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INDUCTION OF ESTRUS AND FERTILITY IN ANESTROUS EWES[1]

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Several potential methods of overcoming the seasonality of reproduction in sheep are available: (1) selecting individuals (or breeds) exhibiting estrous periods throughout the year; (2) artificial modification of environmental factors (temperature and light) affecting estrus; and (3) administration of hormones to induce estrus and fertility during the non-breeding season. Of the three, the latter has the advantages of immediate applicability (when perfected) and concomitant synchronization of breeding and lambing dates.

Numerous workers have investigated the hormonal induction of estrus and conception in the anestrous ewe. Although a single injection of a gonadotrophin such as pregnant mare's serum (PMS) will induce ovulation in a large proportion of anestrous ewes, this phenomena is accompanied by estrus only if PMS is preceded by a period of progesterone injections (Dutt, 1953). Fertility at the induced heat period is generally quite low, particularly during mid-anestrum (approximately 33%). Also, estrous response and fertility are lower in lactating than in non-lactating animals (Gordon, 1958).

Robinson (1960) stated that two of the major problems in controlled sheep breeding are (1) simplification of the progesterone injection procedure and (2) determination of factors affecting fertility following treatment. The studies reported herein were designed to evaluate the efficacy of an orally effective progestin and various combinations and sequences of hormone therapy on the "estrus - ovulation - conception" complex in anestrous ewes.

General Procedures

Thirteen trials were conducted during the non-breeding season

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over a 4-year period (1961-64), including a total of 857 ewes. Various breed and age groups were utilized, with lactating and non-lactating ewes included in the studies. Factors such as breed, age, and lactation status were balanced among the treatment groups.

Hormones utilized in these studies were (1) 6-chloro-Δ^6-dehydro-17-acetoxyprogesterone (CAP), a potent orally effective progestin; (2) ethynyl estradiol 3-methyl ether (EE), an orally effective estrogen; (3) estradiol; (4) progesterone; and (5) pregnant mare's serum gonadotrophin (PMS)[4]. Treatment normally consisted of a 10- to 16-day period of progestin administration followed by a single injection of PMS. The effect of estrogen in this basic treatment was evaluated in some trials by simultaneous administration (in the form of EE) with the progestin or as a single injection (estradiol) following PMS. In the later trials a part or all of the basic treatment was repeated to effect a second synchronous estrus.

CAP and EE were administered in .23 to .45 kg. of ground corn by once- or twice-a-day feeding for the specified number of days. An aqueous solution of PMS was administered subcutaneously 24 or 36 hours after the final progestin feeding. Estradiol was injected subcutaneously at the same time or 36 hours following PMS. Estradiol was administered as the Na salt or dissolved in either corn oil or sesame oil. Progesterone was dissolved in corn oil and injected subcutaneously.

In most cases estrous activity was not determined until the end of the treatment phase, although in a few trials vasectomized or aproned rams were utilized to check estrus before and during the treatment period. Occurrence of estrus following treatment was evaluated twice a day (a.m. and p.m.), unless otherwise stated. Each time of observation for estrus is referred to as a "period," and this is the unit of time used to measure the length of estrus. Therefore, a "period" is roughly equivalent to a half day. Intact rams were used to breed ewes at the induced estrous periods, with approximately an equal number of ewes on each treatment mated to each ram. Each ewe was normally mated once during each heat period. Treatment effects were evaluated by laparotomy, slaughter, or from lambing data.

Untreated control animals were not involved in any trials. In the earlier experiments "positive" controls (received daily injections of progesterone) were utilized as a standard. One group of animals received CAP as a single injection.

Data were statistically evaluated by chi-square or analysis of variance techniques depending on the nature of the observations. The method of unweighed means was utilized in factorial treatment arrangements involving unequal subclass numbers (Snedecor, 1956).

To provide continuity of thought specific procedures involved in each trial will be accompanied by results.

[4]Lyopholyzed PMS supplied by Ferring AB, Malmo, Sweden.

Methods and Results

1961

Three trials were conducted during April and May of 1961 involving 163 Hampshire, Columbia, and grade ewes. Two of the trials were conducted at the South Dakota Agricultural Experiment Station Sheep unit and the third trial in a private flock through cooperation of the owner. These trials were designed to ascertain the level of CAP for optimum results and the effect of estradiol subsequent to PMS.

Trial 1. Five treatments with 13 ewes per treatment were started on April 10. Treatments imposed were: 20, 10 or 5 mg. CAP per ewe daily in 0.23 kg. of corn (fed once daily for 15 days) in treatments 1 to 3, respectively; single injection of CAP (subcutaneously in oil) in treatment 4, six ewes receiving 5 mg. and seven ewes 10 mg.; and 10 mg. progesterone daily (subcutaneously in oil) for 15 days in treatment 5. All ewes received 1,000 I.U. PMS 24 hours after the last progestin treatment with the exception of treatment 4, where PMS was injected 15.5 to 16.0 days after the single CAP injection. Half of the ewes on each treatment received 0.75 mg. Na salt of estradiol (subcutaneous injection) 36 hours following PMS administration. In treatment 4, only ewes receiving the 5 mg. injection of CAP received estradiol. Two ewes from each treatment were laparotomized 7 days after PMS injection and their ovaries examined. Fertility was evaluated from lambing data.

Table 1 summarizes the results of this trial. Estrous response was quite poor in all groups receiving CAP orally except at the 5 mg. level *when* followed by an injection of 0.75 mg. estradiol. All ewes that received a single 5 mg. CAP injection followed by estradiol were observed in heat, whereas none of the ewes injected with 10 mg. CAP and no estrogen were in heat. The "standard" treat-

Table 1. Response of Anestrous Ewes to Various Progestin-Estradiol Treatments

Progestin, mg.	20 CAP[a]		10 CAP[a]		5 CAP[a]		5 CAP[b]	10 CAP[b]	10 Progesterone[a]	
Estradiol, mg.	0	.75[c]	0	.75[c]	0	.75[c]	.75[c]	0	0	.75[c]
Total No.	6	7	6	7	6	7	7	6	6	7
No. in heat[d]	0	1	1	1	0	6	7	0	5	7
Days, PMS to heat		3.0	4.0	3.0		3.1	2.3		2.3	2.4
Periods in heat[e]		1.0	1.0	1.0		1.0	1.9		1.2	2.3
No. lambing	0	0	0	0	0	0	1	0	3	1

[a]Dose administered daily, 6-chloro-$\triangle^6$-17-acetoxyprogesterone (CAP) orally and progesterone by subcutaneous injection. All received 1,000 I.U. pregnant mare's serum (PMS) 24 hours after last progestin treatment.

[b]Single subcutaneous injection in oil; 1,000 I.U. PMS injected 15.5 to 16.0 days after CAP injection.

[c]Administered as a subcutaneous injection 36 hours after PMS.

[d]Significant differences between progestin treatments ($P<.01$), estrogen treatments ($P<.01$), and progestin $\times$ estrogen interaction ($P<.05$).

[e]"Period" refers to each heat check, e.g., two "periods" per day.

ment (15 daily injections of progesterone) resulted in estrus in 12 of 13 ewes. Significant differences ($P<.01$) were obtained between progestin treatments and between estrogen treatments for occurrence of estrus. A significant interaction ($P<.05$) was also observed between these two factors, probably resulting from the inability of estradiol to induce estrus in the 10 and 20 mg. oral CAP treatments.

Examination of ovaries revealed that ewes receiving CAP orally or a single 10 mg. CAP injection did not respond to PMS (2 of 7 ovulated), whereas the three ewes examined which received a single 5 mg. CAP injection or daily 10 mg. progesterone injections had ovulated. Although number of ewes involved in this comparison is small, the results suggest that 5-20 mg. CAP orally or 10 mg. injection inhibited the ovarian response to PMS. Only five of 37 ewes bred produced lambs.

Trial 2. Fifty-four Hampshire and Columbia ewes were used in the second trial with treatments starting May 5. Two groups of 22 ewes each received either 1 or 3 mg. CAP per ewe daily in .23 kg. of ground corn by once-a-day feeding for 16 days. The third group of 10 ewes received 16 daily injections of 10 mg. progesterone per ewe. All animals received 1,000 I.U. PMS 24 hours after the final progestin treatment, and half of the ewes on each treatment were injected with 0.5 mg. estradiol (Na-salt) 36 hours later.

Following hormone administration all ewes were checked twice daily for occurrence of estrus and bred at each period observed in heat. No laparotomies were performed, but lambing data were obtained. Length of estrus was not recorded in this trial.

Results are presented in table 2. Proportion of ewes in estrus on the two CAP treatments approached that observed in the group receiving progesterone injections. The addition of 0.5 mg. estradiol significantly increased the number of ewes exhibiting estrus ($P<.01$).

Ewes receiving CAP had longer intervals between PMS and estrus than those receiving progesterone injections ($P<.01$), and this interval was the greatest at the higher

Table 2. Response of Anestrous Ewes to Oral and Parenteral Progestins with Supplementary Estrogen

Progestin, mg.	3 CAP[a]		1 CAP[a]		10 Progesterone[b]	
Estradiol, mg.	0	0.5[c]	0	0.5[c]	0	0.5[c]
No. ewes	11	11	11	11	5	5
No. in heat[d]	6	10	6	11	4	5
Days, PMS to estrus[e]	4.2	2.8	2.5	2.9	2.0	2.2
No. ewes lambing	1	2	3	4	4	1

[a]Daily CAP (6-chloro-Δ^6-17-acetoxyprogesterone) levels in feed for 16 days.
[b]Daily dose of progesterone (subcutaneous) for 16 days.
[c]Administered as a subcutaneous injection 36 hours after PMS.
[d]Significant differences between estradiol levels, $P<.01$.
[e]Significant differences between progestin treatments, $P<.01$; significant progestin $\times$ estradiol interaction, $P<.01$.

level of CAP. A significant interaction between progestin and estradiol treatments was also detected for this trait ($P<.01$). Administration of estradiol to ewes on the 3 mg. CAP level shortened the interval to estrus, whereas estradiol tended to increase this interval in the other two treatments.

Of 42 ewes observed in heat and bred, 15 conceived and produced term lambs. No significant treatment effects were detected for proportion of ewes lambing. An additional 11 ewes returned to estrus approximately one cycle later and five of these lambed from "repeat" matings.

Trial 3. This trial, involving two groups of 27 ewes each, was conducted in cooperation with a private breeder. Only Hampshire ewes were involved. Treatments consisted of either 2 or 4 mg. CAP per ewe daily for 15 days (starting May 5) followed by 1,000 I.U. of PMS 24 hours following the final progestin feeding. Approximately half of the ewes on each treatment received a subcutaneous injection of 0.5 mg. estradiol (Na-salt) 36 hours after PMS.

Ewes were mated at the induced heat period, but length of estrus was not evaluated.

No significant treatment effects were observed for proportion of ewes in estrus (table 3). However, there was a tendency for more ewes in heat on the 2 mg. CAP treatment and in ewes receiving supplemental estrogen.

Interval from PMS to estrus was significantly affected by both variables ($P<.01$). Ewes receiving the higher level of CAP exhibited a longer interval to estrus, and estradiol administration shortened this interval. The effect of estradiol was more pronounced at the higher CAP level; therefore, a significant interaction was observed for this trait ($P<.01$).

None of the ewes lambed as a result of mating at the induced heat period. Only two rams were available in this trial and one of the rams bred the majority of ewes observed in heat. Therefore, ram fertility may have been a factor in this particular study.

Table 3. Response of Anestrous Ewes to an Oral Progestin Followed by Supplementary Estrogen

CAP, mg.[a]	2		4	
Estradiol, mg.[b]	0	0.5	0	0.5
No. ewes	13	14	13	14
No. in heat	10	13	9	11
Days, PMS to estrus[c]	2.0	1.7	2.7	1.7

[a]6-chloro-Δ^6-17-acetoxyprogesterone (CAP), fed at indicated levels in corn for 15 days. 1,000 I.U. PMS injected 24 hours after last CAP feeding.

[b]Estradiol injection (subcutaneous) administered 36 hours after PMS.

[c]CAP treatments, $P<.01$; estradiol treatments, $P<.01$; CAP $\times$ estradiol interaction, $P<.01$.

1962

Eight trials (trials 4-11) were conducted during May-July; two of the trials were at the Agricultural Experiment Station and the remaining six in cooperation with private breeders. Involved in these studies were 303 ewes. Breeds were Hampshire, Suffolk, Columbia, Southdown, and grade ewes. Lactating ewes were included in the two Experiment Station trials. Factors evaluated in these trials were level

of CAP (0.5-1.0 mg.) with or without ethynyl estradiol 3-methyl ether (EE), interval between progestin treatment and injection of PMS, and the effect of a supplementary estrogen injection subsequent to PMS.

Trial 4. Ninety-eight Hampshire, Columbia and grade ewes were included in this trial. Treatment variables were levels of CAP:EE (0.5:0, 0.75:0, 1.0:0, 1.0:2.0 mg.); interval from last CAP:EE administration to injection of 1,000 I.U. PMS (24 or 36 hours); and level of estradiol injection 36 hours after PMS (0 or 0.5 mg. as the Na-salt). CAP:EE combinations were fed in .45 kg. of ground corn by twice-a-day feeding for 15 days. Treatments were started on May 22. The ewes were checked prior to the start of the trial with vasectomized rams; none exhibited estrous activity during this pretreatment phase. Lactating ewes (n = 36) had lambed 13 to 28 days prior to initiation of hormone feeding.

Table 4. Response of Anestrous Ewes to Various CAP:EE Combinations

CAP:EE (mg.)[a]	0.5:0.0	0.75:0.0	1.0:0.0	1.0:2.0
Total No. ewes	24	25	25	24
No. in heat	14	12	17	10
Days, CAP:EE to estrus[b]	3.7	3.7	3.8	4.5
Periods in estrus[c]	2.6	2.8	2.5	1.9
No. ewes lambing	1	1	3	1

[a]CAP = 6 chloro-Δ^6-dehydro-17-acetoxyprogesterone; EE = ethynyl estradiol 3 methyl ether. Fed in ration for 15 days.
[b]Significant treatment effect, $P<.01$.
[c]See footnote "e", table 1.

Ewes were checked for estrus twice daily after treatment and mated at each heat period observed in estrus. Fertility was evaluated from lambing data.

Results are presented in tables 4 and 5. Treatments did not significantly affect proportion in heat, length of estrus, or number ewes lambing. Therefore, results were summarized over PMS interval, estradiol level, and lactation status for conciseness of presentation (table 4). Although not statistically significant, a larger proportion of ewes receiving estradiol were in heat (.63 vs. .45) and a larger proportion of lactating than non-lactating ewes were in estrus (.61 vs. .50). Length of estrus tended to be shorter in lactating ewes (2.3 vs. 2.6 periods). Only 11% of ewes bred produced lambs (6 of 53); therefore, the statistical evaluation of fertility is not too meaningful.

Combinations of CAP:EE, interval to PMS, and estradiol level all significantly affected interval from PMS to estrus. The inclusion of 2.0 mg. EE in the CAP feeding regime

Table 5. Effect of Time of PMS and Estradiol Injections on Days from CAP:EE to Estrus

Estradiol (mg.)[b]	Hours, CAP:EE to PMS[a] 24	36	Estradiol average
0.0	3.5	4.7[c]	4.1[d]
0.5	3.5	4.0	3.7
PMS average	3.5	4.3[c]	3.9

[a]Hours elapsing between last CAP:EE feeding and injection of PMS (1,000 I.U.).
[b]Injected 36 hours after PMS.
[c]PMS × estradiol interaction, $P<.05$.
[d]$P<.05$.
[e]$P<.01$.

lengthened the average onset of estrus ($P<.01$, table 4). As indicated in table 5 delaying the PMS injection until 36 hours after last CAP feeding resulted in a delay of estrus of approximately 0.8 day ($P<.01$), and injection of 0.5 mg. estradiol hastened the onset of estrus ($P<.05$). A significant interaction between PMS and estradiol was observed ($P<.05$), apparently resulting from the increase in interval to estrus exhibited by animals receiving PMS 36 hours after final CAP with no supplemental estrogen. A significant interaction ($P<.05$) was also detected between CAP:EE and PMS. Injection of PMS 36 hours after CAP:EE resulted in a slightly shorter interval to estrus at the 0.75:0 level, but a greatly lengthened interval at the 1.0:2.0 combination. The 24 hour PMS group showed a general increase in this interval as dose of CAP:EE increased.

Trial 5. A group of 78 grade (predominately Columbia) ewes were divided into eight groups and each group was allotted to one of the following treatment combinations: CAP, 0.75 or 1.00 mg. per ewe daily; EE, 0 or 2.0 mg. per ewe daily; interval between final CAP:EE and 1,000 I.U. PMS, 24 or 36 hours. All ewes received 0.5 mg. estradiol (Nasalt) 36 hours after the PMS injection. Methods of hormone administration were identical to Trial 4. CAP:EE feeding started on May 25 and continued for 15 days. Lactating ewes ($n = 27$) were divided as equally as possible among the eight treatment combinations. These ewes were 26 to 110 days postpartum at the start of hormone treatment.

Following hormone administration, ewes were checked twice daily for estrus and mated with an intact ram at each period observed in heat. Lambing data were obtained as in previous trials.

Level of EE fed did not significantly affect any of the variables measured; therefore, data were summarized over EE levels for presentation.

Considering all ewes treated, 71% (55 of 78) were in estrus subsequent to treatment (table 6). More ewes receiving PMS at the 36-hour interval were in heat ($P\sim .07$). Ewes receiving the higher level of CAP had a longer interval to onset of estrus (0.4 day, $P<.05$). Also, ewes receiving PMS 36 hours after CAP did not return to estrus until approximately 0.4 day after the 24-hour group ($P<.05$).

The only factor exerting a significant effect on length of estrus was lactation status; lactating ewes exhibited a shorter estrus than "dry" ewes (1.6 vs. 2.2 periods, $P<.05$).

Only 25% of ewes mated produced lambs (14 of 55). None of the factors studied exerted an apparent effect on fertility, although small numbers limited the statistical evaluation of this trait.

Trials 6-8. These trials were conducted with cooperative breeders and involved a total of 63 ewes of Hampshire, Suffolk or Southdown breeding. The only treatment variable imposed was level of CAP: 0.75 or 1.00 mg. per ewe daily for 15 days. As in previous trials, CAP was administered in .45 kg. of ground

corn by twice-a-day feeding. All ewes received 1,000 I.U. PMS 24 hours after final CAP feed, and 0.5 mg. estradiol (Na-salt) 36 hours after PMS injection. Treatment was started in one trial on May 23 and in the remaining two on June 22.

Ewes were mated naturally at the induced heat periods. Length of estrus was not determined.

Results are summarized in table 7. No significant differences between treatments or between farms were observed; therefore, data were pooled over farms for presentation. Considering both treatments, 87% of all ewes were observed in heat. However, only one ewe reportedly lambed from the induced mating and treatment identification was lost on this individual.

Trial 9. Sixteen ewes of mixed breeding were utilized in this cooperative trial. Four treatment combinations were studied: 0.75 or 1.00 mg. CAP per ewe daily for 15 days; 24- or 36-hour interval between final CAP and injection of 1,000 I.U. PMS. Other procedures were similar to the previous field trials. CAP feeding was initiated on May 25.

The only significant treatment effect was interval to PMS injection. Ewes receiving PMS 36 hours after CAP exhibited estrus 0.5 day later than the 24-hour group (3.8 vs. 3.3 from last CAP feed, $P<.05$).

Estrus was observed in 15 of the 16 ewes. Two ewes lambed from matings at the induced heat period, but treatment identification was lost.

Trial 10. This cooperative trial was conducted with two groups of 14 ewes, each composed of seven

Table 7. Response of Anestrous Ewes to Two Levels of CAP

CAP, mg.[a]	0.75	1.00
Total No. ewes	31	32
No. in heat	28	27
Days, PMS to estrus[b]	2.6	2.4
No. ewes lambing[c]		

[a]Level of CAP (6-chloro-Δ^6-17-acetoxyprogesterone) fed daily per ewe for 15 days.
[b]1,000 I.U. PMS administered 24 hours after last CAP feeding.
[c]Only one ewe lambed, and treatment identification was lost.

Table 6. Effect of Level of CAP, Interval to PMS, and Lactation Status on Reproductive Phenomena of Anestrous Ewes

CAP, mg.[a]	0.75				1.00			
PMS[b]	24		36		24		36	
Lactation status	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
No. ewes	12	8	12	7	13	7	14	5
No. in heat[c]	6	5	10	6	7	6	11	4
Days, CAP to estrus[d]	3.2	3.1	3.6	3.8	3.4	3.9	3.8	3.9
Periods in estrus[e]	1.8	1.5	2.1	1.5	2.7	1.5	2.3	1.8
No. ewes lambing	1	2	2	2	3	2	2	0

[a]Level of CAP (6-chloro-Δ^6-17-acetoxyprogesterone) administered daily for 15 days.
[b]Hours between last CAP feeding and injection of 1,000 I.U. PMS.
[c]Significantly more ewes on 36 hour PMS interval in heat, $P\sim.07$.
[d]Significantly longer interval; 1.00 mg. CAP, $P<.05$; 36 hour PMS, $P<.05$.
[e]Significantly shorter estrus period for wet ewes, $P<.05$. See footnote "e", table 1 for definition of "periods."

Hampshire and seven Suffolk ewes. One group received 1.0 mg. CAP daily for 15 days, the other 1.0 mg. CAP plus 2.0 mg. EE for the same period. PMS was injected 24 hours after final CAP and 0.5 mg. estradiol 36 hours later. Other procedures were similar to the above trials.

Estrus was observed in 27 of 28 ewes. Seven ewes subsequently lambed from mating at the induced heat period, three on 1.0:0.0 CAP:EE combination and four on 1.0:2.0 CAP:EE. Length of estrus was significantly longer in the latter treatment (3.8 vs. 2.5 periods, $P<.05$).

Trial 11. The final field trial in 1962 involved 20 Suffolk ewes. Half received 0.75 mg. CAP daily and half 0.75 mg. CAP plus 2.00 mg. EE for 15 days. CAP treatment was started on May 25. PMS and estradiol treatments were identical to Trial 10. Breeding practices were consistent with previous trials. One exception to the general procedures noted above was that ewes were checked for estrus at 4-hour intervals subsequent to hormone administration, thereby allowing for rather precise estimates of the length of estrus.

Fifteen of the 20 ewes were observed in estrus; one ewe on each treatment lambed from breeding at the induced heat period.

Length of estrus was significantly shortened by the addition of 2.0 mg. EE to the treatment regime (29 vs. 56 hours, $P<.01$).

1963

The study conducted in 1963 (Trial 12) was designed to evaluate (1) the effect of a "double" PMS injection (second injection of PMS approximately one estrous cycle after first), (2) the effect of a relatively high level of estradiol (5.0 mg. vs. 0.5 mg.) subsequent to PMS, (3) the response of yearling as compared to parous ewes, and (4) the effect of lactation on treatment response. A total of 245 ewes were involved in this study—60 grade yearling ewes and 185 parous Hampshire, Columbia, Suffolk and grade ewes. Lactating ewes ($n = 98$) were 13 to 74 days postpartum when hormone administration began (April 11).

All ewes received 1 mg. CAP in 0.45 kg. of ground corn once daily for 10 days. PMS (1,000 I.U.) was administered to all ewes 36 hours after last CAP feeding. The ewes were then divided into four groups on the basis of parity, breed, lactation status and weight. Two groups received estradiol (in sesame oil) injections 24 hours following the PMS, one group receiving 0.5 and the other 5.0 mg. per ewe. The remaining two groups received a second PMS injection (1,000 I.U.) 16 days after the initial PMS, followed by either 0.5 or 5.0 mg. estradiol 24 hours later.

Rams with marking harnesses were placed with the ewes following estradiol injections, and ewes were checked twice daily for indications of mating. Ewes scheduled to receive "double" PMS were not exposed to rams following the initial PMS injection. In addition yearling ewes were checked for estrus with vasectomized rams for 20 days prior to beginning of treatment.

Ovarian information and fertility data were obtained from yearling

ewes at slaughter approximately 30 days after mating. Lambing data were obtained from the parous ewes.

Results are presented separately for yearling and parous ewes. A large proportion of yearling ewes (88%) was observed in estrus following treatment (table 8). None of the main treatment effects significantly affected this trait; however, the PMS × estradiol interaction was significant ($P<.01$). Only 9 of 15 ewes receiving "double" PMS and 5.0 mg. of estradiol were observed in heat, whereas all but one ewe in the other groups were in estrus.

Table 8. Estrus and Fertility of Yearling Ewes Following CAP-PMS-Estradiol Treatments

No. PMS injections[a]	1		2	
Estradiol, mg.[b]	0.5	5.0	0.5	5.0
No. ewes	15	15	15	15
No. in estrus[c]	14	15	15	9
Days, PMS to estrus[d]	2.2	1.9	2.4	2.8
Periods in estrus[e]	1.9	2.0	1.8	3.8
No. pregnant	8	10	8	6
Embryos/ewe	2.2	1.6	1.9	1.8
No. corpora lutea[f]	4.8	2.2	3.9	2.3
Embryonic survival (%)[g]	56	88	59	93

[a]All ewes received 1 mg. CAP in 0.45 kg. feed daily for 10 days; followed by 1,000 I.U. PMS (subcutaneously) 36 hours after last CAP feeding. Second PMS injection (1,000 I.U.) administered 16 days after first.

[b]Estradiol (in sesame oil) administered 24 hours after PMS.

[c]PMS × estradiol interaction, $P<.01$.

[d]Significantly longer for two PMS injections, $P<.05$.

[e]Significantly longer, 5.0 mg. estradiol, $P<.05$; PMS × estradiol, $P\sim.05$. See footnote "e", table 1 for definition of period.

[f]Significantly larger, 0.5 mg. estradiol, $P<.05$.

[g]Significantly larger, 5.0 mg. estradiol, $P<.01$.

The interval from PMS to estrus was lengthened in ewes receiving "double" PMS ($P<.05$). Length of estrus was greater in ewes receiving the higher dose of estradiol ($P<.05$). Ewes treated with double PMS and 5.0 mg. estradiol had a much longer estrus period than the other treatment combinations resulting in a significant interaction ($P\sim.05$).

Conception rate (60%) and number of embryos per ewe (1.9) were not significantly affected by treatments. However, there was a trend for more embryos at the 0.5 mg. level of estradiol.

Ovulation rate, as reflected by number of corpora lutea in pregnant ewes, averaged 3.3 for all animals. Significantly more ova were shed in ewes receiving the lower dose of estradiol (4.3 vs. 2.2, $P<.05$).

Embryonic survival rate at 30 days of gestation was evaluated by dividing the number of embryos by the number of corpora lutea for each *individual* ewe and expressing this as a percentage. Only data from pregnant ewes were included in this calculation. This method of calculation prevents one animal from disproportionately affecting the overall results. Considering all ewes, average embryonic survival was 74%. This parameter was significantly higher in ewes receiving 5.0 mg. estradiol ($P<.01$).

Estrus was observed in 86% of parous ewes treated (table 9). The single PMS injection resulted in a higher proportion in estrus

($P<.05$), as did the 5.0 mg. level of estradiol ($P<.01$). No significant difference with respect to lactation status was noted.

Interval from PMS to estrus was longer in lactating ewes ($P<.05$). Also, lactating ewes exhibited a shorter estrous period ($P<.05$). Ewes receiving 5.0 mg. estradiol were in estrus longer than those on the lower estrogen treatment ($P<.01$).

Fertility was quite poor in all groups. Only 14% (21 of 154 ewes) of ewes mated lambed. Five ewes were sold or died before lambing started, hence the discrepancy between ewes mated and number remaining at end of gestation. A "double" PMS treatment resulted in significantly more ewes lambing ($P<.01$). Differences in ewes lambing also favored the low level of estradiol (approached significance at 5% level). Therefore, the best treatment combination, with respect to proportion ewes lambing, was "double" PMS and 0.5 mg. estradiol (11 of 33 ewes lambing).

Lactation also exerted a significant depressing effect on fertility ($P<.01$). Only 4 of 77 lactating ewes mated produced lambs.

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Trial 13. The final experiment was designed to evaluate three treatments involving "double" PMS injections. The basic treatment (I) consisted of 1 mg. CAP per ewe daily for 10 days, 750 I.U. PMS 24 hours after last CAP, with the sequence repeated beginning 4 days after the initial PMS injection. Therefore, two PMS injections were separated by 14 days, and each was preceded by a 10-day period of progestin administration. Treatment II was identical to treatment I except that the initial CAP feeding period was eliminated. Treatment III was similar to Treatment II with the addition of a 0.5 mg. estradiol injection at the same time as initial PMS and

Table 9. Estrus and Fertility of Parous Ewes Following CAP-PMS-Estradiol Treatments

No. PMS injections[a]	1		2		Lactation status	
Estradiol, mg.[b]	0.5	5.0	0.5	5.0	Dry	Wet
No. ewes	47	47	45	46	87	98
No. in estrus[c]	39	47	33	40	78	81
Days, PMS to estrus[d]	2.2	2.2	2.5	2.2	2.1	2.4
Periods in estrus[e]	1.7	2.5	2.2	2.2	2.4	2.0
No. ewes lambing[f]	3	2	11	5	17	4

[a]All ewes received 1 mg. CAP in 0.45 kg. feed daily for 10 days; followed by 1,000 I.U. PMS (subcutaneously) 36 hours after last CAP feeding. Second PMS injection (1,000 I.U.) administered 16 days after first.
[b]Estradiol (in sesame oil) administered 24 hours after PMS.
[c]Significantly more ewes in estrus, single PMS, $P<.05$; 5.0 mg. estradiol, $P<.01$.
[d]Longer in lactating ewes, $P<.05$.
[e]Longer in ewes receiving 5.0 mg. estradiol, $P<.01$; in non-lactating ewes, $P<.05$. See footnote "e", table 1, for definition of period.
[f]More ewes lambing—"double" PMS, $P<.01$; dry ewes, $P<.01$. Approaches significance at 5% level for estradiol levels.

one day following final PMS. CAP was mixed with 0.27 kg. of ground corn and fed once daily, starting May 8. All PMS injections (subcutaneously in 0.9% NaCl) were given on the same date. Estradiol was administered subcutaneously in sesame oil.

Treatments I and III involved 45 ewes each and Treatment II 46 ewes. Animals consisted of 107 parous ewes (Hampshire, Suffolk, Columbia and grades) and 29 yearling ewes (grades). All parous ewes had lambed the previous fall and winter, and lambs were weaned approximately a month before the trial started. Ewes were allotted to the treatments on the basis of breed, weight, age and postpartum interval.

Daily checks for estrus (with vasectomized or aproned rams) were started April 3 and continued throughout the trial. Ewes in estrus following the final PMS injection were mated to intact rams. Grade, parous ewes were slaughtered either 4 days after the final PMS injection or approximately 30 days subsequent to breeding to evaluate ovarian conditions and early embryonic survival. The remaining ewes were retained for lambing data.

Results of this trial are summarized in table 10. Due to the small number of animals observed in estrus (particularly on Treatments I and II, the observations were pooled into the following "breed" groups: (1) purebred ewes – all

Table 10. Response of Anestrous Ewes to Several Repeated Hormone Treatments

Treatment[a]	I			II			III		
Breed group[b]	P	G	Y	P	G	Y	P	G	Y
No. ewes	21	15	9	22	14	10	21	14	10
First PMS:[c]									
No. in estrus	1	3	5	1	0	0	5	3	1
Second PMS:[d]									
No. in estrus[e]	2	0	1	4	0	9	19	10	10
Days, PMS to estrus[f]	3.5	--	3.5	4.3	--	2.6	2.1	2.0	2.0
Periods in estrus[g]	1.0	--	1.0	1.3	--	1.1	1.6	2.1	2.8
Ewes lambing[h]	2(2)	--	1(1)	2(4)	--	4(9)	1(18)[i]		5(10)

[a]I = 1 mg. CAP (6-chloro-Δ^6-17-acetoxyprogesterone) per ewe, days 1-10; 750 I.U. pregnant mare's serum (PMS) day 11; 1 mg. CAP per ewe, days 15-24; 750 I.U. PMS day 25.
II = same as I with initial CAP omitted.
III = same as II with 0.5 mg. estradiol on day 11 and day 26.

[b]P = purebred Hampshire, Suffolk and Columbia ewes; G = grade ewes; Y = yearling ewes.

[c]Observations following initial PMS injection; significant treatment differences, $P<.05$; treatment × breed interaction, $P<.05$.

[d]Observations following final PMS injection.

[e]Treatment effects, $P<.01$; breed effects, $P<.01$; treatment × breed interaction, $P\leq.05$.

[f]Significant treatment effects, $P<.01$.

[g]Significant treatment effects, $P<.01$. See footnote "e", table 1, for definition of "period."

[h]Significant treatment effects, $P<.05$. Numbers in parenthesis are number of bred ewes retained for lambing information.

[i]One of 10 ewes pregnant at slaughter 30 days after mating.

Hampshire, Suffolk and Columbia ewes; (2) grade ewes – all parous, grade ewes; (3) yearling ewes. The purebred ewes were of intermediate ages (3-6 years old), and the parous, grade ewes were older (5-8 years old). Therefore, this classification roughly divided the ewes into age groups.

Estrous response following the initial PMS injection was significantly affected by treatment ($P<.05$). Only one of 46 ewes on Treatment II (no initial CAP) was observed in heat, whereas nine ewes in each of the other treatments were in heat. A significant ($P<.05$) breed × treatment interaction was also detected. The largest proportion of purebred ewes was observed in heat on Treatment III (24%), whereas more yearling ewes (56%) were in estrus on Treatment I.

Interval from PMS to estrus and length of estrus were not statistically evaluated due to the small number of observations. However, no major differences between treatments were apparent for these traits.

Both treatment and breed group significantly affected the occurrence of estrus following the final PMS injection ($P<.01$). Treatment III resulted in the largest proportion of ewes in estrus (87%), whereas only 7% of ewes from Treatment I were in heat. More yearling ewes were observed in estrus (69%) than the other two breed groups. In the purebred group 39% were in heat, whereas only 23% of grade ewes returned to estrus following final PMS.

An interaction between treatment and breed group for occurrence of estrus was also indicated in the analysis ($P\cong .05$). This was apparently due to the differential response among breed groups on Treatment II; 90% of yearling ewes on this treatment were in heat, but only 18% and 0% of purebred and grade ewes, respectively.

Interval from final PMS to estrus was shorter on Treatment III (2.1 days) than on Treatment I (3.5 days) and Treatment II (3.1 days). Differences were significant at the 1% level of probability. Length of estrus was also significantly affected by treatment ($P<.01$). Ewes on Treatment III were in heat an average of 2.0 periods, whereas on Treatments I and II ewes were in heat 1.0 and 1.2 periods respectively. Breed differences for interval to estrus and length of estrus following final PMS were not statistically evaluated, since no grade ewes were observed in heat on Treatments I and II.

Grade ewes not returning to estrus following final PMS were slaughtered 4 days after injection ($n = 33$). Examination of ovaries from these ewes indicated that treatments did not induce follicular maturation and ovulation in most cases. Based on number of corpora lutea, only 3 of 33 ewes slaughtered had ovulated from the second PMS injection, and only 58% had follicles 5 mm. or greater in diameter. It appeared that ovulatory response was greater following the initial PMS injection – 27% had corpora albicantia estimated to have resulted from ovulations following initial PMS. No differential treatment effects were noted.

Grade ewes exhibiting estrus

were slaughtered approximately 30 days after breeding. Ten ewes were in this group, and all were on Treatment III. Only one of these ewes was pregnant at slaughter, possessing two normal embryos. An additional six ewes had regressing corpora lutea (1-3 per ewe) which probably resulted from induced ovulations following final PMS. Therefore, 70% of this group apparently ovulated following the second PMS injection. Nine of the 10 ewes had follicles 5 mm. or greater in diameter.

Lambing response was quite poor in the remaining ewes. Only 15 of 44 ewes mated (34%) produced lambs. Treatment I resulted in the highest proportion of ewes lambing (3 of 3), whereas only 6 of 28 ewes lambed on Treatment III. Although treatments differed significantly ($P < .05$), the small numbers involved limit the accuracy of this evaluation. Fifty percent of yearling ewes and 21% of purebred ewes lambed. This difference was significant at the 10% level.

DISCUSSION

Experiments conducted during 1961 indicated that CAP is a relatively potent oral progestin, and that levels as low as 1 mg. per ewe daily for 15-16 days followed by PMS resulted in estrus in a majority of ewes treated. Reducing this dosage to 0.5 or 0.75 mg. per ewe did not result in increased performance (1962). Since the possibility of an inadequate amount of CAP being consumed is increased as dose decreases, a level of 1 mg. per ewe daily was used in subsequent trials. In comparison, Brunner *et al.* (1964) found that approximately 50-60 mg. of 6-methyl-17-acetoxyprogesterone (MAP, another orally active progestin) per ewe daily was required for comparable estrous response. However, Pursel and Graham (1962) reported that 10 mg. MAP daily resulted in 7 of 10 ewes exhibiting an induced estrus. For comparison, approximately 10 mg. progesterone daily (by injection) is required to "prime" the anestrous ewe for estrus and ovulation when progesterone is followed by PMS administration (Robinson, 1959).

The inclusion of an orally effective estrogen, ethynyl estradiol 3 methyl ether (EE), in the CAP treatment did not appear to improve either estrous response or fertility (Trials 4, 5, 10 and 11), and therefore it was eliminated from subsequent trials.

There was an indication that a 36-hour interval between CAP and PMS injection resulted in a larger proportion of ewes in estrus (Trial 5); however, this effect was not observed in other trials comparing interval to PMS injection. The most consistent effect of delaying PMS injection until 36 hours after CAP was a delay in the onset of estrus. This delay was roughly comparable to the additional time elapsing between PMS injections (approximately 0.5 day).

Although a relatively large proportion of treated ewes responded with a synchronous estrus (70% or more in most trials), fertility was quite low. Considering all trials conducted during 1961-62, only 15% of all ewes mated (52 of 342) produced lambs. Even if the trials in which

other factors may have been involved (rams, Trial 3) or where the accuracy of lambing information may be questioned (Trials 6-8) are eliminated, only 21% (51 of 244) ewes lambed. This proportion varied from 11%-36% in the various studies. Other workers have reported low fertility following similar progestin-PMS treatments (Gordon, 1958; Pursel and Graham, 1962; Allen and Lamming, 1960; Braden and Moule, 1962).

Observations by Gordon (1958) indicated that fertility may be normal in ewes experiencing a spontaneous estrus subsequent to an induced estrus. In experiments designed to evaluate this theory, Gordon (1963) reported that a repeated progestrone-PMS therapy (PMS injections separated by an interval equivalent to approximately one estrous cycle) resulted in an apparently normal conception rate (68%). Therefore, trials conducted at this station in 1963 and 1964 were designed to evaluate the efficacy of this treatment sequence. Although the earlier trials involved a 15-16 day period of CAP feeding, it was deemed desirable to reduce this period if possible, especially in a treatment involving repeated administration of the basic hormones. The work of Gordon (1958, 1963) indicated that the period of progestin administration could be shortened to 7 days without adverse effects on estrus and fertility. This supposition has been more recently substantiated by the work of Brunner *et al.* (1964). Therefore, a 10 day CAP feeding period was chosen, and although not contemporarily tested against other lengths of treatment, results indicated that this was a satisfactory "priming" period.

Although fertility was generally improved in these trials, it did not approach the levels observed by other workers (Gordon, 1963, Brunner *et al.*, 1964). Best results were obtained with yearling ewes (50%-60% conception rate) and poorest with lactating ewes (5% conception rate). Length of postpartum interval in lactating ewes may have been a factor contributing to fertility (Wagner, 1964), although the low conception rate in this study prevented an evaluation of this factor.

Lactating ewes generally benefited the most from repeated treatments; yearling ewes the least. In general, yearling ewes exhibited the best response to treatments of any of the age groups. This general relationship has also been observed by Gordon (1958).

The inclusion of supplementary estrogen in the treatment regime following PMS generally resulted in an increase in the number of ewes in estrus, a decrease in interval to estrus, and an increase in length of estrus.

Although not a consistent effect, there was an indication that 0.5 or 0.75 mg. estradiol subsequent to PMS may have been slightly detrimental to fertility. This effect was more clearly demonstrated at a higher level of estradiol in parous ewes (Trial 12). Again, an age difference was observed for the effect of estrogen on fertility. Yearling ewes had a similar conception rate on either 0.5 or 5.0 mg. estradiol, but embryonic survival rate was actually improved on the higher dose. Conversely, parous ewes

demonstrated a decrease in fertility (as evaluated by lambing data) on the higher dose. Gordon (1963) presented evidence indicating that administration of 50 mcg. estradiol after breeding improved fertility in yearling ewes.

In Trial 12 in which ovulation rate was determined at slaughter, 5.0 mg. estradiol significantly depressed ovulation rate in yearling ewes as compared to 0.5 mg. Robinson (1962) also reported that injections of small quantities of estradiol with gonadotrophin tended to suppress ovulation in some cases.

The poor estrous response in ewes not receiving supplemental estrogen in the final trial is difficult to explain. Slaughter results obtained from the old, grade ewes indicated that ovulatory response to PMS was very poor, and suggested that the PMS may have lost some potency or was incorrectly labeled. A sample of the PMS was retained and bioassayed against a standard PMS preparation. Results of the bioassay indicated that the PMS utilized in the last experiment was approximately 50% of the expected potency. Therefore, the dose of PMS administered was only approximately 500 I.U., and may have accounted for the poor estrus and ovulatory response obtained. However, Robinson (1959) reported a satisfactory ovulatory response from as little as 250 I.U. PMS in progesterone primed anestrous ewes.

SUMMARY

A total of 857 anestrous ewes were involved in 13 trials conducted during 1961-64. An orally effective progestin (6-Chloro-$\triangle^6$-17-acetoxyprogesterone, CAP) was fed in the ration for 10 to 16 days, followed by 750 to 1,000 I.U. PMS and 0.5 to 5.0 mg. estradiol subsequent to the PMS. "Double" PMS treatments were evaluated in two experiments. The inclusion of an orally effective estrogen (ethynyl estradiol 3-methyl ether, EE) in the CAP treatment was evaluated in some of the trials.

Approximately 1 mg. CAP per ewe daily resulted in a satisfactory estrous response in most trials. A post-treatment estrus was normally observed in 70% or more of ewes receiving this dose of CAP. Addition of EE to the CAP treatment did not improve estrous response or fertility.

Injection of 0.5 to 5.0 mg. estradiol subsequent to PMS resulted in a larger proportion of ewes in estrus, a shorter interval from CAP to estrus, and a longer heat period. Fertility may have been adversely affected by supplementary estrogen, particularly in parous ewes receiving 5.0 mg. injections. Ovulation rate was suppressed in yearling ewes receiving 5.0 mg. estradiol when compared to 0.5 mg. However, embryonic survival was enhanced in yearling ewes receiving 5.0 mg. estradiol.

Fertility (conception rate) was quite variable, but was definitely lower than that normally observed during the regular breeding season. Age of ewe exerted a large influence on fertility. Yearling ewes exhibited the highest fertility (50%-60% conception rate), whereas lactating ewes had the poorest fertility (5% conception rate).

Repeated PMS treatments were most effective in mature and lactating ewes.

LITERATURE CITED

Allen, D. M. and Lamming, G. E. 1960. The induction of breeding activity in lactating ewes during anoestrus. J. Reprod. and Fertility 1:213.

Brunner, M. A., William Hansel and D. E. Hogue. 1964. Use of 6-methyl-17-acetoxyprogesterone and pregnant mare serum to induce and synchronize estrus in ewes. J. Animal Sci. 23:32.

Braden, A. W. H. and G. R. Moule. 1962. The induction of ovulation in anoestrous ewes. Australian J. Expt. Agric. and Animal Husb. 2:75.

Dutt, R. H. 1953. Induction of estrus and ovulation in anestrual ewes by use of progesterone and pregnant mare serum. J. Animal Sci. 12:515.

Gordon, I. 1958. The use of progesterone and serum gonadotrophin (P.M.S.) in the control of fertility in sheep. II. Studies in the extraseasonal production of lambs. J. Agric. Sci. 50:152.

———. 1963. The induction of pregnancy in the anoestrous ewe by hormone therapy. III. The use of repeated progesterone-pregnant mare's serum therapy. J. Agric. Sci. 60:67.

Pursel, V. C. and E. F. Graham. 1962. Induced estrus in anestrous ewes by use of progestogens and follicle stimulating hormone. J. Animal Sci. 21:132.

Robinson, T. J. 1959. The estrous cycle of the ewe and doe. In Cole, H. H. and P. T. Cupps (editors), Reproduction in Domestic Animals. Academic Press, New York.

———. 1960. Advances in controlled sheep breeding. Proc. New Zealand Soc. of An. Prod., p. 42.

———. 1962. Comparative studies of several gonadotrophin, progestin and oestrogen treatments in the anoestrous ewe. J. Endocrin. 24:33.

Snedecor, G. W. 1956. Statistical Methods. Iowa State College Press, Ames, Iowa.

Wagner, J. F. 1964. Hormonal control of reproductive activity in the ewe. USDA Misc. Pub. 1005.