SOME EFFECTS OF PREGNANT MARE'S SERUM
ON THE TARGHEE EWE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the
Degree Doctor of Philosophy in the Graduate School of
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Prolificacy of the ewe flock is one of the major factors in determining the profitability of a sheep enterprise. In addition to being prolific, each ewe also must be capable of raising her progeny. The underlying purpose of these two factors is maximum production, that is, the production of the greatest number of pounds of lamb per ewe per year. To attain such production, the industry has at its disposal a number of means. The most permanent mean is to select a breed or strain of sheep that has an inherent capacity for fecundity. Future genetic modification of such a breed or strain toward a more fecund capacity necessarily would be slow due to low heritability of this trait and to genetic homeostasis.

Age and longevity are other factors to be considered when attempting to increase fertility of a breed or strain. It is known that mature ewes tend to produce and raise more lambs than yearling ewes. Thus, if the number of replacement yearling ewes can be reduced and the flock can be comprised of mature fertile ewes, the overall effect would be increased lamb production.

Prolificacy can be associated with the time of the breeding season when mating takes place. Ewes generally have a higher ovulation rate near the middle of the breeding season. Twin ovulations are most frequent at this time. The seasonality in ovulation rate is indicative
of latitudes away from the equator. Sheep kept in the region of the equator show an irregular pattern in ovulation rate, as observed by McDonald (1961) and Hafez (1962).

In Ohio and surrounding states, some breeding programs are initiated in late summer. These programs have as their objectives early conception followed by early lambing and a favorable economic situation in the spring market. This practice possibly could reduce the prolificacy of the ewes involved if conception precedes the period of peak ovulation rate. In addition, late summer and early fall breeding programs can be detrimental to lamb production if associated with high ambient temperatures. It has been demonstrated by Allison and Ulberg (1961) and Dutt (1963) that exposure of ewes to high ambient temperatures (32°C) post coitus can cause a reduction in embryo survival.

Another factor which can distort a seasonal peak in ovulation is nutrition. A standard, but not well explained, management practice used to increase fertility levels is flushing. This practice normally involves feeding the ewe so that the animal is gaining weight prior to and during the breeding season, according to Kammlade and Kammlade (1955). The results of this practice can be highly variable.

The initial objective of this study was to determine whether a commercial gonadotrophin, pregnant mare's serum, could be used to increase prolificacy in the ovine female. Other objectives involved establishing an injectable level of gonadotrophin that would provide the highest economic return in terms of lamb production. In addition, a specific day of the estrous cycle had to be selected for exogenous hormone injection.
The criteria used in the selection of the gonadotrophin were that the hormone be available, relatively inexpensive and adaptable to a single injection.
LITERATURE REVIEW

The following review of literature encompasses the effects of exogenous gonadotrophin treatment in the ovine, the bovine and the rodent species. Two exogenous gonadotrophins are considered, pregnant mare's serum (PMS) and human chorionic gonadotrophin (HCG). The latter gonadotrophin has been included in the review only when the experiment cited embodies both PMS and HCG. In addition, the review of literature briefly covers various factors that can affect PMS potency. A review of progesterone levels in the body fluids and various tissues of the ovine female is included as a basis for the discussion of Objective III, D. on page 17.

Ovine Species

In an attempt to induce ovulation and estrus in sheep, Cole and Miller (1932) administered the gonad-stimulating hormone (GSH) or mare serum to ewes during anestrus. They observed that the administration of 50 ru of GSH induced ovulation but not estrus. However, if a second injection was given after a 16 day interval, both ovulation and estrus resulted. If copulation was permitted after the double injection scheme, a high percentage of the matings were fertile.

Hammond Jr. et al. (1942) administered extracts of horse pituitary gland to a band of black face ewes. The extract was given a few days prior to the regression of the corpora lutea. The injection resulted in
superovulation in the following estrus, with as many as twelve concurrent ovulations. In an attempt to reproduce these results, PMS was administered to a similar band of ewes. The results of the PMS experiment were unsuccessful. One reason for the failure possibly was the inferior quality of the ewes.

Pregnant mare's serum or both PMS and unfractionated aqueous extract of desiccated pituitary were used by Murphree et al. (1944) to treat four anestrous Montana finewool type ewes. The four ewes produced eight corpora lutea and eight ova. Contrary to these results, nine ewes of similar breeding injected with a fractionated, trypsin digested pituitary extract produced 84 corpora lutea and 25 unfertilized ova.

Wallace (1955) attempted to augment the fertility of 261 mature Romney ewes utilizing PMS. The first phase of the experiment consisting of four treatments, 000, 250 IU, 500 IU and 1000 IU of PMS administered on the 12th or 13th day of the cycle, resulted in respective ovulation rates of 1.17, 1.50, 2.07 and 4.33 ovulations per ewe. The second phase of the experiment integrated two variables, flushing and the injection of 650 IU of PMS on the 12th or 13th day of the cycle. The results of this phase did not indicate an association between the nutritional state of the ewe and the PMS treatment. It was noted also that differences in strains of sheep may be important in the ovulation response to PMS injection.

In 1958 Gordon carried out an experiment in which three forms of serum gonadotrophin were utilized. The dosage level ranged from 250 IU to 1000 IU. The experiment involved a total of 2,504 experimental ewes, nine breeds and a time span of four breeding seasons. The treated ewes
produced an average of 1.81 lambs per ewe, while the control ewes bore 1.51 lambs per ewe. For 567 ewes injected with 250 IU to 600 IU of PMS, the maximum number of lambs per litter was four. Some litters containing five or more lambs were produced in a group of 712 ewes injected with 750 IU to 1000 IU of PMS. Survival of lambs beyond the first day was inversely related to initial litter size. Percentages of lambs failing to survive beyond one day for litter sizes of one through five were 6.5, 10.5, 20.9, 38.9 and 90.6 percent respectively. At comparable dosages, the different forms of serum gonadotrophin used to induce superfecundy ranked in decreasing potency as follows: whole serum, freeze-dried serum and purified serum. At any given dosage level of PMS, the increase in fertility level induced by the treatment was greatest where the flock fertility level was low.

Averill (1958) utilized PMS treatment for the production of living sheep zygotes. The experiment involved three breeds, Border Leicestar, Welsh Mountain and Suffolk, a total of 179 ewes. The four treatments imposed upon the ewes were control (no treatment), 700 IU, 1100 IU and 1300 IU of PMS. The mean number of ovulations per ewe per treatment was 1.53, 2.77, 6.28 and 9.07 respectively. The results of the experiment indicated that a higher proportion of zygotes were found in ewes which had ten ovulations or less.

Belevickii (1959) treated each of 309 Karakul ewes with 1200 mu of PMS for three successive years. A total of 185 ewes served as a control group throughout the experiment. The number of lambs born to ewes artificially inseminated within six days after injection was greater than to the control group. The respective lambing percentages were
147.2 and 113.7 percent. It was determined by laparotomies that after 75 to 95 days of pregnancy, the ewes treated with PMS had fewer embryos than the number of corpora lutea present. In comparison, the number of embryos for the non-treated ewes corresponded to the number of corpora lutea.

Allen and Lamming (1961) investigated the interaction of PMS injection, nutrition and reproduction in Kerry Hill ewes. PMS was given subcutaneously at a level of 600 IU on the 12th or 13th day of the estrous cycle. Flushing was carried out on a rye grass, white clover pasture. When the ewes were flushed for one estrous cycle, the non-PMS treated ewes had a mean ovulation rate of $1.83 \pm 0.17$ compared to $2.17 \pm 0.17$ for the PMS treated ewes. Ewes flushed for two estrous cycles had a mean ovulation rate of $2.17 \pm 0.31$ for non-PMS treated ewes and $2.50 \pm 0.34$ for the PMS treated ewes. The differences in both treatments proved highly significant.

One hundred and five Mutton Merino ewes were utilized by Döcke (1961) in one phase of an experiment that involved injections of both PMS and the luteinizing hormone (LH). The ewes were injected with 400 ru, 800 ru, 1600 ru and 2400 ru of PMS either 12 or 14 days post estrus. One half of the ewes were injected with 250 IU of LH post coitus in the ensuing estrus. A total of 21 ewes served as a control. The results of the experiment indicated that in the fertile Mutton Merino, the exogenous hormone treatment lowered the fertility of the ewes irrespective of the hormone level. Lambing rates for the injected group were as low as 57.1 percent compared with 128.2 percent for the control group.
Grant et al. (1961) reported the effect of post estrous treatment with PMS on sheep prolificacy. A reversal trial covering two breeding seasons was established to determine the effect of 400 ru of PMS injected 14 days post estrus. Thirty Dorset ewes were utilized in this trial. The fertility level of the control group versus the treated group was 129 and 208 percent; 146 and 191 percent respectively for the two year period.

In a second trial, 26 Shropshire ewes were used to determine the time sequence in which PMS could be effective in increasing the lambing percentage. The treatments were control (no treatment), 400 ru of PMS injected 15 days post estrus and 400 ru of PMS injected 12 days post estrus. The respective lambing percentages for these three treatments were 160, 150 and 240 percent. The test indicated a slight decline in fertility level when the PMS was injected 15 days post estrus. However, an increase in the fertility level occurred when the injection took place 12 days post estrus.

Hancock and Hovell (1961) conducted an experiment involving the transfer of sheep ova. During the experiment, a limited number of donor ewes (four) were synchronized by daily intramuscular injections of 10 mg to 12.5 mg of progesterone in oil. PMS was administered on the last day of the progesterone injections. To the remaining 27 donor ewes, a super-ovulating dose of gonadotrophin was administered on the 12th or 13th day of the cycle. The dosage of PMS varied in proportion to body weight. Welsh ewes received 600 IU of PMS, while Lincoln ewes received 1100 IU of PMS. From the total band of 31 ewes, 222 corpora lutea were counted.
One hundred and thirty-five ova were recovered of which 93 were cleaved. On the basis of transformed percentages of ova shed and fertilized, there was no significant correlation between the number shed and the proportion of recovered ova that were fertilized.

At the University of Gottingen, Haring et al. (1961) attempted to increase the number of multiple births in sheep by the use of PMS. The experiment involved five commercial flocks and one pedigree flock. The breeds of sheep used were Würtemberg Merino, German Blackheaded Mutton and Leine. A total of 189 ewes were treated with 500 IU of PMS on the 14th or 15th day post estrus. The lambing percentage for the treated ewes averaged 132.8 percent compared with 107.5 percent for the control group. However, it was noted that in highly productive flocks there was no increase in conception rates.

Working at the Research Institute at Reyhjavík, Iceland, Pálsson (1962) reported a series of experiments lasting four, five and six years. The experiments were conducted to study the effect of PMS injection 12 or 13 days post estrus in Icelandic ewes. Dosage levels for the PMS injections ranged from 250 IU to 750 IU. The results showed a significant increase in multiple births when ewes received 500 IU of PMS or more. The 250 IU level appeared to have no effect. It was shown that repeated treatment with PMS in successive years had no detrimental effect on superfecundation. However, it was demonstrated that the mortality rate for lambs born to treated ewes was higher than for those born to control ewes, 10.4 percent versus 4.2 percent respectively.

Gossett et al. (1963) conducted three experiments over four consecutive years, utilizing Rambouillet and mutton breed crossbred ewes.
In the first experiment, groups of four ewes were injected with 600 IU, 800 IU or 1000 IU of PMS 14 days post estrus. The ewes were sacrificed during the subsequent estrous period, 48 hours after copulation. The average number of ova recovered was 1.75, 2.25 and 5.00 for the respective treatments. It was noted that the fertilization rate was reduced in the presence of more than the normal number of ova per ovulation. It was noted further that the average number of ova fertilized per ewe was similar for all ewes regardless of treatment.

A second band of ewes was sacrificed 40 days after mating. Prior to mating, they were divided into three groups and treated as follows: control (no treatment), 800 IU of PMS and 1000 IU of PMS. The average number of corpora lutea per ewe per treatment was 1.55, 2.57 and 3.37 respectively. However, the number of live embryos per ewe per treatment was 1.28, 1.57 and 1.63 respectively.

Four groups of ewes were allowed to go to term in a third experiment. The groups had been treated previously with 000, 500 IU, 800 IU or 1000 IU of PMS. On the average, the groups produced 1.57, 1.69, 1.60 and 1.83 lambs per ewe respectively. None of the differences were significant.

Slee (1964) conducted an experiment involving multiple mating and superovulation in sheep. Throughout the experiment, specific doses of PMS were given by intramuscular injection on the 13th day after the onset of estrus. The PMS was a freeze-dried purified preparation. The results of this experiment were variable. Six Wiltshire ewes injected with 450 IU of PMS produced 1.33 lambs per ewe. Six Lincoln ewes receiving 750 IU of PMS produced 2.16 lambs per ewe. Ten blackface ewes when
treated with 750 IU of PMS produced 2.80 lambs per ewe, whereas ten untreated ewes of the same breeding produced 1.50 lambs per ewe.

General review articles dealing with the effects of PMS have been compiled. Phillips et al. (1945) presented a review of literature treating exogenous hormone stimulation of estrus and ovulation in sheep and goats. Hancock (1962) reviewed literature pertaining to fertilization in farm animals. Included in the review is a section on fertilization in the hormone-treated ovine and bovine female.

Bovine Species

Gordon et al. (1962) administered subcutaneous doses of PMS to 525 cows. The dosage level ranging from 800 IU to 2000 IU was injected 16 to 17 days post estrus. Of the 416 cows which ovulated, 173 produced more than one ova. A total of 191 cows were permitted to go to term. One hundred and forty-seven cows bore single calves, while the remaining 44 had multiple births.

Schilling and Holm (1963) attempted to induce limited multiple ovulation in cattle with varying proportions of progesterone, luteinizing hormone and PMS. Of the 13 cows which received only PMS (2000 IU to 2500 IU), one cow failed to ovulate, eight cows ovulated one ova and four animals ovulated three or more ova. Considering all three treatments, the cows receiving only PMS had the largest number of multiple ovulations.
Rodent Species

Greenwald (1962) observed that hamsters injected with 30 IU of PMS on day one of the estrous cycle were superovulated. It was noted that both the duration of ovulation and the cycle length were prolonged with PMS injection. If 30 IU of PMS were injected on day two or three, cycle length was increased and the number of ova shed was approximately one third that of the superovulated animals. Similar treatment on day four resulted in the ovulation of ten ova, one seventh that of the superovulated females. It was concluded that the ovary of the hamster normally has ten large follicles on day one, but has a reserve of 25 follicles that can be stimulated by PMS injection.

Edwards and Fowler (1960) utilized PMS, human chorionic gonadotrophin and a combination of these gonadotrophins to induce superovulation in mice. In the segment of the experiment concerned with PMS, the results indicated that 3 IU of PMS induced ovulation but not superovulation in diestrous mice autopsied 24 hours after injection. Sixty to sixty-two hours after the injection, thirty percent of the mice exhibited estrus and had superovulated. A positive correlation was found between mating and ovulation in these mice. Further investigation revealed that the corpora lutea in the superovulated-mated mice were functional, and that fertilization and embryonic development were normal.

Various placental gonadotrophins were utilized by Lamond (1960) to induce ovulation in mature diestrous albino mice. The dose response lines of the gonadotrophins, PMS and human chorionic gonadotrophin, were parallel over a limited part of the range. High doses of HCG appeared to inhibit ovulation, while high doses of PMS brought about superovulation.
in the diestrous mice. This study in combination with results obtained previously by the author indicated that the ratio of follicle stimulating hormone and luteinizing hormone in PMS is of the order of five to one.

Imamichi et al. (1961) studied the effects of hypophysectomy, sham operation and ether anesthesia on ovulation induced by human chorionic gonadotrophin or PMS in adult rats. The gonadotrophin was injected immediately after the operation or the inhalation of ether. Twenty hours after the treatment, the tubal ova were inspected by transmitted light. The results of the experiment indicated that there was a weak inhibitory effect on induced ovulation by either the sham operation or the ether anesthesia. Hypophysectomy showed a more pronounced inhibitory action on rats receiving PMS than on those receiving HCG. This indicated a PMS-hypophysis-gonad axis, PMS being more dependent on hypophyseal gonadotrophin secretion than HCG. The potency of PMS to induce ovulation was one tenth that of HCG.

McCormack and Meyer (1962) found that the number of ovulations induced in 25 day-old rats by an injection of PMS was reduced significantly by the administration of sodium barbital at 1:30 pm on the 24th day of life. However, if the injection of sodium barbital was postponed until 5:30 pm on the 24th day of life, the number of ovulations approached that of the non-barbiturate treated controls. The data was interpreted to mean that the ovulating hormone was released from the hypophysis between 1:30 and 5:30 pm on the 24th day of life.

Quinn and Zarrow (1964) treated twenty-four day-old Purdue rats with 30 IU of PMS to induce superovulation. The results of the experiment indicated that the induced ovulation could be blocked by
hypophysectomy and by such pharmacological agents as atropine sulfate, chlorpromazine, sodium nembutal and N-(9-fluorenyl)-N-ethyl-beta-chloroethylamine hydrochloride. It was concluded that PMS-induced ovulation in the immature rat appeared to involve a neuroendocrine mechanism.

Igarashi and McCann (1964) have demonstrated an increase in plasma level of follicle stimulating hormone (FSH) in ovariectomized, estrogen, progesterone blocked rats by an injection of stalk-median eminence. The authors had demonstrated previously an increase in plasma luteinizing hormone level using an extract of the stalk-median eminence. It was shown that extracts from the cerebral cortex had no effect on plasma FSH level. It was concluded that the stalk-median eminence extract stimulated the secretion of FSH from the adenohypophysis.

Factors Affecting PMS Potency

In 1930 Cole and Hart tested the potency of pregnant mare's serum for its content of adenohypophyseal hormones. The criterion used to determine serum potency was the increased weight of ovaries of immature rats after injection of the serum. Non-pregnant mares and mares pregnant up to the 37th day gave a negative test. Variation in individual mare's serum potency was encountered between 37 and 42 days of pregnancy. Mares that were 43 to 80 days pregnant possessed the greatest serum potency. Beyond 80 days of pregnancy, the serum elicited uterine and vaginal changes without stimulating an increase in ovarian weight.

Graham et al. (1960) showed that PMS lost its biological activity when incubated with hydroquinone and quinones. Further, it was established that the hydroquinones had to be oxidized to quinones to
inactivate PMS. Relative anti-gonadotrophic activity of the quinones showed p-benzoquinone to be more active than the mono-substituted quinones. The di- and tetra-substituted quinones had very little activity.

Presson and Melander (1961) studied the effect of the pH of the PMS injection vehicle on the uterine weight assay of immature rats and mice. Citrate, phosphate or borate buffers were used to vary the pH of the injection vehicle from pH 4 to pH 10. It was noted that the greatest uterine weight response occurred at pH 7, with the least response at pH 4. It was shown that as the level of PMS approached the higher dosage (0.5 IU for mice and 10 IU for rats), the pH of the carrier was less critical.

**Progesterone in the Ovine Female**

By chemical assay methods, Edgar (1953) established that progesterone could be detected in the blood of the vein draining the active ovary in the non-pregnant and pregnant ewe. Using the same assay method, no detectable progesterone was found in the ovarian venous blood of a ewe with inactive ovaries. Negative results in the detection of progesterone in the peripheral blood of the non-pregnant and pregnant ewe were noted also.

Edgar and Ronaldson (1958) reported blood levels of progesterone in cyclic and pregnant ewes. The blood sample was taken from the vein draining the active ovary. On the third day of the cycle, only a detectable amount of progesterone was found. The mean concentration of progesterone increased to about 1.8 mcg/ml on the seventh day. This level was
maintained until the 16th day of the cycle but decreased to 0.15 mcg/ml on the 17th day. Until the 17th week of pregnancy, the blood from an active ovary possessed the same mean level, 1.8 mcg/ml, as an active cyclic ovary.

Short and Moore (1959) determined the amount of progesterone and 20 alpha hydroxypregn-4-en-3-one in the peripheral blood and placenta of ewes. During pregnancy, the peripheral blood plasma had a mean concentration of 0.41 mcg of progesterone per 100 ml of blood and 0.37 mcg of 20 alpha hydroxypregn-4-en-3-one per 100 ml of blood. Placental tissue of intact and ovariectomized ewes had progesterone concentrations that varied from 4 mcg to 9 mcg/kg of tissue and 20 alpha hydroxypregn-4-en-3-one concentrations that varied from 9 mcg to 12 mcg/kg of tissue. Both progesterone and 20 alpha hydroxypregn-4-en-3-one occurred at the higher concentrations in the ovariectomized ewes.
EXPERIMENTAL

The objectives of this experiment were to determine the following:

I. Whether pregnant mare's serum (PMS) could be used to increase the fertility level of Targhee ewes.

II. The dosage level of PMS and a time schedule for injection that would satisfy a criterion established in the Introduction, "highest economic return in terms of lamb production."

III. The effects of various dosage levels of PMS administered on the 13th day of the cycle in relation to:
   A. Type and number of follicles.
   B. Type and number of corpora lutea.
   C. Fertilization capacity of ova.
   D. Progesterone content of corpora lutea.

Breed, Number and Allotment of Ewes

At the initiation of the study in August 1961, a total of 105 Targhee ewes on pasture were assigned to the project. The animals ranged in age from one to eight years. The basic experimental design involved seven treatments: four levels of PMS given 13 days post estrus, two levels of PMS given 15 days post estrus and a control group.
Each treatment group consisted of 15 ewes allotted randomly within each age group.

In 1962, 26 of the original 105 Targhee ewes were reassigned for further experimentation. Fourteen ewes of similar breeding were added to this group, providing a total of 40 animals. Sixteen of these animals were divided into four equal groups. Three groups were treated with PMS, while one group served as a control. Laparotomies were performed to examine the effect of the exogenous hormone treatment on size, number and type of follicle and on number and type of corpora lutea. Four ewes, selected from the group of 40, were subjected to laparotomy and cannulation of the oviduct in an attempt to recover the ovum or the zygote. The remaining 20 ewes were divided into four equal groups and treated with various levels of PMS. Thirteen days post coitus the ewes were sacrificed, reproductive tracts were removed and luteal tissue was recovered for subsequent chemical analysis.

Management and Feeding of the Ewe Flock

In the 1961 experiment, the ewe flock was rotated on three basic pastures in the following sequence: a ladino clover pasture, a legume pasture containing volunteer white dutch clover and a blue-grass pasture. During this period, the ewes received additional mixed hay when pasture conditions warranted supplementary feed. Water and a phenothiazine-salt mix (1:9) were provided in an open-end shelter. The ewes were drenched with phenothiazine for parasite control while on pasture.

In the 1962 experiment, the flock was quartered in the barn in an area adjacent to the laboratory facilities. Throughout this period
the ewes received a mixed hay allowance, while also having access to legume pasture. Both water and the 1:9 phenothiazine-salt mix were provided at the barn.

Gonadotrophin

The exogenous gonadotrophin used in this study was a commercial pregnant mare's serum, Gonadin by Lockhart. Two separate lots were obtained from the distributor for each experiment, 1961 and 1962. The manufacturer's stated potency was used to determine the various dosage levels employed in this study.

Procedure

The 1961 experimental design involved seven treatments: 560 ru, 700 ru, 840 ru and 980 ru of PMS given 13 days post estrus; 700 ru and 840 ru of PMS given 15 days post estrus; and a control group.

To establish information on estrous cycle manifestations prior to treatment and breeding, vasectomized rams wearing Jourgensen harnesses with crayons were used. Thirteen days prior to the first PMS injection and thereafter, the vasectomized rams were employed under observation twice each day, 8:00 am and 4:00 pm, to determine the estrous animals in the flock.

Injections with PMS began on September 7th. The injection route was a subcutaneous injection in the axillary region. All treated ewes exhibiting estrus after the above date were bred to one of four Columbia rams. Initially, the 15 ewes in each treatment group were randomly allotted to one of three Columbia rams, so that the ratio of five ewes
per ram per treatment was satisfied as nearly as possible. During the experiment, one ram from the original group of three was declared infertile by a routine semen check. As the result, a fourth ram was substituted.

To insure breeding, one observed copulation was recorded when the ewe was placed with the ram. As a routine procedure, the ewe remained with the ram for the duration of the estrous period. If a ewe failed to conceive on the first exposure, one estrous period was skipped and the above injection procedure, depending on treatment, was repeated in the subsequent estrous cycle. One ewe from the re-treated group experienced an anaphylactic reaction upon the second injection of PMS. Recovery was effected by the administration of epinephrine.

The last injection date was November 13th. Thereafter, the flock was returned to the physiology section of the main barn. Lambing procedures started on January 28th.

The 1962 experimental procedures involved three phases. The 13 day post estrus injection schedule was followed in all phases. This schedule was selected on the basis of the data from the 1961 experiment and literature values of Gordon (1958), Hancock and Hovell (1961) and Pálsson (1962).

During the first phase, the objective was to secure information regarding follicular and luteal development in relation to dosage level of PMS. Four treatments were used: 560 ru, 980 ru and 1400 ru of PMS, and a control group. Seventy-two hours after the estrous period subsequent to the injection, a laparotomy was performed.
In preparation for the laparotomy, the animal was fasted for 24 hours. At the time of the operation, sodium pentobarbital was administered intravenously via the jugular vein. The dosage level of sodium pentobarbital was \(1/5\) grain per pound of body weight. Approximately one half of the calculated dose was administered rapidly and the remaining portion was injected slowly until no further forearm or eye reflex was evoked.

After the desired state of narcosis was reached, the ewe was placed on a table equipped with a central longitudinal trough. The legs of the animal were secured. The operative field was clipped and the area scrubbed. Then a solution of 70 percent ethyl alcohol was applied to the area, followed by a mild tincture of iodine (a 2 percent solution of iodine combined with a 2.4 percent solution of sodium iodide in 50 percent ethyl or isopropyl alcohol). To prevent regurgitation and aspiration of ruminal fluids and to aid in the exteriorization of the reproductive organs, the hind quarters of the animal were elevated at a 15° angle by tilting the table. Thereafter, the operating procedure was that of a median line laparotomy, as described by Casida (1959). The operation was performed using sterile technique.

During the operation, once access was gained into the abdominal cavity, both ovaries and the respective horns were exposed. For each ovary, the size and type of every follicle and the type of every corpus luteum was recorded. Size was determined by measuring the exposed diameter of the follicle with a pair of calipers. The criteria used for classifying the follicular types were as follows:

1. Follicle translucent in appearance
2. Same as type one, except for a small transparent area on follicular surface
3. Liquor folliculi dark red

The corpora lutea, as described by Hadek (1958), were classified using the following standards:

1. Follicular cavity filled with blood
2. Corpus luteum dark red
3. Corpus luteum red to pink
4. Corpus luteum pinkish-yellow
5. Corpus luteum white

These classifications have been confirmed since then by Restall (1964).

Upon completion of the operation, the ewe was placed in a confined area in the barn. After several days of observation, the ewe was allowed to return to the flock.

The second phase of the 1962 experiment was the cannulation of the oviduct and recovery of the ovum or zygote. As in the first phase, a median line laparotomy was performed. To ensure recovery of the ovum or zygote, the operation took place between 54 and 72 hours post estrus, a time sequence suggested by Anderson (1938) and by Edgar and Asdell (1960). During the operation the ovaries, oviduct, horns and a small segment of the uterus were exposed. The fimbria was detached from the ovary and the lumen to the oviduct was located. A polyethylene tube, 0.2 cm in diameter by 15 cm long was inserted approximately 2 cm into the oviduct. The tube, as designed by Dr. D. Redman and the author, was flanged at one end to permit its retention in the oviduct without any ligation. A 5 cm watch glass was used at the distal end of the tube to collect the washings.

To wash the tract, gentle pressure was applied to the utero-tubal junction of the horn with thumb and forefinger. Normal saline solution
at body temperature was introduced into the junction with a 10 cc syringe and a 22 gauge needle (refer to Figures 1 and 2). Washings from the tract were examined using a stereomicroscope with a light source from the sub-stage reflecting mirror. A non-segmented ovum was classified as unfertilized; a segmented ovum, as a zygote. These classifications were carried out during the operation, as the washings were recovered. After the operation, the ewe was handled in the same manner as in the first phase.

The third phase involved the collection of luteal tissue through a sacrifice program 13 days post coitus. The quantitization of progesterone extracted from the luteal tissue required the development of a suitable semi-micro method of analysis. The tissue was analyzed for its progesterone content in relation to treatment, either 560 ru, 980 ru or 1400 ru of PMS given 13 days post estrus, or a control.

In the procedure for sacrifice, the ewe was stunned through use of electrical shock and then the jugular vein was severed. Upon death of the animal, the abdominal cavity was opened immediately and the complete reproductive tract was removed. The ovaries were removed from the tract and sectioned. Upon separation from all adhering ovarian tissue, the luteal tissue was placed immediately in 50 ml of 95 percent ethyl alcohol, maintained at 0°C and stored for future chemical analysis.

The semi-micro analysis for progesterone involved the homogenization and extraction of the luteal tissue, column chromatography, thin-layer chromatography and the quantitization of progesterone using a fluorometric technique. To avoid procedural errors, all glassware was placed in a solution of concentrated sulfuric and nitric acids (3:1 v/v).
Figure 1. Stage Prior to Insertion of Polyethylene Tube into Fimbria
Figure 2. Flushing Ova from Oviduct
overnight. Prior to use, the glassware was rinsed in distilled water and air dried. All solvents were glass distilled.

Prior to the homogenization of the luteal tissue from each ewe, the tissue was divided and weighed. One portion was placed in a tared aluminum weighing can. The can was placed in a gravity air-fed drying oven (95° to 97°C) until the tissue was dried to a constant weight. The average moisture content of equivalent sections of luteal tissue was 73 percent, the range extending from 52 percent to 82 percent. Although most literature values of hormone content have been based on wet tissue weight, the progesterone assay in this experiment is reported on a dry tissue weight basis.

The remaining portion of tissue was placed immediately in a test tube with approximately 8 ml of the ethyl alcohol in which it was stored. A Potter-Elvenhjen Homogenizer was used to homogenize the tissue. The homogenate was transferred quantitatively into a 500 ml round-bottom flask. Additional ethyl alcohol was added to the remaining storage ethyl alcohol to bring the volume of solvent in the flask to 75 ml. The homogenate was allowed to reflux for two hours.

After refluxing, the supernatant was decanted into a second 500 ml round-bottom flask. The storage container was rinsed with seven 10 ml aliquots of ethyl alcohol which were added to the solute remaining in the initial flask. The tissue sample was refluxed then for an additional hour. The time sequence employed in the refluxing procedure was suggested by Hansel (in litt.).

The supernatants were combined and taken down to dryness under vacuum. The use of a flash evaporator led to contamination from bearing
oil and stopcock grease, a problem similar to that encountered by Hansel (in litt.). Therefore, it was necessary to resort to an evaporator with Teflon-glass construction, the Model C-High Vacuum Evaporator (California Laboratory Equipment Company). After evaporation, the ethanolic extract was ready for column chromatography.

The chromatographic procedure of Loy et al. (1957, 1960) was employed. A slurry of 5 gm of adsorption alumina, 80-200 mesh (Fisher Scientific Company), in petroleum naphtha (Skelly Oil Company) was poured into a chromatographic column (10 mm x 250 mm) fitted with a porous glass plug. The ethanolic extract was redissolved in 20 ml of petroleum naphtha by allowing the material to stand for one half hour. This solution was applied to the column followed by two 15 ml aliquots of petroleum naphtha used to rinse the extract flask. The column then was developed with 100 ml of petroleum naphtha, followed by 100 ml of 10 percent chloroform-petroleum naphtha and finally by 100 ml of 25 percent chloroform-petroleum naphtha. The last fraction was collected in a 200 ml round-bottom flask. Prior to use, the petroleum naphtha was purified using sulfuric acid and saturated aqueous potassium permanganate.

Following the separation of the steroids from the lipid fraction of the luteal tissue, the steroid eluent was evaporated to dryness using the high vacuum evaporator. To concentrate the eluate for further chromatographic separation, the material was transferred quantitatively from the round-bottom flask to a 5 ml tube with methyl alcohol. After the transfer was complete, the methyl alcohol was evaporated to dryness under a stream of nitrogen.
Previous experiments, Bush 1952, Staples and Hansel 1961, used paper chromatography to separate steroids. To avoid disadvantages inherent in the paper chromatographic system, a prolonged time per run (often 12-16 hours) and a low, variable recovery rate, thin-layer chromatography was selected for this experiment.

The chromatographic plates were prepared by spreading a slurry of Silica Gel HF254 (Brinkman Instruments Incorporated) over a glass plate measuring 200 mm x 200 mm. A Fixed Thickness Chromatofilm Assembly (Research Specialties Company) was used to spread the slurry to a thickness of 250 microns. The plates were activated by exposure in an air-conditioned room, 20°C, for a minimum of three hours.

Using chloroform as the solvent, the entire sample was spotted quantitatively on one chromatoplate, along with a progesterone standard. The solvent system of Bennett and Heftmann (1962), chloroform, methyl alcohol and water, was modified in the proportions 980:20:1 to provide effective polarity for separating the steroids. When development occurred to within 2.5 cm from the top edge, the chromatoplate was removed from the tank and allowed to air dry. The progesterone spots were located using a Mineralight with a wave length of 2537 angstroms.

After detection, the progesterone was extracted from the chromatographic silica gel using a method devised during this experiment. The progesterone-containing silica gel was scraped with a metal spatula from the chromatoplate into a centrifuge tube. Upon the addition of 1 ml of methyl alcohol, the contents of the tube were mixed using a Vortex mixer. The tube was allowed to stand for 15 minutes. Then the suspension was centrifuged at 1500 rev/min for 10 minutes. The supernatant was decanted
into a 25 ml Erlenmeyer flask. The preceding procedure was repeated twice and the three washings combined. The methyl alcohol-progesterone solution was taken to dryness under a stream of nitrogen. A blank was prepared by scraping silica gel which contained no organic fluorescing material from the chromatoplate and treating it in an identical fashion.

Fluorescence of progesterone was determined by the method of Touchstone and Murawee (1960). Upon evaporation of the methyl alcohol, 0.5 ml of 2 N methanolic potassium hydroxide (2 N KOH) was added to the progesterone extract. The solution was permitted to stand for 10 minutes. After being heated in a 60°C water bath for 30 minutes, the solution was allowed to cool to room temperature. Then 0.5 ml of 88 percent sulfuric acid was added and this mixture was permitted to stand at room temperature for 15 minutes.

Fluorescence was measured using a Model 110 Turner Fluorometer. The filter system used in conjunction with the fluorometer was modified to enhance the fluorescence of progesterone. A narrow-pass primary filter which peaked at 360 millimicrons was substituted into the system, as suggested by Phillips (in litt.). In use with the narrow-pass secondary filter which peaked at 495 millimicrons, the author found it necessary to introduce a one percent transmission filter to decrease sensitivity.
RESULTS

The main objective of the 1961 experiment was to determine the effect of PMS injection on the fertility of Targhee ewes. Upon inspection of Tables 1 and 2, it will be noted that the highest lambing percent occurred in the control group. The greatest discrepancies, as seen in Table 1, occurred in the treatment groups of 980 ru at 13 days and 840 ru at 15 days post estrus. In comparing the lambing percent of pregnant ewes with the lambing percent based on live lambs, the difference found in column four resulted from one ewe giving birth to a set of dead triplets. The variation found in column six between the lambing percent of exposed ewes and that of pregnant ewes resulted from two ewes being non-pregnant. One of these ewes was exposed to a fertile ram late in the breeding season, but copulation was not observed.

Table 1. Lambing Percent in Relation to Treatment of Ewes with PMS, 13 and 15 Days Post Estrus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days post estrus</th>
<th>13</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMS, ru</td>
<td>560</td>
<td>700</td>
</tr>
<tr>
<td>No. of ewes exposed</td>
<td>14</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Lambing percent</td>
<td>121</td>
<td>129</td>
<td>113</td>
</tr>
<tr>
<td>No. of pregnant ewes</td>
<td>13</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Lambing percent</td>
<td>131</td>
<td>129</td>
<td>121</td>
</tr>
<tr>
<td>Lambing percent based on live lambs</td>
<td>123</td>
<td>129</td>
<td>114</td>
</tr>
</tbody>
</table>
Table 2. Birth Condition in Relation to Treatment of Ewes with PMS, 13 and 15 Days Post Estrus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days post estrus</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS, ru</td>
<td>560</td>
<td>700</td>
<td>840</td>
</tr>
<tr>
<td>No. of singles</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Sets of twins</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sets of triplets</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*aBorn prematurely and dead at time of parturition.

The data from Table 1 for ewes exposed were subjected to an analysis of variance, refer to Table 3. Although the initial data, Table 1, tended to indicate a difference in fertility levels between the treated groups and the control, the analysis of variance revealed a non-significant difference among treatments.

Table 3. Comparisons Among Means for the Number of Lambs Born to Exposed Ewes Treated with PMS 13 and 15 Days Post Estrus

<table>
<thead>
<tr>
<th>Analysis of Variance</th>
<th>Source of variation</th>
<th>df</th>
<th>ss</th>
<th>ms</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>6</td>
<td>1.81</td>
<td>.3016</td>
<td>.86 ns</td>
</tr>
<tr>
<td></td>
<td>Control vs. treated</td>
<td>1</td>
<td>1.01</td>
<td>1,1000</td>
<td>3.14 ns</td>
</tr>
<tr>
<td></td>
<td>Among treated</td>
<td>5</td>
<td>.71</td>
<td>.1420</td>
<td>.46 ns</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>93</td>
<td>32.43</td>
<td>.3487</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>99</td>
<td>34.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lamond (1960) indicated that PMS may have an effect on the cycle length as well as on the fertility level. In this study, it was observed that the average length of the first estrous cycle of the experimental period was 16.8 to 17.6 days for the treated groups and 16.8 days for the control group. The average length of the estrous cycle for repeat breeders was 15.0 to 18.0 days for the treated groups and 16.1 days for the control group. Refer to Table 4.

Table 4. Average Length and Range of Estrous Cycle in Relation to Treatment with PMS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days post estrus</th>
<th>13</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMS, ru</td>
<td>560</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>840</td>
<td>980</td>
</tr>
<tr>
<td></td>
<td></td>
<td>700</td>
<td>840</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

First cycle:
Number of observations: 13
Average length**: 17.6
Range: 16-20

Number of observations: 14
Average length**: 17.1
Range: 16-21

Number of observations: 15
Average length**: 16.8
Range: 15-18

Number of observations: 15
Average length**: 17.0
Range: 14-23

Number of observations: 14
Average length**: 16.9
Range: 16-19

Number of observations: 14
Average length**: 17.1
Range: 16-18

Number of observations: 12
Average length**: 16.8
Range: 16-19

Second cycle:
Number of observations: 1
Average length**: 15.0
Range: 16-19

Number of observations: 1
Average length**: 18.0
Range: 16-19

Number of observations: 4
Average length**: 17.2
Range: 16-19

Number of observations: 4
Average length**: 17.2
Range: 15-17

Number of observations: 3
Average length**: 16.0
Range: 16-17

Number of observations: 1
Average length**: 16.0
Range: 16-17

Number of observations: 6
Average length**: 16.1
Range: 15-17

*Estrous cycle length was calculated from the first day post estrus to the first day of the subsequent estrus.

A more direct approach in determining whether the injection of PMS had influenced the length of the estrous cycle was to note the time elapsing between the injection and the beginning of the subsequent period. If 17 days was taken as the average length of the estrous cycle and if the injections were given 13 and 15 days post estrus, then the majority of ewes normally should have shown signs of estrus four days and two days
later respectively. In referring to Graphs I and II, it will be observed that the onset of estrus followed the normal pattern. For the first treatment group the average incidence of estrus occurred at 4.1 days, and at 1.9 days for the second treatment group.

The 1962 experiment was divided into three phases. The first phase involved four treatments consisting of four ewes each, the laparotomy of all animals and the inspection of the ovaries of each animal. The size, number and type of follicles and the number and type of corpora lutea were recorded for each ovary.

In the analysis of the data, both follicle number and corpora lutea number have been considered. The results are summarized in Table 5.

Table 5. Follicle\(^a\) and Corpus Luteum\(^b\) Number in Relation to Treatment and Ovary

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>ss</th>
<th>ms</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle number replication</td>
<td></td>
<td>192.3438</td>
<td>64.1146</td>
<td></td>
</tr>
<tr>
<td>Treatment with PMS, day 13 (A)</td>
<td></td>
<td>84.0934</td>
<td>28.0313</td>
<td>1.04 ns</td>
</tr>
<tr>
<td>Error (a)</td>
<td></td>
<td>242.2812</td>
<td>26.9201</td>
<td></td>
</tr>
<tr>
<td>Ovary (B)</td>
<td></td>
<td>.7812</td>
<td>.7812</td>
<td>.024 ns</td>
</tr>
<tr>
<td>A X B</td>
<td></td>
<td>16.1932</td>
<td>5.3977</td>
<td>.226 ns</td>
</tr>
<tr>
<td>Error (b)</td>
<td></td>
<td>286.5256</td>
<td>23.8771</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>822.2184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus luteum number replication</td>
<td></td>
<td>1.25</td>
<td>.4166</td>
<td></td>
</tr>
<tr>
<td>Treatment with PMS, day 13 (A)</td>
<td></td>
<td>1.25</td>
<td>.5833</td>
<td>1.31 ns</td>
</tr>
<tr>
<td>Error (a)</td>
<td></td>
<td>4.00</td>
<td>.4444</td>
<td></td>
</tr>
<tr>
<td>Ovary (B)</td>
<td></td>
<td>.50</td>
<td>.5000</td>
<td>.32 ns</td>
</tr>
<tr>
<td>A X B</td>
<td></td>
<td>3.75</td>
<td>1.2500</td>
<td>.71 ns</td>
</tr>
<tr>
<td>Error (b)</td>
<td></td>
<td>18.75</td>
<td>1.5625</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Types 1 - 3.

\(^b\)Types 1 - 4.
Graph I. Range and Distribution of Days Between Injection 13 Days Post Estrus and Date of Subsequent Estrus
Graph II. Range and Distribution of Days Between Injection 15 Days Post Estrus and Date of Subsequent Estrus
The analysis has indicated that the number of follicles or corpora lutea did not differ significantly among treatments or between the left and right ovary.

Table 6 presents the mean number of follicles and corpora lutea per ovary by treatment groups. The data have indicated that while the treatment group of 560 ru of PMS had the largest number of follicles, it also had the least number of corpora lutea. The largest number of corpora lutea occurred in the treatment group of 1400 ru of PMS.

Table 6. Mean Number of Follicles and Corpora Lutea per Ovary by Treatment Group

<table>
<thead>
<tr>
<th>Treatment with PMS, 13 days post estrus</th>
<th>Control</th>
<th>560 ru</th>
<th>980 ru</th>
<th>1400 ru</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of follicles</td>
<td>8.875</td>
<td>12.625</td>
<td>8.625</td>
<td>9.250</td>
</tr>
<tr>
<td>Mean number of corpora lutea</td>
<td>.875</td>
<td>.750</td>
<td>1.000</td>
<td>1.376</td>
</tr>
</tbody>
</table>

\(^a\)Types 1 - 3.

\(^b\)Types 1 - 4.

In comparing the different types of corpora lutea recorded, Tables 5 and 6, it was necessary to recognize the rapid gross morphological changes which occurred after the graafian follicle ruptured. According to Hadek (1958), the color of the corpus luteum changed from red to pink beginning 72 hours post ovulation. Sixteen days later when the new graafian follicle ruptured, the one-cycle-old corpus luteum appeared whitish-yellow.
Thus in the present experiment, the corpora lutea designated as types one through four represented different stages of the most recent ovulation. Type five corpora lutea were associated with previous ovulations.

With respect to the data in Tables 5 and 6, it was interesting to compare follicular size and type by treatment groups. Graph III shows the diameter of the follicle in relation to treatment. A general and obvious trend appeared, that is, those treatments with the greatest number of follicles possessed fewer follicles of large diameter.

In a like manner, if type of follicle was related to treatment, the 980 ru group possessed the largest number of follicles classified as type two. However, the 1400 ru treatment group which had the largest number of corpora lutea also possessed the greatest number of type one follicles. Refer to Table 7.

Table 7. Follicular Types\textsuperscript{a} (in Percent) and Range in Size\textsuperscript{b} as Related to PMS Treatment, 13 Days Post Estrus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Ewes</th>
<th>Type 1</th>
<th>Range in Size (cm)</th>
<th>Type 2</th>
<th>Range in Size (cm)</th>
<th>Type 3</th>
<th>Range in Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>68</td>
<td>.75-.10</td>
<td>7</td>
<td>.62-.42</td>
<td>26</td>
<td>.45-0\textsuperscript{c}</td>
</tr>
<tr>
<td>560 ru</td>
<td>4</td>
<td>66</td>
<td>.65-.10</td>
<td>3</td>
<td>.95-.35</td>
<td>31</td>
<td>.63-.11</td>
</tr>
<tr>
<td>980 ru</td>
<td>4</td>
<td>55</td>
<td>.50-.0</td>
<td>13</td>
<td>.82-.31</td>
<td>32</td>
<td>1.02-0</td>
</tr>
<tr>
<td>1400 ru</td>
<td>4</td>
<td>79</td>
<td>.78-.11</td>
<td>1</td>
<td>.70</td>
<td>19</td>
<td>.72-.20</td>
</tr>
</tbody>
</table>

\textsuperscript{a}See pages 21 and 22 for definition of follicular types.

\textsuperscript{b}Follicular diameter.

\textsuperscript{c}Zero (0), nonmeasurable due to minute size.
Graph III. Diameter of Follicle in Relation to Treatment, Based on Four Ewes per Treatment Group
The objective of the second phase of the 1962 experiment was to determine whether treatment with PMS had an effect on the fertilization capacity of the ovum. As stated in the procedure, the ovum or zygote recovery took place between 54 and 72 hours post estrus. Therefore, timing was essential. Resulting from the lack of personnel, the operation had to be cancelled on several occasions. However, three of four recoveries attempted were successful. The results are summarized in Table 8.

Table 8. Number of Ova or Zygotes Recovered by Cannulation of the Oviduct

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal No.</th>
<th>Number of Corpora Lutea</th>
<th>Number of Ova or Zygotes</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>980 ru, day 13</td>
<td>568</td>
<td>2</td>
<td>2 zygotes</td>
<td>4 cells each</td>
</tr>
<tr>
<td>980 ru, day 13</td>
<td>724</td>
<td>3</td>
<td>1 ovum</td>
<td>non-segmented</td>
</tr>
<tr>
<td>Control</td>
<td>96</td>
<td>1</td>
<td>1 zygote</td>
<td>8 cell stage</td>
</tr>
<tr>
<td>Control</td>
<td>400</td>
<td>1</td>
<td>1 zygote</td>
<td>8 cell stage</td>
</tr>
</tbody>
</table>

With regards to ewe number 724, one ovum was recovered from the right fallopian tube. The right ovary possessed one active corpus luteum. However, cellular debris accumulated in the area of the fimbria of the left fallopian tube, prohibiting the expulsion of the ova. The left ovary of this animal possessed two active corpora lutea.

The third phase of the 1962 experiment involved the quantitization of progesterone extracted from luteal tissue. Tissue was obtained from
both PMS-treated ewes and control ewes. A composite sample of luteal tissue from each ewe was analyzed by the semi-micro method described in the procedure. The minimum quantity of luteal tissue which could be analyzed by this method was approximately 80 mg on a dry weight basis. In this experiment, one ewe from each treatment group had less than the minimum quantity of tissue on the dry weight basis.

Of the figures shown in Table 9, the numerator represents the average total progesterone content of the luteal tissue by treatment and classification. The denominator represents the range of the total progesterone content of samples in each category. Both numerator and denominator are expressed in mcg/mg of dry tissue.

Table 9. Average Total Progesterone Content of Classified Luteal Tissue from Control and PMS-Treated Ewes, 13 Days Post Estrus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>560 ru</th>
<th>980 ru</th>
<th>1400 ru</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 3</td>
<td>164.37</td>
<td>142.21</td>
<td>152.47</td>
<td>118.83</td>
</tr>
<tr>
<td></td>
<td>164.37</td>
<td>123.18 - 161.00</td>
<td>117.27 - 186.67</td>
<td>118.83</td>
</tr>
<tr>
<td>Type 4</td>
<td>3.75</td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 3d</td>
<td>10.00</td>
<td>25.00</td>
<td>16.75</td>
<td>25.00</td>
</tr>
<tr>
<td>with Atrium</td>
<td>10.00</td>
<td>25.00</td>
<td>6.50 - 27.00</td>
<td>25.00</td>
</tr>
<tr>
<td></td>
<td>35.00</td>
<td>27.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aNon-adjusted figures, based on 80% recovery rate.

*bSee pages 21 and 22 for classification.

*cMal-function of flash evaporator caused loss of one sample.

dSample acquired definite yellow color after addition of 88% H₂SO₄.
The Type 3\textsuperscript{d} classification of luteal tissue involved three separate samples. The yellow color developed after the addition of the 88 percent sulfuric acid to the progesterone extract in 2 N KOMe. Since the 2 N KOMe was prepared prior to each analysis, it has been theorized that the color resulted from a contaminate in the KOMe or its container. From the analysis, it appeared that the yellow color quenched the intensity of fluorescence.
DISCUSSION

A review of the literature section dealing with the ovine species has revealed experiments in which the results of PMS treatment could be classified. Averill (1958), Belevickii (1959), Allen and Lamming (1961), Grant et al. (1961), Pálsson (1962) and Gossett et al. (1963) established that PMS injection increased the ovulation rate and/or prolificacy of treated ewes as compared with untreated ewes. The experimental results of Wallace (1955), Gordon (1958) and Haring et al. (1961) indicated that strain differences may be important in explaining the ovulation response to PMS injection, since PMS was effective in increasing prolificacy in some cases but not in others. The report by Döcke (1961) indicated that treatment of ewes with PMS lowered the fertility of the animals irrespective of the gonadotrophin level.

The findings of this experiment have shown that injection of Targhee ewes with PMS, at various dosage levels 13 and 15 days post estrus, did not increase significantly the fertility level of the animals. However, it will be noted, Table 1, that the ewes treated with PMS had a lower lambing percent than the control ewes. This depression of the fertility level in the treated ewes, although non-significant, was consistent. The lack of significance could be the result of the small number of animals (15) per treatment group. It should be pointed out that the Targhee breed used in the experiment had as a strain average a
lambing rate equal to or greater than 1.5 lambs per ewe. On this basis, the lowered fertility of the PMS-treated ewes has been considered pertinent.

Information provided by laparotomies showed that neither follicle number nor corpus luteum number was increased significantly by PMS treatment 13 days post estrus, Table 5. However an examination of the data in Table 6 revealed a nearly inverse relationship between the mean number of follicles and the mean number of corpora lutea of PMS-treated ewes. The lowest dosage level of PMS, 560 ru, appeared to stimulate follicular development. The highest dosage, 1400 ru, seemed to exhibit both a follicle stimulating capacity and a luteinizing effect. The transition dosage level appeared to be 980 ru of PMS. This treatment group possessed the highest percent of large follicles, Graph III, and the largest number of follicles approaching Type 2, Table 7. If the type two follicle denoted one which had approached the point of ovulation but had failed to rupture, this treatment level could represent a point where PMS still reflected its follicle stimulating capacity but not its luteinizing effect.

With respect to the luteinizing effect of PMS, Lamond (1960) demonstrated induced ovulation by the injection of PMS into hypophysectomized animals. If induced ovulation occurred in this experiment, an alteration of cycle length might be expected. The data in Graphs I and II and in Table 4 provided no indication that this occurred, since the average cycle length approximated the normal 17 days.

In an attempt to account for the consistently lower lambing rates for ewes treated with PMS 13 and 15 days post estrus, the fertilization
capacity of the ova of both treated and control ewes was determined. This part of the experiment was based on the assumption that the ovulation rate of the treated ewes did not differ significantly from the control ewes. The basis for the assumption was derived from the data in Table 5. The results in Table 8 indicated that ova from PMS-treated ewes were capable of being fertilized. These results were in agreement with the findings of Hancock and Hovell (1961), Robinson (1961), Dickinson et al. (1962), Woody and Ulberg (1963) and Slee (1964).

In a further attempt to elucidate the reduced fertility level of the PMS-treated ewes, a quantitative determination of the progesterone content of the luteal tissue from both treated and control ewes was conducted. To analyze the small quantities of tissue obtained, existing quantitative procedures were modified. The results of the analysis, Table 9, revealed a range of 117 micrograms to 186 micrograms of progesterone per milligram of dry tissue for the Type 3 luteal tissue for treated ewes. The value for one control ewe, 164 mcg/mg, was within this range.

The progesterone content of the luteal tissue classified as Type 4 and Type 3 with atrium could not be accounted for directly. According to Restall (1964), the Type 4 corpora lutea could be older than the Type 3 corpora lutea. However, since the animals were sacrificed 13 days post coitus, age difference of the luteal tissue should be minimal. The corpora lutea classified as Type 3 with atrium appeared in all treatment groups, as well as in the control group. Hadek (1958) reported that an atrium in the center of the corpus luteum could be found throughout the first 16 days of luteal development. Thus there was no evidence
to indicate that these structures were atypical, other than their low progesterone content. The Type 3\textsuperscript{d} classification has been discussed in the results section and will not be considered here.

The various types of luteal tissue, except for Type 4, were found evenly distributed in all treatment groups and did not appear to be associated with any one treatment. On the basis of the analysis, there was no indication that the corpora lutea of the PMS-treated ewes differed in progesterone content from the control ewes. The data were limited by the small number of animals available from the breeding flock for sacrifice. Another factor which reduced the number of animals within any one group was the classification of the luteal tissue. This division of numbers was evident only after the completion of the chemical analysis, when the analysis data were compared with the observations made at the time of sacrifice.

To summarize the results of the experiment, it has been shown that PMS injection 13 and 15 days post estrus was ineffective in increasing the fertility level of Targhee ewes. There was no indication that PMS treatment increased significantly the number of follicles or corpora lutea. The consistent depression of fertility in the PMS-treated ewes could not be attributed to the incapacity of the ova to be fertilized or to the progesterone content of the luteal tissue. The results have suggested that for PMS to increase the fertility level of sheep, it must manifest itself in the increased production of ova and their subsequent ovulation.

By the review of literature cited earlier in the discussion, it has been demonstrated that PMS treatment of the ovine species resulted in
various responses. The majority of the published literature indicated that PMS treatment increased the fertility level of the ewe. However, several reports showed that in some instances, PMS treatment failed to duplicate these findings. This response variation could be attributed to breed or strain differences of sheep, location, the ineffectiveness of the particular placental gonadotrophin, or the interaction of two or more of these elements. However to interpret any interaction, it would be necessary to consider the following factors: the chemical and physiological properties of PMS; the mode of action of PMS; the endogenous levels of the follicle stimulating hormone (FSH) and the luteinizing hormone (LH) in sheep as related to season and the reproductive cycle; and ultimately, the environmental factors affecting the synthesis, secretion or storage of the hypophyseal gonadotrophins FSH and LH.

The common factor in all experiments discussed has been the gonadotrophic activity of pregnant mare's serum. According to Lamond and Lang (1961), the gonadotrophic activity existed as a single molecule. Lamond (1960) demonstrated that the molecule exhibited the properties of both FSH and LH in the ratio of five to one. The findings of Catchpole et al. (1935) suggested that this gonadotrophic substance could remain in the blood of injected animals for several days.

The mode of action of PMS after its introduction into the circulatory system of the ewe has been questionable. Lamond and Lang (1961) suggested that PMS was capable of stimulating the ovary of the recipient ewe directly. However in the rat, a PMS-hypophysis-gonad axis was proposed by Imamichi et al. (1961) and Quinn and Zarrow (1964). The latter theory was developed through the use of various neuroendocrine
blocking agents, such as atropine sulfate, chlorpromazine and Nembutal.
Attempts to elucidate the mode of action of PMS could be complicated further by a "feed-back" mechanism inherent in the endocrine system. Such a mechanism could involve the hypothalamus-hypophysis-gonad axis, as suggested by Cowie and Folley (1955). Presently, evidence has been too limited to permit definite conclusions on the mode of action of PMS in the ovine species. However, a combination of both previously stated theories could be a possibility.

Endogenous gonadotrophin levels, particularly FSH and LH, could be a factor in the response to PMS injection. Notwithstanding inherent breed differences, which will be discussed later, the ovine species is considered seasonally polyestrous. Any attempt to interpret the seasonality of the estrous response in terms of the hypophyseal gonadotrophins FSH and LH involves the consideration of two theories. Warwick (1946), Lamond et al. (1959) and Hutchinson and Robertson (1960) have indicated that the total gonadotrophic hormone content of the hypophysis has remained constant throughout both the breeding and the non-breeding seasons.

The second theory has been based on the experiments of Kammlade et al. (1952) and Thibault et al. (1966), which have demonstrated a higher total gonadotrophic hormone content in the hypophysis of sheep during the non-breeding season than during the breeding season. According to their studies, the non-breeding season or anestrus has been explained as a period in which the ovary becomes refractory to high gonadotrophic hormone levels and consequently is inactive. However, there is evidence which has indicated that the ovaries are only
relatively inactive during anestrus. Follicular type ovaries in sheep during anestrus have been observed by Kammlade et al. (1952) and by Hutchinson and Robertson (1960). The findings of Daniel (1960) have demonstrated "sub-level cycling" in anestrous ewes by the observation of the arborization patterns of the cervical vaginal mucus. The patterns associated with the "sub-level cycling" have been those of the proestrus observed during the breeding season. This information also may provide indirect evidence of follicular development during the anestrous period.

On the basis of these observations and in conjunction with the theories proposed by Fevold (1944) and Catchpole (1964), it is possible to theorize that adequate FSH is present in the ewe during anestrus to stimulate follicular development. However, there is no evidence indicating the presence of sufficient LH to act synergistically with FSH to promote the production and secretion of adequate amounts of estrogen, which will result in estrus, or to cause ovulation. This theoretical approach to gonadotrophin levels during anestrus has been confirmed by the findings of Kammlade et al. (1952).

Kammlade et al. (1952) have reported a significant decrease in the hypophyseal gonadotrophic activity at the time of the first estrus of the breeding season. Recently, Santolucito et al. (1960) have determined the FSH and LH content in the ewe hypophysis during various stages of the estrous cycle. They have observed that hypophyseal FSH and LH have decreased significantly between 4 hours and 35 hours after first acceptance, returning to proestrous levels by day 15 of the cycle. On the basis of their observations, they have concluded that a rapid release of these gonadotrophins is the cause of ovulation rather than an alteration...
in the ratio of FSH and LH. They have proposed also that the assumption that LH is the ovulating hormone in the ovine may not be valid.

More recently, Pelletier and Ortavant (1964) have established a type of diurnal rhythm in the hypophyseal release of FSH and LH. Their study has shown that the FSH and LH content of the ovine hypophysis has been greater at the end of a period of darkness than at the end of a period of darkness followed by a period of light. Light and other environmental factors are to be covered in greater detail later in the discussion. However, the report of Pelletier and Ortavant has been introduced to summarize the observations available on the cyclic patterns for the release of FSH and LH in the ovine.

If the results of Kammlade et al. (1952), Thibault et al. (1966), Santolucito et al. (1960) and Pelletier and Ortavant (1964) were superimposed upon one another, a purely speculative graph could be derived. Such a graph would depict the hypophyseal content of FSH and LH in the ovine species, showing the highest concentration of these gonadotrophins during the month of June or July for the Northern Hemisphere. As the breeding season approached and estrus was initiated, the gonadotrophin content would decrease rather sharply. Thereafter a gradual decline would continue until December or January, at which time a reversal would occur. An increase in concentration would follow, culminating in the highest concentration in June or July. During the estrous cycles, the hypophyseal content of FSH and LH would decrease rapidly between 4 hours and 35 hours after first acceptance and then return to proestrous levels by day 15 of the cycle. Associated with the possible seasonal and estrous cycle variations in the hypophyseal content of FSH and LH,
a type of diurnal rhythm could facilitate the gradual release of the
gonadotrophins, irrespective of their levels, with light implicated as
the possible triggering mechanism.

Of the experiments cited, the reports of Kammlade et al. (1952)
and Thibault et al. (1966) have suggested that hypophyseal FSH and LH
are separate hormones, not one glycoprotein complex as PMS. This view
has been substantiated further by the reports of Li and Pederson (1952)
and Li and Evens (1948) in which ovine FSH and LH have been classified
as glycoproteins. FSH has been assigned a molecular weight of 67,000
and an isoelectric point of 4.5; LH, a molecular weight of 100,000 and
an isoelectric point of 4.6.

As noted earlier in the report of Pelletier and Ortavant (1964),
environmental factors appear to be associated with the synthesis,
secretion and/or storage of the hypophyseal gonadotrophins FSH and LH.
These factors include light, temperature, exteroceptive stimulus and
nutrition.

Much of the basic research regarding the interrelationship of light
and the endocrine system has been conducted on the rat. Fiske (1941) has
shown that young male and female rats exposed to 15 to 100 days of light
possessed a higher hypophyseal content and secretion of FSH associated
with a lower content and secretion of LH than rats kept in constant dark-
ness. Ovaries of the rats exposed to light for 15 to 100 days were large
and contained both follicles and corpora lutea. However, the ovaries of
rats exposed to light for 250 days were small and follicular. The
reports by Fiske (1941) and Everett (1942) have demonstrated that rats
kept under constant illumination showed vaginal cornification, whereas rats kept in constant darkness were in diestrus.

The mediation of light stimulus in the rat appears to involve the retina, the optic nerve and the hypothalamus. Critchlow (1963) has suggested that the mediation of continuous light stimulus is via the retino-hypothalamic connection which passes through the chiasm (suprachiasmatic nuclei) and preoptic region. The study of Fiske and Greep (1959) has demonstrated that young rats kept in constant illumination for 56 days have shown definite secretory activity in the supraoptic nucleus. If the above pathway is correct, then the association of the hypothalamic area and the adenohypophysis probably involves the hypophyseal portal system, as suggested by Cowie and Folley (1955).

Current research involving photoperiodism and its regulation of the estrous cycle in rats has encompassed the epiphysis cerebri, the pineal gland, and a number of its metabolic products, particularly 5-hydroxytryptamine (5-HT) and 5-methoxy N-acetyltryptamine (5-MAT). Fiske et al. (1960) and Wurtman et al. (1961, 1963b, 1964a) have demonstrated in rats that exposure to constant light was correlated with a decrease in pineal weight, uterine and ovarian hypertrophy and an increased incidence of the proestrus, the estrous or the metaestrus phase of the estrous cycle. Associated with the decrease in pineal weight, there was a corresponding decrease in the activity of hydroxyindole-0-methyl transferase (HIOMT) and a reduction in the concentration of 5-MAT, its metabolic product, according to Reiss et al. (1963a), Wurtman et al. (1964a) and Axelrod et al. (1965). Wurtman et al. (1964a) have demonstrated that sympathetic innervation of the pineal gland by
bilateral superior cervical ganglionectomy or the removal of both eyes resulted in the inability of the rats to respond to constant illumination as previously stated. However, Wurtman et al. (1963b, 1964a) have demonstrated that rats exposed to continuous darkness had heavier pineal glands and greater HIOMT activity than their littermates exposed to normal illumination.

Pinealectomy in the rat has been associated with physiological responses similar to those found upon exposure to constant light. Wurtman et al. (1959) and Reiss et al. (1963b) have demonstrated that pinealectomy caused hypertrophy of the ovaries, the hypophysis and the adrenals and an increase in the running activity of the rat. However, the injection of 5-MAT into intact animals decreased ovarian weight and reduced the incidence of estrus, according to the findings of Wurtman et al. (1963a). Adams et al. (1965) have reported that injection of 5-MAT increased the secretion of LH from the hypophysis in the intact animal. The introduction of tritium labeled 5-MAT into the circulatory system of cats by Wurtman et al. (1964b) has demonstrated a concentration of this material in the pineal gland, the iris-choroid, the ovary, the hypophysis and the peripheral nervous structures. However in the rat, Wurtman et al. (1964b) have shown that the uptake of tritium labeled 5-MAT by the ovaries was depressed by the exposure of the animals to constant light.

The experimental evidence presented thus far has implicated the pineal gland as a mediator for the influences of illumination in the rat. The rate limiting step seems to be the effects of illumination on the activity of the 5-methoxy N-acetyltryptamine synthesizing enzyme.
hydroxyindole-O-methyl transferase. The "hormonal" association between the pineal gland and the other organs appears to be 5-methoxy N-acetyltryptamine.

To summarize the effects of light on the hypophyseal gonadotrophins FSH and LH in the rat, it is possible to conclude that synthesis, secretion and/or storage of the gonadotrophins is positively correlated with the length of the photoperiod. The proposed pathways involved in the mediation of light stimulus in the rat are retina-hypothalamus-adenohypophysis-gonad, retina-pineal-adenohypophysis (LH secretion)-gonad, and/or retina-pineal-gonad. The latter pathway could include the hypophyseal gonadotrophins via a "feedback" mechanism involving the steroids.

As stated previously, there are several environmental factors that could influence the hypophyseal gonadotrophins FSH and LH. Of these factors, light appears to play the dominant role under normal environmental conditions. However, it has been demonstrated by Browman (1943) that rhythmic changes in temperature have regulated the estrous cycle in rats, when maintained under conditions of constant light or darkness. Studies with sheep by Dutt and Bush (1955) have indicated that ewes maintained at temperatures of 7°C and 9°C during the summer have shown signs of first estrus 54 days earlier than control ewes, daylight length for both groups a constant.

Optimal reproductive ability in sheep is dependent upon thyroxine secretion, with a lowering of the reproductive ability associated with a depression of the secretion rate, as established by Ryle (1962, 1963a, 1963b, 1963c) and Brooks et al. (1964). An inverse relationship exists
between temperature and thyroxine secretion rate, as demonstrated by the studies of Hoersch et al. (1961a, 1961b) and Brooks et al. (1962). Furthermore, there is an interaction between temperature and light on thyroid activity. Hoersch et al. (1961b) have noted that a progressive increase in illumination from 4 hours to 12 hours daily has depressed thyroid activity, but that 12 hours of light or more has stimulated thyroid secretion rate. This biphasic effect of light has been in evidence under both 10°C and 32°C. Although temperature has been implicated in the hormonal control of reproduction in sheep, the specific physiological effects are not known.

Numerous experiments have indicated a relationship between exteroceptive stimulus and the hypophyseal gonadotrophins FSH and LH. Whitten (1956, 1957) has noted an initiation of estrus in non-cycling female mice after the introduction of the male. Under such conditions, mating showed a characteristic peak on the third day after the male was introduced. To the contrary, Whitten (1959) has demonstrated a decrease in the frequency of estrus in female mice caged as a group after having been caged individually. The studies of Bruce (1959, 1960a, 1960b) have shown that pregnancies in mice failed when a strange male was introduced 24 hours after copulation. The experiment of Shelton (1960) with Angora goats has shown that the presence of the male with the does prior to the start of the breeding season had a stimulating effect upon the initiation of estrus and ovulation. This phenomenon also has been demonstrated in sheep by Smith et al. (1958), Watson and Radford (1960) and Lamond (1962).

The mechanism of exteroceptive stimulus in mammals is presently in dispute. In mice the mechanism suggested is neither visual nor tactile
but olfactory stimulation. Little information is available concerning
the mechanism in goats and sheep. However in the study of Thibault
et al. (1948), tactile stimulation of the ewe has been demonstrated.
The results of this study have shown that ewes injected with PMS and
allowed to mate have had a significantly higher ovulation rate than
similarly treated, unmated ewes. Such results suggest ovulation condi­
tioned by coitus, as noted by Turner (1955) and Nalbandov (1958).

The last of the environmental factors to be considered is
nutrition. Limited experimental data are available regarding the direct
effect of nutrition on the gonadotrophins. Variation of dietary protein
levels from 15 percent to 30 percent has been without effect on hypophy­
seal gonadotrophic content in the rat. However, diets containing high
levels of casein have been capable of inducing constant estrus, as
reported by Leatham (1966).

The reproductive cycle of the ewe is not influenced greatly by
nutrition. Hunter (1961) and McDonald (1961) have concluded that only
ewes in extremely poor condition show reduced sexual activity. The
practice of flushing, as stated previously, is related to improving the
physical condition of the ewe, thereby influencing the fertility level.
However, the results of this practice are variable. Although present
information is indicative of an association between nutrition and
hypophyseal gonadotrophins, there is no specific experimental evidence
concerning the ewe.

To reiterate, nutrition, exteroceptive stimulus, temperature and
light are factors involved in endogenous gonadotrophic activity. Based
on the theories presented, the specific role played by endogenous FSH
and LH in the regulation of the seasonally polyestrous response and the estrous cycle of the ovine is open to conjecture. Inherent breed differences with respect to the incidence of estrus, reflected in the length or lack of anestrus, suggest a variation of breed response to FSH and LH. Although there is no information presently available, if the pineal gland were shown to be functional in the ovine, new concepts of hormonal regulation of estrus in this seasonally polyestrous animal may be forthcoming.

It is in the presence of these factors that the exogenous gonadotrophin PMS has been introduced in this experiment and in those cited earlier in the discussion. The fact that PMS is a single glycoprotein molecule with a fixed ratio of FSH and LH activity, may account for its inability to stimulate similar responses in different breeds under different environmental conditions. It is apparent from the above mentioned theories and factors that conclusive evidence concerning basic concepts must be forthcoming before any attempt is made to elucidate the effects of exogenous hormone treatments, such as PMS.
SUMMARY

Treatment with pregnant mare's serum (PMS) at various dosage levels 13 days and 15 days post estrus did not increase significantly the fertility level of Targhee ewes. Evidence has been presented to demonstrate that neither follicle number nor corpus luteum number was increased significantly by PMS treatment 13 days post estrus.

In an attempt to account for the consistently lower fertility level of ewes treated with PMS 13 days and 15 days post estrus, ova recovery and progesterone analysis of luteal tissue was conducted. Limited data have indicated that ova from the PMS-treated ewes were capable of being fertilized. There was no evidence that the corpora lutea of treated ewes differed in progesterone content from control ewes.

Various theories and factors have been discussed which may be pertinent to the results of PMS treatment.
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