley, R. D., B. W. Baxter, W. A. Chamley, I. A. Cumming, H. A. Jonas and J. K. Findlay. 1974. FSH and LH response to gonadotropin-releasing hormone during the ovine estrous cycle and following progesterone administration. Endocrinology 95:937.

Kaltenbach, C. C., T. G. Dunn, T. K. Kiser, L. R. Corah, A. M. Akbar and G. D. Niswender. 1974. Release of FSH and LH in beef heifers by synthetic gonadotrophin releasing hormone. J. Anim. Sci. 38:357.

Kittok, R. J., J. H. Britt and E. M. Convey. 1973. Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cysts. J. Anim. Sci. 37:985.

Laster, D. B., H. A. Glimp and K. E. Gregory. 1973. Effects of early weaning on postpartum reproduction of cows. J. Anim. Sci. 36:734.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Oxenreider, S. L. and W. C. Wagner. 1971. Effect of lactation and energy intake on postpartum ovarian activity in the cow. J. Anim. Sci. 33:1026.

Pelletier, J. and J. Thimonier. 1975. Interactions between ovarian steroids or progestogens and LH release. Ann. Biol. Anim. Biochim. Biophys. 15:131.

Randel, R. D., R. E. Short and R. A. Bellows. 1976. Suckling effect on LH and progesterone in beef cows. J. Anim. Sci. 42:267. (Abstr.)

Reeves, J. J., A. Arimura and A. V. Schally. 1970. Studies on dose response relationship of luteinizing hormone-releasing (LH-RH) in sheep. J. Anim. Sci. 31:933.

Reeves, J. J., A. Arimura and A. V. Schally. 1971. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pre-treated with estradiol benzoate. Biol. Reprod. 4:88.

Reeves, J. J., A. Arimura, A. V. Schally, C. L. Kragt, T. W. Beck and J. M. Casey. 1972. Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anestrous ewes. J. Anim. Sci. 35:84.

Robard, D., P. L. Rayford, J. A. Cooper and G. T. Ross. 1968. Statistical quality control of radioimmunoassays. J. Clin. Endocrinol. Metab. 28:1412.

Saiduddin, S., J. W. Riesen, W. E. Graves, W. J. Tyler and L. E. Casida. 1966. Pituitary LH activity in the postpartum dairy cow. J. Anim. Sci. 25:930. (Abstr.)

Short, R. E., R. D. Randel, D. S. Christensen and R. A. Bellows. 1974. Effects on $E_2\beta$ induced LH release in the bovine. J. Anim. Sci. 39:226. (Abstr.)

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York.

Wagner, W. C. and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological findings. J. Reprod. Fert. 18:493.

Wiltbank, J. N. and A. C. Cook. 1958. The comparative reproductive performance of nursed and milked cows. J. Anim. Sci. 17:640.