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Melengestrol Acetate at Greater Doses Than Typically Used for Estrous Synchrony in Bovine Females Does Not Mimic Endogenous Progesterone in Regulation of Secretion of Luteinizing Hormone and 17β-Estradiol[1]

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ABSTRACT

Our working hypothesis was that doses of melengestrol acetate (MGA) greater than those typically administered in estrous synchrony regimens would regulate secretion of LH and 17β-estradiol (E_2) as endogenous progesterone (P_4) does during the midluteal phase of the estrous cycle. We also hypothesized that endogenous P_4 from the CL would interact with MGA to further decrease the frequency of LH pulses and E_2. Cows on Day 5 of their estrous cycle (Day 0 = estrus) were randomly assigned to an untreated control group (CONT, n = 5) or to one of six MGA treatment groups (n = 5 per group): 1) MGA administered orally each day via a gelatin capsule at a dose of 0.5 mg MGA/cow with the CL present (0.5CL); 2) 0.5 mg MGA/cow daily in the absence of CL (0.5NO); 3) 1.0 mg MGA with CL present (1.0CL); 4) 1.0 mg MGA without CL (1.0NO); 5) 1.5 mg MGA with CL present (1.5CL); 6) 1.5 mg without CL (1.5NO). MGA was administered for 10 days (Day 5 = initiation of treatment). To regress CL, cows assigned to groups without CL received injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg) on Days 6 and 7 of their estrous cycle. All cows were administered $PGF_{2\alpha}$ at the end of the 10-day treatment period. During the treatment period, daily blood samples were collected to determine concentrations of E_2. Serial blood samples were collected at 15-min intervals for 24 h on Days 8, 11, and 14 to determine pattern of LH secretion. Frequency of LH pulses on Days 8, 11, and 14 was greater ($p < 0.05$) in cows without CL (0.5NO, 1.0NO, and 1.5NO) than in cows with CL (0.5CL, 1.0CL, 1.5CL, and CONT). Mean concentrations of LH were greater ($p < 0.05$) in cows from the 0.5NO group on Days 8 and 11 and were greater ($p < 0.05$) in cows from the 0.5NO, 1.0NO, and 1.5NO groups on Day 14 as compared to cows with CL. Overall mean concentrations of LH across Days 8, 11, and 14 were greatest ($p < 0.05$) in cows from the 0.5NO group and were also greater ($p < 0.05$) in cows from the 0.5NO, 1.0NO, and 1.5NO groups as compared to cows in the 0.5CL, 1.0CL, 1.5CL, and CONT groups. Mean concentrations of E_2 during the treatment period were greater ($p < 0.05$) in cows from the 0.5NO group than in cows from either the 1.0NO or the 1.5NO group; these values were also greater ($p < 0.05$) in cows of the 0.5NO, 1.0NO, and 1.5NO groups as compared to cows of the 1.0CL and CONT groups. Therefore, we reject our working hypothesis because doses of MGA greater than those typically used in estrous synchrony protocols did not suppress LH and E_2 to the same extent that endogenous P_4 does. In addition, MGA treatment when CL were present did not result in a further suppression of LH pulse frequency or of E_2 as compared to the values in control cows with functional CL.

INTRODUCTION

Melengestrol acetate (MGA), an orally active synthetic progestin, has been utilized to synchronize time of ovulation and time of behavioral estrus in cattle [1–4]. However, treatment with MGA has often been associated with reduced fertility when cows are inseminated at the synchronized estrus that ensues immediately after termination of MGA administration [1–6]. The possible factors that may influence conception rates at the synchronized estrus after use of MGA include an increased size of dominant ovarian follicle(s) [7, 8] that do not ovulate during the MGA treatment period [7–9]. Associated with the increased size of dominant ovarian follicle(s), increased concentrations of 17β-estradiol (E_2) in peripheral circulation during and immediately after MGA treatment have also been reported [10–13]. A reduction in size and weight of the CL on Day 3 of the estrous cycle after MGA treatment has also been reported [9].

Few studies have evaluated the influence of MGA on secretory pattern of gonadotropins, particularly LH. In general, the pattern of pulsatile secretion of LH in the bovine female is modulated by the ovarian steroids E_2 and progesterone (P_4) [14, 15]. Administration of low doses of P_4 (2–3 ng/ml in plasma) to cows in the absence of CL was reported to result in a greater frequency of LH pulses and increased concentrations of E_2 compared to those of control cows with functional CL [13, 16]. Onset of the preovulatory surge of LH was subsequently shown to be earlier after removal of P_4 treatment in cows receiving a low dose as compared to those receiving greater doses of P_4 (7–10 ng/ml in plasma) [13, 16]. Treatment with lower doses of P_4 for a duration sufficient to extend the estrous cycle has resulted in alterations in patterns of ovarian follicular dy-

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TABLE 1. Frequency of LH pulses during serial blood collections on Days 8, 11, and 14 post estrus.

Treatment[b]	n	Frequency of LH pulses/24 h[a]			
		Day 8	Day 11	Day 14	Overall mean
0.5CL	5	5.0[d]	4.4[e]	3.4[d]	4.3[d]
0.5NO	5	16.6[c]	18.0[c]	17.6[c]	17.4[c]
1.0CL	5	3.4[d]	3.6[e]	3.6[d]	3.5[d]
1.0NO	4	16.8[c]	13.8[d]	13.0[c]	14.5[c]
1.5CL	4	7.8[d]	5.8[e]	7.3[d]	6.9[d]
1.5NO	4	13.0[c]	12.8[d]	13.0[c]	12.9[c]
CONT	4	6.0[d]	4.5[e]	4.5[d]	5.0[d]
Pooled SEM		1.3	1.3	1.6	0.8

[a]Determined with Pulsar software.
[b]See *Materials and Methods* for definitions of abbreviations and explanation of treatment regimens.
[cde]Numbers with differing superscripts within column differ ($p < 0.05$).

namics and prolonged maintenance of the largest follicle that was dominant at the time of administration of P_4; coincidental increases in concentrations of E_2 were also observed [17].

We previously reported [13] that treatment of cows with MGA in the absence of CL at a dose of 0.5 mg/day, which is the dose used in estrous synchrony programs, resulted in frequency of LH pulses and concentrations of E_2 in plasma that were similar to those observed in cows treated with low doses of P_4. Frequency of LH pulses and concentrations of E_2 were greater in cows treated with MGA than in cows with functional CL that were in the midluteal phase of their estrous cycle.

Therefore, our working hypothesis was that doses of MGA greater than those presently used in estrous synchrony programs would regulate frequency of LH pulses and concentrations of E_2 in a manner similar to that of endogenous P_4 from CL during the midluteal phase of the estrous cycle. We also hypothesized that endogenous P_4 from the CL would interact with MGA to further decrease the frequency of LH pulses and peripheral concentrations of E_2.

MATERIALS AND METHODS

Experimental Protocol

Thirty-five mature nonlactating beef cows of a composite breed type (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, 1/4 Red Poll) exhibiting normal estrous cycles were used in this study (3–11 yr of age; 489 ± 12 kg BW, mean ± SEM). Stage of the estrous cycle was synchronized in all cows before initiation of treatments by administration of two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 25 mg; Lutalyse Sterile Solution; Upjohn Co., Kalamazoo, MI) 11 days apart. Cows on Day 5 of their estrous cycle (Day 0 = estrus) were randomly assigned to an untreated control group (CONT, n = 5) or to one of six MGA treatment groups (n = 5/group): 1) MGA (MGA 200 Premix, Upjohn Co.) administered orally each day via a gelatin capsule at a dose of 0.5 mg MGA/cow with CL present (0.5CL); 2) 0.5 mg MGA/cow daily without CL (0.5NO); 3) 1.0 mg MGA with CL present (1.0CL); 4) 1.0 mg MGA without CL (1.0NO); 5) 1.5 mg MGA with CL present (1.5CL); 6) 1.5 mg without CL (1.5NO). The gelatin capsule was used to ensure that cows received specific doses of MGA relative to the treatment groups to which they were assigned. Treatments were initiated on Day 5 of the estrous cycle and cows received the assigned dosage of MGA every morning (0800 h) for 10 days. Cows assigned to the CONT group received an empty gelatin capsule at the same time. Therefore, cows from the CONT group were in the luteal phase (from Day 5 to Day 15) of the estrous cycle during the treatment period. Cows assigned to the groups without CL received injections of $PGF_{2\alpha}$ (25 mg) on Days 6 and 7 for regression of the CL to eliminate endogenous P_4 from the CL. During the initial estrous synchronization before treatments began, four cows that exhibited behavioral estrus were assigned to 1.0NO, 1.5CL, 1.5NO, and CONT groups (one cow per group); however, these cows did not respond to the injections of $PGF_{2\alpha}$ 11 days apart at the initial estrous synchronization before the treatment period as determined by examination of profiles of P_4 concentration from Day −3 to Day 50. Data for these cows were, therefore, eliminated from the statistical analyses.

On Days 8, 11, and 14 after estrus, catheters were inserted in the jugular vein of all cows, and blood samples were collected at 15-min intervals for 24 h. These blood samples were used to determine pulse frequency of LH. On Day 15, simultaneously with the last dose of MGA, all cows received an injection of 25 mg $PGF_{2\alpha}$ (Hour 0). Blood samples were collected at 3-h intervals beginning at Hour 40 (Day 17) and continuing through Hour 169 (Day 22). These blood samples were used to determine the interval to preovulatory surges of LH. Blood samples collected at 15-min and 3-h 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 until assayed for LH.

Daily blood samples were collected in tubes treated with a 30% solution of EDTA (50 μl for a 10-ml blood sample; Fisher Scientific, Fair Lawn, NJ) from all cows throughout the treatment period and for 35 days post-treatment (from

Day −3 to Day 50) in order to evaluate plasma concentrations of P_4 and E_2 during the treatment period and the estrous cycle following cessation of treatments. In an attempt 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 collected and stored at −20°C until assayed for P_4 and E_2. In addition, all cows were observed for behavioral estrus three times a day at approximately 0600, 1200, and 1800 h from Day −5 to Day 50.

RIAs

Concentrations of LH in all samples collected serially on Days 8, 11, and 14 were analyzed by RIA [18, 19]. Intra- and interassay coefficients of variation for LH assays were 2.7 and 10.0%, respectively. Assay sensitivity was 12 pg/ml of serum. Concentrations of FSH in all pooled serum samples were analyzed by RIA [19, 20]. Intra- and interassay coefficients of variation for FSH assays were 2.3 and 4.7%, respectively, and assay sensitivity was 56 pg/ml of serum. Concentrations of P_4 in plasma samples collected throughout the experiment were analyzed by RIA [16]. Intra- and interassay coefficients of variation for P_4 assays were 2.3 and 17.6%, respectively; assay sensitivity was 312 pg/ml of plasma. Concentrations of E_2 in plasma samples collected throughout the experiment were also analyzed by RIA [13]. Intra- and interassay coefficients of variation for E_2 assays were 3.9 and 18.2%, respectively, and assay sensitivity was 0.6 pg/ml of plasma.

Data Reduction and Statistical Analysis

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, Urbana, IL). The G values used for the Pulsar program were 6.8, 4.4, 1.6, 1.3, and 10.0 for G(1) through G(5), respectively. Interval to the initiation of the preovulatory surge of LH was defined as the number of hours between administration of the last dose of MGA and/or injection of $PGF_{2\alpha}$ and the initiation of a continuous high-amplitude rise in concentrations of LH. Area under the curve for the profile of P_4 during the treatment period (Days 5–15) was determined by measurement with a planimeter. The length of the estrous cycle following cessation of treatment was determined by examination of profiles for P_4 and E_2 concentrations and by observations for behavioral estrus during this period.

Data on the pulsatile secretion of LH, the concentrations of P_4 and E_2 on Days 8, 11, and 14, area under the curve for the profile of P_4, interval to the preovulatory surge of LH, and length of estrous cycle following cessation of treatment were analyzed as a completely randomized design [21]

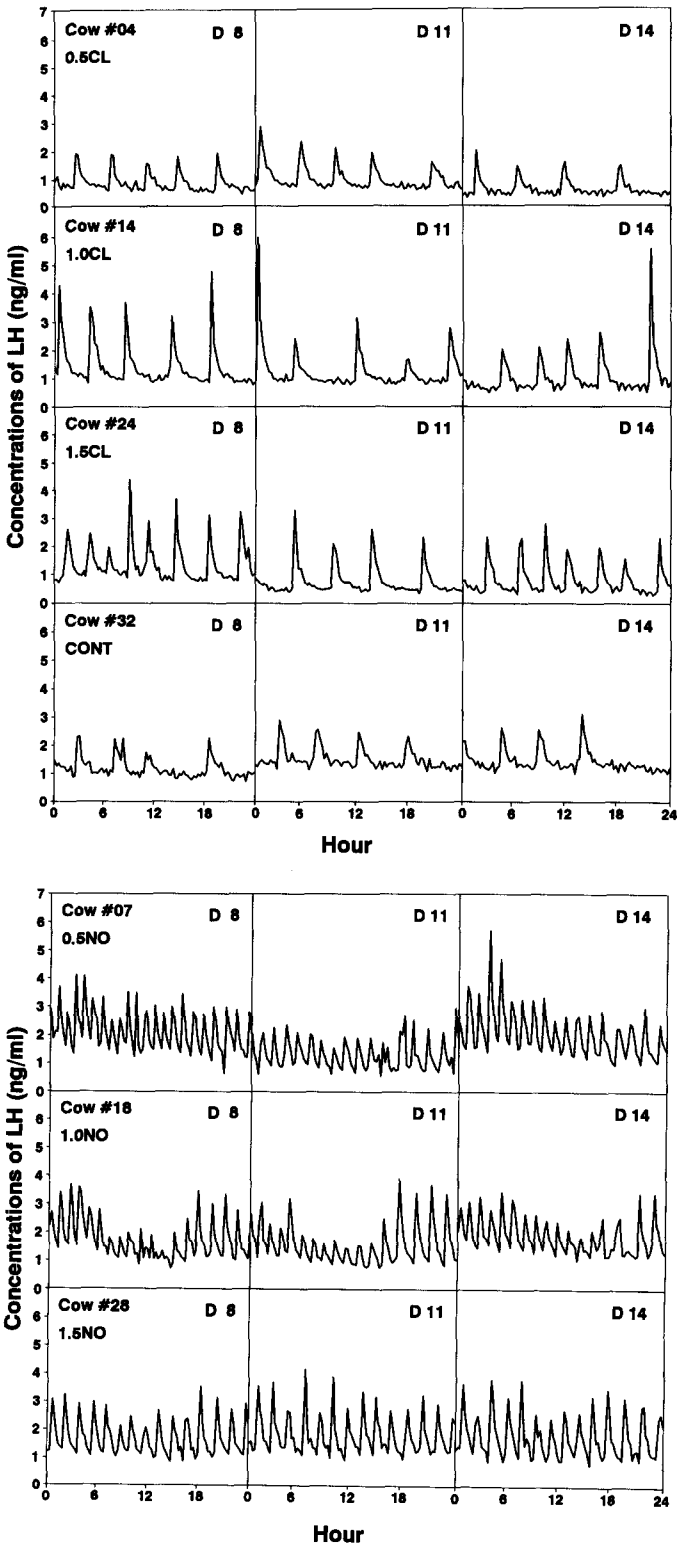

FIG. 1. Secretory profile of LH (15-min intervals for 24 h) from individual representative cows from each treatment group during serial blood collections on Days 8, 11, and 14 after estrus.

TABLE 2. Mean concentrations of LH during serial blood collections on Days 8, 11, and 14 post estrus and mean concentration of FSH during treatment.

Treatment[b]	n	Mean concentrations of LH (ng/ml)[a]				Mean FSH during treatment (ng/ml)[c]
		Day 8	Day 11	Day 14	Overall mean	
0.5CL	5	1.03[f]	1.00[ef]	0.89[e]	0.97[f]	1.92
0.5NO	5	2.60[d]	2.13[d]	2.10[d]	2.27[d]	2.07
1.0CL	5	1.11[ef]	0.94[f]	1.01[e]	1.02[f]	1.83
1.0NO	4	1.62[de]	1.42[ef]	1.75[d]	1.60[e]	2.13
1.5CL	4	1.27[ef]	0.84[f]	1.10[e]	1.07[f]	1.88
1.5NO	4	1.64[de]	1.58[de]	1.71[d]	1.64[e]	1.85
CONT	4	1.08[ef]	1.17[ef]	0.95[e]	1.06[f]	1.97
Pooled SEM		0.17	0.19	0.18	0.10	0.13

[a]Determined with Pulsar software.
[b]See *Materials and Methods* for definitions of abbreviations and explanation of treatment regimens.
[c]Concentrations of FSH did not differ among treatment groups or across days of treatment; therefore, data were pooled.
[def]Numbers with differing superscripts within column differ ($p < 0.05$).

by ANOVA using the general linear models procedure of SAS [22]. Treatment means were compared by orthogonal contrasts and also Duncan's new multiple range test [21]. Pulse frequencies and mean concentrations of LH across Days 8, 11, and 14 and concentrations of E_2, P_4, and FSH during the treatment period were analyzed through the use of a mixed model procedure (Proc Mixed) of SAS [23]. The fitted model included treatment and day as fixed effects and cow as a random effect. To account for the covariance between observations from the same animal at different days, different options of covariance structures for residuals available in the Repeated Statement of Proc Mixed were considered, and the model with the best fit was chosen to analyze data. Comparison of means was performed by using Proc Mixed of SAS [23], which employs the *t*-test and also Duncan's new multiple range test [21].

RESULTS

Hormone Concentrations during Serial Blood Collection Periods

During the serial blood collection on Days 8, 11, and 14 after estrus, LH pulses occurred more frequently ($p < 0.05$) in the cows treated with all doses of MGA when CL were absent (0.5NO, 1.0NO, and 1.5NO) as compared to cows with CL present (0.5CL, 1.0CL, 1.5CL, and CONT; Table 1). Frequency of LH pulses did not differ ($p > 0.10$) among cows with CL present (0.5CL, 1.0CL, 1.5CL, and CONT). The number of LH pulses observed during each of the three serial blood collections was correlated within individual cows ($r = 0.912$; Fig. 1). Mean concentrations of LH were greatest ($p < 0.05$) in cows from the 0.5NO group on Days 8 and 11; they were greater ($p < 0.05$) in cows from the 0.5NO, 1.0NO, and 1.5NO groups on Day 14 as compared to MGA-treated cows that had CL and as compared to cows from the CONT group (Table 2). Overall mean concentrations of LH across Days 8, 11, and 14 were greatest ($p < 0.05$) in cows from the 0.5NO group; they were also greater ($p < 0.05$) in cows from the 0.5NO, 1.0NO, and 1.5NO groups as compared to cows of the 0.5CL, 1.0CL, 1.5CL, and CONT groups (Table 2). Amplitude of LH pulses was not different ($p > 0.10$) among cows of all treatment groups on either Days 8, 11, or 14 (Table 3). Mean concentrations of FSH during the treatment period did not differ ($p > 0.10$) among cows of all treatment groups (Table 2).

On Days 8 and 11, mean concentrations of E_2 were greater ($p < 0.05$) from the 0.5NO group as compared to the cows

TABLE 3. Amplitude of LH pulses during serial blood collections on Days 8, 11, and 14 post estrus.

Treatment[c]	n	Amplitude of LH pulses (ng/ml)[a,b]		
		Day 8	Day 11	Day 14
0.5CL	5	1.52	1.58	1.28
0.5NO	5	2.16	2.31	2.00
1.0CL	5	2.44	1.63	1.67
1.0NO	4	1.47	1.42	1.55
1.5CL	4	1.52	1.72	1.75
1.5NO	4	1.83	1.89	2.24
CONT	4	1.65	1.62	1.67
Pooled SEM		0.50	0.47	0.35

[a]Determined with Pulsar software.
[b]Amplitude of LH pulses did not differ among treatment groups on Day 8, 11, or 14 post estrus.
[c]See *Materials and Methods* for definitions of abbreviations and explanation of treatment regimens.

TABLE 4. Mean concentrations of E_2 during serial blood collections on Days 8, 11, and 14 post estrus.

Treatment[a]	n	Mean concentrations of E_2 (pg/ml)			Mean E_2 during treatment (pg/ml)
		Day 8	Day 11	Day 14	
0.5CL	5	8.4[d]	3.9[e]	14.2[bc]	6.9[cd]
0.5NO	5	20.8[b]	26.5[b]	22.7[b]	18.4[b]
1.0CL	5	10.3[d]	3.6[e]	6.5[c]	6.4[d]
1.0NO	4	13.8[bc]	12.8[cd]	15.6[bc]	11.1[c]
1.5CL	4	8.6[d]	9.5[de]	12.4[bc]	7.0[cd]
1.5NO	4	14.4[bc]	17.4[c]	13.2[bc]	11.0[c]
CONT	4	8.3[d]	5.4[de]	5.5[c]	6.5[d]
Pooled SEM		2.8	2.5	3.6	1.4

[a]See *Materials and Methods* for definitions of abbreviations and explanation of treatment regimens.
[bcd]Numbers with differing superscripts within column differ ($p < 0.05$).

with CL present. Mean concentrations of E_2 during the treatment period were greater ($p < 0.05$) in cows from the 0.5NO group than in cows from either the 1.0NO or the 1.5NO group; these values were also greater ($p < 0.05$) in cows of 0.5NO 1.0NO, and 1.5NO groups as compared to cows of 1.0CL and CONT groups (Table 4). Mean concentrations of P_4 at the end of treatment (Day 14) were greater ($p < 0.05$) in cows from 0.5CL, 1.0CL, 1.5CL, and CONT groups than cows from the 0.5NO, 1.0NO, and 1.5NO groups (Table 5). Area under the curve for the profile of P_4 during the treatment period (Days 5–15) was greater ($p < 0.05$) in cows from 0.5CL, 1.0CL, and CONT groups compared to cows of 1.0NO and 1.5NO groups, while cows of the 1.5CL group did not differ from those of the other groups ($p > 0.10$; Table 5). There was a nonsignificant dose response among cows treated with MGA in the absence of CL, with area under the curve for the P_4 profile decreasing as dose of MGA increased (0.5NO, 227.0 ± 113; 1.0NO, 134.7 ± 24; and 1.5NO, 105.1 ± 30 arbitrary units). These results indicated that the CL in all cows assigned to the no-CL groups had regressed as a result of the two injections of $PGF_{2\alpha}$ on Days 6 and 7. During the treatment period, hormone profiles (E_2 and P_4) and observations for behavioral estrus indicated that none of these cows exhibited behavioral estrus or ovulated during treatment.

Interval to the Preovulatory Surge of LH

After cessation of treatments on Day 15, initiation of the preovulatory surge of LH was earlier ($p < 0.05$) in cows from the CONT (90 ± 10 h) and 0.5CL (95 ± 10 h) groups than in cows from the 1.0CL (143 ± 9 h) and 1.5CL (158 ± 12 h) groups (Table 6). Only one cow from each group treated with MGA in the absence of CL (0.5NO, 1.0NO, and 1.5NO) had initiated a preovulatory surge of LH within the 169-h blood sampling period after cessation of treatments. Interestingly, cows exhibiting the preovulatory LH surge that had received MGA in the absence of CL had intervals much like those of cows given the same dose of MGA in the presence of CL (Table 6).

Estrous Cycle Following Treatments

Duration of the estrous cycle (number of days between behavioral estrus) after treatments was shorter ($p < 0.05$) in cows from the 1.5NO group compared to cows in other groups (0.5CL, 0.5NO, 1.0CL, 1.5CL, and CONT; Table 6). Abnormal function of CL was observed in eleven MGA-treated cows during the estrous cycle following treatments, and eight of these eleven cows were from the groups treated with MGA in the absence of the CL (Table 6). Five cows had a shortened estrous cycle of less than 14 days (Fig. 2c). One

TABLE 5. Mean concentrations of P_4 during serial blood collections on Days 8, 11, and 14 post estrus and area under the curve for the profile of P_4 during the treatment period.

Treatment[a]	n	Mean concentrations of P_4 (ng/ml)			Area[b] (units)
		Day 8	Day 11	Day 14	
0.5CL	5	5.00[cd]	5.69[c]	6.71[c]	681.1[cd]
0.5NO	5	0.56[e]	0.61[d]	0.44[d]	227.0[de]
1.0CL	5	6.33[c]	6.68[c]	5.04[c]	808.0[c]
1.0NO	4	0.63[e]	0.52[d]	0.38[d]	134.7[e]
1.5CL	4	2.83[de]	3.70[cd]	4.42[c]	451.5[cde]
1.5NO	4	0.49[e]	0.51[d]	0.22[d]	105.1[e]
CONT	4	4.40[cd]	6.36[c]	7.38[c]	762.1[c]
Pooled SEM		1.09	1.28	1.16	129.3

[a]See *Materials and Methods* for definitions of abbreviations and explanation of treatment regimens.
[b]Area under the curve for concentrations of P_4 during treatment period as expressed in arbitrary units.
[cde]Numbers with differing superscripts within column differ ($p < 0.05$).

TABLE 6. Interval to the preovulatory surge of LH, duration of the estrous cycle and luteal function after cessation of treatment.

Treatment[a]	Interval to LH surge (h)[b,c]	Duration of the estrous cycle after cessation of treatment (d)[d]	Abnormal P_4 profile following treatment	
			Number of cows with shortened estrous cycle[e]	Number of cows with possible luteinized follicle[f]
0.5CL	95 [g] (n = 4)	19.3[g] (n = 4)	1	1
0.5NO	(85)[i] (n = 1)	17.0[g] (n = 3)	1	2
1.0CL	143 [h] (n = 5)	20.0[g] (n = 5)	1	0
1.0NO	(142)[i] (n = 1)	16.3[gh] (n = 4)	2	0
1.5CL	158 [h] (n = 3)	19.8[g] (n = 4)	0	0
1.5NO	(157)[i] (n = 1)	10.5[h] (n = 4)	1	2
CONT	90 [g] (n = 4)	19.0[g] (n = 4)	0	0
Pooled SEM	10	2.1		

[a]See *Materials and Methods* for definition of abbreviations and explanation of treatment regimens.
[b]Number of cows initiating the preovulatory surge of LH during the 169 h blood collection after cessation of treatment.
[c]Interval of time from treatment cessation to initiation of the preovulatory surge of LH.
[d]The length of the estrous cycle following cessation of treatment was determined by examination of profiles for P_4 and E_2 concentrations and observations for behavioral estrus during this period.
[e]Cows had a shortened estrous cycle less than 14 days following treatment.
[f]Cows had a delayed initiation of the estrous cycle for more than 20 days post treatment and had more than 1 ng/ml of P_4 in plasma, indicating the possibility of a luteinized follicle in these animals.
[g,h]Numbers with differing superscripts within column differ ($p < 0.05$).
[i]One cow from each treatment group initiated a preovulatory surge of LH during 169 h blood collection.

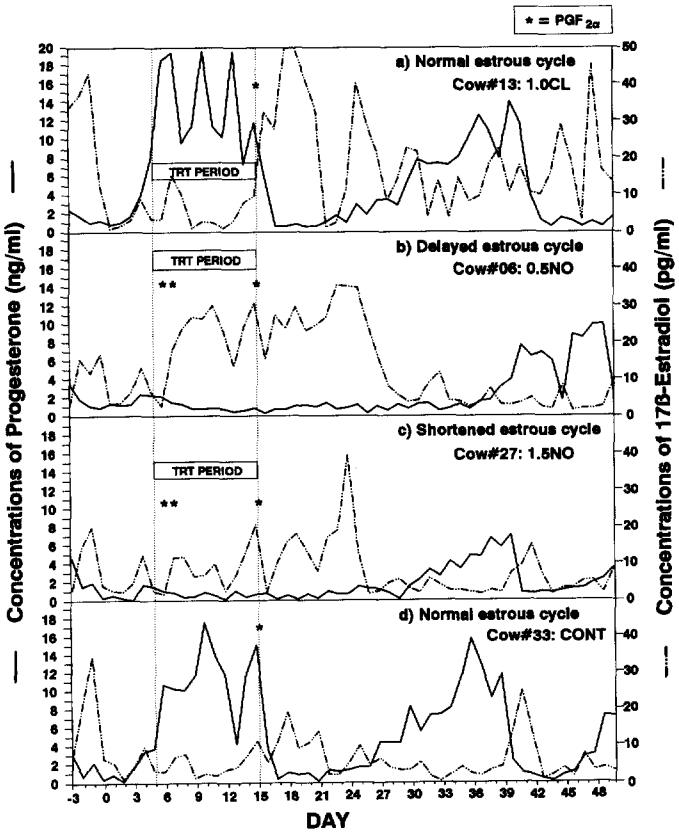

FIG. 2. Secretory profile of P_4 and E_2 from individual representative cows, during and after the treatment period, for (a) a normal estrous cycle after treatment with MGA (Cow #13; 1.0CL); (b) delayed initiation of the next estrous cycle with a possible luteinized follicle (Cow #06; 0.5NO); (c) a shortened estrous cycle, less than 14 days (Cow #27; 1.5NO); and (d) a normal estrous cycle (Cow #33; CONT).

cow had reduced concentrations of P_4 (2–3 ng/ml) in plasma with a shortened estrous cycle of less than 14 days. Another five cows had delayed initiation of luteal function (more than 20 days post-treatment). These cows had 1–4 ng/ml of P_4 in plasma over an extended period of time after the cessation of treatment, and profiles of P_4 from these cows were unlike those for the typical estrous cycle (Fig. 2b compared to the control in Fig. 2d). In contrast, profiles of hormones in a typical estrous cycle from an MGA-treated cow and a control cow are shown in Figure 2, a and d, respectively.

DISCUSSION

In the present study, a greater frequency of LH pulses was observed in cows treated with all doses of MGA in the absence of CL (0.5NO, 1.0NO, and 1.5NO) during each of the three serial blood collections (Days 8, 11, and 14). This greater frequency of LH pulses is characteristic of the pattern of LH pulses normally observed during the follicular phase of the estrous cycle [24–26]. In addition, mean LH pulse frequencies pooled across all three serial blood collections in cows among the no-CL groups revealed a nonsignificant dose response trend with LH pulse frequency decreasing as dose of MGA increased (0.5NO, 17.4 ± 0.5; 1.0NO, 14.5 ± 1.0; and 1.5NO, 12.9 ± 0.6 LH pulses/24 h). In contrast, frequency of LH pulses did not differ among cows treated with doses of MGA in the presence of CL or among controls (0.5CL, 1.0CL, 1.5CL, and CONT). During this period, mean concentrations of P_4 were greater in cows from the 0.5CL, 1.0CL, 1.5CL, and CONT groups than in those

treated with MGA in the absence of the CL (0.5NO, 1.0NO, and 1.5NO). This is in agreement with the secretory pattern of LH from previous studies that utilized treatment with either high or low doses of P_4 [13, 16] or MGA in the absence of CL [13].

It has been reported that effects of synthetic progestins on the pattern of LH pulse frequency were abolished by the presence of CL [27]. These results indicate that the CL is the major factor controlling the secretory pattern of LH during the luteal phase of the estrous cycle. P_4 is the major secretory product of the CL, and MGA failed to modulate the release of LH in the same manner as a functional CL secreting P_4. Therefore, the doses of MGA used in the present study do not mimic the ability of endogenous P_4 to suppress the frequency of the LH pulse. However, all doses of MGA effectively blocked both the preovulatory surge of LH and ovulation. From these results, we postulate that MGA mimics the negative feedback of P_4 in blocking the preovulatory surge of LH but not in suppressing pulsatile secretion of LH, although there is a possibility that much higher doses of MGA might be able to suppress the pulsatile secretion of LH.

On Days 8, 11, and 14, the greatest mean concentrations of E_2 were observed in cows from the 0.5NO group. This greater E_2 probably resulted from greater frequencies of LH pulses in these cows (20.8:16.6, 26.5:18.0, and 22.7:17.6, pg of E_2/ml:LH pulses/24 h, respectively) as compared to cows of other groups. This is in agreement with results from a previous study [13] in which concentrations of E_2 and frequency of LH pulses were positively correlated. These greater concentrations of E_2 were similar to those observed during the late follicular phase of the estrous cycle just before the behavioral estrus and the preovulatory surge of LH [13, 28–30]. However, the greater concentrations of E_2 did not induce behavioral estrus during the treatment period in the present study. Therefore, the dose of MGA that blocks behavioral estrus is less than the dose required to suppress pulsatile secretion of LH to levels observed during the midluteal phase of the estrous cycle. An increase in plasma concentrations of E_2 in cows treated with the synthetic progestin in the absence of CL may be the result of less suppression on the LH pulse frequency, which resulted in enhanced ovarian follicular development and greater concentrations of E_2. Treatment with MGA has been shown to result in increased size of ovarian follicles [7, 8], estrogenic capacity [31], and concentrations of E_2 in plasma of cows [11, 12] via less suppression on the frequency of LH pulses [13].

Use of real-time ultrasonography has indicated that treatment with MGA for a duration sufficient to extend the estrous cycle resulted in the development of ovarian follicles to larger sizes and in the persistence of dominant follicles for longer periods than are typical for dominant follicles during the luteal phase of the estrous cycle [32, 33]. These larger ovarian follicles that develop during progestin-based estrous synchrony protocols have been termed "persistent follicles." The development of persistent ovarian follicles was first described during treatment with low doses of P_4, in the absence of CL [17], that dramatically altered the pattern of ovarian follicular dynamics. Persistent follicles have always been associated with greater concentrations of E_2 in the peripheral circulation and a greater frequency of LH pulses [34]. Interestingly, results from the present study indicated that secretion of FSH may not have a role in the formation of persistent follicles, at least in cows treated with MGA. Since the earlier studies, there have been numerous reports of the development of persistent follicles after treatment with various progestins [34–37]. Previous studies indicated that ovulation of a persistent follicle, brought about by treatment with either a low dose of P_4 [37, 38] or norgestomet in the absence of CL [39], resulted in reduced fertility at the synchronized estrus. Therefore, persistent ovarian follicles resulting from treatment with MGA may be a major factor contributing to reduced fertility rates if cows are inseminated at the first behavioral estrus that ensues after the termination of feeding this synthetic progestin.

The timing of the preovulatory surge of LH was earlier in cows from the CONT and 0.5CL groups than in cows from the 1.0CL and 1.5CL groups after cessation of treatments. Only one cow from each group treated with MGA in the absence of CL (0.5NO, 1.0NO, and 1.5NO) had initiated the preovulatory surge of LH within the 169-h blood sampling period. The intervals to the preovulatory surge of LH in these cows were very similar to those of cows given MGA in the presence of the CL. In general, the interval to the preovulatory surge of LH was prolonged as the dose of MGA increased. These results indicate that clearance of MGA may be closely associated with interval to the preovulatory surge of LH after treatment with MGA. These observations are explained by the fact that MGA is stored in adipose tissue of the body and released very slowly after the administration of MGA is ended [40].

After treatment ended, the duration of the estrous cycle was shorter in cows from the 1.5NO group compared to cows from the 0.5CL, 0.5NO, 1.0CL, 1.5CL, and CONT groups. Abnormal function of CL was also observed in eleven MGA-treated cows, eight of these from groups treated with MGA in the absence of CL. This is in agreement with previous reports of shortened estrous cycles [4] and subfunctional CL with reduced production of P_4 [13] after treatment with MGA. It has also been reported that MGA resulted in reduced size and weight of CL at Day 3 but not at Day 13 of the subsequent estrous cycle [9].

In five cows, initiation of the estrous cycle was delayed for more than 20 days post-treatment and concentrations of P_4 in plasma were greater than 1 ng/ml before the beginning of the next estrous cycle. Interestingly, we observed the same phenomenon in a previous study [13]. These results suggest the possibility that the elevated concentrations of LH associated with MGA administration may induce lu-

teinization of ovarian follicles that could account for the production of P_4 observed in the present study [8]. Another consideration that could explain this observation is that an impaired or abnormal oocyte may reside in persistent ovarian follicles. This notion is supported by the observation that removal of the oocyte from Graafian follicles in situ promotes spontaneous luteinization and production of P_4 by ovarian follicles in rabbits and rats [41–43]. It has also been reported that the mouse oocyte inhibits production of P_4 by the cumulus oophorus in vitro [44]. Thus, prolonged periods of chronically elevated concentrations of E_2 during and after treatment with MGA may cause damage to the oocyte, which could promote spontaneous luteinization and production of P_4 by these ovarian follicles. These luteinized ovarian follicles may persist for longer periods, thereby explaining the delayed initiation of the next estrous cycle after treatment with MGA.

In summary, the doses of MGA used in the present study did not suppress pulsatile secretion of LH as does endogenous P_4 from the CL. Alterations in ovarian biosynthetic processes are indicated by the increased secretion of E_2 during and immediately after treatment with MGA. The increased frequency of LH pulses associated with MGA treatment in the absence of CL might result in the development of persistent follicles and the ovulation of abnormal oocytes, accounting for the reduction in fertility observed in cattle bred at the synchronized estrus. Treatment with MGA possibly causes luteinization of ovarian follicles and induced abnormal luteal function. Therefore, data from the present study indicate that if MGA is used alone, even at greater doses than presently used for estrous synchronization, fertility at the synchronized estrus may be compromised.

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