

**THE ESTRUAL CYCLE IN ROMNEY EWES**

**OF**

**HIGH AND LOW FERTILITY**

**by**

**WALTER GEORGE CADMUS**

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APPROVED:

Redacted for Privacy

Professor of Animal Husbandry

In Charge of Major

Redacted for Privacy

Head of Department of Animal Husbandry

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean, Graduate School

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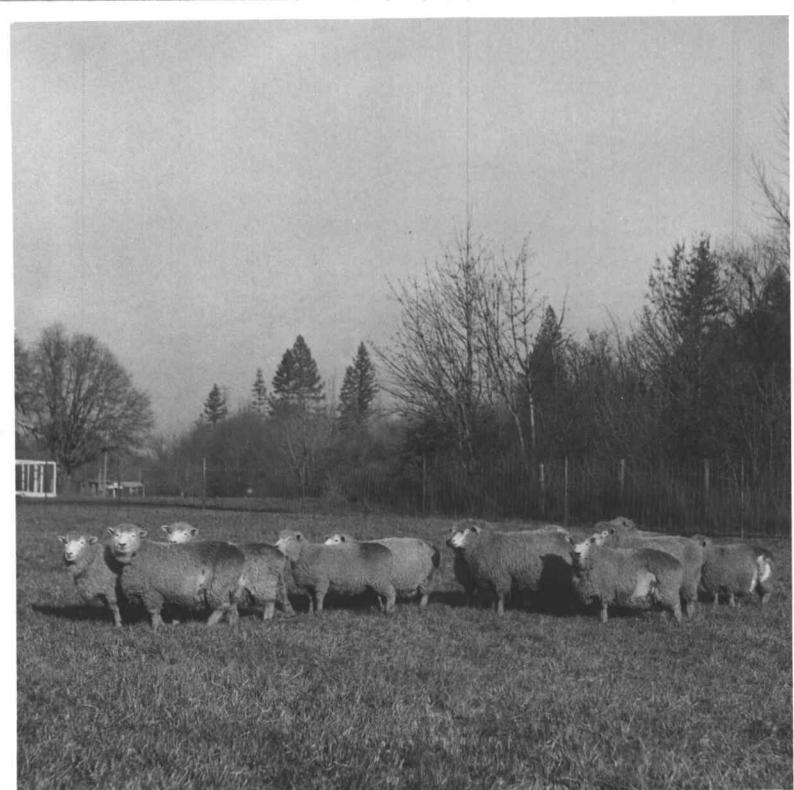
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**Experimental Flock on Pasture**

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INTRODUCTION

Sheep husbandry is a highly important livestock enterprise in the Willamette Valley of western Oregon. In recent years the enterprise has expanded in the Valley in contrast to a declining sheep population in the section of Oregon east of the Cascades. The problems of efficient sheep production are assuming major economic proportions in this area.

Much study and effort have been expended in recent years to improve husbandry methods and to raise the economic level of sheep production in the Willamette Valley. Most of this work has been in the line of developing suitable forages, control of parasites, and economic surveys and analyses of the sheep industry. Satisfactory economic gains have resulted from these studies; but if progress is to be continued, efforts must be directed along lines hitherto given little coordinated attention. It would appear at the present time that improvement of fertility offers a path to the most rapid increase of production efficiency.

One of the predominating breeds of this area is the Romney which is well adapted to the forage and climatic conditions. Original importations of the Romney to America were made in the Willamette Valley. Approximately 9,000 registered Romneys are found in purebred flocks of the Pacific coast, and they are used widely in the West as

foundation stock for grade flocks. However, one of the criticisms of this breed is a late and long breeding season. If this reproductive problem can be corrected, the increased economic return that would be realized is quite apparent.

Correspondence with breeders on the East coast, South America, and New Zealand reveals that late and long lambing seasons are also a problem with Romneys in these areas. This would indicate that late and long lambing is not a local or ecological factor, but a factor characteristic of the breed, or at least strains within the breed.

Investigations concerning fertility and related phenomena in sheep have been carried on for a number of years. The majority of the conclusive and well recognized studies have been conducted by a relatively small number of investigators. Much of the emphasis to date has been placed on attempting to alter the breeding season of ewes by the administration of hormones. The study of fertility in sheep has received much less emphasis than it has in some species such as dairy cattle. This is probably due to the relatively small individual value of sheep and the methods of handling them. An effort has been made here to review available material and to apply the techniques and information gained to the individuals under observation in this problem.

It is the purpose of this study to determine if there are inherent differences in the breeding seasons and capacities of individual Romney ewes. The results will be evaluated, and an effort will be made to apply them in establishing and selecting lines of the desired fertility level in the Romney breed.

## REVIEW OF LITERATURE

Investigations carried on in this project were divided into five related phases. A survey of breeders' records and analysis of pedigrees and records of ewes used in the project, indications and frequency of estrus, ovulation in conjunction with estrus, vaginal smears, and relative gonadotropic hormone potency of the pituitary glands of the experimental ewes. The review of literature and results of the experiment will be presented in that order.

Most of the breeds of sheep of economic importance in this country have a limited single breeding season. McKenzie and Phillips (52, p. 139) observed that with purebred Shropshire, Hampshire, and Southdown ewes the breeding season began the last of August with the Hampshires showing estrus about ten days earlier than the other two breeds. Cole and Miller (16, p. 45) doing work in California with these same breeds showed that the average breeding date of Hampshires was August 17, with the Shropshires breeding September 1, and the Southdowns September 10. They also showed Rambouillet ewes bred in mid July and Romney ewes in mid September. The latter was the latest season of any of the breeds studied. Romney-Rambouillet crossbred ewes had an average breeding date of August 21, which was midway between the two parent breeds. Hammond (36, p. 98) in working with Suffolk, Border Leicester, and Cheviot ewes in Great Britain found the breeding season to be from October to early March with occasional estrus as early as August. Kelley and Shaw (42, p. 18-27) established in Australia that the breeding period occurs at a definite season within breeds, and that some breeds are earlier than others, while the Dorsets will breed

and have two lambing seasons a year. McKenzie and Terrill (53, p. 81) in dealing with Hampshire, Shropshire, Southdown, Rambouillet, and grade ewes found the breeding season to be from September to January. Thompson (64, p. 478-479) in his study of breeding seasons of Merino and three groups of half-breeds in Australia stated that the normal breeding season was from November 15 into January. He also observed that of all those studied, the Merino-Romney cross had by far the latest breeding season.

Unlike the cow and sow, the ewe exhibits few or no visible indications of estrus, and the detection is almost impossible in the absence of a ram. Young (72, p. 145) reports that a ewe in heat may eat less and stand apart from the others. Generally, however, ewes make no indication other than standing for the ram. Urination at time of teasing is not an indication of heat, (72, p. 145) and (53, p. 13). Some slight swelling of the vulva and slight discharge are usually observed, but this cannot be relied upon (52, p. 140). The general criterion of heat accepted by workers in this field is the ewe allowing the ram to mount. Thompson (64, p. 481) found that ewes excited the interest of rams before and after they would accept service. Polovtzeva and Fomenko (57, p. 250) in using the vaginal smear as the criterion of estrus observed that ewes were selected by rams in late proestrus, or the transition stage to estrus. Grant (31, p. 2) states that the mutual behavior of the ram and ewe is the most reliable indication of heat.

McKenzie and Terrill (53, p. 15-16) present data for the duration of 1,235 estrual periods which show a range of three to seventy-three hours with a mean of 29.33 hours. Cole and Miller (16, p. 44-45) report the average length of estrus as thirty to forty

hours. Kardymovic, Marsakova, and Pavljueuk (40, p. 35) observed that the end of estrus occurred thirty to forty-six hours after its initial onset. McKenzie and Phillips (52, p. 140-141) found that the average length of 247 estrual periods was 26.8 hours. Kelley (41, p. 130) states that 50 percent of the range Merino ewes observed had short estrual periods of 19 hours or less. In a comparison of Hampshire, Southdown, and Shropshire ewes, McKenzie and Phillips (52, p. 140-141) found that the Hampshire had an average period of 30.7 hours, which was significantly different than the Southdown with an average of 24 hours. Shropshires fell midway between these two breeds with an average of 26.3 hours. They also found a significant difference between Hampshire lambs with an average of 21.2 hours and Hampshire yearlings with an average of 29.7 hours.

Length of the estrual cycle is generally measured from the beginning of one period to the beginning of the next and has a range of 13 to 21 days with an average of 17.3 days (17, p. 205). Dry (21, p. 386-387) found the average interval in Romney ewes to be a little under 17 days. Ewes returning to heat showed consistency in the length of cycle. Gill (30, p. 306-307), also working with Romney ewes confirms these findings. He observed a normal interval of 15 to 18 days with a flock average of 17 days. Hammond (36, p. 98) found the cycle to average 17 days with the first cycles of the season shorter and more consistent than those occurring later in the season. Kelley (41, p. 130), in working with Dorsets and Merinos of high and low fertility, found a 15 to 19 day range with an average cycle of 17 days. No significant difference as to age or breed was found by McKenzie and Phillips (52, p. 142).

In their study of Shropshire, Hampshire, and Southdown ewes, a range of 8 to 49 days was observed with 16.6 days as the average length of cycle. McKenzie and Terrill (53, p. 16) present data showing that of 1,038 estrual cycles, 938 were within the range of 14 to 19 days in length with a mean of 16.72 days. Thompson (64, p. 482) states that a cycle of 16 to 19 days with an average of 17 days is normal in Merino and half-breeds in Australia. Cole and Miller (16, p. 47) found that if nutrition is normal, the cycle rarely falls outside the 15 to 19 day range. Polovtzeva and Fomenko (57, p. 250) in a study involving 641 ewes found the estrual cycle to be 16.3 days.

Sperm in the reproductive tract of the ewe remains viable for only a short time (68, p. 143). The period in which ova are capable of being fertilized is also short. Green and Winters (33, p. 465) estimated that the survival of sperm and unfertilized ova was not more than 24 hours in the genital tract of the ewe. If a program of artificial breeding is being followed, or if a large number of ewes are being hand bred to a valuable ram, it is essential to know when ovulation occurs in order to inseminate the ewe at the optimum time.

By performing laparotomies and actually observing ovulation, McKenzie and Terrill (53, p. 30-31) established the time of ovulation to be near the end of estrus. They observed ovulations as early as 12 hours and later than 41 hours after the onset of estrus. Green and Winters (33, p. 465) stated that the ewe ovulates late in the estrual period as the animal is passing from heat. Anderson (2, p. 69) in performing laparotomies on Merino ewes found that a minimum of 21 to 25 hours must elapse between onset of heat and ovulation. Kardymovic,

Marsakova, and Pavljucuk (40, p. 35) found the optimum time for breeding to be 18 to 26 hours after the onset of heat, and preferably near the end of that period. Hall (35, p. 25) found indications in the ewes studied that ovulation occurred during the latter part of estrus. McKenzie and Phillips (52, p. 142) obtained excellent results by breeding ewes 14 hours after the beginning of estrus.

The fact that silent heat, or ovulation without heat, frequently occurs is shown by Kelley and Shaw (42, p. 19). In their studies of Merino, Dorset, and Border Leicester ewes, they found from 10 to 80 percent of the ewes exhibiting silent heat during various years. Generally, ovulation without heat occurs as the first ovulation of the breeding season. Silent heat has also been reported by Grant (32, p. 802) as occurring regularly in the month before heat, and sometimes during the breeding season. McKenzie, Allen, et al (50, p. 280) and Cole and Miller (16, p. 47) have observed silent heat in ewes. McKenzie and Terrill (53, p. 46) also noted heat without ovulation in the latter part of the season. McKenzie, Allen, et al (50, p. 280) noted 8 percent of the ewes showing estrus without ovulation.

Twinning is not uncommon in sheep, and occasionally triplets are born, but the frequency of multiple births varies among breeds (55, p. 837-841) and (49, p. 13). Zavadovskii and Paduceva (73, p. 131) observed an average of 1.54 corpora lutea in normal ewes. McKenzie and Terrill (53, p. 31-50) observed ovulation rates of 1.04 to 1.52 among groups of ewes. Two cases of triple ovulation were observed. Mature ewes had a higher ovulation rate than yearlings and ewe lambs. Ewes on a low plane of nutrition had a lower ovulation rate than ewes on a high

plane of nutrition. They also noted that the twin ovulations generally occurred during the first half of the breeding season. Marshall and Potts (49, p. 13) and Nichols (55, p. 840) report that ewes conceiving early in the season have the greatest number of multiple births.

Hall (35, p. 15) found the gestation period of Rambouillet ewes to range from 149 to 152 days. The five-year average gestation for ewes artificially inseminated was 150.3 days, and for those bred naturally was 150.2 days. Gill (30, p. 307) working with Romneys found the duration of pregnancy to range from 145 to 153 days with a weighted average of 148.75 days. Dry (20, p. 387) also working with Romneys gave an average gestation period of 148.7 days and a range of 144 to 153 days. Hammond (36, p. 102) found that gestation was longer for twins than for singles.

The fact that sheepmen have long had interest in methods of changing the occurrence of estrus and rate of ovulation is evidenced by the old and common practice of flushing ewes preparatory to breeding. The practice is so well known and advised that it might well be termed a basic law of successful sheep husbandry. Clark (12, p. 133) found that flushing causes an increased ovulation rate if the ewes are not in a high condition to start with. He also pointed out that flushing appeared to have no material effect in bringing ewes into heat faster if the estrual cycle had already been established. Grant (32, p. 802) postulated that flushing may convert the first silent heat of the season into normal estrus. Marshall and Potts (49, p. 6) report that flushing increased lamb yields and earliness of breeding. McKenzie and Phillips (52, p. 140) noted that taking ewes from a low level of

nutrition to a high level influenced estrus. Nichols (55, p. 835-838) found flushing would increase yields.

Within the past twenty years much work has been conducted in an effort to alter the breeding season and ovulation rate of ewes by means of hormone administration. To date the results have been variable and a technique which will give dependable and satisfactory results has yet to be developed, (56, p. 165).

Anderson (3, p. 328-334) induced estrus in 84 of 113 Merino ewes within 21 days by intramuscular injections of five doses of 1,000 units of estradiol benzoate over a period of three days. A regular breeding season was initiated in 73 of the 84 ewes that responded. At first the interval between heat periods was abnormal, but normalcy was gradually reached. Ovulations were confirmed, but it was not definitely established that they occurred as a result of the injections.

Bell, Casida and Darlow (6, p. 441-449) tested the effect of estradiol alone and estradiol plus progesterone on the genital tracts of spayed ewes during October. All ewes displayed heat one to two days after injection. The changes in the vaginal smear were due to estrogens and influenced very little by progesterone. Frank and Appleby (29, p. 257-258) by injecting stilbesterol produced heat in eleven out of twelve ewes. However, the estrual cycle was not initiated and the ewes failed to ovulate. Thirteen ewes ovulated as a result of one or more injections of PMS (pregnant mare serum). Estrus rarely occurred from single or daily injections of PMS, but four out of six ewes given doses 16 days apart came into estrus following the second injection, and one became pregnant.

PMS did not influence estrus induced by stilbesterol, but stilbesterol inhibited ovulation produced by PMS.

McKenzie and Bogart (51, p. 17-18) found whole PMS more effective than PU in stimulating estrus. Seventy percent of the injected ewes exhibited estrus as compared to 30 percent of the controls during the same period. Two hundred fifty to five hundred R.U. gave the best results with 1,500 R.U. producing too much luteinization. Injections 16 days apart gave the best results. Zavadovskii and Paduceva (73, p. 131) compared the effect of PMS, urine of pregnant women, and prolan in stimulating ovulation. An increase in the percentage of twinning and percentage of barren ewes resulted. Injected ewe ovaries showed an average of 2.23 C.L. as compared to 1.54 C.L. in the controls. PMS gave slightly better results than the others.

Van Der Noot, Reecs and Skelley (65, p. 372-373) produced estrus in ten aged ewes with injections of 250 R.U. of PMS 16 days apart. All ten ewes mated and six conceived. Of 25 yearling ewes, ten came into estrus and mated after the first injection and seven more after the second injection of PMS. Only five of the yearlings lambed. These same workers (66, p. 313-317) injected 177 ewes with 250 Rat Units of PMS. Twenty ewes mated after one injection and 5 lambed. One hundred fourteen ewes mated after the second injection and 65 lambed. They observed that young animals did not respond as well as old, and the physical condition had some effect on the results.

Koger (43, p. 166-169) force bred 46 ewes after injections of 250 R.U. of PMS and crude pituitary extract. Seven of these ewes lambed. Estrogen was used in conjunction with PMS in an effort to open

the cervix. The resulting increase in conceptions was too small to be significant. Cole and Miller (16, p. 80) found estrin produced heat without ovulation and that estrin plus gonad stimulating hormone inhibits ovulation. Cole, Hart and Miller (14, p. 374-380) produced estrus in eight of 118 ewes with a single injection of PMS. All eight ewes were bred and none became pregnant. Of 170 ewes given two injections of PMS 17 days apart, 58 showed estrus, but only 17 conceived. Superovulation was produced with 600 to 700 I.U. of PMS. Enough estrogen to introduce sexual receptivity given alone or in conjunction with PMS inhibited ovarian activity.

Warwick and Casida (70, p. 169-173) administered follicle stimulating extracts of sheep pituitary subcutaneously and followed with luteinizing or unfractionated extracts intravenously during the last few days of the estrual cycle. This treatment lengthened the estrual period an average of 0.76 day in 35 ewes, but individuality in the length of the inter-estrual interval was maintained. Of nine ewes given this treatment during anestrus, only one responded.

Murphree, Warwick, Casida and McShan (54, p. 19-21) injected anestrus ewes, follicular ewes (twelve days after beginning of estrus) and luteal ewes (three days after beginning of estrus) with follicle stimulating extract. The proportions of corpora lutea represented by eggs recovered from the reproductive tract were lower in the luteal and anestrus animals than in the follicular animals. They suggest that this indicates a greater tendency toward entrapment of the ova in the first two groups of ewes. Casida, Dutt and Meyer (10, p. 24-33) compared the effect of FSH extract and unfractionated

pituitary extract on ewes at different stages of the estrual cycle (4, 8, and 12 days.) Neither extract gave any practical gain in controlling time of estrus. In general, the greater physiological disturbances followed the use of the unfractionated extract. Casida, Warwick and Meyer (11, p. 23-27) injected ewes subcutaneously for four days with follicle stimulating extract beginning on the fourteenth day of the cycle. On the seventeenth day an intravenous injection of luteinizing extract was given and the ewes were bred. Two to five days after breeding 70 percent of the eggs ovulated were represented by live embryos. Thirty to 37 days after breeding only 0.8 percent of the eggs ovulated were represented by live embryos. Inadequate uterine development or abnormalities induced in the ova due to rapid development of the follicles were advanced as possible causes of death of the embryos.

Bludovov and Dubrova (7, p. 378-379) gave injections of gravidan to 107 barren ewes that did not show heat. Thirty percent of these came in heat within two days. The remainder were force bred and 56 percent of them produced lambs. Ovulation was also produced in pregnant ewes by gravidan injection.

Another possible method of altering the breeding season of ewes is control of light. Dawson (18, 907-910) has demonstrated that by controlled illumination domestic cats will come into estrus during their normal anestrual season. Sykes and Cole (63, p. 250-252) by controlling the light hours during the day caused five out of eight ewes to breed in June. Litton (46, p. 39) calls attention to a practical breeder of Southdowns who has had apparent success with light control.

The success of early workers in establishing the stages of estrus of laboratory animals by the use of vaginal smears has stimulated considerable interest as to the application of the technique in sheep and other farm animals. Allen (1, p. 321) cites the cellular content of the vaginal smear as a reliable criterion of estrus in the mouse. Long and Evans (47, p. 18-21) found four orderly stages in the vaginal smear of the rat. Each stage is a reliable indication of the subdivisions of the cycle in the living animal. Hartman (37, p. 377-379) was able to detect the definite stages of estrus in the opossum by examination of the cellular content of the vaginal smears. Stockhard and Papanicolaou (62, p. 236-240) observed four definite stages of estrus in the guinea pig. Each stage had a characteristic cellular content of the vaginal smear. Bassett and Leekley (4, p. 6-7) observed definite cycles in the cellular content of vaginal smears of foxes. They found that receptivity in vixens reached a peak at or near the period of greatest vaginal cornification and when the percentage of leukocytes present in the smears is very small.

Evans and Cole (25, p. 74-78) found the vaginal smear to be of doubtful value in testing for estrus in the dog. Definite cellular changes in the smears were noted, but they observed some individual variation. The changes from one type of smear was not as clear cut or abrupt as has been found in the smaller experimental animals.

Wilson (71, p. 425-427) noted variations in the vaginal smear of the sow during different phases of the estrual cycle. These variations were not sharply defined and it was not felt that the vaginal smear in itself affords a reliable means of determining the exact phase

of cycle in this species. Cole (13, p. 276-285) in a study of the vaginal smear of the cow concluded that the presence of mucus is the best criterion that the vaginal smear of the cow affords.

Grant (32, p. 802) observed that vaginal smears in the ewe undergo changes prior to ovulation without heat. Kardymovic, Marsakova and Pavljucuk (40, p. 36) report that conception in ewes was highest in the individuals bred when the vaginal mucus was clear and transparent. They were unable to diagnose the stage of estrus by use of the vaginal smear. Gunn (34, p. 105) found the vaginal smear of the ewe to have small value in the determination of heat. Examination of smears prior to insemination was unreliable.

Polovtzeva and Fomenko (57, p. 250) in examining the ovaries of ewes found complete agreement between vaginal smears and ovarian condition. They recommended vaginal smears as a reliable and simple method for deciding the appropriate moment for mating ewes. Hawkins and Darlow (38, p. 274-277) have shown a correlation in ewes between the histology of the reproductive tract and vaginal smears taken at various stages of the reproductive cycle. Darlow and Hawkins (17, p. 205-207) describe cyclic changes in the vaginal smear of the ewes. The presence or absence of leukocytes is described as the main distinguishing feature of the various phases.

Cole and Miller (15, p. 50) and (16, p. 46-65) describe six stages of the vaginal smear in ewes and the corresponding stages of the estrual cycle. They maintain that vaginal smears of the ewe indicate the stage of sexual activity and believe the signs are practically infallable. They found that smears of the ewe were not as clear

cut as in smaller animals, but would allow one to follow the cycle fairly well. It was also shown that except during the breeding season the vaginal smear was not a reliable criterion of estrus.

The variable results obtained from the study of vaginal smears of ewes may have been due in part to methods of taking and fixing the smears. Environmental factors may also have affected the results. Evans (24, p. 651-652) has shown that vitamin A deficiency will cause constant cornification of cells in the vaginal smear of the rat. This symptom appears much earlier and more constant than xerophthalmia. Emery and Schwabe (22, p. 153-154) and Wade and Doisy (67, p. 707-709) have demonstrated that pressure as well as irritation of the vaginal mucosa will cause characteristic estrual smears to appear in the vagina of castrate and lactating rats. Estrual smears were induced by the frequent use of cotton swabs or glass rods. Although smearing changed vaginal epithelium, the uterus was not affected. Emery (21, p. 106) produced frequent and prolonged vaginal smears in the rat by the removal of one ovary.

Japp (39, p. 244) states that the main difference between a good and a poor laying hen seems to be either a difference in the gonad stimulating power of the pituitary or a difference in the ability of the ovary to respond to stimulation. A comparison of the potency of the Follicle Stimulating Hormone of the anterior pituitary and whole pituitary extracts of sheep has been made by Fevold, Hisaw, Hellbaum and Hertz (27, p. 710-723). Relatively pure FSH extract increased the ovaries of immature rats and rabbits 400 to 1400 percent as compared to

150 percent increase following the administration of entire pituitary extract. Warwick (69, p. 4-9) demonstrated that the gonadotropic potency of pituitary glands from crossbred Lincoln-Rambouillet ewes was significantly higher than that of pituitaries from purebred Hampshire ewes. However, no comparison of fertility or breeding season was made between these two groups. Stephens and Allen (61, p. 582-583) by underfeeding guinea pigs so as to cause them to lose 20 to 30 percent of their weight in two weeks found the animal to develop atrophic ovaries with virtual disappearance of all follicles. Administration of a pituitary extract caused luteinization and the ovaries did not return to normal. Animals put back on full feed for two weeks without other treatment showed evidence of ovarian recovery. They advance the theory that the ovarian change may be due in part to the inability of the pituitary to produce sufficient hormones to maintain the ovaries during a low diet. Smith (60, p. 198-203) demonstrated with rats that two-thirds or sometimes more of the anterior pituitary can be removed without disturbing the sex cycle or normal reproduction. However, in large mammals the margin of safety may not be as large because the relationship between the weight of the anterior pituitary and the body weight is not as close as it is in the rat.

Work has been done to show that in the absence of active gonads the gonadotropic content of the anterior pituitary increases. Riley and Fraps (58, p. 541) found the anterior pituitaries of hens with follicles in the regressive or quiescent phases had a higher gonad stimulating effect than those from hens in full production. Evans and Simpson (26, p. 373-374) found castrate pituitaries were six times as potent as normal pituitaries when injected into rats. The cryptorchid male

pituitary gave results intermediate between normal and castrate males. Rat pituitaries showed an increased size and hormone storage period of several months following castration. Engle (23, p. 102-105) transplanted anterior pituitaries from castrate and entire animals into rats. The castrate pituitaries caused ovarian growth four to nine times as large as those stimulated by normal pituitary transplants. The hypophysis of the castrates was also larger and heavier than in normal animals.

In conjunction with methods of testing pituitaries through the use of laboratory animals some work has been done to determine the effect of different methods of storing and preparing pituitaries.

Kupperman, Elder and Meyer (44, p. 24-26) testing with rats, found that dessication of sheep pituitaries by alcohol or acetone resulted in a 50 percent loss of potency. This loss could not be explained by analysis of the alcohol or acetone residue. Glands stored in a frozen state for 80 days showed no significant loss of potency. Air-dried glands gave no loss of potency after storage for one year. Breneman (8, p.192) testing with chicks found acetone-dried pituitaries just as effective as fresh glands.

Various test animals have been used to analyze the potency of pituitary glands. However, the trend seems to be toward the use of immature birds as they respond to small doses and do not require so much material. Breneman (8, p. 191) has established a "chick unit" as measurement for pituitary potency. Using ten or more test animals gonad weight increases of 35 percent over the average gonad weight of the controls was considered to constitute a "chick unit" (C.U.). Schockaert (59, p. 507) found that injections of beef pituitary substances elicited

marked weight increases in testicles of immature ducks and fowl. Lahr, Riddle and Bates (45, p. 682-690) found extreme growth in testes of immature pigeons as a result of beef pituitary injections. Jaap (39, p. 245) found that injections of sheep pituitary extracts in chicks caused greater response in the testes than in the ovaries. Domm and Van Dyke (19, p. 349-350) obtained hypertrophy of cockerel testicles by injections of purified sheep pituitary extract.

By using young chicks as test animals very satisfactory results have been obtained in a short period of time. Byerly and Burrows (9, p. 366-367) injected chicks over a period of 96 hours and performed necropsy 24 hours after the final injection. A marked increase in testis weight was noted. Jaap (39, p. 245) elicited satisfactory response by injecting chicks for a period of four to six days. Breneman (8, p. 191) established his "chick unit" by giving five injections in a 96-hour period. Warwick (69, p. 3-4) obtained satisfactory testicular response in day-old chicks by injecting sheep pituitary powder suspended in distilled water for a period of five days.

#### MATERIALS AND METHODS

In the summer of 1946 a survey was made of the records of six breeders of purebred Romney sheep in the Willamette Valley. Information as to the management of each flock was obtained at this time. Breeders of Romneys in Virginia and South America were contacted through correspondence. A discussion relative to the lambing season of Romneys in New Zealand was held with an animal physiologist from that country. Also available for study were detailed records of the Oregon State College Romney flock. Statistical analysis was applied to the accumulated data in an effort to discover a simple method of selection for early lambing.

Two groups of purebred Romney ewes were selected for close observation. Group A consisted of 6 seven-year-old ewes selected from a flock of high producers. These six ewes themselves had a lifetime average lambing rate of 122 percent with an average lifetime lambing date of February 11. Group B consisted of eight ewes, five, six, and seven-year-olds, selected from a flock in which difficulty has been experienced in obtaining early lambs. The available records for group B are not as accurate as for group A, but they show a lifetime lambing rate of 47 percent with an average lambing date of March 22. The reproductive history of these two groups of ewes is tabulated in Table I, page 32. To provide a basis for the study of inheritance of these animals, a pedigree study was made. Each ewe was traced back for five generations with special attention being given to the birth dates of the various ancestors.



Ewe 42A



Ewe 44A



Ewe 46A



Ewe 48A



Ewe 49A



Ewe 50A



Ewe 4239B



Ewe 176B



Ewe 106B



Ewe 107B



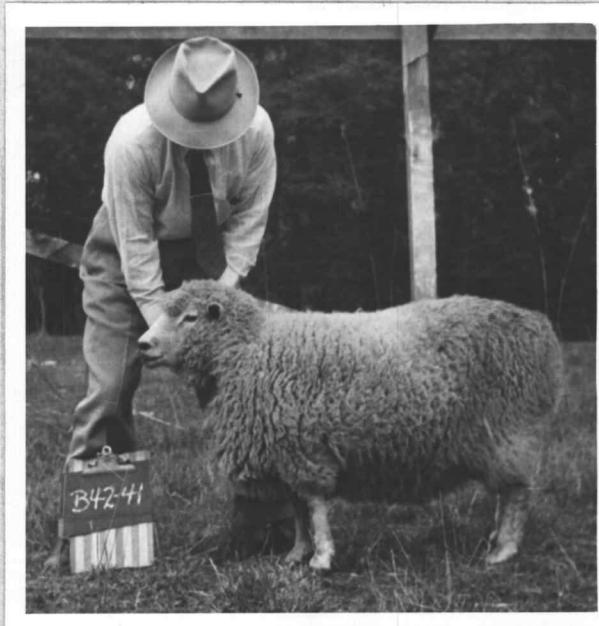
Ewe 112B



Ewe 100B



Ewe 58B



Ewe 4241B



Teaser Ram



Ewe 44A, nine days after laparotomy

On July 22 an active yearling ram was placed with these 14 ewes. The ram was provided with a leather apron to prevent copulation. A mixture of mineral oil and oil pigment was applied to the ram's brisket daily and the color was changed every 17 days. The ram was kept with the ewes constantly to avoid the possibility of missing an estrual period.

The ewes were all placed on the same level of nutrition a week before the ram was turned in. The ration consisted of one-fourth pound of grain daily (equal parts by weight of oats, wheat, and barley) and native and seeded grass pastures, supplemented with alfalfa hay whenever necessary to maintain the ewes in a thrifty condition.

Beginning on August 15 vaginal smears were taken on alternate days and on days ewes were marked by the ram. Smears were taken with the ewe in a standing position. A speculum was employed and a sample of mucus was taken from the mouth of the cervix with a glass rod. Immediately after the mucus had been placed on a glass slide, the smear was fixed in a solution of equal parts of 95 percent alcohol and ether for five to fifteen minutes. The slide was then rinsed successively in 70 and 50 percent alcohol and distilled water. The slides were stained in Harris' hematoxylin, OG6 and EA50. These latter two stains are smear stains prepared by the Ortho Pharmaceutical Corporation.

Immediately following the second estrual period of each ewe, as indicated by the ram, laparotomy was performed in order to observe the condition of the ovaries. Nembutal was administered as a general anesthesia at the time of the operation.

During the latter part of October some of the ewes were inseminated and the embryos of those conceiving were removed by surgery after a development of three weeks. Observation was made as to the interval from the time of embryo removal to the return of estrus.

Ten days after estrus the ewes were castrated. This was during the last part of November and the first part of December. Ninety days after castration, the ewes were slaughtered. Within 15 minutes after slaughter the pituitary glands were removed and placed in an excess of acetone. After returning to the laboratory, usually a matter of three to four hours, as much of the adhering connective tissue as possible was removed. The glands were then cut into small cubes and placed in fresh acetone. Two additional changes of acetone were made at intervals of 2½ hours. After remaining in the acetone for 72 hours, the gland was removed and drying was completed in a dessicator containing P<sub>2</sub>O<sub>5</sub>.

After remaining in the dessicator for ten days to two weeks the individual glands were removed and ground with a mortar and pestle until fine enough to pass through a 140-mesh (to the inch) screen. The pituitary powder was then weighed and stored in a tightly stoppered vial until ready for use.

Assays of the individual glands were carried out with chicks and the potency of the glands measured in "chick units" as established by Breneman (8, p. 191). Day-old leghorn cockerels were used and the increase in testis weight was the end point. A total of 10 mg. of pituitary powder was suspended in 1.25 cc. of physiological saline and injected daily in five equal doses over a period of five days. The

control group was given equal injections of pure physiological saline. Injections were given subcutaneously in the flank on alternate sides. Preparatory to injection, the area was swabbed with alcohol. Fresh suspensions were prepared daily and prior to injection bacterial cultures were made. These cultures indicated that all suspensions had the same relative bacterial content.

Eleven groups of injected chicks and one group of controls were used. They were kept on hardware cloth to prevent possible intake of hormones from the droppings. Commercial chick starter was fed and fresh water provided at all times. The various groups were identified by water color painted on the back. Injections were made with a 1 c.c. tuberculin syringe and a 20-gauge needle.

Twenty-four hours after the last injection the chicks were killed and the testicles immediately weighed on a chain-o-matic balance. Necropsy of each chick and weighing of testicles required about one minute. Chicks were killed in the order of one from each group alternately.

## RESULTS

Records of six breeders of purebred Romneys in the Willamette Valley showed that not all had experienced difficulty with late lambing. A breeder in Virginia reported as of September 22, 1946, that only 9 of 25 purebred Romneys had been bred. On the same farm 27 of 29 mutton type or "down" ewes had bred by that date. A good deal of artificial insemination is done with sheep in Uruguay and a report from one of the inseminators in that country states that one of the difficulties encountered with the Romney breed is a late and prolonged estrual season. The Romney breed has been greatly developed in New Zealand. When Doctor L. R. Wallace, Chief Nutrition Officer of New Zealand, visited the United States last summer, he reported that lambs being dropped late in the season were not uncommon within the Romney breed.

Of the flocks surveyed in this locality the one from which the group A ewes were selected has the best record for early lambing. The lambing season for 41 ewes in 1945 ranged from January 24 to March 6; only two yearlings lambed after February 19. In 1946 the lambs from 48 ewes were dropped between January 27 and February 26. Again only six ewes lambed after February 19, and they were all yearlings that had been bred to a ram lamb. The breeder of this flock raises all of his own ewes and has selected his rams from one flock. It is the practice within this flock to flush the ewes on sudan grass four weeks before turning the ram in. In addition, the yearling ewes are usually given some dry land eastern Oregon alfalfa and the rams are grained. This operator has a large farm with plenty of good pasture available at all times.

Table I

## Reproductive History of Experimental Ewes

Ewe	Birth Date	Ave. Date of Conception	Average Lambing Date	Barren Seasons	Number of Lambs	Lambing Percentage
42A	Mar. 20	Sept. 21	Feb. 16	0	10	166
44A	Mar. 1	Sept. 21	Feb. 16	0	6	100
46A	Feb. 26	Sept. 10	Feb. 5	0	6	100
48A	Feb. 22	Sept. 13	Feb. 8	0	9	150
49A	Mar. 1	Sept. 18	Feb. 12	0	6	100
50A	Mar. 12	Sept. 14	Feb. 9	0	7	116
4239B	April 17	Nov. 10	April 7	0	6	100
176B	Mar.	Oct. 17	March 15	2	4	66
106B	Mar.	Oct. 12	March 10	2	3	60
107B	Mar.	Oct. 29	March 27	4	1	20
112B	Mar. 25	Jan. 11	June 8	3	1	25
100B	Mar.	Oct. 12	March 10	2	2	50
58B	Feb.	Oct. 11	March 9	3	1	25
4241B	Feb.	---	---	4	0	0

Table II

Differences in Lambing Seasons  
of Romney Ewes Bred to Romney and "down" Rams

Year	No. of Ewes	Breed of Ram	Day In First Lamb.	Days to First Lamb.	Length of Lamb. in 180 Days	% of ewes in Season	% of ewes Lamb. in 195 Days	% of ewes Lamb. in 210 Days
1945	99	Romney	9/4	149	81	57	72	88
	50	"down"	9/10	152	48	85	100	
1946	118	Romney	9/3	143	91	63	75	88
	23	"down"	9/5	143	34	100		

Of all the flocks studied, the one from which the group B ewes were selected has the largest number of late lambs. Lambs were still being dropped in May of 1946. This is a relatively new flock, and until the past two years has consisted entirely of purchased ewes from breeders in this locality. Expensive imported rams have been used almost exclusively in this flock. As of April, 1946, there were eleven ewes out of 70 that had not lambed. Some of these ewes were not barren, but lambed in May. Of the 59 ewes lambing by April, only 22 lambed by February 19. Breeding is done without flushing the bucks and no new pasture is provided for breeding. Some oats are provided during breeding and alfalfa is sometimes fed. The ewes of this flock are quite variable as to type, and it is quite evident that they are not kept on as high a plane of nutrition as the ewes in the flock previously discussed.

One of the breeders interviewed has a program of dividing his ewes into two groups. Romney rams are used on one group of ewes for the production of breeding stock, and a mutton or "down" type ram is used on the other group for market lamb production. The age and nutrition for these two groups of ewes were at the same level. A ewe may have been bred to a Romney ram one year and a "down" ram the next. Results from the use of the two types of sires are tabulated in Table II, page 32.

None of the breeders interviewed make a practice of putting the ram with the ewes before the first of September. A few breeders have in the past tried putting the ram in the middle of August, but were unable to have enough ewes served before September to warrant continuing

Table III

Conception Rate  
And Recovery From Abortion

Ewe	Times	Number	Interval From Conceptions	Days From Abortion to Conception	Days From Estrus to Return of Estrus	C.L. at Castration
Not Aborted						
42A	1	1				
44A	0	0	--	--	10	1
46A	1	1	23	9	10	1
48A	2	1	23	14	10	1
49A	1	1	23	16	10	1
50A	1	1	24	6	9	1
4239B	1	1	25	18	10	1
176B	1	1	27	28	10	1
106B	1	1	21	No return	--	1
107B	2	0	--	--	10	0
112B	1	1	23	No return	--	0
100B	2	0	--	--	10	1
58B	2	0	--	--	10	1
4241B	3	0	--	--	10	2

this practice. By turning the ram in too early, there were still individual ewes that conceived late in the season. The result was an even longer lambing operation than normal.

No correlation was found between the birth date and the subsequent lambing dates of an individual. An effort was made to correlate the date of a ewe's first lambing with her subsequent lambing dates. No correlation was found. An analysis of variance was computed to determine if ewes of a flock will maintain a relative order of lambing within the flock throughout their lifetime. If ewes do maintain a relative order of lambing, then it could be assumed that individuals of a flock lambing late in the season should be culled if a flock of early lambing ewes is to be developed.

The statistical analysis gave satisfactory indication that a late lambing ewe of a flock will lamb late each year in comparison with the other ewes of the flock. The order of lambing in the flock analyzed is given for 1944, 1945, and 1946 in Table IV, page 36. An "f" of 2.22 for the variation between ewes is necessary for a high degree of significance. An "f" value of 3.86 was found for the variation between these 24 ewes.

Additional support to the rather conclusive evidence that individuals maintain a relatively constant order of lambing is furnished by a detailed study of Table IV, page 36. For purposes of computation, a converted variable value was substituted for each numerical rank with the first 12 ewes receiving plus values and the last 12 receiving minus values. Converted values were taken from Fisher and Yates (28, Table XX). In the total converted variable values the

Table IV

The Lambing Order of Twenty-four Romney Ewes  
Over a Three-Year Period

	1944		1945		1946		Total
	Numerical	Converted	Numerical	Converted	Numerical	Converted	Converted
	Rank	Value	Rank	Value	Rank	Value	Value*
1	1.95	.4	.96	3	1.24	4.15	
2	1.37	21	-1.14	13	- .32	-.09	
2	1.37	3	1.24	5	.805	3.415	
4	1.04	4	.96	5	.805	2.805	
5	.805	6	.665	1	1.95	3.420	
5	.805	16	- .37	13	- .32	.115	
7	.60	9	.315	13	- .32	.595	
8	.48	17	- .54	13	- .32	-.38	
9	.37	8	.48	13	- .32	.53	
10	.26	6	.665	2	1.50	2.425	
11	.16	19	- .73	20	- .96	-1.53	
12	0	21	-1.14	23	-1.50	-2.64	
12	0	9	.315	7	.60	.915	
14	- .16	2	1.50	4	1.04	2.38	
15	- .37	11	0	10	.156	-.214	
15	- .37	17	- .54	10	.156	-.754	
15	- .37	11	0	19	- .73	-1.10	
18	- .60	11	0	13	- .32	-.92	
19	- .73	1	1.95	9	.37	1.59	
20	- .88	20	- .88	10	.156	-1.604	
21	-1.04	23	-1.50	20	- .96	-3.50	
22	-1.24	15	- .26	22	-1.24	-2.74	
23	-1.50	11	0	8	.48	-1.02	
24	-1.95	24	-1.95	24	-1.95	-5.85	

\*Minimum significant difference in totaled converted values at 5 percent level is 3.86. At the 1 percent level, approximately 5.00.

minimum significant difference at the 5 percent level is 3.86. At the 1 percent level the minimum significant difference is approximately 5.00. In the event of ewes lambing on the same day equal credit was given. The following four points should be noted:

1. Four of the five ewes to lamb first during 1944 remained in the first five for the three-year average.
2. Four of the five ewes to lamb late during 1944 remained in the last five for the three-year average.
3. The first ewe to lamb in 1944 had the highest average for the three years.
4. The last ewe to lamb in 1944 lambed last in 1945 and 1946.

Pedigree study of these two groups of ewes afforded little information. The number of ewes studied was too small to allow statistical analysis of the pedigrees. All of the group A ewes had a common sire and trace through this sire to an imported ram. This imported individual also appears in the pedigrees of all of the group B ewes. Five ewes of group B showed an inbreeding coefficient to this common ram. 42-41B and 176B had an Fx of 18.75 percent, 106B and 107B which were full sisters had an Fx of 12.5 percent, ewe 100B had an Fx of 9.37 percent. In computing the coefficient of inbreeding (Fx), the method set forth by Lush (48, p. 365-367) was used. There was no apparent correlation between the birth dates of the experimental ewes and those of their respective ancestors.

Ewe 42-39B was marked by the ram on July 26 and again on August 12. Ewe 112B was marked by the ram on August 4 and again on August 19. A regular estrual cycle was not initiated on any of these dates and laparotomy in early September failed to confirm ovulation.

Occurrence of the first estrus and ovulation in the flock were observed in ewe 48A on August 28. The latest initial occurrence of estrus and ovulation was in ewe 42-39B on October 23. Onset of the estrual cycle with confirmed ovulation had occurred in all group A ewes by September 26. Figure II, page 39 shows the first occurrence of ovulation and estrus in the experimental ewes. Solid bars indicate the time after September 1 each year when conception took place according to lambing dates and allowing 148 days as the period of gestation. The open bars indicate when these same ewes first actually showed heat and ovulation during the fall of 1946.

Assuming that each observed corpus luteum represented an ovum shed, the ovulation rate for 18 estrual periods in group A was 1.28. For 21 estrual periods in group B the ovulation rate was 1.38. This is not a significant difference. Silent heat was definitely confirmed in only two ewes. Both of these individuals were in group B. No difference in the frequency of ovulation was noted between the right and left ovary. Ewe 42A was bred during the first heat and although two corpora lutea were present, birth was given to a single lamb.

Five ewes of group A were inseminated. Four of these ewes conceived after one insemination and one ewe conceived after the second insemination. All 8 of the group B ewes were inseminated and four conceived after one insemination each. Three of the ewes in this group

Figure I  
Length of Estrual Cycles

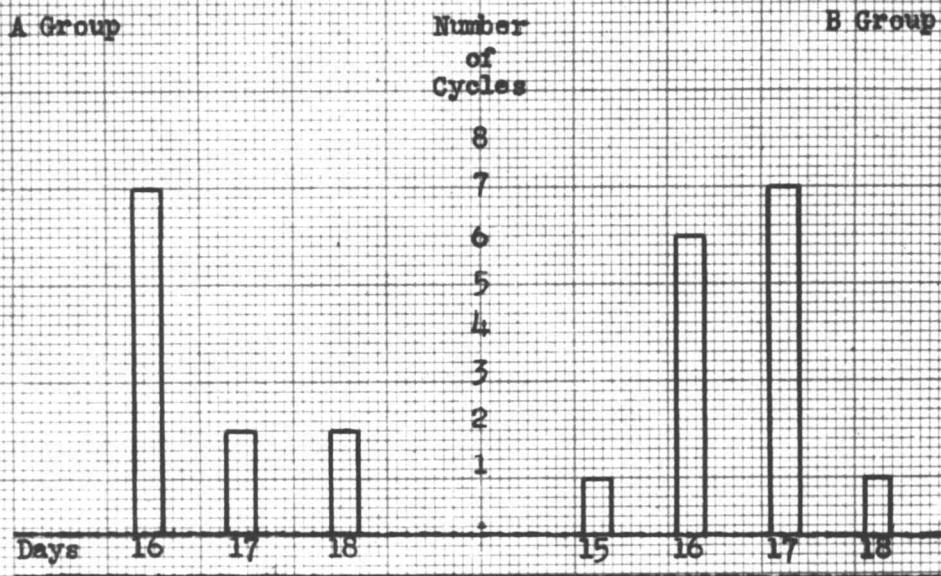
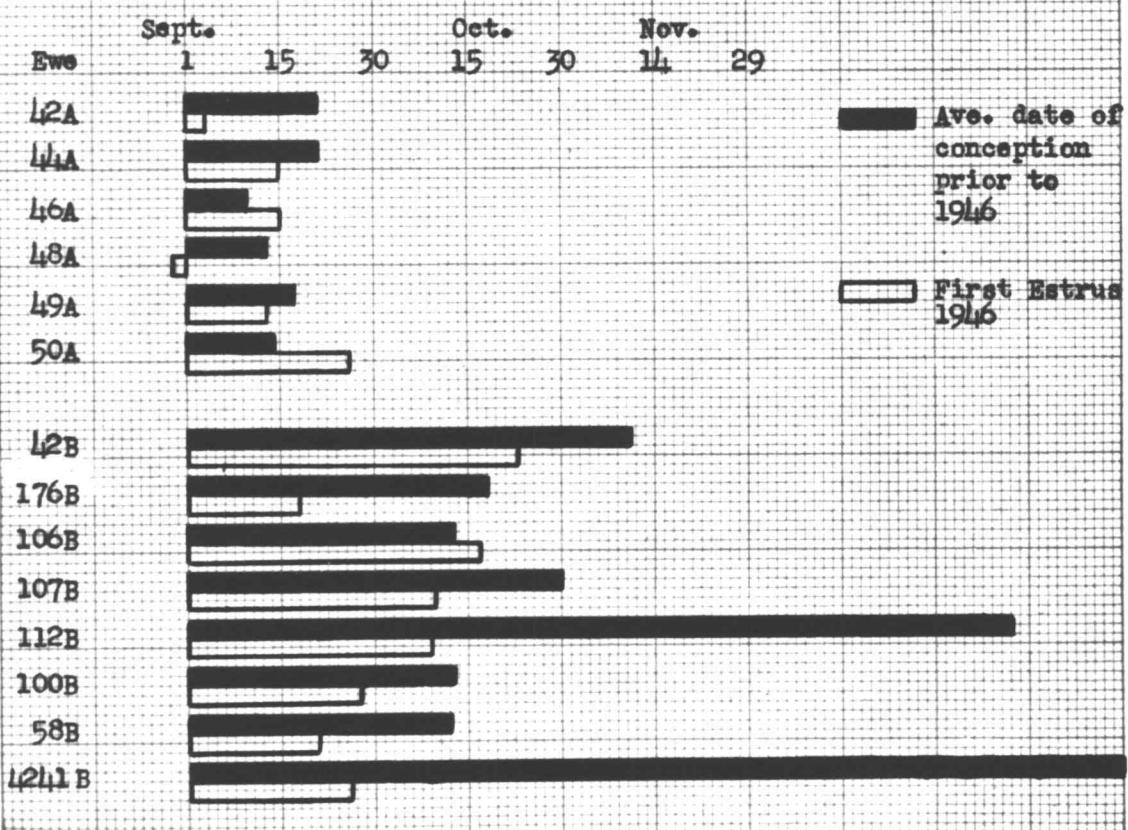


Figure II  
The Onset of the Breeding Season  
in the Experimental Ewe

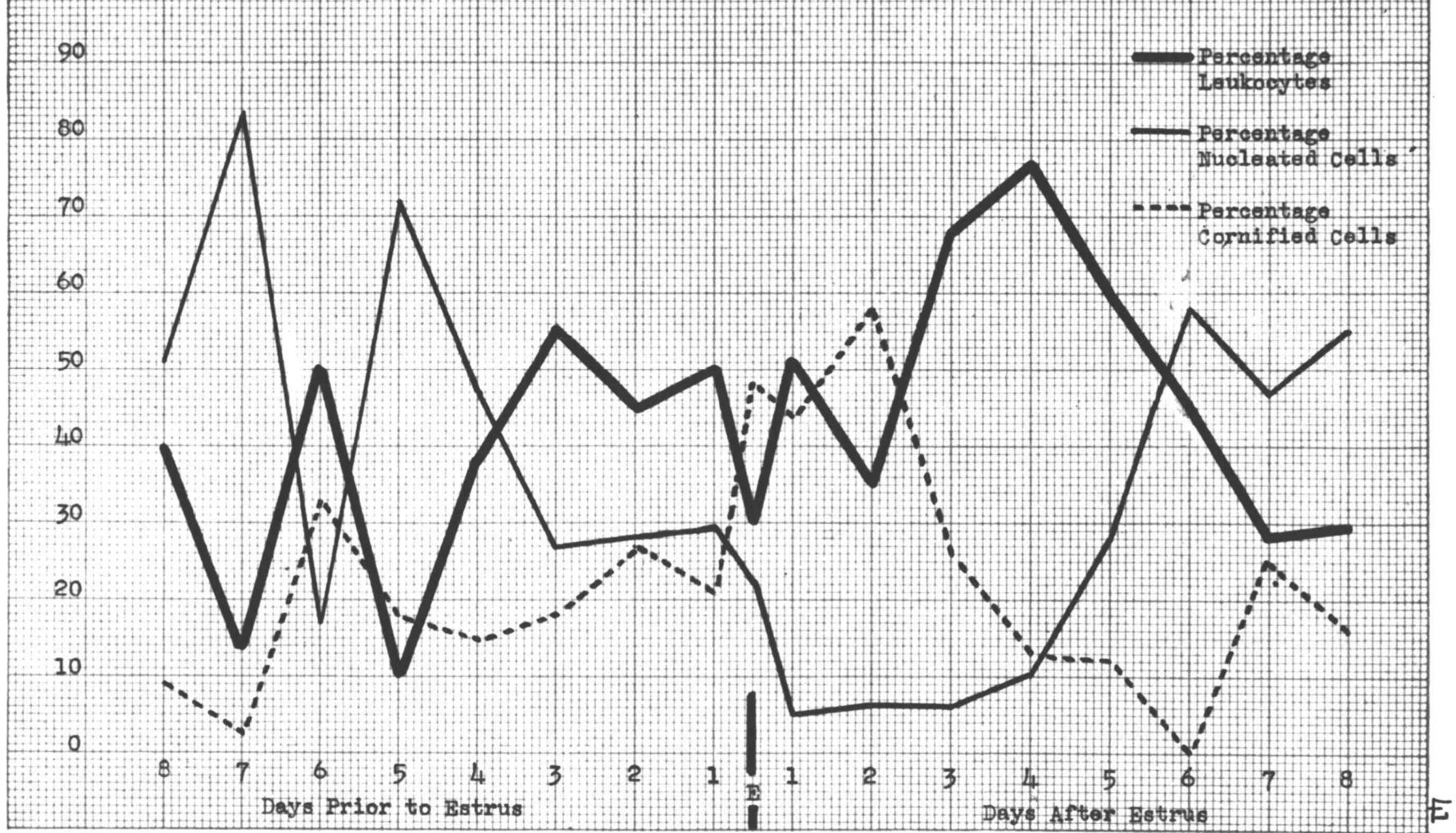


were inseminated twice and one was inseminated three times. None of the latter four ewes conceived. The resulting conception rate was 83.3 percent for group A and 30.7 percent for group B. These data are tabulated in Table III, page 34.

All four of the group A ewes showed estrus and ovulated within an average of 11.25 days after induced abortion. Two of the group B ewes ovulated and came into estrus on an average of 23 days (Table III, page 34) after abortion was induced. The remaining two ewes of group B did not return into estrus and were castrated 30 days after induced abortion. At the time of castration a fresh corpus luteum was observed on the left ovary of one of these ewes, Table III, page 34.

In obtaining vaginal smears it was noted that with the exception of the estrual period there was a variation between the physical consistency of the mucus on the cervix and that of the vestibule of the vagina. Cyclic changes in the cellular content, physical properties, and amount of vaginal mucus were observed. Characteristic smears occurred in the various stages of the estrual cycle, but the change from one type to another was gradual and the duration of each smear stage was quite variable. Length of each smear stage was as variable in an individual as it was between individuals. Although the cellular content followed a definite trend, there was a good deal of variation in actual percentage of content of each type of cell. Judging from observations made with the experimental ewes, only three main types of smears were isolated.

Figure III  
Relationship Between Percentages  
of Leukocytes, Nucleated, and Cornified Cells in Vaginal Smears



The most consistent and characteristic smear observed occurred during estrus. At this stage of the cycle voluminous amounts of clear, thin, viscous mucus appeared in the vaginal tract. This smear was characterized by the sudden appearance of large numbers of cornified cells, Figure III, page 41. Leukocytes and nucleated cells were present in approximately equal proportions. Toward the end of estrus, the mucus began to thicken and turn white. In some instances white flakes were observed in the early stages of this thickening process.

Metestrus was characterized by the appearance of a thick, dry, cheesy smear occurring between the second and sixth days following estrus. The appearance and duration of this smear was quite variable. The cellular content of the smear at this stage was the most striking of those observed. It was marked by extreme numbers of cornified cells and leukocytes accompanied by a virtual disappearance of nucleated cells (Figure III, page 41). The leukocytes continued to increase reaching a maximum concentration four days after estrus. As the leukocytes increased, the cornified cells decreased and the nucleated cell content began to rise.

No striking difference between the smears taken at diestrus and proestrus was noted. The physical consistency and amount of mucus during the last seven or eight days of the cycle were quite variable. Generally, medium to small amounts of thin, clear, or milky mucus were observed at this time. The physical appearance of mucus during these stages was comparable to that seen during early pregnancy. A high content of nucleated epithelial cells was characteristic of smears

Table V  
Testicular Responses of Day-Old Chicks  
To Injections of 10mg. of Sheep Pituitary Powder

Ewe	Number of Chicks	Average Testis wt.	Standard Error of the Mean	"t"
4239B	11	26.16	1.73	.3466
4241B*	8	17.77	2.03	2.3800
58B	8	26.66	2.03	.4870
107B	9	22.91	1.91	.7403
112B**	6	25.30	2.34	.0452
176B	8	22.46	2.03	.8677
44A	6	25.15	2.34	Standard for Comparison
46A	11	22.37	1.73	.9543
48A	9	21.02	1.91	.3734
49A	9	22.21	1.91	.9716
50A	7	22.01	2.17	.9030
Control	6	11.15	2.34	4.2245

\* Ewe had never lambred.

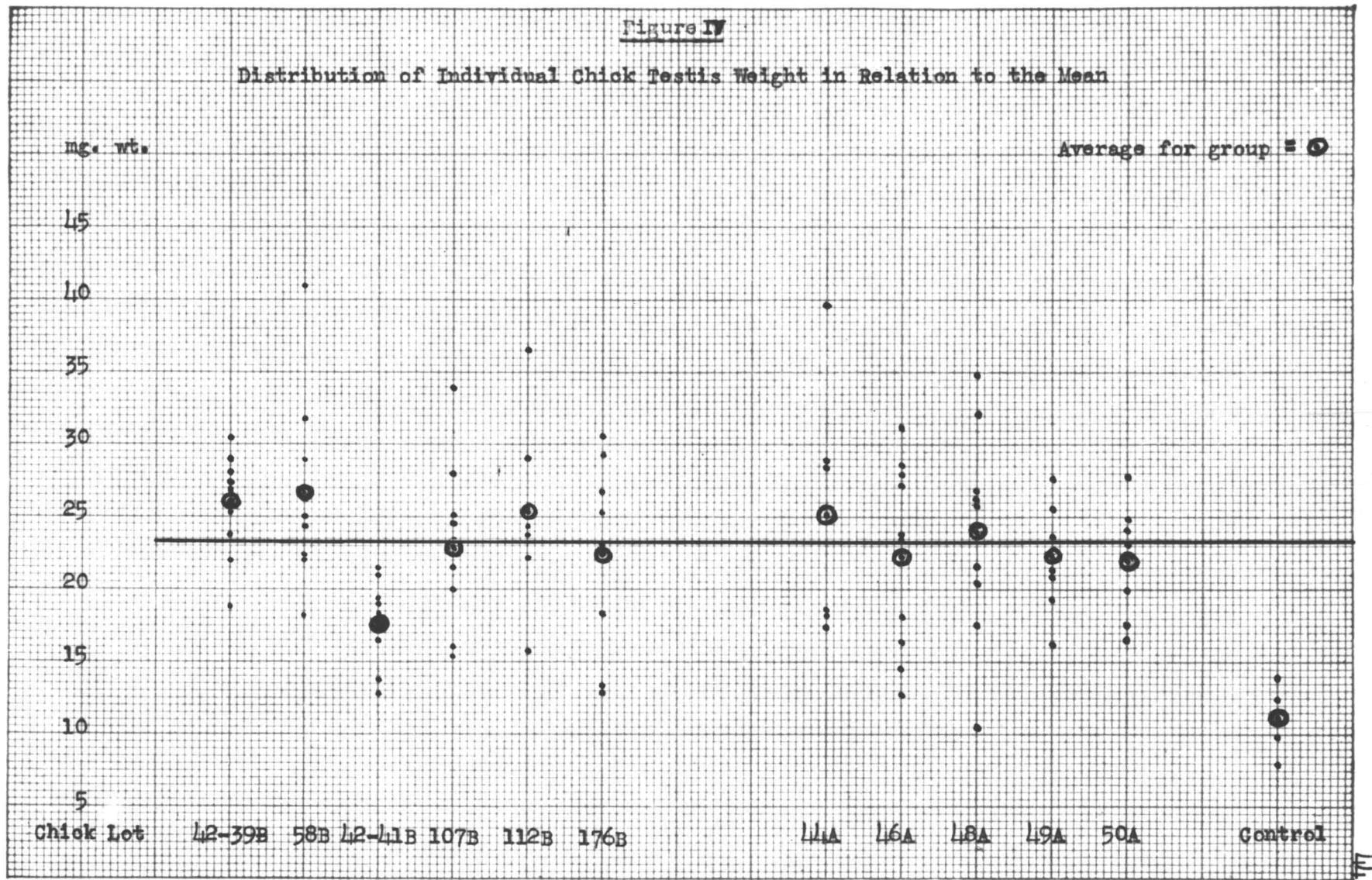
\*\* Not castrated prior to removal of pituitary.

5 percent significance level of "t" with 86 degrees of freedom is 2.00

1 percent significance level of "t" with 86 degrees of freedom is 2.66

Figure IV

Distribution of Individual Chick Testis Weight in Relation to the Mean

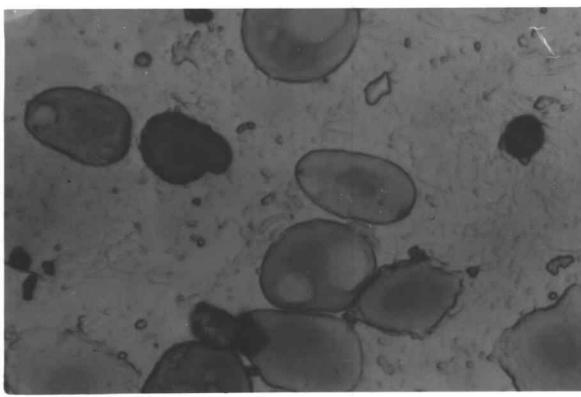


taken at these stages. Fluctuation of leukocytes and cornified cell content was shown by these smears with a rise in leukocyte content three days before estrus.

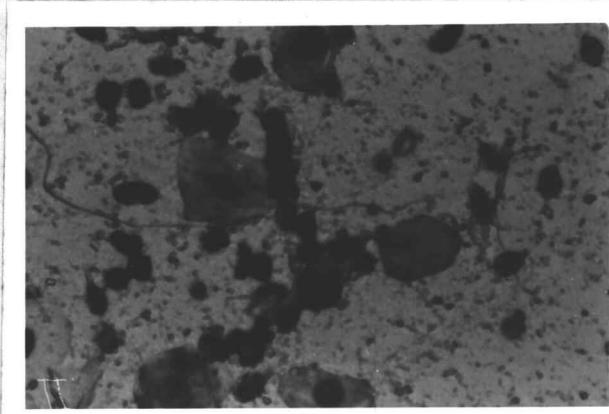
The cellular composition of smears taken at various stages of the estrual cycle is shown graphically in Figure III, page 41. Plates I to IV are microphotographs of typical smears obtained from the experimental ewes during their estrual cycles.

Results of the pituitary test on day-old chicks are tabulated in Table V, page 43. A highly significant increase of testicular weight was obtained in all of the injected chicks. The only significant difference of pituitary potency observed between ewes was in the case of ewe 42-41B. This ewe has no record of ever lambing, and injection of her pituitary elicited significantly low response. The pituitary of ewe 112-B was removed before castration and gave a response comparable to that of the ewes which had been castrated for a period of 90 days. In computing "t," ewe 44A was used as a basis of comparison for the other individuals. Figure IV, page 44 illustrates the distribution of individual testicular weights of the experimental chicks in relation to the mean weight of the group.

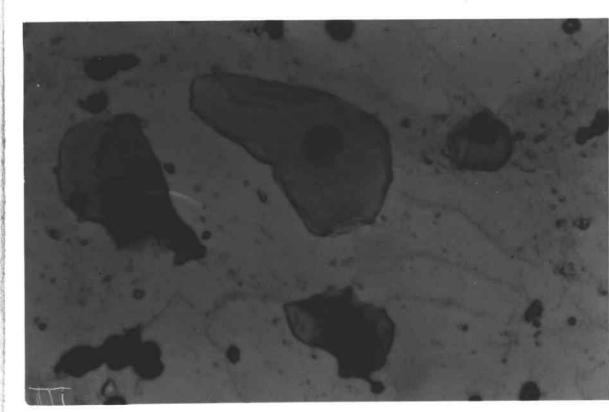
## Plate I



Vaginal smear (48A) five days before estrus.  
Note large proportion of nucleated cells. X 150

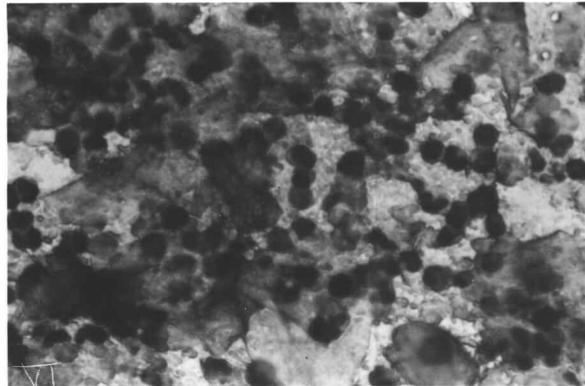


Vaginal smear (48A) three days before estrus.  
Note leukocytes. X 150

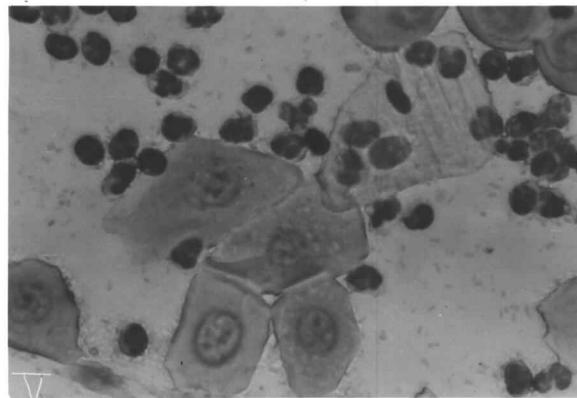


Vaginal smear (44A) estrus.  
Large cornified cells and absence of nucleated cells. X 150

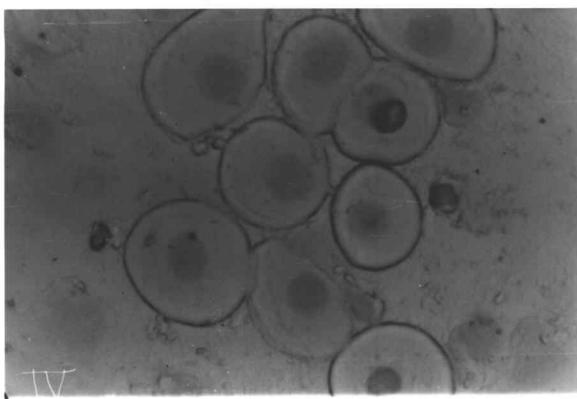
## Plate II



Vaginal smear (50A) three days after estrus.  
Masses of cornified cells and leukocytes. X 150

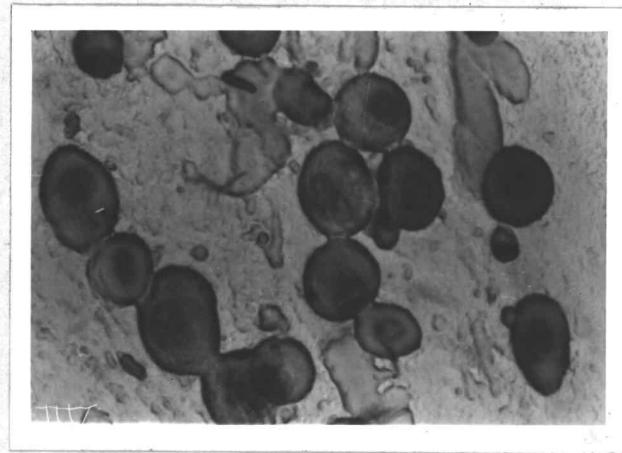


Vaginal smear (50A) four days after estrus.  
Cornified cells disappearing,  
large percentage of leukocytes still present. X 150

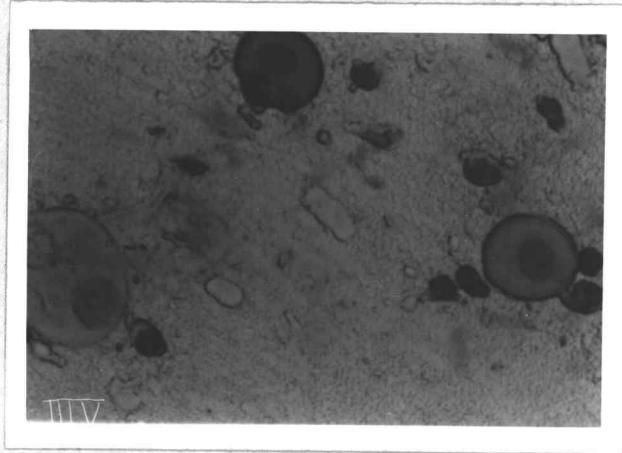


Vaginal smear (49A) seven days after estrus.  
Consists almost entirely of nucleated cells. X 150

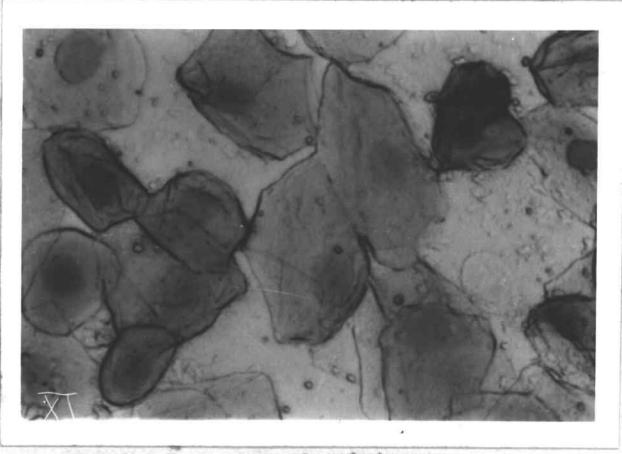
## Plate III



Vaginal smear (176B) five days before estrus.  
Mainly nucleated cells. X 150

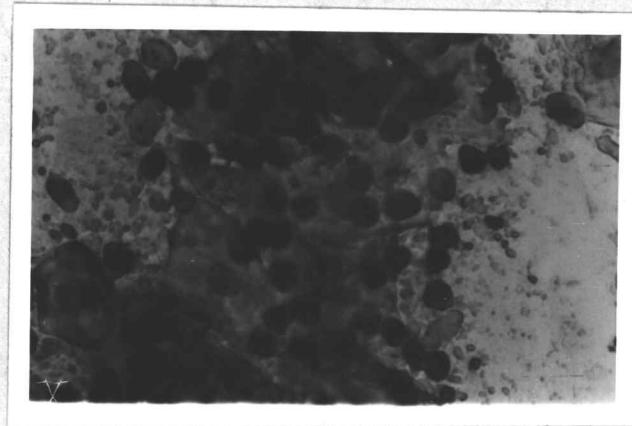


Vaginal smear (176B) two days before estrus.  
Few leukocytes appearing. X 150

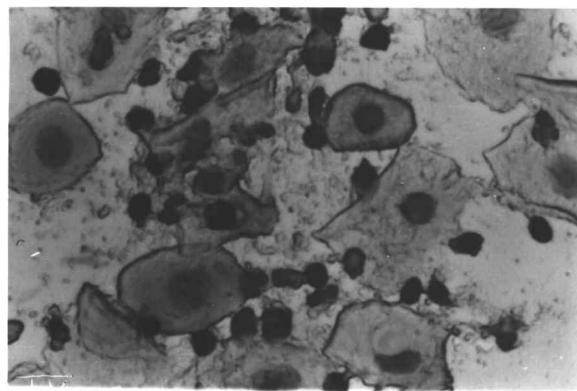


Vaginal smear (176B) estrus.  
Large cornified cells with few nucleated cells. X 150

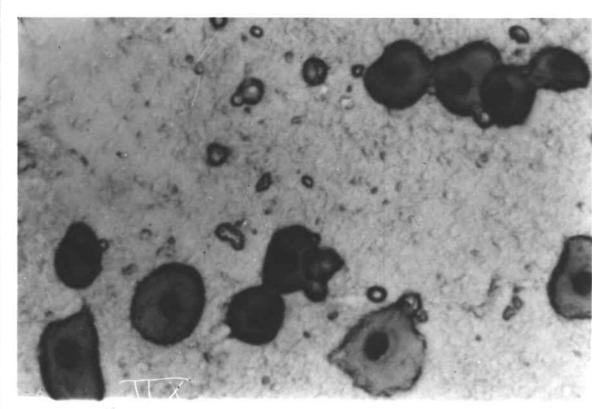
## Plate IV



Vaginal smear (100B) two days after estrus.  
Cornified cells with large percentage of leukocytes. X 150



Vaginal smear (176B) four days after estrus.  
Mixture of leukocytes, cornified, and nucleated cells. X 150



Vaginal smear (176B) eight days after estrus.  
Mainly nucleated cells. X 150

### Discussion

Information obtained from different areas indicates that as compared to fine and medium wool breeds, late breeding is a characteristic to be found in Romney sheep. This conclusion has been borne out by investigations of Cole and Miller (12, p. 45) and Thompson (56, p. 478-479).

The review of literature has shown that factors such as nutrition, hormone administration, and light control will modify the onset of the breeding season of sheep, but with the return of normal conditions the seasonal pattern characteristic of each breed re-establishes itself. In some breeds such as the Merino (31, p. 6) and Dorset (42, p. 18-27) ewes have the capacity to breed at almost any season of the year. Bell (5, p. 1) has shown that over half of the observed mature Rambouillet ewes could have been bred as early as June. Investigations with long wool and mutton types indicate that the onset of the breeding season is in the fall (52, p. 139), (16, p. 45), (36, p. 98). Kelley and Shaw (42, p. 27) observed that breeds vary as to earliness of breeding and that this is reflected in crosses of breeds. Thompson (64, p. 480) found that crossbreds usually have a breeding season midway between that of the two parent breeds. Cole and Miller (16, p. 45) found Romney-Rambouillet crossbred ewes to have an average breeding season between that of the two parent breeds.

From information such as this it can be deduced that the time of the onset of sexual activity is an individual breed characteristic, which like other breed characteristics has a genetic basis. As

with all genetic characteristics, variation on either side of the average can be expected to occur.

Data from flocks included in this study indicate considerable variation does occur in the breeding season as computed from the lambing dates of Romney ewes. The fact that the ram may be at fault is indicated in Table II, page 32. Observation of the two groups of ewes in the experimental flock establishes that variation in the occurrence of heat and ability to conceive is found in individual ewes or groups of ewes (Figure II, page 39 and Table I, page 32.)

The two groups of experimental ewes showed a marked difference as to the onset of heat during the fall of 1946. This difference was not as great as that indicated by previous lambing records of the two groups (Figure II, page 39). Onset of heat in the high fertility group was quite consistent as compared to their previous records. In the low fertility group, the onset of estrus was earlier in 1946 than was to be expected from previous lambing records. It is quite possible that being on a high plane of nutrition these ewes displayed heat at a time which had been a "silent heat" period during their previous seasons. Grant (32, p. 802) has advanced the theory that increased nutrition will convert the first "silent heat" into a normal estrual period.

Results obtained from insemination of the two groups, Table III, page 34, indicate that although the low fertility group ovulated at the same rate as the high fertility group, they have a lower percentage of conception. This situation may be due in part to incomplete preparation of the genital tract for implantation of the fertilized ova.

Although fertilization may occur, the fertilized ova fail to implant in the uterus. It can also be postulated that ova shed by this group were not mature or in a condition to bring about complete fertilization.

A difference between the two groups either in the hormone level or capacity of the organs to respond is reflected by the ability to return to estrus after surgical removal of the fetus in those ewes that conceived (Table III, page 34). Incomplete hormonal stimulation in the low fertility group following removal of the fetus may be indicated by the fact that one of the ewes at time of castration had a fresh corpus luteum but had failed to display heat.

Examination of ewe 42-41B revealed an abnormal anatomical structure of the reproductive tract. It was impossible to pass the speculum fully into the vagina of this ewe due to the presence of a strong sphincter muscle anterior to the vestibule. The uterine horns and cervix were under-developed and the cervix was abnormally long laying in the shape of an "S". In this particular case it is quite probable that pregnancy was prevented solely by mechanical impediment since normal ovulation and estrual cycles were displayed.

A comparison of the management and feeding practices found in the two flocks from which the experimental ewes were selected would afford an indication as to the cause of the different levels of fertility. However, since most of the ewes in the low fertility group were purchased as mature animals, it is difficult to compare adequately the two flocks on the basis of lifetime nutrition because the early nutritional history of the low fertility group is unknown. Ewes of the high fertility group were in much better condition at the beginning of the

experiment and were much more uniform in type than those of the low fertility group.

Ewe 42-39B was marked twice by the ram within a period of 24 hours during July 25 and 26 and again on August 12. Examination of the vulva on each of these dates disclosed a slight swelling accompanied by the presence of a liberal amount of thin mucus within the vagina and on the lips of the vulva. A vaginal smear taken on August 16 had the physical characteristics of a typical metestrus smear, but was composed of approximately equal numbers of leukocytes and nucleated epithelial cells. Practically no cornified cells were seen. The breeding season was not initiated on these dates and laparotomy in early September failed to confirm ovulation during these indications of heat. It is possible that the ewe was caught by the ram and marked forcefully. Considering the condition of the vulva and vagina in conjunction with the interval between markings, it is quite likely that heat without ovulation did occur. The vaginal smear taken four days after the second marking would also point to the possibility of an incomplete occurrence of normal estrus. The mucus had the physical properties to be expected, but the cellular content was different than that generally found at the metestrus stage. McKenzie and Terrill (53, p. 47) and McKenzie, Allen, et alii (50, p. 280) report heat without ovulation as occurring in ewes. However, McKenzie and Terrill observed this to occur toward the end of the breeding season. Due to the interval between indicated heat and laparotomy, a corpus luteum may have been missed at the time of the operation. If ovulation did occur, the physiological condition was not at the proper state to allow initiation of a normal breeding season.

Ewe 112B was marked by the ram on August 4 and again on August 19. Vaginal smears taken on the latter date had the physical appearance of true estrual smears, but were composed almost entirely of nucleated cells and a few cornified cells. Leukocytes were practically absent in these smears. A cheesy smear was taken on August 29th. It was composed almost entirely of leukocytes, but contained a few cornified and nucleated cells in approximately equal numbers. This smear displayed one of the heaviest concentrations of leukocytes seen in any of the vaginal smears. No further indications of heat were shown by this ewe until October 9 at which time ovulation was confirmed. Laparotomy in early September failed to confirm ovulation during August as no corpora lutea were seen.

Another possible indication of heat without ovulation was observed in ewe 107B. This ewe was castrated ten days following the third exhibited heat period. The ovaries were closely examined, but no corpora lutea were identified, which would indicate that ovulation had not occurred at the last heat period. Initial onset of the breeding season in this individual was on October 10. Examination of the ovaries at the time confirmed ovulation. Two old yellow bodies were seen on the left ovary which would indicate that at least one "silent heat" had occurred prior to displayed estrus. Insemination at two different heat periods failed to produce pregnancy in this ewe.

These possible failures to ovulate during heat are further indication that in ewes of low fertility either the hormone stimulation is incomplete, or the organs do not properly respond to stimulation. It is significant that no indication of abnormal or incomplete estrual cycles was noted in the high fertility group.

Indication of the inheritance of fertility is shown by a comparison of ewes 107B and 106B. These two ewes are twins that were dropped in March, 1940. Records show that for five seasons ewe 107B lambed once and that was on March 27, 1946. Ewe 106B lambed three times during this same period with an average lambing date of March 10. The first heat shown in the fall of 1946 was on October 10 for 107B, and October 17 for ewe 106B. Ewe 107B displayed heat without ovulation and failed to conceive when inseminated on two different estrual periods. Ewe 106B conceived with one insemination, but failed to return to heat within 30 days after removal of the fetus. At the end of this 30 days the ewe was castrated and one fresh corpus luteum was observed which would indicate that ovulation had occurred, but heat was not detected by the ram.

It is the writer's opinion that comparisons of this nature will afford one of the most valuable aids available in improving the fertility of sheep. Unfortunately, there are few complete records kept by sheepmen that lend themselves to accurate analysis. Most of the available records make dam and sire progeny comparisons over a period of years quite difficult. A system of record keeping that will place some emphasis on fertility and lambing dates is essential if a program for the improvement of fertility in sheep is to attain any degree of success. Such records will entail little more if as much time as is devoted to some systems of record keeping now used by certain breeders. Culling of only a few barren or low fertility ewes would provide more than adequate returns for the time involved in the keeping of workable records. Initiation of such a program could be the responsibility of

the registry association working in conjunction with the Agricultural Extension Service. Stricter requirements for registration would help improve record keeping. As an example, some associations require only the month of birth, not the date, to be given in an application for registry. Surely such laxness does not stimulate interest in the accuracy of record keeping. The association could issue standard record keeping forms which would be subject to inspection at all times. Forms could be set up in such a manner that progeny comparisons could be made quickly and accurately.

Until standard forms are set up by the associations, individual breeders can devise their own record forms. Such forms need not be elaborate, but they should be kept on the basis of lifetime production. A complete and up-to-date record of each female's actual production will provide an excellent basis for the selection of replacement breeding stock. Too often replacement ewes and rams are selected on the basis of physical characteristics without due emphasis placed on the fertility records of their respective dams and sires.

In setting up a system of records the entire reproductive history of each individual should be kept on a single page of the record book. Most records that are being kept today list the ewes in lambing or numerical order for each year. This makes it difficult to evaluate the performance of any single individual. Ewe records should include:

1. Birth date of the ewe.
2. Number of days each year from the time the ram is turned in until the lambing date.

3. Number of times bred each season before settling.
4. Identification of the ram used each year.
5. Number of lambs dropped and the relative rank or order of lambing within the flock each year.
6. Fertility records of progeny.

Other pertinent data could be included according to the desire of the breeder. Records of rams can be as detailed as the breeder deems advisable, but should at least include the percentage of ewes settled and number of services performed during the breeding season. If such data were available, it would not be many years before animals could be selected on the basis of a more accurate knowledge of their fertility background.

The pedigree study provided insufficient data for any conclusions at this time. Tracing of the pedigrees was done with the hope that low fertility and late lambing could be associated with certain lines within the breed. A ram imported into this country in 1929 appeared in the pedigree of all of the ewes in both groups. Five of the eight ewes in the low fertility group have an inbreeding coefficient to this ram ranging from 9.37 percent to 18.75 percent. This may be an indication that concentration of this individual's blood will cause a lowering of fertility. An extensive study of this sire's progeny is necessary before any conclusions of this nature can be made.

Since this imported ram was used very extensively for a number of years, it is quite probable that he appears in the pedigree of most individuals of the Romney breed in this locality. Some of the Romney breeders of this area who have been in the business for a long time recall that several years ago a few lambs were dropped during

the last week of December. At the present time very few Romney ewes lamb as early as the last few days of January. The problem of late lambing in the Romney sheep of this area is a comparatively recent one if the memory of these breeders can be accepted as reliable.

Early importations of Romney sheep into this country may have been of early lambing strains. Subsequent ram importations may have been based on body type and fleece rather than fertility. Consequently, late lambing and low fertility may have been introduced. Further detailed study along this line might prove quite valuable to breeders in the selection of rams for future importation.

In the pedigree study no correlation was noted between the birth dates of individuals and those of their respective ancestors. Before a thorough study of this factor could be made, it would be necessary to have information as to (1) the date rams were turned in with the ewes, and (2) other management and environmental factors that would affect lambing dates.

No statistical significance was found between the birth dates of ewes and their subsequent lambing dates. This is probably due to variability of environmental and management factors from year to year. In such a computation it would also be necessary to determine and evaluate the influence of the ram from year to year.

The relative order of lambing in a group of 24 ewes with the records for three consecutive years was analyzed, Table IV, page 36. Ewes were listed in order of lambing for each of the three years and equal rank given those lambing on the same day. A high significance of variation between these ewes was found. In other words, the individual

order of lambing was closely maintained by each ewe. These results indicate a simple basis for selection toward early lambing.

By culling the last five ewes which lambed in 1944, only the consistently late lambing ewes of this flock would have been disposed of. For the three-year average, four of these ewes were among the last five to lamb. Although the other individual did not remain in the last five, she was near the middle of the lambing season in 1945 and 1946. Some errors will be made if flocks are culled in this manner, but results shown in the group analyzed point to a moderate degree of accuracy. Until some simpler and more accurate method is devised, this basis of culling may be used to advantage in flocks where late lambing is a problem.

The few potential early lambers that might be culled through application of this system will be compensated for by the removal of the very late lambing individuals. Generally, late lambing ewes are of low fertility. This is not always true, but more barren seasons are found in the records of late lambing ewes than in the records of early lambing ewes.

Results obtained in this study indicate that vaginal smears can be used only as a general guide in determining the stage of the estrual cycle in ewes. The technique employed in obtaining vaginal smears did not afford a critical diagnosis of exact phases that have been obtained by workers in the study of laboratory animals. Individual variation in cellular content of the smears at different stages of the cycle was as great within ewes as that observed between ewes of high and low fertility.

Until more critical methods of obtaining and staining smears are developed, the diagnostic value of vaginal smears will be limited. The physical consistency of the vaginal contents can be used as a guide in checking estrus as determined by the ram and in the employment of artificial insemination. Insemination should be done when a voluminous amount of thin, clear mucus is present. Results obtained in this study indicate that such a smear is characteristic in ewes at the height of estrus.

No significant difference in the gonadotropic hormone potency of the pituitary was noted between the high and low fertility groups. All chicks injected with glandular tissue showed a very significant testicular response over the control chicks which were injected with pure physiological saline. The pituitary from ewe 4241B elicited a low testicular response at the 5 percent significance level, Table V, page 43. This individual had not lambred in four seasons and failed to conceive after being bred three times in the fall of 1946. In addition to the apparent low potency of the pituitary, this ewe had an under-developed and abnormally shaped genital tract. It may be postulated that an inherently low pituitary potency resulted in an abnormal development of the genital tract.

Ewe 112B was not castrated prior to removal of the pituitary. Injection of her pituitary into six chicks gave as great a testicular response as that found in the other groups of chicks. This is not in accordance with the findings of other workers who have determined that the gonadotropic potency of the pituitary increases after castration. It is difficult to postulate the cause of our results with this ewe if

results obtained by Warwick (69, p. 4) are accepted. By testing of sheep pituitaries castrated at different seasons, Warwick found that the stored potency of the pituitary is not significantly affected by the season in which castration is done.

Results obtained in the pituitary potency test carried on with the ewes in this experiment would indicate that there was no significant difference in pituitary potency between the high and low fertility groups. More work should be done on critical testing of relative pituitary gonadotropic potency of sheep before any definite conclusions may be reached. The unexpected results obtained with ewe 112B suggest that the technique employed may be subject to doubt.

If accuracy of the pituitary test is accepted, it can be assumed that with the possible exception of ewe 42-41B, the differences in fertility of these ewes were not due to a difference in levels of pituitary gonadotropins. A possible cause of the difference in levels of fertility may be the capacity of the organs to respond. This is a problem that merits investigation since the difference in ability between groups to return to estrus following induced abortion, Table III, page 34, indicates that the group B ewes have either a low level of hormonal stimulation or have an inferior capacity to respond.

"Chick unit" values were not computed for each ewe, because due to errors in sexing of the chicks, not enough individual testicular weights were obtained.

### Summary and Conclusions

1. The breeding season of sheep can be altered by a change of environmental factors or hormone administration.
2. Under normal conditions, the time of onset of the sexual season is a breed characteristic with variations found within the breed. This indicates that fertility and time of breeding and conception are inherited characteristics.
3. These studies indicate that delayed conception and a prolonged lambing season in Romney sheep may be influenced either by the ram or by the ewe.
4. At the time of observed ovulation, there was no significant difference in the ovulation rate between ewes of high and low fertility.
5. The rate of conception in the high fertility group was 83.3 percent and 30.7 percent in the low fertility group.
6. Low fertility ewes may not conceive until several heat periods have occurred. This would indicate either a low receptivity of the genital tract, the production of immature or otherwise faulty ova, or the entrapment of ova in the follicle. Hormonal stimulation may be cumulative in effect, and a preparatory period of sexual activity may be necessary before a condition of complete receptivity in the reproductive organs is reached. Failure of pregnancy to persist may be due to insufficient development of the corpus luteum.
7. Low fertility ewes have less ability to return to estrus after induced abortion than do high fertility ewes.

8. Estrus was displayed in late July and August by two low fertility ewes. Laparotomy failed to confirm ovulation on these dates and the sexual season was not initiated at this time in either of the ewes. Initiation of the breeding season and estrus with ovulation did not occur in these individuals until late fall.

9. Ovulation without estrus and estrus without ovulation were observed in the low fertility group. No abnormal or incomplete estrual periods were seen in the high fertility group.

10. A more complete and accurate system of record keeping should be adopted by sheepmen. Such records should afford comparisons as to the fertility of ewes and rams and fertility of their offspring.

11. Five of the eight ewes in the low fertility group had an inbreeding coefficient to a ram imported in 1929. The inbreeding coefficients to this individual ranged from 9.37 percent to 18.75 percent.

12. No significant correlation was found between the birth dates of individuals and those of their respective ancestors.

13. No statistical significance was found between the birth dates of individual ewes and their subsequent and respective lambing dates.

14. Ewes maintained their relative order of lambing within a flock to a significantly high degree. On this basis records for one lambing season might be used to cull late lambing individuals from a flock.

15. In this study the physical characteristics of the vaginal smears were a more reliable criteria of estrus than the cellular content.

Three distinct physical stages were found to occur in the vaginal contents:

- a. Estrus — voluminous, thin, clear mucus.
- b. Metestrus — thick, dry, cheesy mucus.
- c. Diestrus and proestrus — medium to small amounts of thin, clear, or milky mucus.

The duration and occurrence of each type of smear was quite variable.

16. Leukocytes and cornified cells increase at estrus and continue to increase for two to four days. The smears taken midway between heat periods were characterized by a large proportion of nucleated cells.

17. No significant difference was observed in smears of the high and low fertility ewes. Perhaps a more critical technique would show differences in smears of these ewes.

18. The only ewe to show a significantly lower pituitary potency in comparison to the other individuals was a ewe with an abnormal reproductive tract. This ewe failed to conceive in five different seasons.

19. The pituitary test that was used indicated no significant difference in the gonadotropic potency between the low and high fertility groups.

20. All chicks injected with pituitary material showed a significant testicular response over that of the controls.

21. The pituitary from a low fertility ewe that had not been castrated elicited as much response in the test animals as did pituitaries from ewes that had been castrated.

22. If accuracy of the pituitary test is accepted, it can be assumed that with the possible exception of one individual, the different levels of fertility in the experimental ewes was not due to differences in levels of pituitary gonadotropins. A weakness of the test employed is that only the residual or stored gonadotropins were measured. Further research should be done with sheep to determine if this method of testing reflects the relative pituitary potency to be found during the breeding season.

23. Investigations should be done in comparing the ability of low and high fertility ewes to respond to hormone stimulation.

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