

INFLUENCE OF BREED AND SEASON ON OVARIAN AND PITUITARY RESPONSE IN PROGESTOGEN-PMSG-TREATED EWES

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ABSTRACT

Breed and seasonal effects on LH release, ovarian steroid secretion, and ovulation were evaluated in mature Finnish Landrace (Finn) and Hampshire ewes that received either a progestogen-PMSG treatment in May, July and November (experiment 1) or estradiol-17 β (50 μ g) in May and July (experiment 2). The progestogen-PMSG treatment increased plasma estradiol within 12 hr after the PMSG injection at all three treatment periods and resulted in plasma LH and estradiol profiles similar to those during proestrus in cyclic ewes. Season, but not breed, affected the time from PMSG injection to preovulatory LH surge (56.5 \pm 1.4 hr in November vs 77.1 \pm 3.4 hr in July). Ovulation rate was higher in Finn than Hampshire ewes except in July when it decreased in Finn ewes. Magnitude of the estradiol-mediated LH release was decreased in July in Finn but not Hampshire ewes. Seasonal effects on reproduction in progestogen-PMSG treated ewes appear to be mediated through pituitary gonadotropin secretion with breed differences as to time and/or intensity of the seasonal effect(s).

INTRODUCTION

With few exceptions, the ewe is considered to be a seasonal breeder, exhibiting estrous cycles during the Fall and Winter months. Since the gestation length for the ewe is approximately 150 days, it is conceivable that the ewe could give birth to offspring twice a year if fertile reproductive cycles were initiated during the seasonal anestrus period.

Treatment of both seasonally anestrus (out-of-season mated) and naturally cyclic ewes with a flurogestone acetate (20 mg) impregnated, intravaginal pessary for nine days followed by 20 mg of progesterone and 500 IU of PMSG intramuscularly at pessary removal induced spontaneous ovulation in all of the ewes, but the percentage of ewes conceiving was 76% for out-of-season mated ewes and 100% for cyclic ewes (1). Other researchers, using a variation of the described progestogen-PMSG treatment, have reported conception rates for out-of-season mated ewes that range from 22% to 70%, depending upon the treatment regime, stage of the anestrus period when the treatment was initiated, seasonal changes in the fertility of the rams, breed of sheep or a combination of these factors (2,3,4). As to whether the lower fertility in out-of-season mated ewes is the result of a seasonal influence on the responses of the ovaries and/or pituitary to the progestogen-PMSG treatment, a failure of the treatment to mimic or initiate the endocrine

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events of the natural estrous cycle, or a combination of these two factors remains to be determined. In addition, inherent differences exist between breeds of sheep relative to ovulation rate and timing of the natural breeding season; therefore, suggesting the need for an evaluation of breed and seasonal differences in ovarian and pituitary response between a high prolificacy breed (Finn) and a lower prolificacy breed (Hampshire). The objectives of this study were to 1) evaluate breed and seasonal effects on ovarian and pituitary endocrine responses and 2) to compare endocrine responses associated with ovulation between progestogen-PMSG treated anestrous ewes and naturally cyclic ewes.

MATERIALS AND METHODS

Experiment 1. Five Finn and five Hampshire mature ewes were treated for 9 days with a progestinated intravaginal pessary, containing 20 mg of flurogestone acetate (Synchro-mate, G. D. Searle and Co., Skokie, Illinois), followed by an intramuscular injection of 20 mg of progesterone in corn oil and 500 IU of PMSG in sterile saline at pessary removal (1). The injection of 20 mg progesterone was incorporated into the progestogen-PMSG treatment in the previous fertility study to delay the onset of estrus after pessary removal to provide additional time for removal of debris from the vagina before insemination. Since the objective of the present study was to compare endocrine responses of the ovaries and pituitary during the progestogen-PMSG regulated estrous cycle to those of the natural estrous cycle, the same treatment protocol was employed in experiment 1 even though the ewes were not bred. To evaluate the influence of season (anestrus vs cyclic) on endocrine responses of the ovaries and pituitary, the progestogen-PMSG treatment was administered to the same ten ewes during three seasonal periods; early seasonal anestrus (initiated on April 29), mid seasonal anestrus (July 8) and natural breeding season (November 1). Pessaries were inserted into the cyclic ewes in November on Day 4 of the estrous cycle and removed on Day 13 before or at the time of the demise of the corpus luteum because it had been reported that regression of the corpus luteum during progestogen treatment stimulated LH and estradiol secretion (5). Two additional Finn and Hampshire ewes were added to the experiment in July; however, one Hampshire ewe became ill in November and was removed from the experiment. A natural estrous cycle that occurred in October provided control data on the same 14 ewes for comparison with the progestogen-PMSG treated groups. All of the ewes were laparotomized prior to the April and July treatments and were determined to be anestrus because of the absence of corpora lutea and preovulatory follicles. Additional laparotomies were performed 8 to 10 days after observed or estimated estrus subsequent to the three treatment periods and the control estrus to determine ovulation rate based on number of corpora lutea present. The ewes received a corn silage diet ad libitum for the duration of the study.

Jugular vein blood (20 ml) was collected by venipuncture at 6-hr intervals for six days beginning immediately prior to the PMSG injection, then daily for an additional 10 days. During the control estrous cycle, blood samples (20 ml) were collected daily Day 10 through 13, at 6-hr intervals Day 14 through 2, and then daily Day 3 through 8 of the subsequent cycle. Blood was collected into heparinized syringes, refrig-

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erated and centrifuged, and plasma stored at -10 C until assayed. All of the plasma samples were assayed for LH, but only samples collected at 0800 and 2000 hr and daily samples were assayed for steroids.

Plasma LH concentrations were determined by a double antibody radioimmunoassay (6) and are expressed as ng of NIH-LH-S18/ml of peripheral plasma. The LH antibody (DJB 3-12/11) bound in excess of 90% of the LH-¹²⁵I (LER-1056-C2) at a 1:300 dilution and 45 to 50% at the working dilution of 1:35,000. The ovine anti-rabbit γ -globulin was used at a dilution of 1:120. Estradiol and progesterone were extracted from 10 ml of plasma with diethyl ether, separated on Sephadex LH-20 columns and quantified by radioimmunoassay (5,7).

Experiment 2. Data from experiment 1 indicated that the time required for the induction of LH surges was longer in July than May. Also, a preovulatory LH surge and subsequent corpora lutea were not detected in one Finn ewe after the July treatment period but plasma estradiol concentrations were similar to those for ewes that ovulated. Thus, seven Finn and seven Hampshire mature ewes were given intramuscular injections of 50 μ g estradiol-17 β in corn oil on May 11 (early anestrus) and again on July 12 (mid anestrus) to evaluate the influence of stage of anestrus on estradiol-mediated LH release in the two breeds. Plasma LH concentrations were determined for jugular vein blood samples (6 ml) collected by venipuncture at 0, 9, 12, 15, 18, 21, 24, 27, 30 and 33 hr after the estradiol injection. Blood samples were handled and assayed for LH as in experiment 1.

Assays were evaluated and hormone concentrations were determined by reported procedures (8). The intra-assay coefficient of variation for duplicates and the inter-assay coefficients of variation for two plasma pools included in each assay did not exceed 10% for either the LH, estradiol or progesterone radioimmunoassays. An LH surge was defined as an increase in plasma LH concentration greater than two standard deviations above the mean concentration for 0 through 24 hr after PMSG injection (experiment 1) or for 0, 27, 30 and 33 hr after estradiol-17 β (experiment 2). A split-plot analysis of variance was used to determine breed and seasonal effects and their interaction on the measured parameters. The error mean square to test breed effect was animal within breed. Animal within season x breed was used as the error mean square to test the effects of season and season x breed. Simple correlations were calculated to assess the relationship between preovulatory estradiol concentration and subsequent ovulation rate (9).

RESULTS

Experiment 1. Plasma estradiol concentration was significantly increased within 12 hr after the PMSG injection (figure 1), remained elevated until an LH surge occurred about 3 days later, then dropped precipitously. Plasma LH concentration (figure 2) was low at 0 hr after PMSG injection at the May and July treatment period, but increased to the baseline for the November treatment period within 6 hr after the PMSG injection. The systemic profiles for plasma LH and estradiol during proestrus and estrus were similar between the treated anestrus ewes that ovulated and the cyclic control ewes. The increase in plasma progesterone (figure 1), which followed the injection of progesterone at the time of pessary removal, did not appear to inhibit the stimula-

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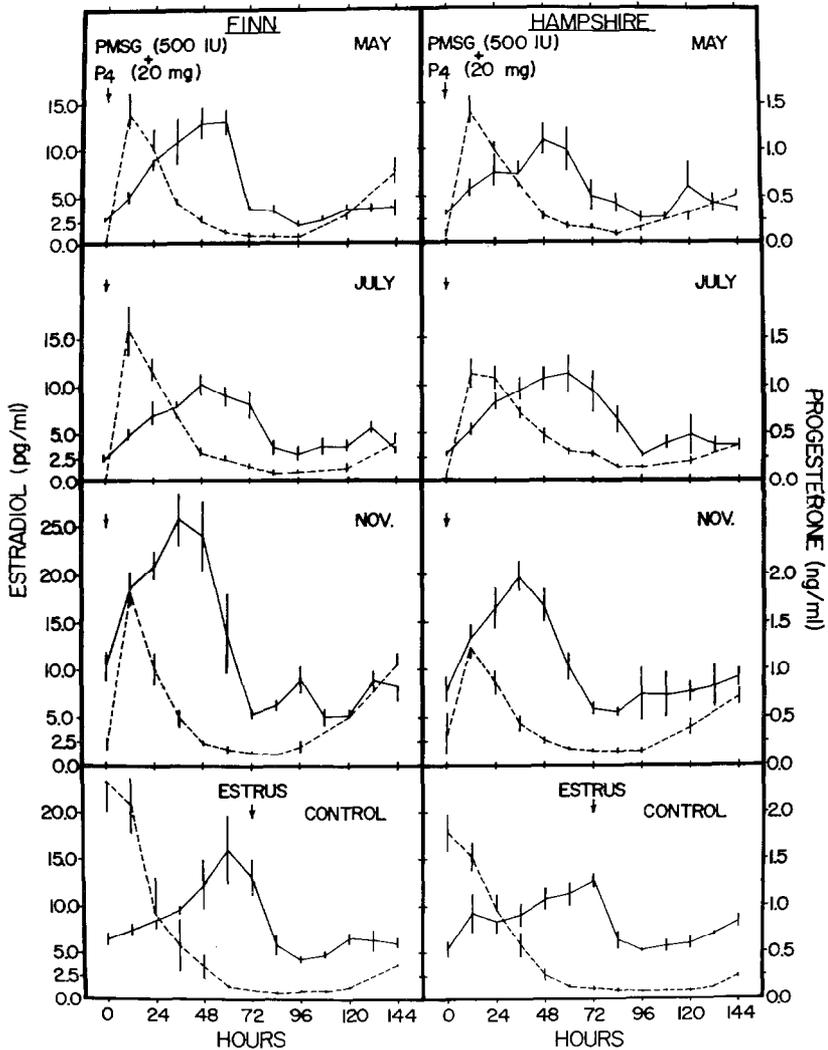


Figure 1. Plasma estradiol (E_2) (solid line) and progesterone (P_4) (broken line) concentrations (mean \pm SE) during proestrus and estrus for Finn and Hampshire ewes ($n = 5$ to 7) that received progestogen-PMSG treatments in May, July and November (Nov.) and no treatment (control) in October.

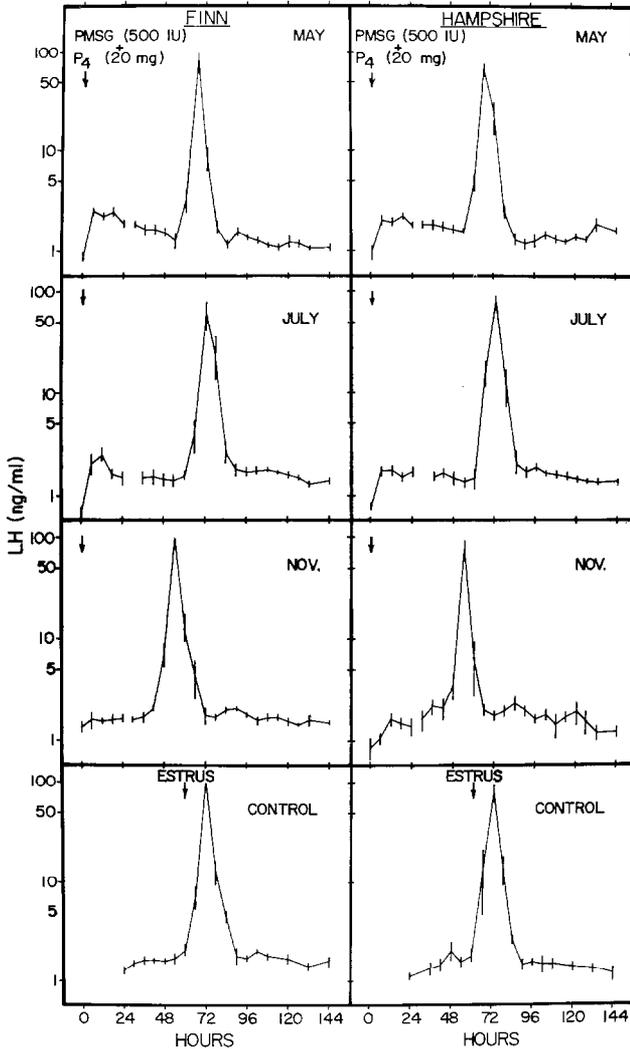


Figure 2. Plasma LH concentrations (mean + SE) during proestrus and estrus for Finn and Hampshire ewes (n = 5 to 7) that received progestogen-PMSG treatments in May, July and November (Nov.) and no treatment (control) in October. Plasma LH values were normalized to PMSG injection except for the periods of LH surges when LH was normalized to the LH peak.

TABLE 1. Breed and seasonal effects on plasma LH and estradiol concentrations and ovulation rate in control and progestagen-PMSG treated Finn and Hampshire ewes^a.

Time	Interval from PMS to LH surge (hr)	Peak estradiol concentration		(pg/ml)	Ovulation rate		
		Finn	Hampshire		Finn	Hampshire	
May	64.8±2.2 ^c	14.8±1.9 ^{d,e(5)}	12.8±1.7 ^{d(5)}	2.0	3.4±.5 ^c	2.2±.2	1.2*
July	77.1±3.4 ^d	11.2±1.0 ^e	13.9±1.3 ^{d(7)}	2.7	2.0±.6 ^{d,f}	1.6±.2	.4
November	56.5±1.4 ^e	28.7±2.9 ^c	20.5±1.4 ^{c(6)}	8.2**	3.0±.4 ^{c,g}	2.0±.3	1.0*
Control ^b	--	17.8±2.8 ^d	13.3±.8 ^{d(7)}	4.5*	2.8±.4 ^{c,d}	1.6±.2	1.2**

^a Number of animals composing a mean are in parenthesis. All three parameters were measured in the same ewes.

^b Control group consists of untreated cyclic ewes sampled from Day 14 through Day 5 (estrus = Day 0) in October.

^{c,d,e,f,g} Mean differences within a column are indicated by different superscripts (c,d,e,P<.01; f,g, P<.05).

* P<.05

** P<.01

tion of estrogen and LH secretion by PMSG. The synthetic progestogen contained in the pessary was not detected by the progesterone assay; therefore, its rate of disappearance and effect on the system after pessary removal could not be evaluated.

Breed and seasonal comparisons for interval from PMSG injection to preovulatory LH surge, peak preovulatory plasma estradiol concentration, and ovulation rate are reported in table 1. Season, but not breed, significantly ($P < .01$) affected the length of the interval from the PMSG injection to the preovulatory LH surge. The shortest interval was in November (56.5 ± 1.4 hr) and the longest interval was in July (77.1 ± 3.4 hr). Ovulation rate (table 1) was significantly higher for Finn than Hampshire ewes (May and November, $P < .05$; control $P < .01$) except in July when ovulation rate was decreased ($P < .05$) for Finn ewes. Finn ewes had higher peak preovulatory plasma estradiol concentrations (table 1) than Hampshire ewes at the control period (17.8 ± 2.8 vs 13.3 ± 1.8 pg/ml, $P < .05$) and at the November treatment period (28.7 ± 2.9 vs 20.5 ± 1.4 pg/ml, $P < .01$), but not at the May and July treatment periods. The positive within treatment correlations between preovulatory estradiol concentration and subsequent ovulation rate were significant ($P < .01$) in Finn ewes for the November ($r = .84$) and the control period ($r = .85$), but were not significant for the May ($r = .16$) or July period ($r = .12$). Preovulatory estradiol concentration and ovulation rate were not significantly ($P > .05$) correlated in Hampshire ewes at the four measured periods. Peak preovulatory estradiol concentration was significantly ($P < .01$) greater in both breeds at the November period than at the control period or at the May or July treatment periods. However, ovulation rate was not increased ($P > .05$) compared to the other three periods. One Finn ewe failed to ovulate subsequent to the July treatment. The plasma estradiol pattern for this ewe was similar to those for the other ewes in the group, but no preovulatory LH surge was detected.

Experiment 2. Fifty μ g of estradiol-17 β produced an LH surge in all seven Hampshire ewes at both the May and July treatment periods (table 2). All seven Finn ewes also had an LH surge at the May treatment. However, the incidence of LH surges tended to be reduced at the July treatment as only four of seven ewes had an LH surge greater than two times the basal concentration. Profiles of the estradiol-mediated LH release in three of the Finn ewes are illustrated in figure 3. Maximum LH concentration, area under the LH peak, and interval from estradiol injection to LH surge (table 2) were similar for the two breeds in May; whereas in July, Finn ewes had a significantly ($P < .05$) longer interval between estradiol injection and LH surge, and significantly ($P < .05$) lower maximum LH concentrations and areas under the LH peak than Hampshire ewes. Stage of anestrus affected maximum LH concentration (table 2) inversely in the two breeds of sheep as indicated by significant ($P < .05$) breed x stage of anestrus interaction. Hampshire ewes had higher ($P < .05$) LH concentrations and Finn ewes had lower ($P < .05$) LH concentrations at the July than at the May treatment period.

DISCUSSION

Data from the present study suggest seasonal differences in LH secretion among ewes subjected to a progestogen-PMSG treatment in May, July and November, or to 50 μ g of estradiol-17 β in May and July. The

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TABLE 2. Effects of breed and season on estradiol-17 β (50 μ g)-mediated LH release in ewes.

Breed and stage of anestrus	No. ewes	Interval from E ₂ to LH peak (hr)	Max. LH conc. (ng/ml)	Area of the peak (cm ²)
Finn				
Early(May)	7 ^a	17.6 \pm .8	88.1 \pm 16.9 ^b	53.3 \pm 8.6 ^b
Mid(July)	6 ^a	21.0 \pm 1.3*	36.0 \pm 19.0 ^c	22.9 \pm 11.1 ^c
Hampshire				
Early(May)	7	16.3 \pm .9	70.7 \pm 12.7 ^b	43.0 \pm 7.7 ^b
Mid(July)	7	15.9 \pm .6	122.2 \pm 11.5 ^b	52.1 \pm 6.5 ^b

^aAn LH release was not detected in one Finn ewe.

^{b,c}Mean differences (P<.05) within a column are denoted by different superscripts. Means \pm SE.

* P<.05

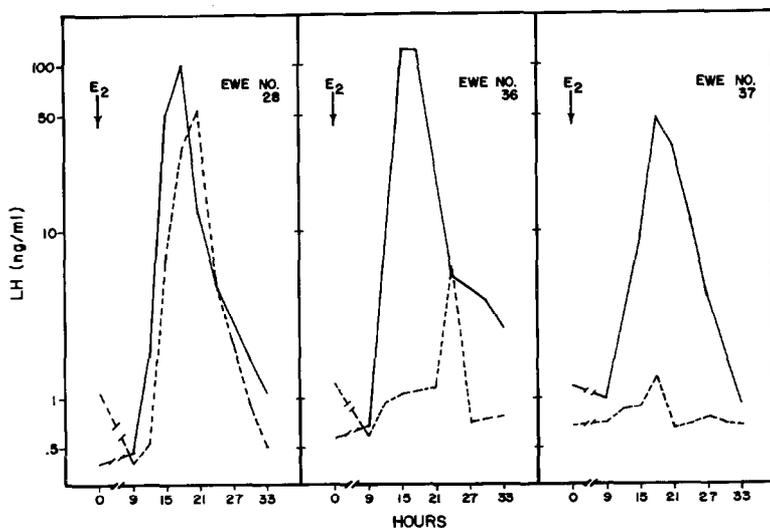


Figure 3. Plasma LH concentrations for three Finn ewes given 50 μ g estradiol-17 β (0 hours) in May (solid line) and July (broken line).

seasonal difference between May and July in LH secretion was more pronounced in Finn than Hampshire ewes as noted by the reduction in magnitude, or absence, of the estradiol-mediated LH release (experiment 2) and by the absence of a preovulatory LH surge in a PMSG-treated Finn ewe in July (experiment 1). However, the preovulatory LH surge was delayed in both breeds during July in experiment 1, as measured by the interval from PMSG injection to LH surge, which suggested a seasonal effect on LH secretion for both breeds in that experiment. The decreased LH response to estradiol in the Finn ewes in July is consistent with results for ovariectomized Finn ewes (10), whereas the lack of a stage of anestrus effect on estradiol-mediated LH release in the Hampshire ewes is consistent with results for intact Columbia ewes (11). Results from previous studies that evaluated seasonal effects on the positive estradiol feedback on LH release are inconsistent as noted by observations that the magnitude of the LH release and the time interval from estradiol treatment to peak LH concentration were unaffected by season (11), that magnitude of the LH release was unaffected but the time interval was longer in anestrus ewes (12), or that magnitude of the release was decreased in anestrus ewes but the time interval was unaffected (13). Because these studies were conducted at different geographical locations in either ovariectomized or intact ewes of different breeds it is difficult to comparatively evaluate the findings of these studies.

The results from experiment 2 indicate a season (stage of anestrus) by breed interaction on the magnitude of the estradiol-mediated LH release. The biological significance of the difference in maximum LH concentrations between May and July for the estradiol-treated Hampshire ewes can not be ascertained. However, the decreased LH secretion in Finn ewes in July may represent decreased positive estrogen feedback on gonadotropin secretion and suggest possible breed differences in magnitude or timing of the seasonal effect on LH secretion. The transition from seasonal anestrus to the natural breeding season occurs 20 to 30 days later in Finn than Hampshire ewes which may contribute to the observed differences in pituitary and ovarian response between the two breeds in July. Clarification of the discrepancy in positive estrogen feedback between Finn and Hampshire ewes in this study and those previously reported (10,11,12,13) may require a more frequent evaluation of LH secretion within the seasonal anestrus period and the breeding season in breeds of sheep with different reproductive patterns. It is unlikely that the decrease in positive estradiol feedback on LH release in July is the result of decreased pituitary LH content as it has been reported that pituitary LH content and release to synthetic LH-RH is the same for anestrus and cyclic ewes (14). Seasonal variation in gonadotropin secretion has also been observed in rams (15,16) and has been experimentally duplicated by increasing and decreasing photoperiod length (17,18). However, ewes subjected to a photostimulation regime of artificial daylength still showed an innate natural seasonal effect on the onset of behavioral estrus after pessary withdrawal (19). Whether seasonal differences in LH secretion for ewes are associated with change in photoperiod length or some other inherent seasonal change(s) can not be deduced from the present study.

The progestogen-PMSG treatment produced significantly ($P < .01$) higher preovulatory plasma estradiol concentrations and a shorter interval

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from PMSG injection to LH surge subsequent to the November treatment period than at the control period, or at the May and July treatment periods. The PMSG was injected in November during the proestrous phase of the estrous cycle; therefore, the enhanced hormonal response may have resulted because of a synergistic action on the ovaries of a simultaneous endogenous and exogenous gonadotropin release. The higher plasma estradiol concentrations were not associated with an increase in ovulation rate. Therefore, the increased preovulatory estradiol concentrations must have resulted from increased stimulation of existing preovulatory follicles or the stimulation of estradiol secretion by nonovulatory follicles.

Finn ewes had a significantly higher ovulation rate than Hampshire ewes at the May and November treatment periods ($P < .05$) and at the control period in October ($P < .01$), but not at the July treatment period ($P > .1$). The seasonal variation in ovulation rate for the Finn ewes is consistent with reported seasonal variation in ovulation rate for PMSG-treated Merino ewes (20), and with observed breed and seasonal differences in ovulation rate and lambing rate for progestogen-PMSG-treated ewes (3,4). Blood samples were collected too infrequently in experiment 1 (6-hr intervals) to determine whether ovulation rate was directly associated with magnitude of the preovulatory LH surge in Finn ewes, but the difference in magnitude of the estradiol-mediated LH release between May and July (experiment 2) could be interpreted as indirect evidence to suggest that the lower ovulation rate in July resulted from decreased LH release and justifies further evaluation of such a relationship.

In experiment 1, ewes were injected with 20 mg of progesterone at pessary removal. Plasma estradiol concentration increased in the presence of elevated progesterone concentrations. Therefore, it appears that any inhibitory effects of progesterone on estrogen secretion are mediated through gonadotropin secretion (5) rather than a direct inhibitory effect on estrogen secretion by the ovaries.

With the exception of one Finn ewe in July, the progestogen-PMSG treatment initiated the endocrine responses required for ovulation and corpus luteum development in seasonally anestrous ewes. Seasonal differences in length of the interval from PMSG injection to the preovulatory LH surge, and decreased ovarian and pituitary response in Finn ewes in July could contribute to previously observed breed and seasonal influences on fertility in progestogen-PMSG-treated ewes (4) and account for the variation among experiments reported in the literature (1,2,3,4). The observed differences in ovarian and pituitary response between Finn and Hampshire ewes suggest possible breed differences in timing and/or intensity of the seasonal anestrous period.

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