Exogenous Progesterone and Progestins as Used in Estrous Synchrony Regimens Do Not Mimic the Corpus Luteum in Regulation of Luteinizing Hormone and 17**β**-Estradiol in Circulation of Cows

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ABSTRACT

Our working hypothesis was that the low concentrations of progesterone (P_4) and synthetic progestins administered in hormonal regimens to control estrous cycles of cows would have similar effects on secretion of LH and 17β-estradiol (E_2). In addition, we hypothesized that concentrations of exogenous P_4 typical of the midluteal phase of the estrous cycle and the corpus luteum (CL) would have similar effects on LH and E_2, and the effects would be different from those of synthetic progestins and low concentrations of P_4. Cows (n = 29) were randomly assigned to one of five treatment groups: 1) one Progesterone Releasing Intravaginal Device (1PRID; n = 6); 2) two PRIDs (2PRID; n = 6); 3) norgestomet, as in Syncro-Mate-B® regimen (SMB; n = 6); 4) melengestrol acetate (MGA; 0.5 mg/day; n = 5); and 5) control (CONT; n = 6). Treatments were administered for 9 days (Day 0 = initiation of treatment). All cows from 1PRID, 2PRID, SMB, and MGA groups were injected with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on Days 2 and 5 of the treatment period to regress CL. Cows in the 1PRID and SMB groups were also administered exogenous estrogen according to the respective estrous synchronization protocol for these products. Daily blood samples were collected from Day 0 to 35 to determine concentrations of P_4. On Day 8, blood samples were collected at 15-min intervals for 24 h to determine pattern of LH secretion. On Day 9, all treatments ceased and cows in the CONT group received injections of $PGF_{2\alpha}$. Blood samples were collected at 1-h intervals after cessation of treatments to determine time of the preovulatory surge of LH. During the treatment period, mean concentrations of P_4 in 2PRID and CONT groups were greater ($p < 0.05$) than in cows from the other groups. Mean concentrations of E_2 in cows from the SMB group were greater ($p < 0.05$) as compared to cows from the other groups during the treatment period. In addition, concentrations of E_2 in cows from the 1PRID group were greater ($p < 0.05$) than in cows from the 2PRID and CONT groups. On Day 8 of the treatment period, frequency of LH pulses in cows treated with SMB was greater ($p < 0.05$) and mean concentrations of LH in cows from the CONT group were lower ($p < 0.05$) as compared to cows from the other groups. Amplitude of LH pulses was not different ($p > 0.05$) among cows receiving the various treatments. Initiation of the preovulatory surge of LH was 20 h earlier ($p < 0.05$) in cows from the SMB group and tended ($p < 0.10$) to be earlier in cows from the 1PRID group than cows in the 2PRID and CONT groups. Four of five cows treated with MGA failed to initiate a preovulatory surge of LH during the sampling period. Concentrations of P_4 during the subsequent estrous cycle were greater ($p < 0.05$) in cows from 2PRID and CONT groups than in cows from the other groups. We accept our working hypothesis that secretion of LH is similar in cows treated with the low dose of P_4 and synthetic progestins. However, cows with the higher dose of P_4 had a different profile of LH as compared to control cows with their corpus luteum in situ. Therefore, we reject this part of our working hypothesis.

INTRODUCTION

Synthetic progestins, melengestrol acetate (MGA) and norgestomet, have been developed to synchronize time of ovulation and time of behavioral estrus in the cow by mimicking the function of corpus luteum (CL) [1, 2]. However, use of synthetic progestins has often been associated with reduced fertility when cows are inseminated during the es-

trus that results from treatment [2–8]. The reason for reduced fertility when MGA (an orally active synthetic progestin) is used may be due to the increased size of dominant ovarian follicle(s) that do not ovulate during the treatment [9–11] and/or to increased concentrations of 17β-estradiol (E_2) in circulation [12–14]. A reduction in size and weight of CL formed after MGA treatment has also been reported [11]. Use of norgestomet has also been shown to cause a reduction in fertility [5–8] that may be associated with variability in timing of onset of the preovulatory surge of LH [15–17] and behavioral estrus [16, 18–20]. The actions of norgestomet on follicular development may be mediated by the increased frequency of LH pulses during the period of norgestomet treatment [21]. An increase in size of ovarian follicles and estrogenic capacity of these follicles has been reported in postpartum beef cows treated with norgestomet [22, 23]. Norgestomet and MGA may alter ovarian follicular development via alteration in the secretory pattern of LH. Ovulation of aberrant follicles may lead to defects in function of the CL, known as a luteal phase defi-

ciency, which has been associated with reduced secretion of progesterone (P_4) and infertility in many species [24–27].

The pattern of pulsatile secretion of LH is modulated by ovarian steroids, E_2 and P_4 [28, 29]. Administration of low levels of P_4 to cows results in a high frequency of LH pulses [30, 31]. Roberson et al. [31] reported that concentrations of E_2 are higher and the onset of the preovulatory surge of LH is earlier after removal of the source of P_4 in cows receiving a low as compared to those receiving a high dose of P_4. These results indicate that lower doses of P_4 alter ovarian activity (increased secretion of E_2) during the treatment period and after P_4 withdrawal [31].

Few studies have involved administration of synthetic progestins (MGA and norgestomet) in the regimens used to control stage of the estrous cycle and compared the secretory pattern of LH under these hormonal regimens to the way the CL modulates LH secretion. If these hormonal regimens do not modulate secretion of LH as does the CL, then this may help explain the lower fertility that occurs when these hormonal regimens are used to synchronize estrus. Therefore, our working hypotheses were as follows: 1) lower doses of P_4—resulting in 2–3 ng of circulating P_4/ml of plasma—and MGA or norgestomet administered in the hormonal regimens used to synchronize estrus would have similar effects on secretion of LH and E_2; and 2) higher doses of P_4—resulting in 7–10 ng of circulating P_4/ml of plasma—and the midluteal phase CL would have similar effects on secretion of LH and E_2, but the effects would be different from those observed when doses of P_4 were lower and synthetic progestins were administered. Additionally, function of the CL during the estrous cycle subsequent to treatment was characterized.

MATERIALS AND METHODS

Experimental Protocol

Twenty-nine mature nonlactating crossbred beef cows (one-fourth Hereford, one-fourth Angus, one-fourth Pinzgaur, one-fourth Red Poll) exhibiting normal estrous cycles were used in this study (2–5 yr of age; 492 ± 12 kg body weight, mean ± SEM). Cows were randomly assigned to an untreated control group (CONT; n = 6) or one of four treatment groups: 1) one Progesterone Releasing Intravaginal Device with 10 mg E_2 benzoate in a gelatin capsule (PRID; Sanofi Animal Health, Inc., Overland Park, KS), to provide levels of P_4 of 2–3 ng/ml of plasma in circulation (1PRID; n = 6); 2) two PRIDs without E_2 benzoate to provide midluteal phase concentrations (7–10 ng/ml) of exogenous P_4 (2PRID; n = 6); 3) norgestomet, administered in the Syncro-Mate-B® (Sanofi Animal Health, Inc.) regimen, which includes a 6-mg norgestomet ear implant and an i.m. injection of 5 mg E_2 valerate and 3 mg norgestomet at the time of implant insertion (SMB; n = 6); and 4) MGA (The Upjohn Co., Kalamazoo, MI) orally at a level of 0.5 mg/cow daily (MGA; n = 5). Stage of the estrous cycle of

cows assigned to these four treatments was not synchronized before the treatment and was completely random. Stage of the estrous cycle was synchronized in cows from the CONT group before the initiation of treatments by administration of two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; The Upjohn Co., Kalamazoo, MI) given 11 days apart. Therefore, cows in the CONT group were in the luteal phase of the estrous cycle during the treatment period. The treatment regimens used with the SMB, MGA, and 1PRID groups were those recommended when these products are used commercially for estrous synchronization.

Treatments were administered for 9 days. At the initiation of treatment (Day 0), cows in the 1PRID and 2PRID groups received a single PRID. Additional PRIDs were administered to cows in the 2PRID group on Day 1 of the treatment period to avoid excessive increases in P_4 when two PRIDs are administered on the same day. To maintain P_4 at 7–10 ng/ml of plasma in circulation (2PRID), the PRID inserted on Days 0 and 1 of the treatment period were replaced with new PRIDs on Days 5 and 6, respectively. Cows in the SMB group received a norgestomet ear implant and an injection of 5 mg E_2 valerate and 3 mg norgestomet at the initiation of treatment. Cows in the MGA group were fed their initial MGA on the morning (0800 h) of initiation of treatments and received the same amount of MGA via their diet on each day of the treatment period. Cows in the CONT group were on Day 5 (Day 0 = day of estrus) of their estrous cycle and received a sham device intravaginally at initiation of treatment. All cows from the 1PRID, 2PRID, SMB, and MGA groups received injections of $PGF_{2\alpha}$ (25 mg) on Days 2 and 5 of the treatment period to induce regression of the CL to eliminate endogenous P_4.

On Day 8 of the treatment period, catheters were inserted in the jugular vein of all cows and blood samples were collected at 15-min intervals for 24 h. All cows were fed in the middle of the 24-h bleeding period. Therefore, MGA was fed in the middle of this period. These blood samples were used to compare pulse frequency of LH of cows in the different treatment groups. On Day 9 of the treatment period, PRIDs and norgestomet implants were removed from cows in the 1PRID, 2PRID, and SMB groups; the last feeding of MGA was given to cows in the MGA group, and cows in the CONT group received an injection of $PGF_{2\alpha}$ (25 mg). Blood samples were collected at hourly intervals for 75 h (1PRID, 2PRID, and SMB groups) or 100 h (MGA and CONT groups) after removal of PRIDs, norgestomet implants, last feeding of MGA, and the injection of $PGF_{2\alpha}$. These blood samples were used to determine timing to the preovulatory surges of LH, FSH, and pattern of E_2 during the follicular phase that followed progestin or P_4 withdrawal or the injection of $PGF_{2\alpha}$.

Blood samples collected at 15-min and hourly intervals were allowed to clot at room temperature and then stored at 4°C for 24 h. Samples were then centrifuged at 1520 × g for 15 min, and serum was decanted and stored at −20°C

TABLE 1. Mean concentrations of P_4, area under the curve for the profile of P_4 in circulation, and duration of the luteal phase following treatment.

| Treatment[a] | n | Treatment period | | Subsequent estrous cycle[b] | | |
		Mean P_4 (ng/ml)	Area[c] (units)	Mean P_4 (ng/ml)	Area[c] (units)	Luteal phase[d] (d)
1PRID	6	3.9[e]	623.3[e]	4.3[e]	691.1[e]	16.3
SMB	6	1.4[f]	216.7[f]	2.7[e]	493.0[e]	15.3
MGA	5	2.4[f]	344.9[f]	3.4[e]	513.0[e]	16.4
2PRID	6	10.1[g]	1542.1[g]	7.3[f]	1376.5[f]	18.7
CONT	6	9.0[g]	1301.4[g]	7.6[f]	1106.4[f]	18.3
Pooled SEM[h]		2.7	216.2	2.0	399.3	2.9

[a]See *Materials and Methods* for definition of abbreviations and explanations of Progesterone Releasing Device with 10 mg E_2 treatment regimens.
[b]Estrous cycle following removal of all treatments.
[c]Area under the curve for concentrations of P_4 expressed in arbitrary units.
[d]Interval between first rise in P_4 above 1 ng/ml and decline in P_4 below 1 ng/ml of plasma.
[e,f,g]Numbers with differing superscripts within column differ ($p < 0.05$).
[h]Pooled standard error of mean (SEM).

until assayed for LH, FSH, and E_2. All serum samples collected at 15-min and hourly intervals were pooled for 4-h periods within each cow to determine concentrations of FSH and E_2.

Blood samples were collected daily from all cows to evaluate plasma concentrations of P_4 (sample tubes treated with 30% solution of EDTA, 50 μl/10 ml of blood sample; Fisher Scientific, Co., Fair Lawn, NJ) throughout the treatment period, and then were collected every other day for 26 days post-treatment for the estrous cycle subsequent to treatment. To avoid possible degradation of P_4 in blood, these samples were placed on ice immediately and plasma was separated from blood cells by centrifugation at 1520 × g for 15 min within 1 h of collection. Plasma was then harvested and stored at −20°C until assayed for P_4.

Radioimmunoassays

Concentrations of LH in all samples collected serially were analyzed by radioimmunoassay [32], validated in our laboratory [33], using rabbit antiserum against ovine LH (TEA-RAOLH #35) provided by Dr. J.J. Reeves (Washington State Univ., Pullman, WA), highly purified ovine LH (LER-1374A) as radiolabeled tracer, and NIH-LH-B7 as standard. Intra- and interassay coefficients of variation for LH assays were 2.9 and 11.6%, respectively. Concentrations of FSH in all pooled serum samples were analyzed by radioimmunoassay [34], validated in our laboratory [33], using rabbit antiserum against ovine FSH (JAD-RAOFSH #17-6,7,9) provided by Dr. J.A. Dias (New York State Dept. of Health, Albany, NY), and highly purified ovine FSH (LER-1976-A2) as radiolabeled tracer and standard. Intra- and interassay coefficients of variation for FSH assays were 2.2 and 4.5%, respectively. Concentrations of P_4 in plasma samples collected throughout the experiment were analyzed by radioimmunoassay, as validated in our laboratory [31]. This procedure used a monoclonal antibody (02-9B4-94) to P_4-11-BSA

(BiosPacific, Emeryville, CA), P_4-11α-hemisuccinate-TME provided by Dr. A. Belanger (Le Centre Hospitalier de l'Universite Laval, PQ, Canada) as radiolabeled tracer, and P_4 (Sigma Chemical Co., St. Louis, MO) as standard. Intra- and interassay coefficients of variation for P_4 assays were 6.3 and 12.3%, respectively.

Concentrations of E_2 in all pooled serum samples were analyzed by radioimmunoassay which utilized an antiserum (Lilly lot #022367) provided by Dr. N.R. Mason (Lilly Research Laboratories, Indianapolis, IN), ^{125}I-E_2 (IMS.135; E_2-6[O-carboxymethyl]oximino-^{2}[^{125}I]iodohistamine, Amersham Corporation, Arlington Heights, IL) as radiolabeled tracer, and E_2 (Sigma Chemical Co., St. Louis, MO) as standard. Assay validation and procedures using this antiserum have been reported [35-37]. Antiserum to E_2 was used at a dilution of 1:1 000 000. Serum samples were extracted twice with 2 ml of diethyl ether. Recovery of [^{3}H]E_2 averaged 87.4 ± 2.3% in eight assays, and sample values were corrected for extraction efficiency. Recovery of added mass (0.1, 0.2, and 6.4 pg E_2) from 200 μl of serum from each of three independent serum samples averaged 99.4 ± 2.9%. Assay determinations of 100 and 200 μl of sample from each of ten independent serum samples were highly correlated (100 and 200 μl, r = 0.969). The amount of E_2 in each of the unknown samples was determined with a Four Parameter Curve Fitting Program [38]. Intra- and interassay coefficients of variation for E_2 assays were 2.6 and 19.3%, respectively.

Mean concentrations of LH (ng/ml) in serum samples, frequency of pulses of LH (pulses/24 h), and amplitude of pulses of LH (ng/ml) were determined through the use of algorithms (Pulsar software modified for the IBM-PC by J.F. Gitzen and V.D. Ramirez, Univ. of Illinois, Urbana, IL). Interval to the initiation of the preovulatory surge of LH was defined as the number of hours between cessation of treatments and the initiation of a continuous high-amplitude rise in concentrations of LH. Area under the P_4 release curve
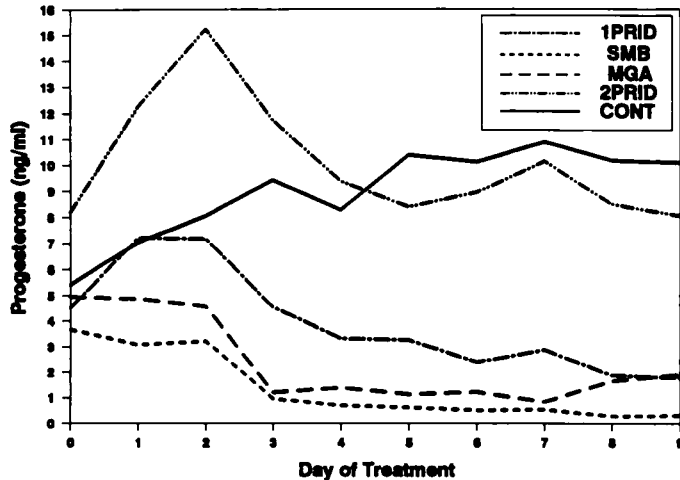
FIG. 1. Mean concentrations of progesterone in plasma samples during the treatment period (1PRID, n = 6; SMB, n = 6; MGA, n = 5; 2PRID; n = 6; CONT, n = 6). Pooled standard error of mean was 2.1 ng/ml.

during the treatment period and the estrous cycle following cessation of treatments was determined by measurement with a planimeter. The length of the luteal phase during the estrous cycle subsequent to treatment was determined by calculating the number of days between the first rise in P_4 above 1 ng/ml early and decline below 1 ng/ml of plasma late in the estrous cycle.

Statistical Analysis

Data were analyzed as a completely random design [39]. Data regarding the pulsatile secretion of LH on Day 8 of treatment, interval to the preovulatory surge of LH, and mean concentrations of E_2 and P_4 during the treatment period and the subsequent estrous cycle after cessation of treatment were examined by analysis of variance (ANOVA) using the general linear models procedure of SAS [40]. Treatment means were compared by orthogonal contrasts [40]. Area under curve for P_4 during the treatment period and the subsequent estrous cycle were analyzed by ANOVA using the general linear models procedure of SAS [40].

RESULTS

Hormone Concentrations during Treatment Period

During the 9-day treatment period, mean concentrations of P_4 (Table 1) in cows treated with two PRIDs and cows from the CONT group were higher ($p < 0.05$) than in cows from the 1PRID, SMB, and MGA groups. Cows treated with two PRIDs had similar mean concentrations of P_4 as compared to cows from the CONT group. Area under the curve for P_4 (Table 1) during the treatment period was greater ($p < 0.05$) in cows treated with two PRIDs and cows from the CONT group than cows from the other groups. Mean concentration of E_2 (Table 2) in cows from the SMB group

TABLE 2. Mean concentrations of E_2 during the treatment period, on Day 8 of treatment, and on Day 9, at the time of cessation of treatments.

| | | Mean concentrations of E_2 | | |
| | | During treatment[b] (pg/ml) | Day 8[c] (pg/ml) | Day 9[d] (pg/ml) |
Treatment[a]	n			
1PRID	6	8.4[f]	7.6[f]	7.7[fg]
SMB	6	35.3[e]	16.5[e]	17.8[e]
MGA	5	6.9[fg]	10.4[f]	12.2[ef]
2PRID	6	3.9[g]	2.5[g]	4.3[g]
CONT	6	3.0[g]	2.1[g]	3.2[g]
Pooled SEM[h]		5.1	4.6	4.5

[a]See *Materials and Methods* for definition of abbreviations and explanation of treatment regimens.
[b]During 9-day treatment period.
[c]At serial blood collection on Day 8 of treatment.
[d]At time of treatment cessation on Day 9.
[e,f,g]Numbers with differing superscripts within column differ ($p < 0.05$).
[h]Pooled standard error of mean (SEM).

(35.3 ± 11.1 pg/ml) was higher ($p < 0.05$) compared to that of cows from the other groups. Mean concentration of E_2 in cows from the 1PRID group (8.4 ± 1.4 pg/ml) was higher ($p < 0.05$) than in cows from the 2PRID and CONT groups (3.9 ± 0.6 and 3.0 ± 0.3 pg/ml, respectively) during the treatment period. These higher concentrations of E_2 in cows from SMB and 1PRID groups were due to their treatment with E_2 valerate or E_2 benzoate, respectively. Figures 1 and 2 depict mean concentrations of P_4 and E_2, respectively, for all treatment groups during the treatment period.

Hormone Concentrations during Serial Blood Collection Period

On Day 8 of treatment, mean concentrations of LH (Table 3) were lowest ($p < 0.05$) in cows from the CONT group (0.48 ± 0.06 ng/ml). Frequency of LH pulses (Table
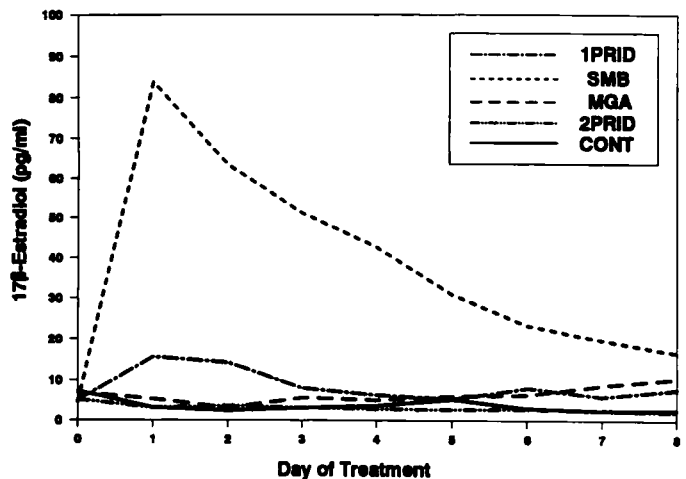

FIG. 2. Mean concentrations of 17β-estradiol in plasma samples during the treatment period (1PRID, n = 6; SMB, n = 6; MGA, n = 5; 2PRID; n = 6; CONT, n = 6). Pooled standard error of mean was 14.92 pg/ml.

TABLE 3. Mean concentrations of P$_4$, mean concentrations of LH, and amplitude and frequency of pulses of LH on Day 8 of treatment.

Treatment[a]	n	Mean P$_4$ (ng/ml)	Mean LH[b] (ng/ml)	LH frequency[b] (pulses/24h)	LH amplitude[b] (ng/ml)
1PRID	6	1.9[c]	0.8[c]	11.8[d]	1.1
SMB	6	0.3[c]	1.1[c]	18.8[c]	1.1
MGA	5	1.7[c]	1.1[c]	13.4[d]	1.5
2PRID	6	8.5[d]	0.8[c]	9.5[d]	1.3
CONT	6	10.2[e]	0.5[d]	2.5[e]	1.7
Pooled SEM[f]		1.4	0.1	3.0	0.8

[a]See *Materials and Methods* for definition of abbreviations and explanation of treatment regimens.
[b]Determined with Pulsar software.
[c,d,e]Numbers with differing superscripts within column differ ($p < 0.05$).
[f]Pooled standard error of mean (SEM).

3) was highest ($p < 0.05$) in cows from the SMB group (18.83 ± 1.11 pulses/24 h) and was lowest ($p < 0.05$) in cows from the CONT group (2.50 ± 0.43 pulses/24 h) as compared to cows in other groups. Frequency of LH pulses did not differ ($p > 0.05$) between cows from the 1PRID, MGA, and 2PRID groups (11.83 ± 1.35, 13.40 ± 1.91, and 9.50 ± 1.23 pulses/24 h, respectively). Amplitude of LH pulses (Table 3) was not different ($p > 0.05$) among the five groups of cows. Figure 3 depicts data from individual
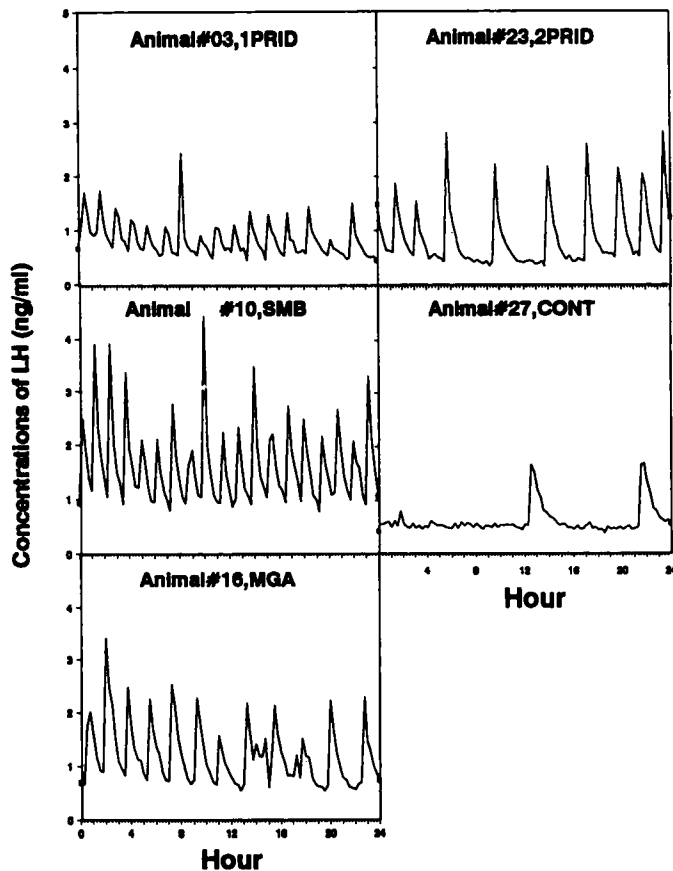

FIG. 3. Secretory profile of LH from individual representative animals from each treatment group during serial blood collection on Day 8 of treatment.

representative cows from each treatment group for pulsatile secretory profile of LH in circulation on Day 8 of treatment. Mean concentrations of P$_4$ (Table 3) were higher ($p < 0.05$) in cows treated with two PRIDs (8.5 ± 0.9 ng/ml) and cows from the CONT group (10.2 ± 0.6 ng/ml) as compared to cows in the other groups. During serial blood collection, mean concentrations of E$_2$ (Table 2) in cows from the 1PRID and SMB groups (7.6 ± 2.0 and 16.5 ± 3.0 pg/ml, respectively) were higher ($p < 0.05$) than in cows from the 2PRID and CONT groups (2.5 ± 0.3 and 2.1 ± 0.2 pg/ml, respectively).

Hormone Concentrations after Cessation of Treatment

After cessation of treatments on Day 9, initiation of the preovulatory surge of LH (Table 4) was 20 h earlier ($p < 0.05$) in cows from the SMB group (45 h) and tended ($p < 0.10$) to be earlier in cows from the 1PRID group (51 h) than in cows from the 2PRID (65 h) and CONT (65 h) groups. The preovulatory surge of FSH was observed concomitantly with the LH surge (data not shown). Four of five cows treated with MGA failed to initiate a preovulatory surge of LH within the 100-h blood sampling period after treatment removal.

Mean concentrations of E$_2$ (Table 2) were higher ($p < 0.05$) in cows from the SMB group (17.8 ± 2.5 pg/ml) as compared to cows from the 1PRID, MGA, 2PRID, and CONT groups (7.7 ± 1.5, 12.2 ± 3.3, 4.3 ± 0.5, and 3.2 ± 0.4 pg/ml, respectively) at the time of cessation of treatments. A continuous increase in concentrations of E$_2$ was observed in cows that initiated the preovulatory surge of LH. Figure 4 depicts mean concentrations of E$_2$ from cows in the 1PRID, SMB, 2PRID, and CONT groups during the follicular phase of the estrous cycle subsequent to cessation of treatments.

Mean concentrations of P$_4$ and area under the curve for P$_4$ (Table 1) during the estrous cycle subsequent to treatments were higher ($p < 0.05$) in cows treated with two PRIDs and cows from the CONT group than cows from the 1PRID, SMB, and MGA groups. Duration of the luteal phase (Table 1) was not different ($p > 0.10$) between cows from the five treatment groups.

Treatment[a]	Number of animals[b]	Interval to LH surge (h)[c]
1PRID	4	51[a][f]
SMB	4	45[e]
MGA[d]	1	(68)
2PRID	4	65[f]
CONT	5	65[f]
Pooled SEM[g]		8.16

[a]See Materials and Methods for definition of abbreviations and explanation of treatment regimens.
[b]Number of animals initiating the preovulatory surge of LH during the 75 or 100 h after cessation of treatment.
[c]Interval of time from treatment cessation to initiation of the preovulatory surge of LH.
[d]Four of five cows treated with MGA did not initiate a preovulatory surge of LH during the 100-h blood collection.
[e,f]Numbers with differing superscripts within column differ (p < 0.05).
[g]Pooled standard error of mean (SEM).

DISCUSSION

Synchronization of estrus with synthetic progestins (norgestomet and MGA) in groups of cows has often been associated with reduced fertility at the synchronized estrus. Results from the present study indicate that synthetic progestins administered in the regimen used to synchronize estrus do not mimic the function of CL in regulation of pulsatile secretion of LH and ovarian activity. During the serial blood collection on Day 8 of treatment, frequency of LH pulses was highest in cows from the SMB group, whereas frequency did not differ among cows from the 1PRID, MGA, and 2PRID groups. This higher frequency of LH pulses observed in cows from the SMB group is more characteristic of the pattern normally observed during the follicular phase of the estrous cycle [41, 42] and is in agreement with previous results [21]. An increase in LH pulse frequency with lower doses of P_4 has also been reported previously [30, 31]. In contrast, frequency of LH pulses was lowest for cows in the CONT group in the present study. This was in agreement with results from previous research [31, 41, 43].

Cows from the 2PRID group failed to exhibit an LH pulse frequency similar to cows from the CONT group. Similar results have been observed previously in our laboratory [31]. Mean plasma concentrations of P_4 during the treatment period were similar in cows from the 2PRID (10.1 ng/ml) and CONT (9.0 ng/ml) groups. At the time of serial blood collection (Day 8), cows from the 2PRID group had lower ($p < 0.05$) concentrations of P_4 (8.5 ng/ml) than cows from the CONT group (10.2 ng/ml) that were in the midluteal phase of their estrous cycles. There are several possible explanations for the difference in LH pulse frequency between cows receiving exogenous P_4 that results in circulating concentrations of P_4 typical of the midluteal phase (2PRID) and cows in the midluteal phase of the estrous cycle (CONT). In the present study, PRIDs were replaced with new PRIDs at 5-day intervals in cows from the 2PRID group. This regimen was based on data obtained from our

previous study [31], which showed that concentrations of P_4 begin to decline after 5 days from insertion of PRIDs. Consequently, new PRIDs had been in place for 2 and 3 days when serial blood samples were collected on Day 8 of treatment in the present study. However, even after 2 and 3 days from the time of replacement of PRIDs, mean concentrations of P_4 had declined (on Day 8 of treatment) in cows from the 2PRID group. In contrast, cows from the CONT group had continuously increased concentrations of P_4 as the luteal phase progressed (Fig. 1). These differences in concentrations of P_4, either increasing or declining, may contribute to the pattern of pulsatile secretion of LH observed in our study. The 1.7-ng difference in concentration of P_4 on the day of serial blood collection may also contribute to differences in pulsatile secretion of LH between these two groups of cows. Another possibility is that the CL produces a factor(s) other than P_4 that may influence LH pulse frequency. Yet another possibility is that the profile of P_4 in circulation has less variation when the source of P_4 is exogenous as compared to that produced by the CL and that this variation is related to modulation of LH secretion. However, comparison of profiles of P_4 in circulation in cows from the 2PRID and CONT groups lead us to believe that there is equal variance in P_4 concentrations in circulation among animals from these two groups.

Mean concentrations of E_2 were not different among cows from the 2PRID and CONT groups either during the treatment period or on the day of serial blood collection. These results indicate that the differing patterns of LH in circulation were not caused by major shifts in P_4:E_2 ratio, which has been proposed as a determining factor in the pattern of LH in circulation [28, 29]. While it is clear that P_4 is a
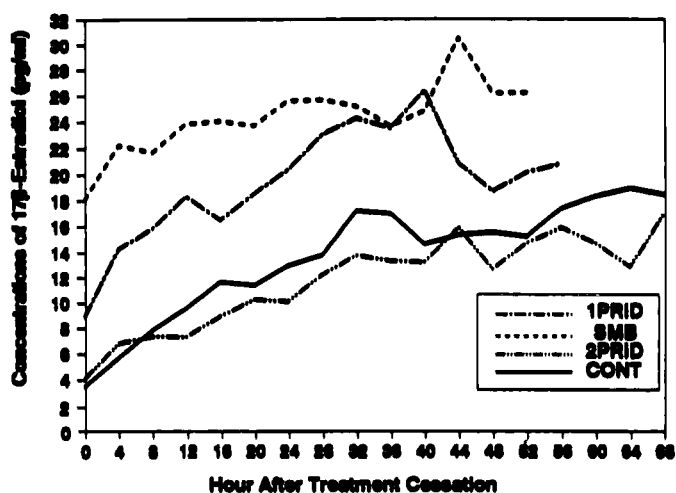

FIG. 4. Mean concentrations of 17β-estradiol in pooled serum samples (every 4 h) after treatment cessation (1PRID, n = 4; SMB, n = 4; 2PRID, n = 4; CONT, n = 5; n = number of animals initiating the preovulatory surge of LH during the 75 or 100 h after cessation of treatment). Only one animal treated with MGA had a preovulatory surge of LH during the period of blood collection after cessation of treatment. Thus, data from this group are not depicted. Pooled standard error of mean was 7.9 pg/ml.

modulator for the pulsatile secretion of LH, other factors produced by the CL may modulate the pattern of LH in circulation.

On Day 8 of the treatment period, mean concentrations of E_2 were greater in cows from the SMB group than in other groups and were similar in cows from the 1PRID and MGA groups. In contrast, mean concentrations of E_2 were significantly lower in cows from the 2PRID and CONT groups as compared to other treatment groups. The concentrations of E_2 and frequency of LH pulses were positively correlated ($p < 0.0001$); SMB (16.5:18.8) > MGA (10.4:13.4) > 1PRID (7.6:11.8) > 2PRID (2.5:9.5) > CONT (2.1:2.5, pg of E_2/ ml plasma:LH pulses/24 h, respectively). The differences in concentrations of E_2 in circulation in cows treated with synthetic progestins may be caused by a greater LH pulse frequency which in turn may alter ovarian follicular development and further increase concentrations of E_2. It has been reported that cows treated with norgestomet have an increased frequency of LH pulses [21] and elevated circulating concentrations of E_2 [22], which are associated with increased size, estrogenic capacity [22], and number of LH receptors [23] of the largest ovarian follicle. Treatment with MGA has been shown to increase size of ovarian follicles [9, 10] and concentrations of E_2 in circulation of cows [13, 14]. Interestingly, effects of synthetic progestins on pattern of LH pulse frequency are abolished by the presence of the CL [44].

In general, increasing concentrations of E_2 after a decline in P_4 will induce a preovulatory surge of LH in cows [45, 46]. In the present study, concentrations of E_2 were greater in cows from the 1PRID or SMB groups than cows from the 2PRID or CONT groups throughout the follicular phase following cessation of treatments on Day 9. Subsequently, the initiation of the preovulatory surge of LH occurred earlier in cows from the 1PRID or SMB groups. These results indicated that follicular maturation might have been in a more advanced stage in cows from the 1PRID and SMB groups as compared to cows from the 2PRID and CONT groups. Results from cows in the 1PRID group are in agreement with those of Roberson et al. [31] who reported that cows treated with a half PRID without the gelatin capsule containing E_2 had an increased concentration of E_2 in circulation during the treatment period. In that study, cows treated with a half PRID also had increased E_2 during the early portion of the follicular phase following cessation of treatment and an earlier preovulatory surge of LH as compared to cows from a 2PRID or control group [31]. Several studies have been reported in which cows treated with norgestomet initiate preovulatory surges of LH 30–48 h after implant removal [15–17], which is in agreement with results of the present study.

Four of five cows treated with MGA failed to initiate a preovulatory surge of LH within the 100-h blood sampling period following cessation of treatment. The failure of initiation of the preovulatory surge of LH in cows treated with

MGA might be associated with circulating concentration of P_4. Three of four MGA-treated cows that did not initiate the LH surge had more than 1 ng/ml of P_4 in circulation throughout the treatment period. Despite two injections of $PGF_{2\alpha}$, concentrations of P_4 were not maintained at basal concentrations in these cows. We have no explanation for this phenomenon at this point; however, MGA may cause luteinization of the cells [10] that make up the ovarian follicles to the point that production of some P_4 occurs. All cows (38%) that did not initiate a preovulatory surge of LH during the blood sampling period (75 or 100 h) had increased concentrations of P_4 within 10 days after cessation of treatment. These cows presumably had a delayed preovulatory surge of LH beyond the hourly blood collection period (75 or 100 h, depending on treatment).

Recently, Sirois and Fortune [47] demonstrated, by using real-time ultrasonography, that subtle changes in the hormonal milieu (levels of P_4) can dramatically alter the pattern of ovarian follicular dynamics. Administration of the low doses of P_4 at the time of natural luteolysis causes cessation of normal ovarian follicular recruitment and atresia. This results in a prolonged development of the ovulatory (or largest) follicle, which is dominant at the time of natural luteolysis. It was also reported that greater concentrations of E_2 are observed in cows treated with low doses of P_4 as compared to nontreated cows during the treatment period. According to these observations, ovarian follicular maturation in cows treated with the lower doses of P_4 in the present study might have been more advanced at the time of cessation of treatments. Thus, the shortened interval to the preovulatory surge of LH could have resulted. It has been suggested that aberrant ovarian follicular development is closely associated with function of subsequent CL [26]. Murdoch et al. [27] demonstrated that premature induction of ovulation by intrafollicular injection of gonadotropins results in luteal phase deficiency in the ewe.

These observations allow us to hypothesize that a prolonged development of the dominant ovarian follicle results when low concentrations of P_4 or synthetic progestins persist in circulation. The prolonged dominance of this ovarian follicle may lead to development and ovulation of an abnormal oocyte that is less fertile. Defects in function of CL after ovulation of this abnormal follicle may also occur. Another reason for the reduced fertility in cows treated with synthetic progestins is that alterations in their hormonal milieu (hyperestrogenic environment) may occur that lead to abnormal uterine conditions [48] and contractions [49, 50]. Doses of P_4 that mimic circulating concentrations typical of the midluteal phase of the estrous cycle may have the potential to synchronize estrus without reducing fertility.

In summary, the low levels of P_4 and synthetic progestins (norgestomet and MGA) administered in the regimen used to synchronize estrus alter the pattern of pulsatile secretion of LH and apparent ovarian activity, which is indicated by

the increased secretion of E_2 during the later part of the treatment period and subsequent follicular phase. These treatments also induced apparent luteal phase deficiency during the estrous cycle subsequent to treatment. These observations suggest that abnormal development of ovarian follicles by the alteration in secretion pattern of LH could lead to production of an abnormal oocyte that would be less fertile and ovulation of an abnormal follicle that may lead to altered function of the subsequent CL. The 2PRID treatment that resulted in midluteal phase concentrations of P_4 did not mimic the functions of midluteal phase CL in modulating the pattern of LH in circulation. Further studies are needed to investigate in more detail the follicular development and CL function associated with treatment with synthetic progestins. Based on data from our present research, synchronization of estrus with levels of P_4 that mimic midluteal concentrations may lead to improved conception rates at synchronized estrus, with conception rate comparable to those observed at spontaneous estrus.

ACKNOWLEDGMENTS

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