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Changing Dose of Progesterone Results in Sudden Changes in Frequency of Luteinizing Hormone Pulses and Secretion of 17β-Estradiol in Bovine Females[1]

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ABSTRACT

The aim of the present study was to elucidate the time course according to which changes in circulating concentrations of progesterone influence pulsatile secretion of LH and secretion of 17β-estradiol. Our working hypothesis was that changing the dose of progesterone would result in changes in frequency of LH pulses and secretion of 17β-estradiol within 72 h. Five days after behavioral estrus, thirty-three cows were randomly assigned to one of five groups: 1) control, no treatment (CONT, n = 5); 2) treatment with two progesterone-releasing intravaginal devices (PRIDs) for 11 days (2PRID, 5–6 ng/ml plasma progesterone, n = 7); 3) treatment with a 0.5 PRID for 11 days (0.5PRID, 1–2 ng/ml plasma progesterone, n = 7); 4) treatment with 2 PRIDs for 8 days followed by treatment with a 0.5 PRID for 3 days (2–0.5PRID, n = 7); and 5) treatment with a 0.5 PRID for 8 days followed by treatment with 2 PRIDs for 3 days (0.5–2PRID, n = 7). Cows subject to PRID treatments received injections of prostaglandin $F_{2\alpha}$ on Days 1 and 2 (Day 0 = day of initiation of PRID treatments, fifth day of the estrous cycle in CONT cows) to lyse the existing corpus luteum. Cows were bled for 12 h at 15-min intervals on Day 7.5 of the treatment period (twelfth day of the estrous cycle in CONT cows). The dose of progesterone was changed on Day 8 in cows that were assigned to the 2–0.5PRID and 0.5–2PRID groups, and blood collections continued an additional 72 h to characterize profiles of circulating concentrations of LH and 17β-estradiol. Cows treated with a 0.5 PRID had a greater ($p < 0.05$) number of LH pulses and higher ($p < 0.05$) concentrations of 17β-estradiol throughout the entire blood collection period than cows in the 2PRID and CONT groups. An increase in the number of LH pulses was detected within 6 h after the change from the high to the low dose of progesterone (2–0.5PRID), and frequency of LH pulses was similar to that of cows in the 0.5PRID group for the remainder of the period of blood collection. LH pulse frequency declined within 6 h after the shift from the low to the high dose of progesterone (0.5–2PRID) and was similar to that of cows in the 2PRID group by 12 h after the dose was changed. Within 6 h after the dose of progesterone was changed, circulating concentrations of 17β-estradiol increased ($p < 0.05$) in cows shifted from the high to low dose (2–0.5PRID) and declined ($p < 0.05$) after the dose of progesterone was changed from low to high (0.5–2PRID). We conclude that changing the circulating concentrations of progesterone concurrently affects frequency of pulsatile LH release and secretion of 17β-estradiol within 6–24 h.

INTRODUCTION

The intricate relationships between circulating concentrations of progesterone, frequency of LH release from the pituitary, and amount of secretion of 17β-estradiol from ovarian follicles have been studied in the bovine female. During the mid- to late portion of the luteal phase of the bovine estrous cycle, increased progesterone is secreted by the corpus luteum [1–3], LH pulse frequency is relatively low compared to early in the luteal phase [4], and relatively low concentrations of 17β-estradiol are secreted from the ovary compared to other stages of the estrous cycle [2, 3, 5].

In sheep, progesterone has a direct, estradiol-dependent inhibitory effect on pituitary release of LH, and estradiol may enhance pituitary response to LHRH [6]. In bovine females, administration of progesterone and 17β-estradiol combined results in lower concentrations of LH in circulation than administration of either alone [7]. This indicates a role for 17β-estradiol in modulating secretion of LH during the luteal phase of the bovine estrous cycle. Therefore, the ovarian steroids, progesterone and 17β-estradiol, modulate LH release by working independently or in concert during the bovine estrous cycle [7–9].

Little information available for the bovine species specifically addresses the time course for changes in LH pulsatile release and secretion of 17β-estradiol that occur as a result of either an increase or decline in progesterone concentrations in circulation. Therefore, the aim of the present study was to elucidate this time course.

MATERIALS AND METHODS

Stage of the estrous cycle of 33 nonlactating cows of composite breeding (MARC III, 1/4 Angus, 1/4 Hereford, 1/4 Red Poll, and 1/4 Pinzgauer; 441 ± 2 kg, average ± SE) was synchronized with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Cows were randomly assigned to treatments on the fifth day of their estrous cycle (Fig. 1). The day treatment was initiated is designated Day 0. Treatment groups were as follows: 1) control, no treatment (CONT, n = 5); 2) treatment for 11 days with

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two progesterone-releasing intravaginal devices (PRIDs; Sanofi Animal Health Inc., Paris, France), 5–6 ng/ml plasma progesterone (2PRID, n = 7); 3) treatment for 11 days with a 0.5 PRID, 1–2 ng/ml plasma progesterone (0.5PRID, n = 7); 4) treatment for 8 days with 2 PRIDs and then for 3 days with a 0.5 PRID (2–0.5PRID, n = 7); and 5) treatment for 8 days with a 0.5 PRID and then for 3 days with 2 PRIDs (0.5–2PRID, n = 7). All cows treated with PRIDs were also treated with $PGF_{2\alpha}$ on Days 1 and 2 to lyse the existing corpus luteum. PRIDs were changed every 4 days in cows in the 2PRID or 2–0.5PRID groups to maintain relatively constant high concentrations of progesterone in circulation during the time of 2PRID treatment. The two PRIDs were changed over a 2-day period (one on each of Days 4 and 5 and/or 8 and 9) to avoid introducing a large "bolus" release of progesterone into circulation. This regimen was also followed for cows in the 0.5–2PRID treatment group: on Day 8 the 0.5 PRID was removed, and 1 PRID was inserted (Hour 0); at Hour 24, a second PRID was inserted.

Indwelling jugular canulae were fitted in all cows on Day 5, and blood samples were collected beginning Day 7.5 for 12 h at 15-min intervals. The dose of progesterone was changed in cows in the 2–0.5PRID and 0.5–2PRID groups at Hour 0 (Day 8), and blood collections continued in all animals for an additional 72 h at 15-min intervals. Blood samples collected every 15 min were allowed to clot at 4°C for 24 h, and serum was decanted after centrifugation. Blood serum was assayed to determine concentrations of LH [10, 11]. All samples were stored at −20°C until assays were performed. Hourly samples were also collected during the entire period of serial blood collection (84 h) into tubes treated with a 30% solution of EDTA (50 μl/tube), and plasma was separated by centrifugation within 2 h of collection to minimize degradation of progesterone. Plasma was assayed to determine concentrations of 17β-estradiol [12] and progesterone.

Concentrations of plasma progesterone (nonextracted) were assayed by using a monoclonal antibody (02-9B4-94) to P4-11-BSA (BiosPacific, Emeryville, CA) at a dilution of 1:30 000, and the assay was validated as follows: serial dilutions of six different pooled samples of bovine plasma were used to evaluate parallelism of two pools of plasma with the standard curve and standard sera. Slopes of dilutions of both plasma pools and the standard curve were not different as determined by Allfit [13] ($p > 0.10$). Plasma volumes used in the assays were 1.25 μl/tube (1:40 dilution) because if 10 μl or more of plasma are used in the assay, increased nonspecific binding results. Recovery of mass of three different levels of progesterone (3.9, 7.8, and 15.6 pg) added to 50 μl of diluted bovine plasma (1:80 and 1:60) of two pools averaged 101.4 ± 6.4%.

Intraassay coefficients of variation were 6.4% and 13.6%, and interassay coefficients of variation were 6.2% and 14.9%, respectively, for the progesterone and 17β-estradiol

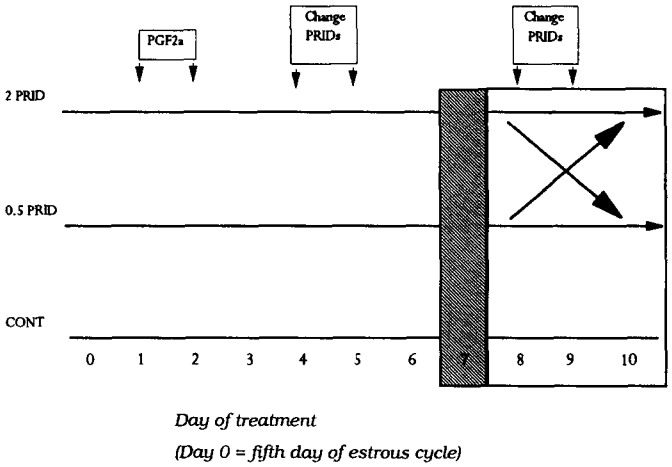

FIG. 1. Diagram of treatment protocol. Shaded block on Day 7 indicates 12-h bleed prior to dose shift; open block including Days 8–10 indicates 72-h blood sampling period during and subsequent to dose shift. Blood samples were collected at 15-min intervals for characterization of LH pulses and at hourly intervals to characterize progesterone and 17β-estradiol concentrations.

assays. Sensitivities of the progesterone and 17β-estradiol assays were 0.08 ng/ml and 0.50 pg/ml, respectively. Intra- and interassay coefficients of variation for the LH assay were 3.1% and 7.8%, respectively. Sensitivity of the LH assay was 30 pg/ml.

Characteristics for LH in circulation were evaluated by determining mean concentrations of LH, frequency of pulses, and amplitude of pulses by use of algorithms (Pulsar [14], software modified for the IBM-PC by J.F. Gitzen and V.D. Ramirez, Urbana, IL). Changes in characteristics of LH in circulation, concentrations of progesterone, and concentrations of 17β-estradiol during the 84-h period of serial blood collection were subjected to analyses that compared changes over time for cows treated with different dosages of progesterone, using a mixed-model analysis for repeated measures over time [15, 16]. The fitted model included the fixed effects of treatment and period of serial blood collection. Hormone data were classified in 6-h time periods during the 84 h of serial blood collection. To account for the covariance between observations from the same cow at different periods, different options of covariance structures for residuals available in the repeated statement of Proc Mixed (SAS [17]) were considered, and the model with the best fit was chosen to analyze the data. Comparison of means was performed by use of Proc Mixed. Significance for all variables was determined as $p < 0.05$.

RESULTS

Control animals had an average of 6.3 ng/ml progesterone, 1.3 pulses of LH within 6-h periods, and 1.6 pg/ml of 17β-estradiol in circulation throughout the 84-h period of serial blood collection (Fig. 2). Cows in the 2PRID group had approximately 4.6 ng/ml of progesterone in circulation,

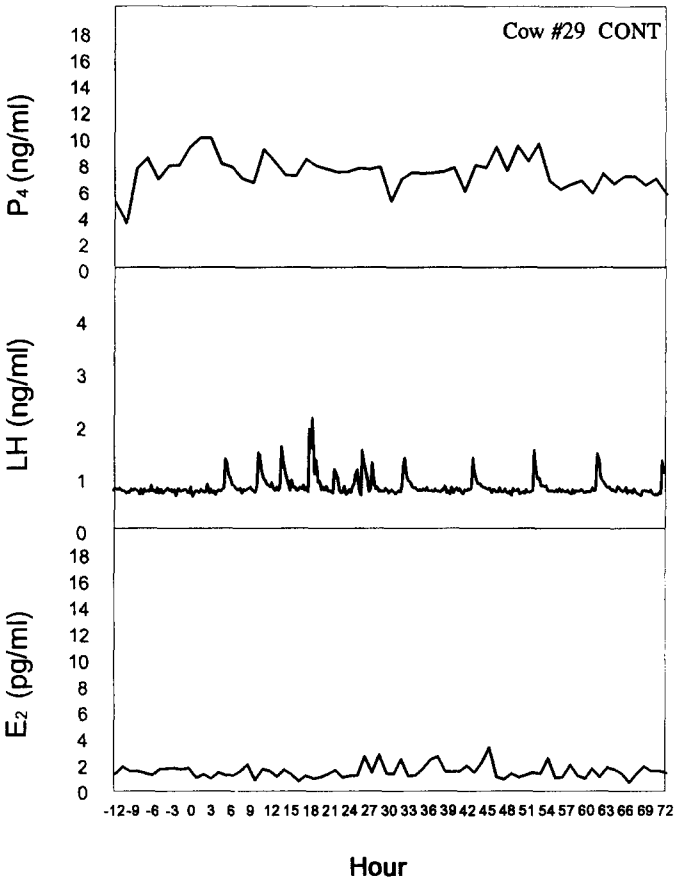

FIG. 2. Circulating concentrations of progesterone (P_4, ng/ml), LH (ng/ml), and 17β-estradiol (E_2, pg/ml) for representative cow (#29) from CONT group. Hour 0 = time of progesterone treatment shift; blood samples were collected at 15-min intervals for characterization of LH pulses and at hourly intervals to characterize progesterone and 17β-estradiol concentrations.

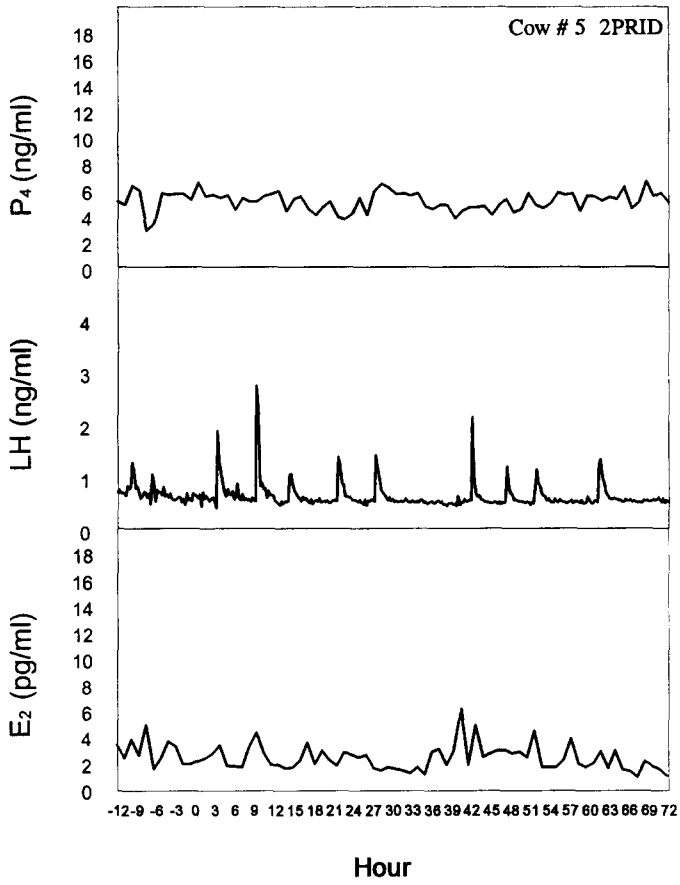

FIG. 3. Circulating concentrations of progesterone (P_4, ng/ml), LH (ng/ml), and 17β-estradiol (E_2, pg/ml) for representative cow (#5) from 2PRID group.

TABLE 1. Mean circulating concentrations of progesterone (ng/ml) across 6-h periods during Days 7–10 of treatment.

	Treatment				
Period (h)	CONT	2PRID	0.5PRID	2–0.5PRID	0.5–2PRID
Day 7					
−12 to 0[a]	6.73[d]	5.18[de]	1.34[g]	5.41[d]	1.45[g]
Day 8					
0 to 6	8.05[c]	5.75[d]	1.27[g]	2.95[f]	2.94[f]
6 to 12	6.94[cd]	4.59[e]	1.45[g]	1.92[fg]	3.38[f]
12 to 18	6.52[d]	4.52[e]	1.32[g]	1.69[g]	3.89[ef]
18 to 24	6.34[d]	4.24[e]	1.38[g]	1.54[g]	4.16[e]
Day 9					
24 to 30	5.93[d]	3.77[ef]	1.20[g]	1.37[g]	4.14[e]
30 to 36	6.29[d]	3.82[ef]	1.20[g]	1.46[g]	7.36[cd]
36 to 42	5.98[d]	4.78[de]	1.16[g]	1.81[g]	8.36[c]
42 to 48	5.95[d]	4.62[de]	1.16[g]	1.42[g]	7.15[cd]
Day 10					
48 to 54	5.81[d]	4.78[de]	1.53[g]	1.99[fg]	7.12[cd]
54 to 60	6.11[d]	4.27[e]	1.26[g]	1.31[g]	6.81[cd]
60 to 66	5.73[d]	4.45[e]	1.30[g]	1.58[g]	6.10[d]
66 to 72	5.01[de]	4.81[de]	1.42[g]	1.36[g]	6.66[d]
SEM[b]	0.64	0.51	0.55	0.51	0.54

[a] Average of the two 6-h periods prior to treatment shift.
[b] Maximum SEM.
[c,d,e,f,g] Numbers with different superscripts differ ($p < 0.05$).

1.3 pulses of LH/6 h, and 3.5 pg/ml 17β-estradiol in circulation throughout the period of blood collection (Fig. 3). Cows in the 0.5PRID group had approximately 1.3 ng/ml of progesterone, 4.1 pulses of LH/6 h, and 12.7 pg/ml 17β-estradiol throughout the period of serial blood collection (Fig. 4). Cows treated with a 0.5 PRID throughout the treatment period had lower ($p < 0.05$) circulating concentrations of progesterone (Table 1), a greater ($p < 0.05$) number of LH pulses (Table 2), and, consequently, higher ($p < 0.05$) concentrations of 17β-estradiol (Table 3) throughout the entire period of blood collection than did cows in the 2PRID and CONT groups.

Hormone profiles for a representative cow in the 2–0.5PRID treatment group are summarized in Figure 5. On Day 7, before the dose of progesterone was changed, circulating concentrations of progesterone were similar to those observed in cows treated with 2 PRIDs throughout the entire treatment period (Table 1). Frequency of LH pulses, however, was elevated in cows in this treatment group (2–0.5PRID) before the change in dose of progesterone, as were concentrations of 17β-estradiol, in comparison to those in cows administered 2 PRIDs throughout the 11-day treatment period. Data for mean concentrations and

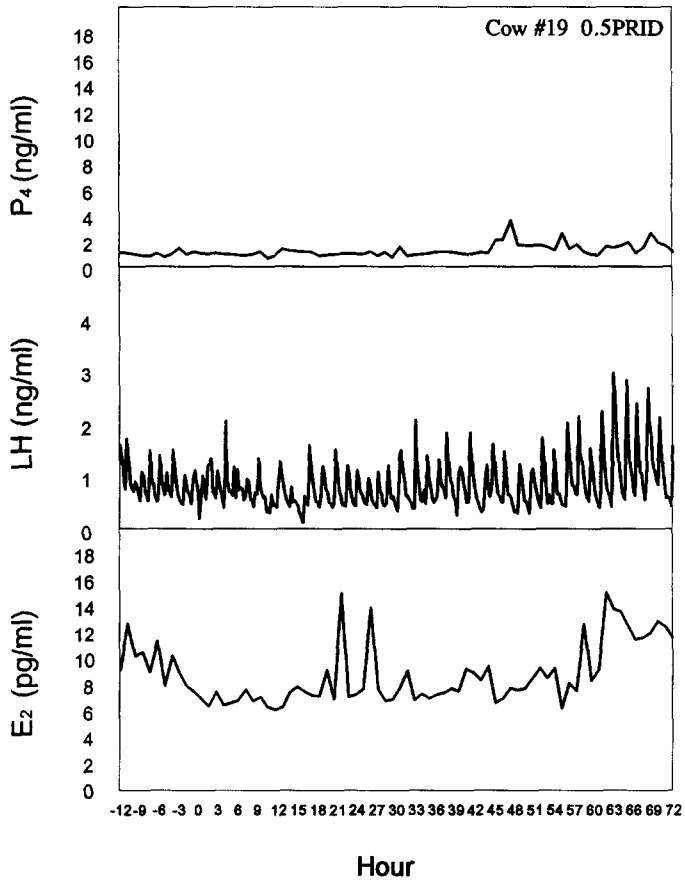

FIG. 4. Circulating concentrations of progesterone (P_4, ng/ml), LH (ng/ml), and 17β-estradiol (E_2, pg/ml) for representative cow (#19) from 0.5PRID group.

amplitude of pulses of LH did not differ among treatments; therefore, data are not reported. Within 6 h of the change in dose of progesterone, circulating concentrations of progesterone declined ($p < 0.05$; Table 1) but were still slightly higher than those of cows treated with a 0.5 PRID throughout the treatment period. The decline in progesterone resulted in an increase ($p < 0.05$; Table 2) in LH pulse frequency that occurred within 6 h of the time at which the dose of progesterone was changed (2–0.5PRID) and was similar to that of cows treated with the low dose of progesterone (0.5PRID) for the remainder of the treatment period. As LH pulse frequency increased in cows shifted from the high to the low dose of progesterone (2–0.5PRID), there was an increase ($p < 0.05$) in circulating concentrations of 17β-estradiol within 6 h after the change in dose of progesterone, and concentrations of 17β-estradiol were similar to those of cows treated with the low dose of progesterone (0.5PRID) for the remainder of the treatment period.

On Day 7, before the change in dose of progesterone, hormone profiles were similar between cows in the 0.5–2PRID group (Fig. 6) and those treated continuously with the low dose of progesterone (0.5PRID; Fig. 4). Within 6 h of the shift (0.5–2PRID), concentrations of progesterone in

TABLE 2. Frequency of release of LH pulses across 6-h periods during Days 7–10 of treatment.

	Treatment				
Period (h)	CONT	2PRID	0.5PRID	2–0.5PRID	0.5–2PRID
Day 7					
−12 to 0[a]	0.20[c]	1.43[cd]	4.36[e]	2.43[d]	3.22[e]
Day 8					
0 to 6	0.40[c]	1.29[cd]	4.29[e]	3.43[e]	2.00[d]
6 to 12	1.20[cd]	1.71[cd]	4.14[e]	3.71[e]	0.86[c]
12 to 18	1.20[cd]	1.71[cd]	3.71[e]	2.43[d]	1.14[cd]
18 to 24	1.40[cd]	1.43[cd]	4.00[e]	2.86[d]	0.86[c]
Day 9					
24 to 30	4.40[e]	1.00[cd]	3.86[e]	3.00[de]	1.00[cd]
30 to 36	1.20[cd]	0.57[c]	4.00[e]	3.14[de]	0.43[c]
36 to 42	1.20[cd]	0.57[c]	4.29[e]	2.86[d]	0.43[c]
42 to 48	0.60[c]	1.14[cd]	4.14[e]	3.43[e]	0.29[c]
Day 10					
48 to 54	1.40[cd]	1.43[cd]	4.14[e]	3.57[e]	0.29[c]
54 to 60	1.40[cd]	1.57[cd]	3.86[e]	3.29[e]	0.71[c]
60 to 66	1.80[cd]	1.57[cd]	4.29[e]	4.00[e]	0.86[c]
66 to 72	1.60[cd]	1.00[cd]	3.71[e]	3.14[de]	1.14[cd]
SEM[b]	0.55	0.47	0.46	0.49	0.47

[a]Average of the two 6-h periods prior to treatment shift.
[b]Maximum SEM.
[c,d,e]Numbers with different superscripts differ ($p < 0.05$).

circulation increased ($p < 0.05$) and remained similar to concentrations in cows treated with the high dose of progesterone (2PRID). The increase in progesterone resulted in a decrease ($p < 0.05$) in LH pulse frequency within 6 h of the time the dose of progesterone was changed. By 12 h after the treatment was changed, frequency of LH pulsatile release in cows in the 0.5–2PRID group decreased further and remained similar to those of cows treated continuously with the high dose of progesterone (2PRID). As frequency

TABLE 3. Mean circulating concentration of 17β-estradiol (pg/ml) across 6-h periods during Days 7–10 of treatment.

	Treatment				
Period (h)	CONT	2PRID	0.5PRID	2–0.5PRID	0.5–2PRID
Day 7					
−12 to 0[a]	1.98[c]	3.59[c]	14.30[f]	7.41[d]	10.31[e]
Day 8					
0 to 6	1.27[c]	3.19[c]	13.44[f]	10.02[e]	4.64[c]
6 to 12	1.82[c]	3.70[c]	13.52[f]	10.06[e]	3.85[c]
12 to 18	1.28[c]	3.42[c]	13.82[f]	10.79[e]	3.80[c]
18 to 24	1.25[c]	3.38[c]	11.99[ef]	9.65[de]	3.80[c]
Day 9					
24 to 30	1.58[c]	3.85[c]	11.78[ef]	10.30[e]	3.69[c]
30 to 36	1.75[c]	3.99[c]	12.69[ef]	10.29[e]	4.03[c]
36 to 42	1.88[c]	3.53[c]	12.27[ef]	11.36[e]	4.86[c]
42 to 48	0.99[c]	3.81[c]	12.64[ef]	10.42[e]	3.68[c]
Day 10					
48 to 54	1.73[c]	3.57[c]	10.71[e]	10.64[e]	3.37[c]
54 to 60	1.16[c]	3.55[c]	9.65[de]	13.14[f]	4.07[c]
60 to 66	1.39[c]	2.96[c]	11.77[ef]	12.89[ef]	3.52[c]
66 to 72	1.83[c]	3.16[c]	14.32[f]	10.65[e]	3.45[c]
SEM[b]	1.76	1.41	1.49	1.49	1.33

[a]Average of the two 6-h periods prior to treatment shift.
[b]Maximum SEM.
[c,d,e,f]Numbers with different superscripts differ ($p < 0.05$).

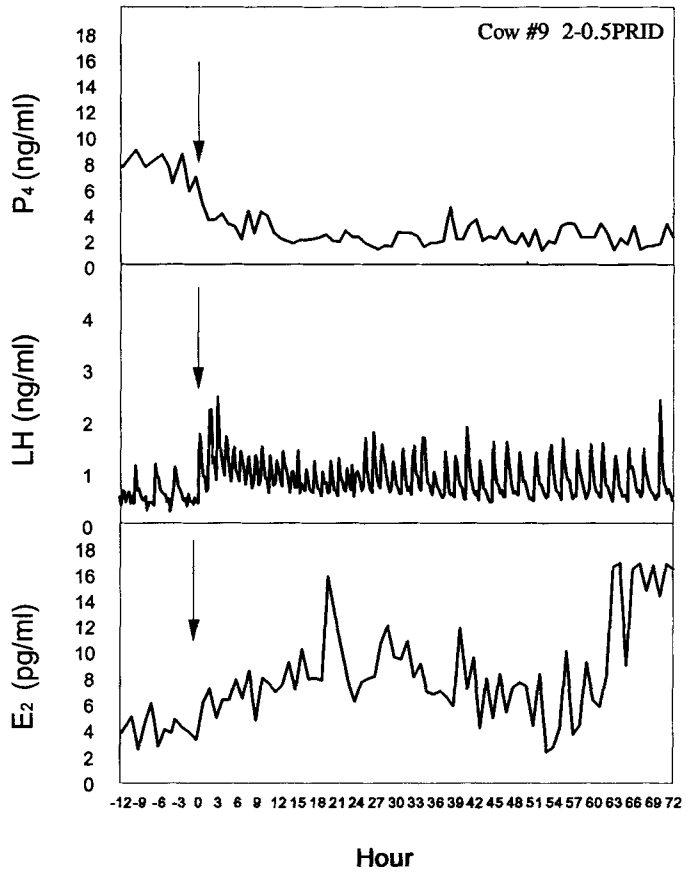

FIG. 5. Circulating concentrations of progesterone (P_4, ng/ml), LH (ng/ml), and 17β-estradiol (E_2, pg/ml) for representative cow (#9) from 2–0.5PRID group. Arrows indicate time of shift of progesterone (Hour 0, Day 8).

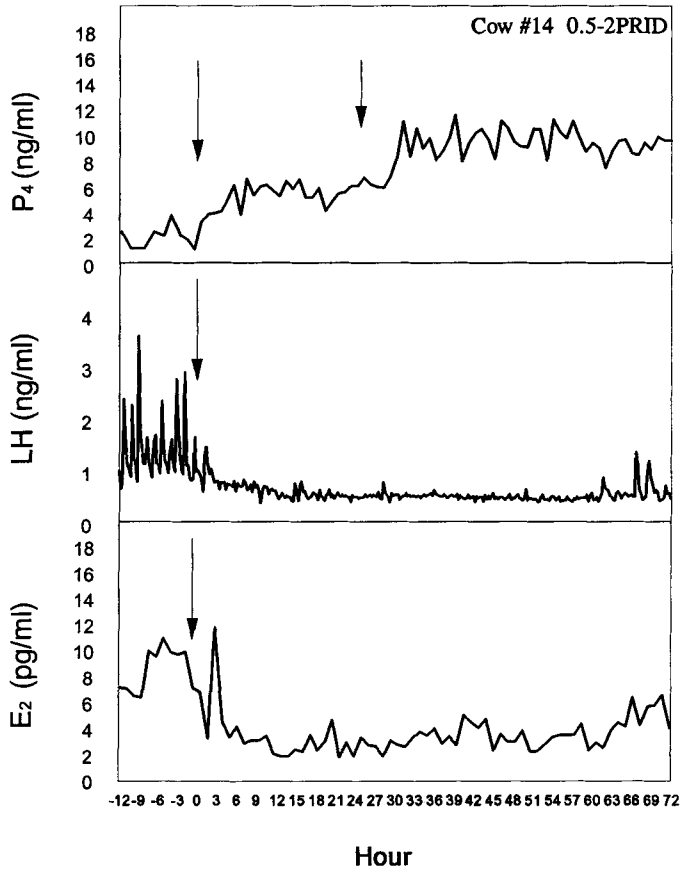

FIG. 6. Circulating concentrations of progesterone (P_4, ng/ml), LH (ng/ml), and 17β-estradiol (E_2, pg/ml) for representative cow (#14) from 0.5–2PRID group. Arrow indicates time of shift of progesterone (Hour 0, Day 8; Hour 24, Day 9).

of LH pulses declined, there was a decrease ($p < 0.05$) in circulating concentrations of 17β-estradiol, again within 6 h subsequent to the time when the dose of progesterone was changed, and circulating concentrations of 17β-estradiol in cows of the 0.5–2PRID group were similar to those in cows treated with the high dose of progesterone (2PRID) for the remainder of the treatment period.

DISCUSSION

Within 6 h after the change from the high to the low (2 to 0.5 PRID) dose of progesterone, a significant increase in pulsatile release of LH was detected. Previous studies have shown that in bovine females, there is an inverse relationship between concentrations of progesterone in circulation and LH pulse frequency [4, 18–20]. In a typical bovine estrous cycle, circulating concentrations of progesterone are higher and frequency of LH pulses are lower during the midluteal phase than in the early luteal phase [4]. Administration of exogenous progesterone that elevated circulating progesterone to concentrations typically observed during the midluteal stages of the estrous cycle resulted in a low frequency of LH pulses as would be typical of this phase of the estrous cycle [20, 21]. A decline in LH pulse amplitude and an increase in LH pulse frequency occurs in cows that progress from the late luteal to the early follicular stage of the estrous cycle [5]. Changes in circulating concentrations of progesterone may differ in cows with normal regressing corpora lutea from those experienced by cows in the present study. Although a direct comparison between natural and induced luteolysis and the sudden change in circulating progesterone caused by the 2–0.5PRID shift may not be appropriate, the associated physiological processes may be similar.

A decrease in LH pulse frequency occurred within 6 h in cows that were shifted from the low to the high dose of progesterone (0.5–2PRID). By 12 h subsequent to the treatment shift, frequency of LH pulses became static. It is known from earlier work that a decrease in circulating concentrations of progesterone during the late luteal phase of the estrous cycle is associated with an increased LH pulse frequency [20]. Additionally, previous studies in cattle and sheep have shown that lower doses of progesterone, characteristic of luteal phase deficiency, resulted in a higher fre-

quency of LH pulsatile release compared to greater doses of progesterone [10, 19, 22–26]. The present study shows that the communication between the ovary and hypothalamic-pituitary axis that occurs as a result of the shift in progesterone concentration is rapid, allowing a change in frequency of LH pulses to occur within 6 h. We were surprised at the sensitivity of the hypothalamic-pituitary-ovarian axis to the change in the circulating concentration of progesterone.

An explanation for the delay of cows in the 0.5–2PRID group in attaining static frequency of LH pulses in the present study is that only 1 PRID was inserted at Hour 0 (on Day 8), and the second PRID was inserted at Hour 24. This protocol was used to avoid administering a supraphysiological "bolus" of progesterone on Day 8. The PRID replacement protocol may in part explain why pulsatile release of LH did not change as abruptly in cows of this treatment group in comparison to cows initially treated with 2 PRIDs and shifted to a 0.5 PRID. However, this explanation does not offer a reason why static frequency of LH release was attained by 12 h after the dose of progesterone was changed. Therefore, concentration of progesterone in circulation may not be as important as the shift in concentration. Progesterone is more effective in suppressing LH pulse frequency and/or the LH surge in the presence of estradiol [18]. However, in the presence of high concentrations of estradiol, progesterone may elicit its inhibitory effect on pulsatile LH release only at elevated concentrations. A threshold most likely exists for each animal that, when reached, elicits a very sudden change in pulsatile LH release and thus secretion of 17β-estradiol from ovarian follicles.

Pulsatile release of LH for cows in the 2PRID and CONT treatment groups differed on Day 7 at the beginning of serial blood collection and again on Day 9. CONT cows had fewer pulses of LH on Day 7 than did cows in the 2PRID group. On Day 9 from Hours 24–30, CONT cows had more pulsatile release of LH than did cows in the 2PRID group. On Day 7 and Day 9, circulating concentration of progesterone was slightly elevated in cows in the CONT group in comparison to cows in the 2PRID group; possibly this slight increase negatively affected pulsatile release of LH. Although pulsatile release of LH differed among the cows in the CONT and 2PRID groups during these two times, circulating concentrations of 17β-estradiol did not; therefore we speculate that this difference is not significant physiologically. There was no related difference in concentrations of 17β-estradiol during Day 7 and Day 9 when pulsatile release of LH differed, but there was a slight difference between concentrations of progesterone during Hours 24–30 on Day 9 of treatment among cows in the CONT or 2PRID groups. Stress is known to inhibit pulsatile LH release; possibly the CONT cows were more susceptible to the stress of cannulation, and the exogenous progesterone treatments allowed for a more controlled hormonal milieu. Cows in the CONT group had fewer LH pulses on Day 7 (Hours 1–6) and then greater numbers of pulses of LH on Day 9 (Hours 24–30). One may speculate that these cows were "catching up" on Day 9 with more LH pulses to make up for the fewer pulses on Day 7. This resulted in differing profiles of pulsatile LH release in these cows, but these differences were not of large enough magnitude to affect circulating concentrations of 17β-estradiol. For cows in the 2–0.5PRID group, LH pulsatile release was elevated before the change in dose of progesterone, as were concentrations of 17β-estradiol, in comparison to cows in the 2PRID group. A greater number of LH pulses within a few cows may account for the elevated average of cows in the 2–0.5PRID group. Therefore, it is possible that interanimal variation was responsible for this difference.

The change in dose of progesterone resulted in concurrent changes in frequency of LH pulsatile release and secretion of 17β-estradiol in the present study. It is likely that higher frequency of LH release in cows with low (0.5 PRID) circulating concentrations of progesterone, as compared to those with higher (2PRID and CONT) circulating concentrations of progesterone, resulted in increased steroidogenic capacity of ovarian follicles and, therefore, greater secretion of 17β-estradiol. When the influence of the circulating concentration of progesterone was evaluated over longer time frames than those in the present study, a higher frequency of LH pulses permitted the dominant ovarian follicle to escape normal atresia, and larger follicles developed [27–29]. Maintenance of growth and dominance of the large ovarian follicle for a prolonged period of time resulted in higher concentrations of 17β-estradiol during the treatment period. The increased 17β-estradiol presumably resulted from enhanced steroidogenic capacity of these large ovarian follicles [30], and results from the present study provide support for this presumption.

In cows, administration of a combination of progesterone and 17β-estradiol results in lower concentrations of LH in circulation than administration of either progesterone or 17β-estradiol alone [7]. This indicates a role for 17β-estradiol in modulating secretion of LH during the luteal phase of the bovine estrous cycle. However, the immediacy of the changes in 17β-estradiol observed in the present study after progesterone doses were changed (within 6 h) suggests a more direct effect than one mediated solely through and subsequent to changes in pulsatile LH secretion. The ovarian steroids, progesterone and 17β-estradiol, modulate release of LH by working independently or in concert during the bovine estrous cycle [7–9]. However, perhaps a shift in circulating concentrations of progesterone also plays an important paracrine role in modulating 17β-estradiol secretion by the ovary. In the ovine female, progesterone has a direct, estradiol-dependent inhibitory effect on pituitary LH release [6]. Additionally, it has been suggested that an up-regulation of pituitary progesterone receptors by estradiol occurs and is associated with progesterone inhibition of LH release in

ewes [31]. Therefore, if circulating concentrations of progesterone have an estradiol-dependent inhibitory effect on pulsatile LH release and 17β-estradiol can increase the number of progesterone receptors at the pituitary, then in the case of animals in the 0.5–2PRID group, the high concentrations of 17β-estradiol may have previously up-regulated progesterone receptors at the pituitary; hence the rapid response (decrease) of pulsatile release of LH when circulating concentrations of progesterone were increased. However, in those cows in the 2–0.5PRID group, it would then follow that the time frame required for a response in LH pulse frequency to take place would be lengthened, and that did not occur in the present study. The shift in progesterone dose resulted in a change in pulsatile release of LH within 6 h of the change for both treatments in which the dose was shifted. We therefore speculate that progesterone modulates pulsatile release of LH by methods that are not presently understood.

The action of progesterone in modulating the frequency of LH pulsatile release probably occurs at the LHRH pulse generator in the hypothalamus. Frequency of LHRH release into the portal vein in ewes is reduced as a result of administration of progesterone [32]. The influence of circulating concentrations of progesterone on frequency of LH pulses and secretion of 17β-estradiol has been evaluated over a longer time after the dose of progesterone was changed in previous research [32, 33]. Progesterone, in high concentrations, acts directly on the bovine median eminence and inhibits the release of LHRH [33].

The uniqueness of the present research is that the sudden changes in both pulsatile release of LH and secretion of 17β-estradiol were evaluated in relation to a shift in circulating progesterone from low to high and high to low doses. The primary hypothesis was that there would be changes in the rate of LH pulses within 72 h after doses of progesterone were changed. We were surprised, however, at the apparent exquisitely sensitive communication between circulating concentrations of progesterone and the rate of LH pulses from the anterior pituitary. We speculate that the communication is at the hypothalamic "pulse generator" for LHRH and that this communication precedes the change in the rate of LH pulses. A further hypothesis was that there would be an enhancement in estrogenic capacity of the ovarian follicles as a result of the increase in frequency of LH pulses. The changes in secretion of 17β-estradiol, however, occurred more rapidly after the changes in frequency of LH pulses than anticipated. Additionally, although the present study was not designed to address this issue, we speculate that the circulating concentration of progesterone may not be as important as the shift in concentration in eliciting the LH and 17β-estradiol response. A threshold most likely exists for each animal that, when reached, elicits a very sudden change in pulsatile LH release and concentrations of 17β-estradiol. Therefore, we speculate that a shift in concentration of progesterone, as seen in the present study, precipitates a cascade of events, culminating in either an inhibition or enhancement of LHRH release, thereby modulating pulsatile release of LH from the anterior pituitary and ovarian follicular function as represented by an increase in 17β-estradiol.

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