

# OVARIAN AND PITUITARY HORMONES IN BLOOD OF PROGESTOGEN-TREATED EWES

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## SUMMARY

Estradiol ( $E_2$ ), estrone ( $E_1$ ), progesterone, LH and prolactin concentrations were determined by radioimmunoassay in blood plasma samples from parous ewes treated with medroxyprogesterone acetate (MAP) or melengestrol acetate (MGA) orally, with MAP by intravaginal sponge, or progesterone by intramuscular injection. Treatment was begun on Day 10 of the estrous cycle and continued for 14 days. Blood samples were collected daily from Day 10 through 13 of the estrous cycle for untreated ewes and from Day 10 through 23 for progestogen-treated ewes, after which the frequency of blood collection was increased to 6-hr intervals until 48 hr after the beginning of estrus.

Corpora lutea (CL) regressed, as indicated by a reduction of plasma progesterone concentration, between Day 13 and 15 of the estrous cycle. As soon as the CL regressed, LH ( $P < .01$ ) and  $E_2$  concentrations increased linearly with time in untreated ewes and in ewes still under treatment with MGA or MAP orally or with progesterone intramuscularly. The increases were not found in ewes treated with MAP

intravaginally. After termination of progestogen treatments on Day 23, plasma  $E_2$  increased ( $P < .01$ ) further, followed by a LH surge  $73.8 \pm 3.3$  hr after the last progestogen administration. For the untreated ewes,  $E_2$  concentrations rose sharply (from 5.8 pg/ml to 22.0 pg/ml) during the 48-hr period before the preovulatory LH surge; this  $E_2$  secretion for untreated ewes (response slope of 5.4) differed ( $P < .01$ ) from those of the progestogen-treated ewes (response slopes of 1.8 to  $-4$ ). Estrone secretion paralleled  $E_2$ , but the  $E_1$  concentrations were about one-third of the magnitude.

(Key Words: Ovarian, Pituitary, Hormones, Progestogen-Treated Ewes.)

## INTRODUCTION

Intravaginal or oral administration of synthetic progestogen reduces, at the ensuing estrus, cervical mucus production (Smith and Allison, 1971), duration of estrus (Smith and Allison, 1971), sperm cell transport (Quinlivan and Robinson, 1969; Hawk and Conley, 1972), ovum fertilization rates (Moore *et al.*, 1967), and conception rates (Hunter, 1968). Because ovarian and pituitary hormones influence these reproductive processes, this study was conducted to determine the effect of progesterone or synthetic progestogenic compounds on ovarian and pituitary hormonal secretion patterns during and after withdrawal of the progestogen treatments.

## MATERIALS AND METHODS

Parous Rambouillet ewes were assigned to one of five treatment groups: 1) no treatment; 2) .3 mg/day of melengestrol acetate (MGA) orally, or 3) 60 mg/day of medroxyprogesterone acetate (MAP) orally (each compound administered in a soybean meal mix contained in a gelatin capsule; 4) a cylindrical sponge impregnated with 60 mg of MAP inserted into

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the anterior vagina; 5) 10 mg of progesterone daily, administered intramuscularly in 1 ml of corn oil. The untreated group contained seven ewes and each of the progestogen groups contained five. Treatments were initiated on Day 10 of an estrous cycle (estrus = Day 0) and continued for 14 days. On the final day of treatment, the progesterone and orally administered progestogens were given at half the usual dosage. Estrus was monitored twice daily for the entire duration of the study with vasectomized rams.

Twenty-five milliliter samples of jugular vein blood were collected daily by venipuncture from Day 10 (immediately before administration of the treatments) through Day 23. Beginning 18 hr after the last hormone administration (Day 24), blood samples were collected at 0100, 0700, 1300 and 1900 hr until 48 hr after the ewe was detected in standing estrus. In the untreated ewes, blood samples were collected daily from Day 10 through 13 of the estrous cycle and at 0100, 0700, 1300 and 1900 hr from Day 14 until 48 hr after the beginning of estrus. Blood samples were collected in heparinized tubes, refrigerated, centrifuged, and the plasma stored at  $-10^{\circ}\text{C}$  until assayed.

*Radioimmunoassay of Estradiol, Estrone and Progesterone.* The details of the procedures employed for extraction with diethyl ether, correction for procedural losses, and separation of progesterone, estradiol ( $\text{E}_2$ ) and estrone ( $\text{E}_1$ ) with column chromatography using Sephadex LH-20 are described by Echternkamp and Hansel (1973). Steroid assays were validated by the quantitation of known amounts of steroids added to distilled water or low steroid plasma. The amount of recovered steroid after correction for procedural losses was not different from amount added. Parallelism was established by determination of steroid concentration in 10, 25 and 50 ml aliquots of two plasma pools.

The estrogen was quantified with antiserum #126-2 that was developed in a ram against an estradiol-17-BSA antigen. The antibody exhibits a 90% cross reaction with  $\text{E}_1$ ; consequently,  $\text{E}_2$  and  $\text{E}_1$  were first separated and then quantified using appropriate standards.

One-tenth milliliter of estradiol- $17\beta$ - $6,7^3\text{H}$  (14,000 DPM), diluted in .1% gelatin, .1M  $\text{Na}_2\text{PO}_4$  pH 7.0 buffer (Buf A) was added to the assay tubes containing the  $\text{E}_2$  or  $\text{E}_1$  eluates or standards previously dried under  $\text{N}_2$  at  $40^{\circ}\text{C}$ . Curves were constructed from standards containing 0, 10, 20, 40, 80, 160, 320, or 480 pg

of  $\text{E}_2$  or  $\text{E}_1$ . The tubes were mixed and refrigerated overnight. The following morning, .1 ml of anti-estradiol serum, diluted 1:100,000 in Buf A was added to the assay tubes, agitated gently and incubated for 30 min at  $40^{\circ}\text{C}$  and then placed in a ice bath. After 90 min in the ice bath, 1.0 ml of dextran coated charcoal [.25% Norit A Charcoal, .025% dextran (37-43,000 M.W.) in Buf A] was pipetted into the tube, mixed gently, and allowed to stand for 10 min at  $4^{\circ}\text{C}$  followed by centrifugation at  $1,100 \times g$  for 10 minutes. The supernatant was decanted into a scintillation vial containing 10 ml of toluene scintillation fluid (4 g PPO, .05 g POPOP, 150 ml ethylene glycol monomethyl ether and 1 liter toluene).

Progesterone was determined by a radioimmunoassay procedure similar to that described by Thorneycroft and Stone (1972). The antibody (anti-progesterone- $6\beta$ , GDN #869) was used at a final dilution of 1:3000. The buffer system was the same as described for the estrogen assay. All progesterone values were corrected for procedural losses.

With the #869 antiserum, the activity of 13 other C-21 steroids relative to progesterone was less than 1% and pregnenolone ( $\Delta^5$ -pregnen- $3\beta$ -ol-20-one) was 3.4% (G. D. Niswender, *personal communication*). In this laboratory, the relative activity of  $\Delta^4$ -pregnen- $20\alpha$ -ol-3-one was also less than 1%, and pregnenolone was less than 5%.

The mean concentration obtained with duplicate determinations in 14 separate assays on two plasma pools was 4.9 and .4 ng/ml with an interassay coefficient of variation of 6.8 and 16.7%, respectively. The mean recovery of cold progesterone added to the plasma pools (2 ng/ml) was 95%. A blank value for buffer was usually not detectable (less than 95% of zero standard) and was never greater than the lowest standard, 40 picograms. The mean intra-assay coefficient of variation was 4.7% (100 unknown samples in three assays). The specificity of the antiserum was sufficient to permit the neutral steroid fraction (the first fraction obtained from the Sephadex LH-20 chromatographic step described for the estrogen assay) to be assayed without further chromatography. The specificity was determined by splitting the neutral steroid fractions from 15 animals and comparing the progesterone values obtained (after correction for procedural losses and sample dilution) by direct assay to values obtained after subjecting the fraction to a

second chromatographic step. In the latter case, the sample was placed on a Sephadex LH-20 column and eluted with iso-octane:benzene:methanol (90:5:5) to resolve progesterone from other neutral steroids (Carr *et al.*, 1971). There was no significant difference ( $P>.05$ ) between the progesterone values obtained by the two methods. Therefore, the second chromatographic step was eliminated.

**LH Radioimmunoassay.** Plasma LH concentrations were determined by use of the double antibody radioimmunoassay for ovine LH described by Niswender *et al.* (1969) with modification described by Lewis *et al.* (1974). A purified ovine LH preparation LER-1056-C2 was used for radioiodination. The LH standard was NIH-LH-S12.

**Prolactin Radioimmunoassay.** Plasma prolactin was determined by use of a double antibody radioimmunoassay similar to that reported by Davis *et al.* (1971). General procedures for iodination and radioimmunoassay were the same as described for LH. In brief, 5  $\mu\text{g}$  of ovine prolactin (NIH-P-S8) was reacted with 10  $\mu\text{g}$  of chloramine T and 1 mCi of  $\text{Na}^{125}\text{I}$  for 30 sec before the addition of 20  $\mu\text{g}$  sodium metabisulfite. The mixture was transferred to a  $.5 \times .2$  cm AG  $1 \times 10$  (Biorad) column to separate prolactin- $^{125}\text{I}$  from  $\text{Na}^{125}\text{I}$ . The prolactin- $^{125}\text{I}$  was then placed on a  $1 \times 30$  cm Sephadex G-100 column and eluted with .05 M  $\text{Na}_2\text{PO}_4$ , pH 7.5. The descending portion of the prolactin- $^{125}\text{I}$  peak was diluted with .1% BSA-PBS to about 50,000 cpm/.1 ml and stored at 4 C.

The antibody DJB 7-0330 was obtained from a rabbit immunized against NIH-P-S8. The antibody bound in excess of 90% of the prolactin- $^{125}\text{I}$  at a 1:300 dilution and 50 to 55% at the working dilution of 1:160,000. The non-specific assay background (counts bound with 1:300 normal rabbit serum, but no antibody) was less than 2% of total counts added.

The second antibody, DJB 5  $\times$  2, was used at a dilution of 1:80. Results are expressed relative to the prolactin standard NIH-S8. Other NIH hormones LH-S12, FSH-S5, GH-S8, or TSH-S5, did not exhibit cross-reactivity when tested at 500 ng/assay tube.

The mean concentration of prolactin (obtained with determinations made at three levels in 27 separate assays) for three plasma pools was 517, 80, and 45 ng/ml with an interassay coefficient of variations of 15, 12 and 13%, respectively. The NIH-P-S8 was quantitatively

recovered from .2 ml of plasma. Each unknown plasma sample was assayed at .025, .05, and .1 ml and the coefficient of variation of the mean was seldom greater than 20%, indicating parallel response of standard and plasma.

**Statistics.** The statistical procedures and standards of Rodbard *et al.* (1968) were used to evaluate the assays. Because the secretion of ovarian and pituitary hormones is affected by the reproductive status of the ewe, the effects of the progestogen treatments on plasma concentrations of ovarian and pituitary hormones were evaluated among treatment groups over the following time periods: 1) before CL regression, 2) during treatment after CL regression, 3) during the 48 hr immediately preceding preovulatory LH release (proestrus), 4) after the preovulatory LH release, 5) overall experiment. Of the parameters measured, the preovulatory LH release was considered to be the parameter in closest association with the onset of estrus (estrus began 0 to 3 hr before LH release).

Treatment effects on plasma hormone concentrations were evaluated for the above time periods by a least-squares analysis of variance (Harvey, 1960). Regression coefficients (response slope,  $b$ , from the equation  $y=a+bx$  where  $y$  is the hormone concentration in ng/ml or pg/ml and  $x$  is time in days) for each hormone were tested for homogeneity (Harvey, 1960) among treatments. Differences in response slopes within each hormone were compared among treatment groups within time periods using the  $t$  test (Steel and Torrie, 1960). Relationships among plasma hormone concentrations were determined by within animal, among animals, and within group correlations for all five time periods.

## RESULTS

**Progesterone Secretion and Regression of Corpora Lutea.** Plasma progesterone concentration declined consistently, in both untreated and progestogen-treated ewes, between Day 13 and 15 of the estrous cycle (compare plasma progesterone between Days 10 and 14 and 15 and 23, table 1). The decline in progesterone secretion occurred 3 to 5 days after initiation of the progestogen treatments (figure 1), and indicated that treatment with progestogen did not change the time of CL regression.

Blood samples were collected daily from progesterone-injected ewes just prior to the

TABLE 1. PLASMA HORMONE CONCENTRATIONS AND REGRESSION COEFFICIENTS (B) DURING AND AFTER PROGESTOGEN TREATMENT (MEAN  $\pm$  SE)

Time and treatment	No. of ewes	E <sub>2</sub>	E <sub>1</sub>	Prog.	LH	ProL.
		pg/ml	pg/ml	ng/ml	ng/ml	ng/ml
<i>Days 10 to 14 (before CL regression)</i>						
Untreated ewes	7	3.4 $\pm$ .2	1.1 $\pm$ .1	3.4 $\pm$ .5	1.0 $\pm$ .2	183.8 $\pm$ 28.6
Treated ewes	21	3.0 $\pm$ .1	.9 $\pm$ .1	3.0 $\pm$ .3	.9 $\pm$ .2	177.2 $\pm$ 12.7
<i>Days 15 to 23 (after CL regression)</i>						
MGA, oral	5					
Mean		5.3 $\pm$ .3	1.1 $\pm$ .1	.2 $\pm$ .1	1.8 $\pm$ .1	234.5 $\pm$ 27.8
B <sup>a</sup>		.23	-.04	-.05	.05	.28
MAP, oral	5					
Mean		3.7 $\pm$ .2	1.0 $\pm$ .1	.2 $\pm$ .1	1.6 $\pm$ .1	212.7 $\pm$ 22.2
B		.07	.00	-.05	.04	-8.70
MAP, sponge	5					
Mean		3.5 $\pm$ .2	1.0 $\pm$ .1	.4 $\pm$ .1	1.2 $\pm$ .1**	146.0 $\pm$ 18.6
B		-.22*	-.07	-.13	.01	-8.93
Prog. IM	6					
Mean		4.7 $\pm$ .2	1.0 $\pm$ .1	1.1 $\pm$ .2	1.5 $\pm$ .1	217.0 $\pm$ 32.7
B		.12	-.01	-.09	.04	-9.11
<i>-48 to -6 hr before LH release</i>						
Untreated	7					
Mean		9.6 $\pm$ 1.0 <sup>bc</sup>	1.9 $\pm$ .2	.2 $\pm$ .1	1.7 $\pm$ .1	98.9 $\pm$ 9.3
B		5.41**	.21	.09	.30	-29.61
MGA, oral	5					
Mean		12.8 $\pm$ .9 <sup>b</sup>	2.5 $\pm$ .2	.5 $\pm$ .4	2.7 $\pm$ .5	162.0 $\pm$ 39.9
B		1.82	-.06	-.02	1.68	-93.11
MAP, oral	5					
Mean		9.1 $\pm$ .6 <sup>bc</sup>	2.1 $\pm$ .1	.1 $\pm$ .0	2.3 $\pm$ .4	104.2 $\pm$ 23.8
B		-.31	.03	.03	1.07	36.43
MAP, sponge	5					
Mean		8.9 $\pm$ .7 <sup>bc</sup>	2.1 $\pm$ .2	.4 $\pm$ .4	2.2 $\pm$ .3	71.3 $\pm$ 7.8
B		1.44	-.44	-.85	.97	17.33
Prog. IM	6					
Mean		7.8 $\pm$ .4 <sup>c</sup>	1.9 $\pm$ .2	.7 $\pm$ .3	1.7 $\pm$ .1	111.1 $\pm$ 16.2
B		-.44	-.55	-.53	.29	-15.79
<i>0 hr (peak LH)</i>						
Untreated	7	7.9 $\pm$ .9	1.6 $\pm$ .1	.8 $\pm$ .6	52.4 $\pm$ 7.0	298.5 $\pm$ 102.2
MGA, oral	5	9.0 $\pm$ 1.5	3.5 $\pm$ .8	.1 $\pm$ .0	39.6 $\pm$ 5.8	359.9 $\pm$ 225.8
MAP, oral	5	10.3 $\pm$ 1.5	2.2 $\pm$ .6	.1 $\pm$ .0	69.6 $\pm$ 7.6	217.3 $\pm$ 119.3
MAP, sponge	5	10.7 $\pm$ 2.1	1.5 $\pm$ .2	.1 $\pm$ .0	50.4 $\pm$ 12.4	267.3 $\pm$ 175.8
Prog. IM	6	9.6 $\pm$ 1.4	1.9 $\pm$ .2	.1 $\pm$ .0	61.5 $\pm$ 8.5	186.1 $\pm$ 80.8
<i>+6 to +48 hr after LH released<sup>d</sup></i>						
Untreated	7					
Mean		5.5 $\pm$ .5	1.4 $\pm$ .2	.1 $\pm$ .1	1.8 $\pm$ .2	80.2 $\pm$ 14.3
B		-.97	-.25	.04	.03	-35.02
MGA, oral	5					
Mean		6.4 $\pm$ .7	1.7 $\pm$ .3	.3 $\pm$ .2	1.6 $\pm$ .2	47.5 $\pm$ 12.6
B		.36	0	.02	.12	-26.88
MAP, oral	5					
Mean		7.1 $\pm$ .7	2.1 $\pm$ .5	.1 $\pm$ .0	1.5 $\pm$ .1	80.4 $\pm$ 11.0
B		-2.14	-.09	.26	.21	-17.44
MAP, sponge	5					
Mean		6.9 $\pm$ .6	2.2 $\pm$ .2	.1 $\pm$ .0	1.3 $\pm$ .1	73.4 $\pm$ 16.6
B		-1.73	.40	.04	.16	-50.61
Prog. IM	6					
Mean		6.9 $\pm$ .9	2.2 $\pm$ .4	.2 $\pm$ .0	1.7 $\pm$ .2	54.6 $\pm$ 7.0
B		.43	.46	-.1	.30	-13.85

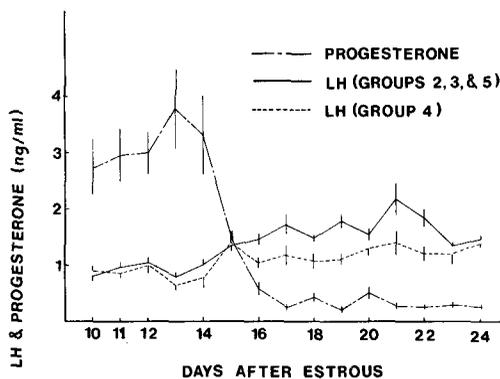


Figure 1. Plasma LH concentrations for ewes treated from Day 10 through 23 after estrus with MGA orally (Group 2), MAP orally (Group 3), and progesterone intramuscularly (Group 5) vs MAP sponge intravaginally (Group 4). Plasma progesterone concentrations were combined for the four progesterone groups.

intramuscular administration of 10 mg of progesterone. Therefore, the plasma progesterone concentration of approximately 1 ng/ml after CL regression (Group 5, table 1) represents the minimal circulating progesterone concentration in the progesterone-treated ewes. Plasma progesterone was less than .5 ng/ml following CL regression in the other groups of progestogen-treated ewes, indicating that the synthetic progestogen did not interfere with the progesterone assay. The mean plasma progesterone concentration for the 48-hr period preceding the LH peak averaged less than .3 ng/ml for all ewes (table 1).

**Estrogen Secretion.** Plasma  $E_2$  concentrations for ewes in each of the progestogen treatment groups increased after CL regression (table 1). The  $E_2$  concentrations for Groups 2, 3, and 5 remained elevated for the duration of the progestogen treatment. However, by about Day 18, the  $E_2$  concentrations for Group 4 (MAP sponge ewes) had returned to the mean concentration of Days 10 to 14 (figure 1), resulting in the regression coefficient for  $E_2$  concentration for Group 4 from Days 15

through 23 being significantly different ( $P < .05$ ) from the regression coefficients for each of the other three progestogen groups (table 1).

The  $E_2$  concentrations for progestogen-treated ewes increased further after withdrawal of the progestogen ( $P < .01$  between mean  $E_2$  concentration of 4.3 pg/ml for Days 15 to 23 and 9.6 pg/ml for the -48- to -6-hr period). The MGA-treated ewes had higher overall  $E_2$  concentration in comparison to the other progestogen treatment groups both during the 48-hr period preceding the LH peak and during the last 9 days of treatment (after CL regression). Plasma  $E_2$  concentrations for the MGA-treated ewes during proestrus (-48- to -6 hr) were significantly ( $P < .05$ ) higher than for the progesterone-treated ewes (table 1).

Proestrus (-48- to -6 hr)  $E_2$  secretion patterns also differed ( $P < .01$ ) between untreated ewes and those in progestogen treatment groups, as indicated by the regression coefficient for  $E_2$  concentration over the 2-day period of 5.4 for the untreated and 1.8 to -.4 for the progestogen groups (table 1). In general, the mean  $E_2$  concentrations for the progestogen-treated ewes increased sharply after progestogen withdrawal, with little further increase during the 48-hr proestrus period. In contrast, the mean  $E_2$  concentrations for the untreated ewes increased from 5.8 pg/ml plasma at -48 hr to 22.0 pg/ml at -6 hr before LH release, as reflected in the sharp response slope. Post-estrus  $E_2$  concentrations (+6- to +48 hr, table 1) were not affected by progestogen treatment.

Plasma  $E_1$  concentrations ranged between 1 and 3 pg/ml and were not affected by the progestogen treatments (table 1). Secretion of  $E_1$  paralleled  $E_2$ , as indicated by a significant ( $P < .01$ ) within animal correlation between  $E_1$  and  $E_2$  (table 2), but the  $E_1$  concentrations were about one-third of the magnitude for  $E_2$ .

The increase in  $E_2$  after the reduction in plasma progesterone concentration resulted in an overall significant ( $P < .01$ ) negative correlation between  $E_2$  and progesterone (table 2).

**LH.** The administration of progesterone or

Footnotes for table 1:

\* $P < .05$  and \*\* $P < .01$  denote significance for mean or regression coefficient comparisons within the same time period.

<sup>a</sup>Regression coefficients are the change in plasma hormone concentration with time (days).

<sup>b,c</sup>Means with different superscripts are significantly ( $P < .05$ ) different.

<sup>d</sup>The means for LH include data from +12 through +48 hr. The LH concentration averaged 10.2 ng/ml at 6 hr, but had decreased to 1.9 ng/ml at 12 hours.

TABLE 2. WITHIN ANIMAL CORRELATIONS OF PLASMA HORMONE CONCENTRATIONS FOR DAYS 15 TO 23, PROESTRUS AND OVERALL EXPERIMENT<sup>a</sup>

Hormone	LH	Prol.	E <sub>1</sub>	E <sub>2</sub>
<u>I. Days 15 to 23</u>				
Prol	.217*			
E <sub>1</sub>	-.039	.036		
E <sub>2</sub>	-.024	.020	.392**	
Prog	-.160	-.029	.432**	-.070
<u>II. -48 to -6 hr before LH</u>				
Prol	-.045			
E <sub>1</sub>	-.244	.095		
E <sub>2</sub>	.015	.015	-.085	
Prog.	-.045	-.045	.340	-.178
<u>III. Overall</u>				
Prol	.396**			
E <sub>1</sub>	.100	-.028		
E <sub>2</sub>	.121	.034	.265**	
Prog	-.090	-.010	-.056	-.241**

\*Correlations significant at  $P < .05$

\*\*Correlations significant at  $P < .01$ .

synthetic progestogens from Day 10 to 24 delayed the preovulatory release of LH after regression of the corpus luteum (Days 15 to 23, table 1). However, there was a significant ( $P < .01$ ) linear increase in basal LH concentration between Days 15 and 23 (figure 1) except for the ewes with MAP sponges (Group 4). The slow increase was followed by the preovulatory LH surge (0 hr, table 1) after withdrawal of the progestogen treatment. The increases in LH and E<sub>2</sub> after CL regression were not simultaneous, as indicated by the nonsignificant correlation between E<sub>2</sub> and LH (table 2).

The interval from the last progestogen administration to the LH surge averaged 73.8 hr for all ewes. The treatment group means  $\pm$  S.E. were as follows: MGA, oral  $75.6 \pm 6.5$  hr; MAP oral,  $57.6 \pm 4.5$ ; MAP sponge,  $81.6 \pm 9.8$  and progesterone I.M.,  $63.0 \pm 3.7$ . Mean values were not significantly different between groups. These time intervals fall within the 2 to 5 day range reported for ewes by Hunter (1968).

The estrous cycle length for the untreated ewes ranged from 16 to 20 days. The mean was 17.9 days, which was about a day longer than the mean length of the previous cycles for the same ewes. The stress of frequent blood sam-

pling may have lengthened the interval from progestogen withdrawal to LH release, as Smith and Robinson (1970) observed a delay of estrus in ewes laparotomized before the onset of estrus.

Proestrous (-48- to -6 hr) and post-estrous (+6- to +48 hr) plasma LH concentrations did not differ significantly among the untreated and treatment groups. The magnitude of the preovulatory LH peak (0 hr, table 1) did not differ significantly among groups, but blood was not collected frequently enough to ascertain definitely that progestogen treatment had no effect on the LH peak.

*Prolactin.* The prolactin concentrations (table 1) were highly variable and were not significantly affected by treatment or by the reproductive status of the ewe. Stress resulting from venipuncture may have accounted for the variability and magnitude of the prolactin concentrations (Bryant and Greenwood, 1968).

The within-animal correlation between prolactin and LH (table 2) was significant for Days 15 through 23 ( $P < .05$ ) and for the overall experiment ( $P < .01$ ). The positive relationship between LH and prolactin was reflected in lower plasma LH and prolactin concentrations for MAP sponge-treated ewes between Days 15 and 23, and the consistent increase in both LH and prolactin at estrus within all five groups.

### Discussion

The changes in peripheral concentrations of E<sub>2</sub> observed for the untreated ewes in

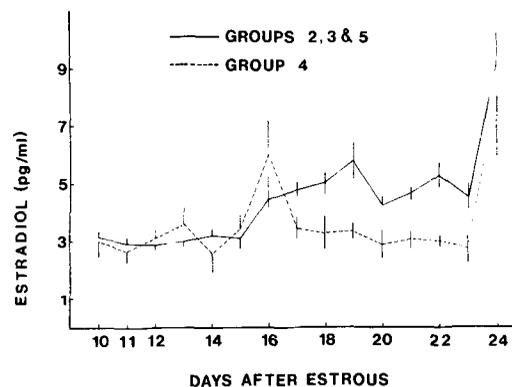


Figure 2. Plasma estradiol concentrations for ewes treated from Day 10 through 23 after estrus with MAG orally (Group 2), MAP orally (Group 3) and progesterone intramuscularly (Group 5) vs MAP sponge intravaginally (Group 4).

this study agree with previously reported ovarian  $E_2$  secretion patterns (Moore *et al.*, 1969; Smith and Robinson, 1970; Cox *et al.*, 1971). The elevated proestrous plasma  $E_2$  concentration presumably mediated the preovulatory LH surge with withdrawal of the progestogen treatment or CL regression in the untreated ewes, since estradiol-17 $\beta$  induced LH release in anestrus ewes (Goding *et al.*, 1969).

Plasma  $E_1$  concentrations tended to parallel those for  $E_2$  ( $P < .01$ , table 2). The magnitude of the  $E_1$  concentrations were approximately one-third of the  $E_2$  concentrations, which confirmed previous reports that  $E_2$  is the predominant estrogen secreted during the follicular phase of the ovine estrous cycle (Moore *et al.*, 1969; Smith and Robinson, 1970).

The treatment of cyclic ewes with progesterone or synthetic progestogen at the dosages used in this study did not prevent an increase in  $E_2$  secretion, presumably of follicular origin, after regression of the CL and while the ewes were still on progestogen treatment (Days 15-23). However, the circulating  $E_2$  concentration during these days did not reach the proestrous concentration. Zimbelman (1963) reported that both the size of follicles and percent of heifers with one or more follicles increased when the MAP treatment continued past the time of CL regression.

Basal LH concentrations also increased in the progestogen-treated ewes after CL regression, but not to the concentration required to induce ovulation or follicular luteinization. Either the elevated  $E_2$  concentrations were not high enough to induce a preovulatory LH surge or the progestogen dosages were adequate to block LH release. Since the administration of progesterone with estradiol will block estradiol mediated LH release (Scaramuzzi *et al.*, 1971), it can be assumed that regression of the CL in the control ewes removed the negative feedback effect of progesterone on pituitary and/or ovarian function but that the exogenous progestogen suppressed a potential LH surge. The lower LH ( $P < .01$ ) and  $E_2$  concentrations for the MAP sponge-treated ewes suggest that this continuous progestogen treatment was more effective in suppressing ovarian and pituitary activity than the daily treatments administered in the other treatment groups.

The lack of a significant correlation between LH and  $E_2$  for Days 15 through 23 indicates that the two hormones did not increase simultaneously; however, the lower  $E_2$  and LH

( $P < .01$ ) concentration for the MAP sponge group (Group 4) still suggest that the  $E_2$  and LH concentration are related. A reduction in the negative feedback of progesterone on the pituitary at Day 13 to 15 probably stimulated LH secretion, which in turn stimulates ovarian  $E_2$  production; however, the possibility of a negative progesterone feedback on ovarian function or receptivity to FSH and LH must be considered.

A comparison of uterine contractions during estrus in untreated ewes and during the ensuing estrus in progesterone-treated ewes indicated that each of the four progestogen treatments used in the present study reduced the number of contractions moving toward the oviducts and increased the number moving toward the cervix (Hawk and Echterkamp, 1973). Near and soon after the end of estrus the direction of uterine contractions in untreated ewe changes, apparently due to declining estrogen secretion, from predominantly toward the oviducts to predominantly toward the cervix (Hawk, 1975). In the present study, comparison of plasma  $E_2$  concentrations before and after the LH surge did not show a difference between the untreated and progestogen groups and no indication of a premature decline in estrogen for the treated ewes. However, the progestogen treatment continued for 8 days after corpus luteum regression, with elevated  $E_2$  and low progesterone concentrations; the prolonged exposure of the uterus to estradiol in the progestogen-treated ewes may have desensitized the uterus to estradiol. Also, plasma  $E_2$  concentrations for the untreated ewes increased sharply throughout the 48-hr (table 1) proestrous period, whereas in the progestogen-treated ewes the  $E_2$  concentration remained fairly constant for 36 hr before the LH surge. The increasing  $E_2$  concentrations throughout the proestrous period in the controls may have been more effective in maintaining movement of uterine contractions toward the oviducts at estrus.

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