A STUDY OF SOME EFFECTS OF PROGESTERONE AND PREGNANT MARES' SERUM (PMS) ON REPRODUCTIVE PHENOMENA IN THE ANOESTROUS ROMNEY EWE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I INTRODUCTION

The breeding seasons of most vertebrate species are determined by two sets of causes, the ultimate and the proximate (Baker, 1937). Bullough (1951) describes the proximate causes of the timing of a breeding season as being threefold; firstly, the internal reproductive rhythm, which usually has an approximate periodicity of one year; seaondly, the environmental variant to which the cycle is attuned; and thirdly, the so-called psychological factors which are, as yet, inadequately studied and in many cases are indistinguisable from the environmental causes. He further points out that the ultimate causes, which are of a teleological nature, were originally invoked in order to explain why a particular time of the year is the most beneficial for the purpose.

It is not surprising that the most easily examined of these factors, namely the environment, has received most attention in the domestic animals. Investigations into the factors controlling the breeding season* in the domesticated ewe have raised high hopes for successful out-of-season breeding plans.

Such studies have particular significance when

*The term 'breeding season' is employed in a restricted sense to indicate that period of the year when mating behaviour is evident and when copulation results in fertilisation. In direct antithesis, the term 'non-breeding season', or 'anoestrus', refers to the remaining portion of the year.

applied to New Zealand where there are major economic benefits to be had by earlier lambing and twice-year lambing. In the North Island, because of its comparatively mild temperature and well-distributed rainfall, there is really no time of the year when lambs could not be reared successfully. The normal procedure is to put the rams to the ewes in March, but there is evidently a desire on the part of more progressive farmers to advance the time of mating by one or two months in order to profit from the high prices for early-season fat lambs. Unfortunately the Romney, which is predominant on fat lamb producing farms, does not normally enter upon the breeding season until March. Here, then, is the broad justification for research into methods of procedure for out-of-season breeding in the Romney ewe.

As a result of a perusal of the literature on the subject, it was evident that present methods of attacking the problem with hormones, notably progesterone and pregnant mares' serum (PMS), had resulted in successful synchronisation of ovulation and oestrus but because of poor lambing results, it was equally clear that little was known about the response of the reproductive tract, per se.

Therefore, a study, to be detailed presently, was undertaken, in which not only was oestrus and ovulation looked upon as physiological endpoints for the treatments used, but further, the histological changes in the reproductive tract and the gonadotrophin content of the pituitary gland were examined.

II THE REVIEW OF LITERATURE

A. The Extent and Significance of the Anoestrous Period

1. Introduction

The seasonal nature of oestrus in the sheep is well established. Table 1 lists some data reported in the literature on the length and occurence of the anoestrous period. For a more complete review see Hafez (1952). It will be observed that the breeding season, as indicated by cyclic recurrence of oestrus, generally commences in the autumn, continues through the winter and ends in the early spring months.

Hammond, Jnr. (1944) pointed out that the breeding season is roughly evenly spaced about the shortest day; but there are differences between breeds in the length and occurence of the breeding season which Hammond (1947), Hafez (1952) and Yeates (1954) attribute to the latitude in which the sheep developed.

Anoestrus, however, is a relative rather than absolute quiescence. Kupfer (1928), Grant (1933), Roux (1936), Loginova (1939), Quinlan et al. (1941), Hammond, Jnr. (1944), Robinson (1950) and Sayed et al. (1952) have cited instances of ewes ovulating and in some cases conceiving in the anoestrous period. Robinson (1951) concluded that there is an inverse relationship between pituitary activity and hours of daylight and that this fundamental relationship accounts for the seasonal nature of the reproductive rhythm

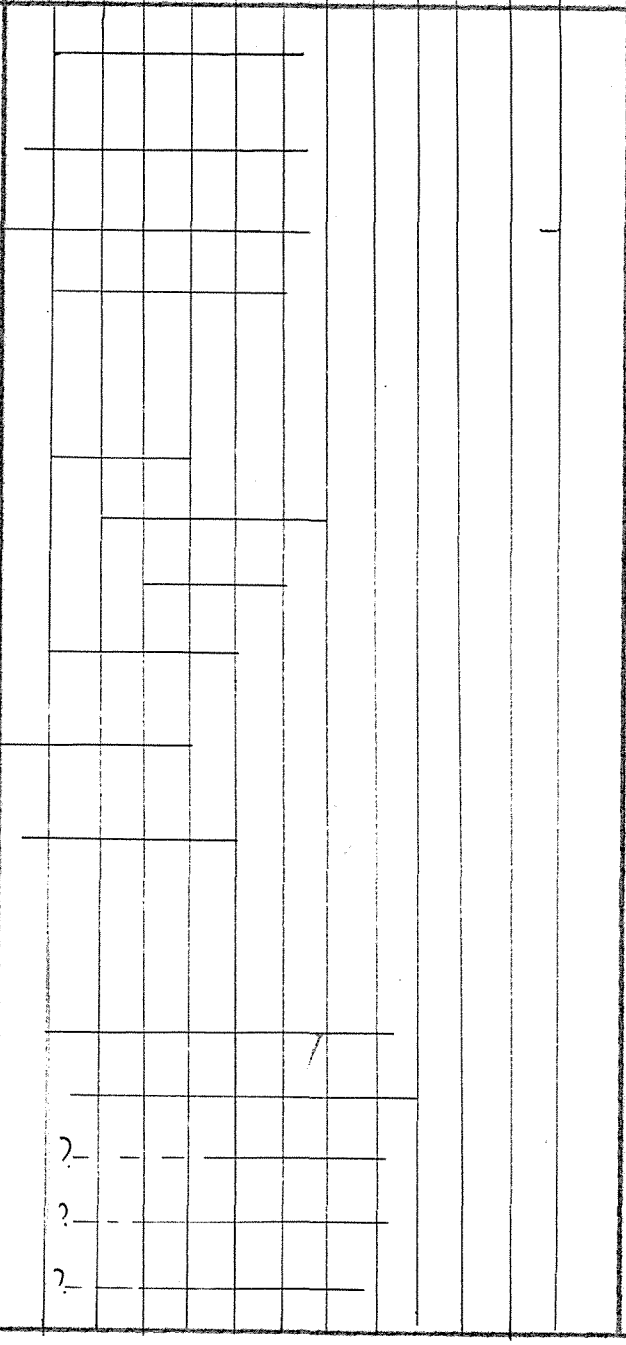
TABLE I

Length of Anoestrus in Different Breeds of Domesticated Sheep

Authority	Breed	Anoestrus - Northern Hemisphere												Length in mths
		J	F	M	A	M	J	J	A	S	O	N	D	
Kupfer, 1928	European													0
Villegas, 1929	European													0
Schott et al., 1939	Corriedale													4
Schott et al., 1939	Dorset													3
"	Dorset/ Tas. Mer.													3
"	Dorset/ Del. Mer.													4
McKenzie & Terrill 1937.	Grade													7
McKenzie & Phillips, 1930.	Hampshire													
Schott et al., 1939	Hampshire													
McKenzie & Terrill 1937	Hampshire													7
Schott et al., 1939	Karakul													
Phillips et al., 1945	Karakul													
Phillips 1947	Karakul													4
Loginova, 1939	Karakul													0
Schott et al., 1939	"/Scottish													
Schott et al., 1939	Merino													1
Cole & Miller, 1935	Rambouillet													
McKenzie & Terrill 1937	Rambouillet													9
Cole & Miller, 1935	Ram/Romney													5
Cole & Miller, 1935	Romney													
Mihaila, 1934	Romney													
McKenzie & Phillips 1930.	Shropshire													
Schott et al., 1939	Shropshire													
McKenzie & Terrill 1937	Shropshire													
McKenzie & Phillips 1930	Southdown													
Schott et al., 1939	Southdown													
McKenzie & Terrill 1937	Southdown													
Hammond, Jnr., 1944	Suffolk/ B.L. - Chev.													6

TABLE 1 (cont.)

Authority	Breed	Anoestrus - Southern Hemisphere J A S O N D J F M A M J	Length in months
Kelley & Shaw, 1939	Dorset		5½
Kelley & Shaw, 1943	Border Leicester		6
Underwood et al., 1944	B.L./Mer.		7
Kupfer, 1928	Merino		5
Quinlan & Mare, 1931	Merino		0
Marais, 1936	Merino		3
Roux, 1936	Merino		5
Kelley, 1939, 1946	Merino		3
Stewart & Moir, 1943	Merino		4
Underwood et al., 1944	Merino		4
Underwood et al., 1944	Merino		4½
Kelley & Shaw, 1943	Merino		0
Walker, 1943	Romney		7
Walker, 1943	Romney		7½
Till, 1950	Romney		
Goot, 1949	Romney		
Webster, 1955	Romney		



exhibited by the ewe.

Recent information suggests that Robinson's thesis cannot be accepted with certainty. Kammlade, Jnr. et al. (1952) studied the gonadotrophin content of the pituitary glands of anoestrous and cycling ewes and concluded that total gonadotrophin content was at as high a leval during anoestrus as during the follicular phase of the oestrous cycle. While Yeates (1954) puts forward a strong case for light as being the major controlling factor in the annual reproductive rhythm of the ewe, Hammond, Jnr. (1954) draws attention to what he considers are errors in the interpretation of the available data. A portion of the studies herein reported is concerned with the pituitary gland gonadotrophin content of the Romney ewe, and this aspect will be reviewed more fully later.

While it must be admitted that light plays an important, albeit unexplained, part in the breeding behaviour of the ewe, it seems fair to say, on the basis of available evidence in the literature, that the factors controlling the phenomenon of anoestrus in the domesticated ewe have not been established.

2. Factors modifying the extent of the anoestrous period

i. Breed - It will be seen from Table 1 that there are considerable differences in the occurrence and length of the anoestrous period in domesticated sheep. Although certain breeds such as the Romney, Hampshire and Shropshire have a definite breeding season extending from

autumn to spring, other breeds, such as some strains of the Australian Merino, appear to be capable of breeding at any time of the year. This may be because of selection towards an early breeding type over many years (Kelley & Shaw, 1943; Underwood et al. (1944). Hafez (1952) pointed out that within the same environment, but from year to year, a particular breed will begin cycling at a constant date, ceteris paribus. Radford & Watson (1955) have observed marked variation, however, by individual ewes in the commencement of their breeding season.

That variations in the length of the breeding season have a genetic basis is supported by the fact that progeny of crosses of different breeds have breeding seasons intermediate in length to those of the parents (Cole & Miller, 1935; Schott et al., 1939; Walker, 1943; Kelley & Shaw, 1943; Hammond, Jnr., 1944; Hafez, 1952). Further support is gained from the observations of Kelley & Shaw (1943) that the onset of the breeding season is different for different strains within the same breed.

ii. Latitude - Marshall (1936) drew attention to the fact that when British breeds of sheep are transported from England to South Africa they finally alter by six months the time of the calendar year at which they breed, so conforming with the seasons in their new environment. This was strong evidence that the seasonal nature of reproduction in sheep must be due largely to some influence of the external environment and Marshall (1936) postulated that if these animals

react to light, it must be to diminution and not to increase, as they are autumn breeders.

A theory of natural selection, based on the conception of characteristic response to the light environment, is held by Yeates (1954) to explain both the time of onset and the duration of the sexual season of sheep evolved in different latitudes, and he points out that the precise details of breeding following transferences, say, from one hemisphere to the other, depend on the time of year of the movement and on the reaction time to light of the individual animal concerned.

iii. Age - It is generally agreed (McKenzie & Phillips, 1930; Cole & Miller, 1935; Roux, 1936; McKenzie & Terrill, 1937; Hammond, Jnr., 1944; Goot, 1949; Hafez, 1951; Bettini, 1953), that ewe lambs and yearling ewes have a restricted breeding season. Early born lambs may show signs of heat in their first autumn but late born lambs do not begin cycling until the following autumn. This restricted season is characterised by a later start and an earlier cessation of cycling.

iv. Plane of nutrition - South African workers (Quinlan & Marè, 1931; Roux, 1936) have shown that malnutrition in the Merino leads to a delay in the onset of the breeding season. Hafez (1952), studying Suffolks in England, concluded that a submaintenance diet did not delay the onset of the breeding season, but that it did increase the number of silent heat periods in the early part of the breeding season.

Grant (1934) and Wallace (1951) believe that the onset can be hastened by flushing but this has not been found to be the case by McKenzie & Phillips (1930), Underwood & Shier, (1941), Briggs et al., (1942), Till, (1950).

v. The presence of the ram - Underwood, Shier & Davenport, (1944) observed that the incidence of lambing in Merino & Border-Leicester Merino half-bred ewes in Western Australia was far from random. They noted a characteristic lag period of approximately 16 days when a few ewes lambed, followed by a period of 8-10 days when a high proportion of the ewes lambed.

Coleman (1950, 1951) reports trials in which a group of Corriedale ewes were run with vasectomised rams for 3 weeks prior to mating with Corriedale rams. Lambing was earlier and lambing % higher than in a control group. Hafez (cited by Robinson, 1951) noted that the introduction of rams to a flock of Suffolk ewes in August hastened the onset of the breeding season when compared with ewes with which a ram had been running all the summer.

A report by Thompson & Schinckel (1952) tended to confirm the hypothesis that the presence of the ram may take the form of an external* stimulus to reproductive activity. The nature of this stimulus was investigated by Schinckel (1954a), who asserted that "the effect of

*The term "exteroceptive stimulus" has been incorrectly used by many workers when referring to an "external stimulus." Thus it is correct to apply the term "exteroceptive" to the organ, e.g. the eye, which receives the "external" stimulus.

the ram on sexual activity of the ewes takes two distinct forms depending on the duration of the association. Under protracted association extending over periods of months, and particularly when this association extends through anoestrus, the effect is one of depression of activity and extension of the period of anoestrus. The effect of sudden association late in anoestrus, when ewes have been separated from rams for long periods, is one of stimulus and the synchronisation of the activity in a high proportion of ewes." Schinckel (1954b) and Riches & Watson (1954) present data in support of the above hypothesis.

Granger (1955) indicates that the presence of the ram during lactation anoestrus may have an effect similar to that shown to occur in late anoestrus.

vi. Other factors - From the studies of McKenzie & Phillips (1930), Kelley (1937, 1939) and Yeates (1949) it would appear that temperature does not play a major part in the control of breeding behaviour in the ewe. Dutt & Bush (1955), however, have brought ewes into season during the normal anoestrous period by subjecting them to cold temperatures (46 - 48 deg. F) for a period of 7 weeks.

Following pregnancy, during which there is normally no signs of oestrus, a lactation anoestrus apparently lasts 5-9 weeks (Robinson, 1951), depending upon the season of the year. Under normal husbandry conditions in New Zealand, where ewes are mated early in the breeding

season, lambing occurs at the end of the season so that lactation anoestrus will tend to merge with the true anoestrus.

B. Methods Used to Alter the Anoestrous Period

1. Introduction

There have been two distinct methods of tackling this problem, namely, the use of light and the use of hormones. These may be looked upon merely as two different ways of approaching the same problem of altering the pituitary-ovarian relationships during anoestrus, which may be termed unfavourable from the point of view of breeding behaviour, to that pertaining during the normal breeding season.

The use of light involves, in practice, a study of different light:dark combinations for the 24-hour period. While a considerable amount of work has been done on small animals, including mink, ferret, birds, etc., (Hammond, Jnr., 1954), the effect of light on the ovine species has received scant attention.

The practise of injecting hormones, singly or in combination, has not led to such spectacular results as have been reported for light despite the much larger volume of literature. Nevertheless it will be noted that since the significance of the spontaneous ovulations unaccompanied by heat which occur in late anoestrus (Grant, 1933) was pointed out by Hammond, Jnr. et al. (1942) there have been

encouraging results.

2. Light

It is well to remember that two factors in particular may moderate, and hamper interpretations of light effects; one is the influence of courtship, mating and parental behaviour; the other is the presumed existence of an inherent rhythm (Hammond, Jnr., 1954).

Sykes & Cole (1944), by artificially first increasing and then decreasing spring day-lengths, obtained pregnancies at an abnormal time of the year. Similar treatment was used by Yeates (1949) in a more elaborate experiment. His conclusion was that the start of the breeding season comes 13-16 weeks after a change to decreasing day-length and ends 14-19 weeks after increasing day-length, irrespective of the absolute day-length at the time of change.

Hart (1950), Hafez (1951,1952) and Terry & Meites (1951) have conducted experiments using various combinations of light and dark. Although these studies show that light has both inhibitory and stimulatory effects there is no clear quantitative evidence of the way sheep summate the effects of light responses (Hammond, Jnr., 1954).

3. Hormone administration

The use of hormones in the control of the breeding cycle has been reviewed by Phillips et al. (1945) and

Robinson (1951). The following is a summary of their findings augmented by a full review of subsequent available literature.

It should be pointed out at this stage that both reviewers drew attention to the apparent divergence from the potency designated by the manufacturer of gonadoprophin preparations. The potency of the dry powder may fall by about 50% during the first two weeks of storage, after which time it remains stable (Li, 1949). Consequently where preparations have not been checked for potency it is possible that large errors may have occurred, and this fact should be realised when considering the results of various investigators.

Cole & Miller (1933) reported that PMS would fairly regularly induce ovulation without heat, and that a second injection 16 days later would cause ovulation accompanied by heat. This experiment was the forerunner of a great deal of work which was directed towards:-

i. increasing the reliability of the method involving the double injection of PMS at 16-day intervals;

ii. obtaining fertilisation and implantation by artificial insemination or forced mating following a single injection;

iii. obtaining heat and ovulation by a single PMS injection either alone or with other hormones (Robinson, 1951).

Results have been generally disappointing from the point of view of induction of heat and the number of conceptions following artificial insemination or forced mating. Exceptions have been noted in breeds with a relatively shallow anoestrus (Litovcenko, O'Neill - quoted by Robinson, 1951; Kelley & Shaw, 1943). The results of Cole et al. (1945) are typical. Over a period of 11 years results ranged from almost complete failure to even better than in the initial work; they record that 7% of ewes came into oestrus after one injection of 125 - 700 i.u. PMS and none conceived, while following two injections the oestrus rate was 34% but only one-third of these conceived.

Nevertheless there seems little doubt that a single injection of PMS will cause ovulation in a large proportion of animals. The threshold level is regarded as 400 i.u. (Robinson 1951) but this will vary with stage of anoestrus, breed of sheep, and type of management.

There are reports of the use of other hormones (oestrogens, progesterone and testosterone) with PMS. Oestrogens have generally yielded poor results (Cole & Miller, 1935; Bell et al., 1941b; Hammond, Jnr. et al., 1942; Frank & Appleby, 1943; Cole et al., 1945; Phillips et al., 1946; Vander Noot et al., 1949). On the other hand, pretreatments with progesterone and testosterone have been quite promising (Hammond, Jnr. et al., 1942; Cole et al.,

1945; Phillips et al., 1946; Thibault et al., 1948; Robinson, 1950).

Robinson (1951) concludes that there is a fairly clear pattern of behaviour following injection of PMS in the anoestrous ewe the various responses being related to the presence or absence of a spontaneous corpus luteum.

Recent work has been directed towards an understanding of the role of progesterone. It has been shown that if progesterone is injected for several days prior to PMS oestrus often accompanies the first ovulation which is induced (Robinson, 1952, 1954e; Dutt, 1953; Dauzier et al., 1954, Raeside, 1955; Edgar, 1955; Lambourne 1955). The reliability of the occurrence of the oestrus response when PMS is given four days after the commencement of the progesterone injection is considerably enhanced by greater subdivision of the dose of progesterone (Robinson, 1954e). A further increase is obtained by prolonging the period of stimulation by three days. Thus, a total of 75 mgms of progesterone, administered over a period of six days in 12 twice-daily injections in oil, followed 40 hours after the final injection by 1000 i.u. PMS will result in oestrus with ovulation, within 40 hours of the gonadotrophin injection, in practically all ewes treated (Robinson, 1955b).

Lambing results in this type of treatment, however, have not been satisfactory (Robinson, 1954a,

1955b; Edgar 1955), indicating that the maternal environment has not reached the same state of receptivity as in the normal breeding season.

The administration of progesterone alone has been followed approximately three days after the final injection by ovulation with and without heat (Dauzier & Wintenberger, 1952; Dutt, 1953, Robinson, 1954e, 1955b; Raeside, 1955). The responses have been promising but the mechanism is obscure.

C. Cyclical Changes in the Reproductive Tract

1. Introduction

Because of the minor nature of the extent of the changes in the reproductive tract of the anoestrous ewe, this section consists of a brief summary of the normal oestrous cycle, followed by a review of the literature dealing with the anoestrous ewe. It is hoped, in this way, to indicate the fundamentally important fact that the reproductive tract of the anoestrous ewe is in a state of relative inactivity.

2. Macroscopic changes in the ovary

During the course of a normal oestrous cycle, the ovary undergoes characteristic changes. In the follicular phase one or more follicles (depending mainly on breed and level of nutrition - Marshall, 1904; Hammond, 1921, Quinlan and Marè, 1931; Cole & Miller, 1935; Roux, 1936; McKenzie & Terrill, 1937; Underwood and Shier, 1941; Hammond, Jnr., 1944;

Robinson, 1950, 1951b; and others) develop to maturity under the influence of the follicle stimulating hormone (FSH) and ovulate under the added influence of the luteinising hormone (LH or ICSH) of the anterior lobe of the pituitary (Robinson, 1954d). The cavity of the ruptured follicle is then invaded by luteal cells thus forming the corpus luteum which reaches its maximum size by the 5th day. Regression follows from the 14th day and the luteal phase gives way to the next follicular phase (Warbritton, 1934; Grant, 1934).

It is generally believed that follicles of nearly every stage of growth up to but not including maturity may be found in each ovary of the ewe throughout the oestrous cycle. Casida & McKenzie (1932) reported evidence of a gradual increase in mean diameter of the follicles, 2 mm. or larger, during the first one-third of the cycle. For the remainder of the cycle there was an irregular but fairly well sustained mean diameter. There were follicles at approximately the mid-period of the cycle, as large as those just prior to ovulation. This latter finding has also been reported by Bierbauer (cited by Casida, 1946).

Grant (1934) could not confirm the observation of Quinlan & Marsd (1931) that rapid enlargement of the follicles destined to rupture at the next oestrus

occurs shortly after ovulation, these follicles continuing to grow, but very slowly, during the remainder of the interoestrous period. Instead he concluded that during interoestrus one or more follicles grow to a certain size i.e. 5-7 mm; when such follicles exist in the ovaries at the time of the previous ovulation they remain little changed in size during interoestrus, but when only very small follicles are present they enlarge up to 5-7 mm. in diameter. The cycle may, however, be complicated by follicular atresia, indetectable macroscopically.

McKenzie & Terrill (1937) agreed with Cole & Miller (1935) that the number of small follicles present in the interoestrous period was quite variable, ranging from none up to 30 in each ovary.

Kammlade, Jnr. et al., (1952) could not find any relation between the number of follicles and the stage in the cycle, but the regression of average follicle diameter on day of cycle was highly significant.

The interesting finding by Phillips et al. (1945) that lot-fed lambs contained significantly more follicles over 4 mm diameter than range-fed animals at the end of the winter feeding period is difficult to interpret.

Mid-anoestrus can be clearly differentiated from early or late anoestrus because in the former the ovary contains no corpora lutea. Thus Grant (1934), Cole & Miller (1935) and Roux (1936) found

that the ovary goes through a series of cyclic changes during early and late anoestrus similar to those occurring in the sexual season. These same workers agree that the ovaries during mid-anoestrus contain small and medium-sized follicles (1 - 5 mm) in about the same numbers encountered during the sexual season. Some follicles may attain a considerable size and it is thought that new follicles are continually formed to replace those lost by atresia. There is, however, no certainty on this point.

Kammlade, Jnr. et al. (1952), taking into consideration only follicles 1 mm in diameter or larger, found no significant change in either number or size during anoestrus. It was noted, however, that although there was no difference in either the average diameter of all follicles or the diameter of the largest follicle between anoestrus and the breeding season, there was a highly significant difference between the two periods in the total number of follicles.

3. Histological changes in the tract

i. Fallopian tubes - As a result of studies on the fallopian tubes in the oestrous cycle, Casida & McKenzie (1932) concluded that the greatest height of the epithelium in both the mid-tube and the fimbriated end occurred during late oestrus and metoestrus. The early luteal phase also provided the most obvious development of the connective tissue stroma of the folds and the clearest definition of the cell boundaries.

Cytoplasmic projections from epithelial cells were most clearly seen in the fimbriated end during metoestrus and in the mid-tube during dioestrus and prooestrus.

The most striking differences observed by McKenzie & Terrill (1937) were changes in the height of epithelium and the phenomenon of cellular extrusion. The epithelium was highest near the time of ovulation. The cytoplasmic projections appeared some time after ovulation and increased in prominence as the height of the epithelium decreased. These changes are in general agreement with those observed in the bovine oviduct (Hammond, 1927; Cole, 1930; Roark & Herman, 1950.)

There are no reports in the literature concerning the oviduct of the ewe during anoestrus.

ii. Uterus - The histological changes occurring in the uterus throughout the oestrous cycle have been studied in detail by Casida & McKenzie (1932), and McKenzie & Terrill (1937). Contributions have also been made by Marshall (1904) and Cole & Miller (1935).

Most obvious changes during the oestrous and prooestrous stages were increase in oedema and vascularity of the stroma. When the corpus luteum is in the period of greatest activity there appears to be an increase in the coiling and branching of the uterine glands; the height of the glandular and surface epithelium appears to be greatest at this time also. Similarly, folding of the epithelium was found to be most marked in the mid-luteal phase.

Regression of the corpus luteum was found to be associated with an increase in numbers of lymphocytes and a decrease in the height of the epithelium.

According to Cole & Miller (1935) the uteri of ewes autopsied in mid-anoestrus are easily distinguished from those autopsied during the sexual season. The uterine glands and all other portions of the uterine mucosa recede to a point not seen at any other reproductive phase of the mature ewe. Both the superficial and glandular epithelium are low columnar.

iii. Cervix - Detailed examination of the mid-cervical region by Grant (1934), Cole & Miller (1935) and McKenzie and Terrill (1937) indicates that the epithelium secretes mucus throughout the cycle. There appear to be differences in the amount of mucus in the cells, however, - the production and storage taking place during dioestrus and the mucus liberated during prooestrus and oestrus. The stroma underlying the epithelium appears to become oedematous during oestrus. The character of the mucus in the cervix varies considerably throughout the cycle.

McKenzie & Terrill (1937) noted a "spent" appearance of the epithelial cells in late luteal and follicular phases.

Cole & Miller (1935) observed that in mid-anoestrus the superficial layer of cells is lower in height than during the breeding season and the underlying epithelial cells are small and inactive.

Likewise the complexity of the cervical glands is reduced.

iv. Vagina - It was reported by Darlow & Hawkins (1931) that rhythmical changes in the character of the vaginal smear occur regularly throughout the cycle, these changes serving as indices of the different phases, prooestrus, oestrus, metoestrus and dioestrus. Hawkins & Darlow (1933), Grant (1934) and Cole & Miller (1935) made a thorough study of the relationship between histological changes in the vagina and the vaginal smear throughout the cycle. Casida & McKenzie (1932) and McKenzie & Terrill (1937) have also studied the cyclical histological changes in the vagina.

While findings concerning the changes in the vaginal smears agree fairly closely there is some doubt as to their reliability as indicators of separate stages in the oestrous cycle. This is perhaps based on the fact that histologic changes are not striking and results reported in the literature are somewhat variable.

In general, oedema of the stroma, congestion of blood vessels and accelerated growth of the epithelium were noted during oestrus. Cornification was usually noted in late oestrus and metoestrus, but its appearance is apparently not as abrupt as that in the goat (Hamilton & Harrison, 1951).

Desquamation of the epithelial cells appeared

greatest in the early luteal phase. Leucocytes have been found to be more numerous in the epithelium during the middle and later stages of the cycle.

Grant (1934) and Cole & Miller (1935) described the vagina during anoestrus and pregnancy. Whereas during anoestrus there was no great difference from that of the prooestrus stage, the vagina in pregnancy consists of about 4 layers of stratified columnar epithelium; there was some evidence of mucus secretion in this period also. The scant, moist vaginal smear obtained during mid-anoestrus contained a few leucocytes and epithelial cells.

Grant (1934) observed that in late anoestrus the vaginal epithelium became thicker and the number of free lymphocytes in the stroma increased, accompanied by an increase in vasularity. Approximately 2-4 weeks prior to the first heat period the vaginal smear showed changes similar to those taking place in the normal oestrous cycle.

D. The Response of the Reproductive Tract to Hormone Administration

In a previous section of this review of literature attention was drawn to the methods used to alter the anoestrous period. Emphasis was placed on the use of oestrus and ovulation, and in some cases pregnancy, as the physiological endpoints by which the success of the treatments was judged. In this section the

emphasis is on the changes which take place in the separate organs of the reproductive tract as a consequence of hormone administration. It will be seen that this particular aspect has been generally neglected in the anoestrous ewe. Consequently it is deemed pertinent to consider changes taking place in the ovariectomised ewe and the ewe in the normal breeding season.

McKenzie & Terrill (1937) observed that injections of oestradiol benzoate (1000 R.U. given in 5 injections of 200 R.U. at 6-hour intervals) brought two castrated ewes into heat in 24 and 36 hours after the first injection. Vaginal, cervical and fallopian tube epithelial linings were all higher in the treated ewes than in the uninjected castrate; also the lumina of the uterine glands became much larger.

Bell et al. (1941a) in an extensive experiment involving 30 spayed ewes obtained essentially similar results with oestradiol. The addition of progesterone appeared to have no effect on the vaginal epithelium, but it maintained the height of cervical and uterine glandular and surface epithelium. It was noted that the addition of progesterone led to a definite increase in the coiling of uterine glands after pre-treatment with oestradiol.

Polovceva & Judovic (1939) found the conditions of the reproductive tract, when ovulation was induced without heat, using PMS, to be similar to that seen in

oestrous ewes, except that tonic uterine contractions were absent and there was a tendency for the cervix to remain closed.

Bell et al. (1941b) noticed that the vaginal smear changes after induction of ovulation with or without heat by PMS, sheep pituitary extract and oestradiol benzoate alone and in combination, were similar to those observed at and after natural oestrus, and were independent of whether or not the animals came on heat or ovulated.

Hammond, Jnr. et al. (1942) found that horse pituitary extract clearly caused an increase in the number of follicles developed in anoestrous ewes. The addition of progesterone to horse pituitary extract did not appear to have any effect on the growth of medium-sized follicles, but examination of the data indicates that two yearlings which received 4 mgms progesterone at the same time and two days before the horse pituitary extract had 8 large follicles each. It will be noted that both showed doubtful signs of heat.

Phillips et al. (1946), in a series of experiments involving ovariectomised ewes receiving oestradiol alone and followed by progesterone, could find no evidence that progesterone acts synergistically with oestradiol injected 16-64 hours prior to the administration of the progesterone. This is in agreement with the findings of Cole et al. (1945).

Casida (1946) reported an unusual finding with regard to the use of oestrogens in the oestrous cycle.

He stated that ovulation, without heat, was obtained most consistently when the oestrogen was administered on the fourth day of the cycle and was obtained rarely at other stages.

Dutt & Casida (1948) showed that 10 mgms of progesterone per day would control oestrus and ovulation in all ewes tested. These injections began on the 4th, 8th, and 12th day of the cycle and all ewes ovulated with heat about three days after the final injection. O'Mary et al. (1950) achieved similar results.

Cole et al. (1945) and Robinson (1950) obtained oestrus in a high proportion of ewes when testosterone propionate was given prior to the PMS injections. Ovulation, however, was either delayed or suppressed.

Robinson (1952), reporting encouraging results using progesterone prior to PMS in the anoestrous ewe, ventured the opinion that the action of progesterone is to develop receptors in organs such as the uterus, these receptors responding to subsequent oestrogen stimulation resulting in oestrous behaviour. In a subsequent paper, Robinson (1954a) showed that such receptors, if present, were not important, since he obtained oestrus in hysterectomised ewes with progesterone followed by PMS. He further observed that the follicle picture in the ovary, after progesterone and PMS administration, was not noticably different from the controls.

Further studies by Robinson (1954b; 1954c; 1955b) have culminated in the very important demonstration that the response of the progesterone-primed ewe to oestrogen

is exceedingly critical.

It is clear that there is much to learn about the true role of progesterone both in the phenomenon of oestrus and the preparation of the tract for reception of the fertilised ovum.

E. The Pituitary Gonadotrophins

1. The role of anterior pituitary lobe gonadotrophins in the anoestrous ewe

It is customary to recognise the presence of three substances produced by the anterior pituitary gland which act upon the ovary. These are the follicle stimulating hormone (FSH), the luteinising hormone (LH or ICSH) and the luteotrophic hormone (sometimes called wither lactogenichormone or prolactin). It has not yet been shown, however, that these hormones, as separate entities, are secreted into the boood stream (Burrows, 1949; Evans & Simpson, 1950).

Hammond (1946) refers to the fact that in the cow and sheep LH predominates and that this possibly accounts for the strongly developed luteal phase in the ovarian cycle of these two animals. Thus the action of PMS which is a potent source of a follicle stimulating hormone, on the anoestrous ewe, whereby ovulation is brought about, is attributed to the interaction of the FSH with the animal's own pituitary gonadotrophins. Detailed histological examination of ewe pituitary glands by Warbritton & McKenzie (1937)

would tend to substantiate Hammond's thesis. Thus they observed a marked decrease in activity of the cells which they presumed to produce FSH during anoestrus, while the cells which were presumed to produce LH remained in a similar state to that found during the early luteal phase of the oestrous cycle. It may be noted, however, that the FSH producing cells were abundant during the oestrous cycle, although their activity was not easily determined.

Working upon the postulate that the pituitary gland of the anoestrous ewe would indicate its potential activity by its ability to produce and store gonadotrophic hormones after spaying, Warwick (1946) interpreted his results as indicating that the anoestrous ewe is as capable of producing high levels of gonadotrophin as the ewe in the normal breeding season.

Cole & Miller (1935), Overfield et al. (cited Warwick, 1946) and Kammlade Jnr. et al. (1952) compared the gonadotrophic activity of anoestrous ewes with normal cycling ewes and obtained evidence that the gonadotrophic potency in the anoestrous ewe is at least as high as that of the ewe in the mid-luteal phase. It was not possible in the above experiments to be certain whether FSH or LH or both caused the observed assay responses.

It may be pointed out that Warwick (1946) obtained a significant difference between breeds; Hampshires being lower than Crossbreds, both in intact and spayed ewes, irrespective of the season.

2. The chick assay for pituitary gonadotrophins

Domm & Van Dyke (1932) and Shockaert (1933) induced precocious testes development of immature male fowls by injection of extracts of the anterior lobe of the pituitary gland; Hamburger (1934) and Asmundson & Wolfe (1935) achieved similar results using PMS.

Breneman (1936) showed that the follicle stimulating extract produced an hypertrophy of the tubules of the testes and the luteinising fraction an increase in the interstitial tissue, in 5-day old chicks.

Byerly & Burrows (1938) described a method for assay of PMS and male day-old chick anterior pituitary gonadotrophins. They obtained a straight line relationship for log dose - log response with PMS. They also pointed out that the gonadotrophic potency of the day-old chick was measurable, and Breneman (1945) found the 5-day old chick to be capable of gonadotrophin production.

The day old chick has also been used by Bates *et al.* (1940); Bates *et al.* (1941); Bergman *et al.* (1939) and Simpson *et al.* (1942) to assay gonadotrophins. Bergman & Turner (1942) give some information on the normal weights of chick gonads over a four year period.

Venzke (1941) described the histological changes in the gonads of the chick prior to hatching, after injections of PMS and Kumarin & Turner (1949) have described the normal histological changes taking place in the testes of the immature cockerel.

Warwick (1946) and Kammlade Jnr., et al. (1952) used a modification of the assay technique of Evans et al. (1940) for studies of the pituitary glands of sheep and Robinson & Nalbandov (1951) used a similar method for swine pituitaries.

III MATERIALS AND METHODS

A. The Experimental Animals

1. Sheep

a. History - One hundred and five (105) Romney ewes were obtained from the College farms for use in this experiment. These ewes were cull animals and comprised part of the normal surplus of the farms. They were culled for old age (the majority being 5 and 6 year ewes), poor teeth and mastitis. Lambing records existed for all ewes and the total number of lambs reared per ewe varied from 3 to 8. The previous season's lambs had been weaned at the middle of November 1954, by which time all but seven ewes had been shorn. These seven were:-

Ewe	Group
81.47	2 c (ii)
56.47	4 b (ii)
405.47	2 a (i)
190.48	2 a (i)
104.48	3 b (i)
145.50	3 a (ii)
6.AG	3 a (ii)

These ewes remained unshorn.

At the commencement of the experiment 82 of the ewes were judged to be in good condition, 23 in fair condition and 4 in poor condition. Ewes in poor condition were:-

Ewe	Group
8 AG	2 a (ii)
136 H	5 a (ii)
150 H	4 a (i)
156 H	2 b (i)

Four aged vasectomised Romney rams were run with the ewes from January 2nd. These rams had been shorn in November and were in good condition and very active. Four

entire Romney rams, 2 years old, were joined with the ewes on January 5th. They were unshorn, but this did not prevent two of them, in particular, showing great interest in the ewes.

b. Management - The ewes were run together on pasture until the first day of the injections, January 2nd, when groups 2 c and 3 c were separated from the main mob and run with a teaser ram for the period of the injections. This was done because these two groups were receiving two injections daily and consequently pasturing them separately facilitated handling. On January 6th these ewes rejoined the main flock. For the period of the experiment the ewes were pastured in a paddock which comprised a gully, a steep face and a small plateau, on which the yards were situated. The main pasture plants were white clover, ryegrass, browntop, sweet vernal and dogstail; the gully being quite green and the remainder rather brown and dry.

2. Chickens

White Leghorn, 1-day old, cockerels were obtained from the College Poultry Farm each Monday afternoon. Actually some of the chicks would be almost 2 days old by then since they were hatched throughout the week-end. They were placed in a standard chick brooder, in a building, in which the temperature was thermostatically controlled. Great care was taken with the feeding and temperature regulation and general hygiene - so much so that the average losses

were less than 10% .

B The Hormones Used

1. Progesterone

Crystalline, pure progesterone was obtained from Organon Laboratories (Batch No: A 1030). Six grams of this was dissolved in 480 ml. peanut oil on January 1st and the mixture kept at room temperature; a further 1.25 grams was dissolved in 100 ml. peanut oil on January 4th. The concentration of progesterone in the peanut oil was thus 12½ mgms/ml.

2. Pregnant Mare Serum (PMS)

The PMS used on both the ewes and the chickens was Gonodotrophin "Gestyl" - 1000iu/vial, product of Organon Laboratories. That administered to the sheep was purchased in November, 1954, but the date of preparation was not recorded. That administered to the chickens was Batch No: 1092, December 10th, 1954, which was purchased in June, 1955.

C The Methods

1. Grouping

Each ewe was ear tagged and a list of the tag numbers taken. The ewes were allotted at random into 10 groups of 10 and one group of 5. The groups and the treatments are as indicated in Table 2. It will be noted from Table 2 that groups 2 c and 3 c were injected twice daily; and that group 5 a received 1 ml. of oil (arachis) per day for 4 days.

2. The injections

Injections commenced on January 2nd at 9.30 am. Morning

injections were commenced thereafter between 8.00 and 8.30 am. and completed by 10.00 am; and evening injections were given between 6.45 and 7.45 pm. (group 2 c and 3 c only).

The hormones were injected subcutaneously in the neck region, alternately on the right and left sides.

3. Detection of oestrus

The vasectomised rams were raddled with blue tupping paste and the others with red. Each ram was marked twice daily until January 12th and thence only once daily until January 20th, by which time all ewes had been slaughtered. The ewes were yarded and inspected twice daily for signs of oestrus. A ewe was listed as showing positive signs of oestrus if she was clearly marked over the rump and tail region.

4. The slaughter and collection of specimens

The five members of each group which were to be slaughtered were selected at random the day before slaughter, and kept in a pen that night. Early next morning they were taken to the local freezing works and were the first sheep to go along the chain. The ewes were killed in batches of fifteen, since this was the largest number than could be handled each day without causing too much inconvenience at the works. As the ewes went along the chain the head and reproductive tract of each was removed and labelled. The pituitary gland of each ewe was then removed as quickly as possible and placed in 10 ml. of acetone. All glands were in acetone within 75 minutes of killing. The ovaries were placed in

TABLE 2

Experimental groups and treatments

Group	Ewe tag numbers	Treatment* Jan 2nd	Jan 3rd	Jan 4th	Jan 5th	Jan 7th	Date of slaughter
1	44H, 101.49, 223.49, 474.49, 520.49	100 mg		100 mg			12/1/55
2 a i	8H, 97H, 405.47, 190.48, 91.49	50 mg		50 mg			10/1/55
2 a ii	8AG, 12AG, 71H, 132H, 258.49	50 mg		50 mg			18/1/55
2 b i	2AG, 128H, 126H, 156H, 411.49	25 mg	25mg	25mg	25 mg		11/1/55
2 b ii	57H, 168H, 8.48, 169.49, 517.49	25 mg	25 mg	25 mg	25 mg		19/1/55
2 c i	42H, 57.49, 204.49, 306.49, 359.49	25 mg	25 mg	25 mg	25 mg		12/1/55
2 c ii	134H, 81.47, 254.49, 269.49 42.52	25 mg	25 mg	25 mg	25 mg		20/1/55
3 a i	11AG, 2H, 76H, 83.49, 383.49	25 mg		25 mg			10/1/55
3 a ii	6AG, 14H, 133H, 149H, 145.50	25 mg		25 mg			18/1/55
3 b i	4AG, 7AG, 49H, 104.48, 458.49	12½ mg	12½ mg	12½ mg	12½ mg		11/1/55
3 b ii	9AG, 15AG, 151H, 159H, 160H,	12½ mg	12½ mg	12½ mg	12½ mg		19/1/55
3 c i	14AG, 18H, 62H, 107.49, 152.51	12½ mg	12½ mg	12½ mg	12½ mg		12/1/55
3 c ii	5AG, 137H, 439.49, 446.49, 464.49	12½ mg	12½ mg	12½ mg	12½ mg		20/1/55

TABLE 2 (Cont.)

Experimental groups and treatments

Group	Ewe tag numbers	Jan 2nd	Jan 3rd	Jan 4th	Jan 5th	Jan 7th	Date of slaughter
4 a i	13H, 115H, 121H, 150H, 329.49	25 mg	25 mg	25 mg	25 mg	800iu	13/1/55
4 a ii	1AG, 9H, 55H, 256.49, 279.49	25 mg	25 mg	25 mg	25 mg	800iu	20/1/55
4 b i	19H, 122H, 110.49, 168.49 280.49					800iu	13/1/55
4 b ii	3AG, 48H, 56.47, 229.49, 385.49					800iu	20/1/55
5 a i	13AG, 40H, 88H, 163H, 415.49	1 ml	1 ml	1 ml	1 ml		11/1/55
5 a ii	16H, 58H, 136H, 88.49, 521.49	1 ml	1 ml	1 ml	1 ml		19/1/55
5 b i	10AG, 56H, 65H, 120H, 11.49						10/1/55
5 b ii	4H, 50H, 109H, 230.49, 356.49						18/1/55

* mg Progesterone
iu PMS
ml Arachis oil
25 (e.g.) divided dose

10% formalin. The following sections of the reproductive tract were also placed in 10% formalin:-

Fallopian Tube	-	approx. ½" from the fimbriated end,
	-	mid region,
	-	approx. ½" from the utero-tubal junction.
Uterus	-	one portion about 1" - 2" from the utero tubal junction, from each horn.
Cervix	-	mid region.
Vagina	-	from the roof of the vagina about 1" from the cervix.

After 24 hours the ovaries and above sections were transferred to buffered formalin, the composition of which was as follows:-

Formalin B.P.	100 mls.
Na_2HPO_4 anhyd.	6.5 gms.
NaH_2PO_4 anhyd.	3.5 gms.
($OrNaH_2PO_4.H_2O$	4.0 gms.)
Distilled Water to	1000 mls.

This mixture has a pH of about 6.8 - 7.0.

5. Examination of Ovaries

All ovaries were examined within three weeks of collection. They were examined again six weeks later by way of a check. Each ovary was examined for number of follicles and corpora lutea. The ovary was sectioned vertically at 2mm. intervals for counting. All follicles less than 2.5 mm. in diameter were neglected. Follicles of 2.5 - 5.0 mm. diameter were classed as small; those of diameter greater than 5 mm. as large. The diameters of the corpora lutea were measured to the nearest millimeter.

6. Preparation of sections for histological examination

The sections remained in the fixing fluid for 10 - 12

days.

a. Blocking

The technique for blocking was as follows:-

i. Wash for 24 hours. The specimens were washed in a special type of grid placed in a Buckner funnel so that the water completely covered the tissues during the continuous washing.

ii.	70% alcohol	22 hours
iii.	85% alcohol	8 hours
iv.	95% alcohol	4 hours
v.	100% alcohol A	1 hour
vi.	100% alcohol B	1 hour
vii.	Alcohol & Benzine 50:50	1 hour

(The benzine used was a commercial product, purchased from the local service station).

viii.	Benzine	9 hours
ix.	Benzine & Paraffin	4 hours

The mixture was 2 parts benzine to 1 part paraffin. The small, flat bottomed vials into which the specimens were now placed were kept warm by placing on top of the oven.

x.	Paraffin A	1 hour
xi.	Paraffin B	1 hour

For paraffins A & B the vials were placed in the oven at 57°C.

xii. Block.

b. Cutting

The sections were cut to a thickness of 8 u. At least 50 sections were cut from each block, and of these 5-6 were fixed on one slide with egg albumen. The sections were dried for 24 hours at room temperature before staining.

c. Staining

It was decided to stain the sections with haematoxylin and eosin. The ingredients of the haematoxylin were as

follows:-

Haematoxylin	1 gram
Distilled water (warm)	250 ml
Iodine (1% in 95% alcohol)	50 ml
Saturated aqueous Ammonium Alum	700 ml

The ingredients were mixed and brought to the boil, then cooled. The eosin was dissolved in 95% alcohol. The acid alcohol used was made by adding 0.2 ml Nitric acid to 100 ml 70% alcohol.

The staining technique was as follows:-

i.	Xylol A	10 minutes
ii.	Xylol B	rinse 1 minute
iii.	Absolute alcohol	2 minutes
iv.	90% alcohol	2 minutes
v.	70% alcohol	2 minutes
vi.	50% alcohol	2 minutes
vii.	Distilled water	2 minutes
viii.	Haematoxylin	40-60 minutes
ix.	Rinse in tap water	few minutes
x.	50% alcohol	2 minutes
xi.	Acid alcohol	½-1 minute
xii.	Tap water	1 minute
xiii.	70% alcohol	2 minutes
xiv.	95% alcohol	2-5 minutes
xv.	Eosin	½-1 minute
xvi.	Rinse in 95% alcohol	
xvii.	Absolute alcohol	1 minute
xviii.	Absolute alcohol and Xylol 50:50	1 minute
xix.	Xylol A	2 minutes
xx.	Xylol B	5 minutes
xxi.	Mount in "Sera" Mounting fluid.	

It was possible to stain specimens from 5 sheep only each day. It was found most convenient to do one sub-group each time. Care was taken to ensure that the technique did not vary from day to day.

7. Examination of sections

The sections were examined under a binocular microscope using a direct source of illumination. At least four sections

of each tissue were available for examination.

a. Vagina

The vaginal sections were examined under low power (x 140). The height of epithelium was measured as accurately as possible. Each of the four sections on each slide was examined along its whole length (about 1 mm). An average estimate was then made of the whole and the gradings made accordingly. The grading system was as follows:-

Leucocytes	+++	100 or more per field
	++	50 - 100 per field
	+	0 - 50 per field
Crypts	S	Shallow crypts ($< 60\,\mu$).
	SS	More than 5 shallow crypts/field.
	D	Deep crypts ($> 60\,\mu$ and up to $400\,\mu$ in places).
	DD	More than 5 deep crypts/field.
Definition of cell membranes	D	Clearly defined in most cells.
	I	Poorly defined in most cells.
Cell protoplasm	G	Globular.
	F	Fibrillar.
Nuclei in the basal cell layer	+	Plump, no crowding.
	0	Indefinite.
	-	Crowded.
Vacuolar degeneration	++	Much
	+	Some.
	0	None.
Cornification	+++	More than 6 layers.
	++	3 - 6 layers
	+	1 - 3 layers
	0	None

Height of Epithelium - measured to the nearest 5 μ, between crypts as far as possible.

b. Cervix

The cervical sections were examined under high power (x 520) and low power (x 140). The factors taken into consideration were:-

Size of epithelial cells.

Position and character of the basal nuclei.

Character of the sub-epithelial stroma, particularly the blood vessels.

c. Uterus

The height of the uterine epithelium was examined under oil immersion (x 840). Three fields were selected as random on each of the four sections on each slide. One measurement of the height of the uterus epithelium, between cotyledons, was taken in each field. The part of the epithelium measured was actually that nearest the eyepiece scale when the field was focussed. Thus 12 measurements for each sheep were taken. A preliminary check indicated that variation within sheep was quite small; consequently 12 readings were considered quite sufficient. The uterine sections were also examined under a low power dissection microscope (x 8.75) and the normal low power (x 140) and high power (x 520).

d. Fallopian tubes (oviducts)

As a result of an accident during the washing process a number of portions of the oviducts were mixed and nearly two-thirds of the total was rendered useless from the point of view of comparisions between groups. The following, however, were examined under high power (x 520) :-

T A B L E 3

Sections of fallopian tubes examined

Group	Number of Sheep Examined in each Group			
	Fimbriated End	Mid-tube	Uterine End	The three portions in the same sheep
1	4	3	3	3
2 a (i)	3	-	3	-
2 c (i)	3	-	3	-
2 c (ii)	4	4	4	3
3 c (ii)	5	4	5	4
4 a (i)	2	4	4	2
4 a (ii)	4	3	4	3
4 b (i)	2	2	2	2
4 b (ii)	4	3	4	3
5 (a+b) (i)	1	4	3	1
5 (a+b) (ii)	5	3	5	3
Total	37	30	40	24

It can be seen that some comparisons are possible, but the limitations are very obvious. In the fimbriated and mid-tube portions 12 measurements/sheep were taken of both the height of epithelium and the width of the stroma; in the uterine end only the height of epithelium was measured.

8. The assay of the pituitary glands

a. Preparation of the pituitary glands for assay

The glands were stored in acetone for 4 months, the acetone being changed at approximately 3-weekly intervals. They were then dried in a vacuum desiccator, trimmed of obvious connective tissue, weighed and ground. After grinding the glands were stored in an ordinary desiccator in a refrigerator until they were assayed in July/August, 1955.

The procedure for assaying consisted, essentially, of suspending the powder in distilled water and injecting the suspension into day-old male chicks. It was quickly discovered that the powder in its present form was not suitable for injection with a small needle (G. 23) because of pieces of extraneous matter blocking the needle. Thus each pituitary gland was reground, put through an 80-mesh sieve and then the required amount was suspended in distilled water on the day of the first injection. Care was taken to ensure that the poorly-wettable powder went into suspension uniformly. During the 4-day period of injections the suspension was stored in a refrigerator.

b. The assay technique

Early trials indicated that 15 mgms of powder per chick injected intraperitoneally would give an adequate response in testes weights. It was decided to use 5 birds for each pituitary gland and to give the powder in 8 doses of 0.2 ml suspension (in distilled water) over a period of 4 days. The chicks were to be killed 24 hours after the last injection. In order to compare separate batches of chicks two levels of PMS (15 iu and 30 iu) were to be given each week. It was assumed that if each batch of chicks gave similar responses to the levels of PMS errors due to differences between batches could be neglected. In the event of there being differences in responses to these levels from week to week, it was expected that some estimate of the difference could be obtained from a dose-response trial carried out with the same batch of PMS.

During the week commencing July 26th, groups 2 a and 3 a were assayed. As will be seen from the results, deaths were heavy and there was considerably variation in body weights. It was clear that the intraperitoneal route was not at all satisfactory. It was then decided that subcutaneous administration should be tried. During the week commencing August 2nd, using a fresh batch of PMS additional dose-response data was obtained. It was also shown at this time that 20 mgms of powder gave a more definite response than 15 mgms, and this level was used thereafter; furthermore, losses were reduced to a negligible level and the body weights of the chicks were much more uniform.

The remaining 80 pituitary glands were assayed according to the following plan:-

Eight injections, each of 0.2 mls of the powder in suspension, were given to each chicken over a period of four days. Five chicks were used for each pituitary; each group of five being identified by dye markings. The chicks were killed on the 5th day with ether or coal gas and their body weights recorded. Each pair of testes was immediately removed and dissected free of extraneous tissue on moistened blotting paper; excess moisture was then removed by blotting on dry paper and the testes were weighed.

In order to be sure that the injection itself was not affecting the chick, during the week commencing August 9th, 20 chicks were injected with distilled water alone.

Table 4 indicates the order in which the pituitary glands were assayed.

T A B L E 4

The Assay Series

Series	Date	Treatment//	Number of Chickens
1	July 26th	*20 pituitary glands (groups 2 a & 3 a)	100
		*15 iu PMS	5
		*30 iu PMS	5
		Control	10
1 a	August 2nd	10 iu PMS	20
		15 iu PMS	20
		20 iu PMS	20
		30 iu PMS	20
		40 iu PMS	20
		10 mgms pooled pituitary powder	10
		*15 mgms pooled pituitary powder	20
		15 mgms pooled pituitary powder	20
		20 mgms pooled pituitary powder	10
		Control	40
2	August 9th	20 pituitary glands (2 b & 3 b)	100
		15 iu PMS	10
		30 iu PMS	10
		Control	10
3	August 16th	20 pituitary glands (2 c & 3 c)	100
		15 iu PMS	10
		30 iu PMS	10
		Control	10
4	August 23rd	20 pituitary glands (5 a & 5 b)	100
		15 iu PMS	10
		30 iu PMS	10
		Control	10
5	August 30th	20 pituitary glands (4 a & 4 b)	100
		15 iu PMS	10
		30 iu PMS	10
		Control	10

// Injected subcutaneously in the leg, alternate side.

* Injected intraperitoneally.

Note: The pooled powder consisted of the remains of groups 2 a and 3 a.

IV RESULTS

A Manifestation of Oestrus

The ewes which showed definite signs of oestrus are listed in Table 5, together with the date of oestrus and the number of hours after the last injection.

TABLE 5

The ewes which exhibited oestrus

Tag Number of ewe	Group	Date of oestrus	Hours after last injection (approx.)
279.49	4 a	8th January	30
9 H	4 a	9th January	40
256.49	4 a	9th January	40
329.49	4 a	9th January	40
55 H	4 a	9th January	50
1 AG	4 a	11th January	90

It can be seen that 6 ewes of 10 in group 4 a, which received 25 mgms progesterone per day for four days followed 50 hours later by 800 iu PMS, came into heat 30 - 90 hours after the PMS injection.

Not one other ewe in the entire experimental flock exhibited oestrus.

B Macroscopic Examination of the Ovaries

1. Ovulations

The total number of ewes which ovulated is listed in Table 6.

TABLE 6

The ewes which ovulated

Tag number of ewe	Group	Number of corpora lutea	Diameters of corpora lutea in mm*
57.49	2 c i	1	5
50 H	5 b ii	2	7 , 8
115 H	4 a i	2	8 , 8
121 H	4 a i	1	5
329.49	4 a i	2	8 , 8
19 H	4 b i	1	5
122 H	4 b i	1	7
110.49	4 b i	2	4 , 6
168.49	4 b i	2	4 , 7
1 AG	4 a ii	1	10
9 H	4 a ii	3	10 , 10 , 15
55 H	4 a ii	2	10 , 10
256. 49	4 a ii	2	10 , 10
279.49	4 a ii	2	8 , 10
229.49	4 b ii	1	10

* The diameters are the approximate mean of two measurements taken at right angles.

The single corpus luteum in group 2 c i must be considered in the same light as the double ovulations in ewe 50 H in the control group; that is, as a spontaneous ovulation. Spontaneous ovulations, unaccompanied by heat, have been observed at the end and the beginning of the breeding season and even throughout the anoestrous period

(Grant, 1933 and others).

a. Groups 4 a and 4 b

The ewes 13 H, 150 H (group 4 a) and 48 H, 280.49 (group 4 b) are not included in the above table because either one or both ovaries of these ewes were lost at slaughter.

i. Number of ewes ovulating. As a result of the histological examination of the reproductive tract it was determined that 13 H and 150 H (group 4 a) had ovulated and that 48 H and 280.49 (group 4 b) had not ovulated. Consequently in Table 7 are included all ewes in both groups.

T A B L E 7

Comparison of number of ewes ovulating in groups 4 a and 4 b

Group	Total number of ewes	Number of ewes which ovulated	Number of ewes which did not ovulate
4 a	10	10	0
4 b	10	6	4
Total	20	16	4

Calculations based on the method described by Fisher (1950, page 96) for this type of data indicate that the treatment applied to group 4 a gave a significantly greater response ($p = 0.0434$) than that applied to 4 b.

ii. Number of corpora lutea per group. Only ewes in which both ovaries were recovered at slaughter are included

in Table 8.

T A B L E 8

Comparison of the total number of corpora lutea in groups 4 a and 4 b

Group	Number of ewes	Total number of corpora lutea per group
4 a	8	15
4 b	8	7

The Null hypothesis in the analysis of the data in Table 8 by chi-square (Snedecor, 1946, page 22) is that there are no differences between the groups in respect to the total number of corpora lutea produced per group. The result was not significant.

$$\chi^2 = 2.27 \text{ for } 1 \text{ df} \qquad (p = 0.15)$$

In view of the difficulties of analysing such meagre data as that presented in Tables 7 and 8, and considering the values for p obtained one cannot entirely refute the suggestion that progesterone augments PMS under the conditions of the experiment. The relationship between the number of ewes showing oestrus, the number of ewes ovulating and the total number of corpora lutea produced, in groups 4 a and 4 b, and the possible consequences from the point of view of successfully breeding ewes out-of-season will be discussed later.

2. The size of the experimental corpora lutea

Table 6 lists the diameters of the corpora lutea in

groups 4 a and 4 b. It will be seen from Table 9 that the average size does not differ greatly from that of normal functional corpora produced during the breeding season (the figures for the latter were derived from the findings of Warbritton, 1934; Grant, 1934; Robinson 1950). Thus the 11-12 day old corpora were about 4 mm greater in diameter than those of 4-5 days.

T A B L E 2

Comparison of normal and experimental corpora lutea

	Experimental		Normal	
	4-5 day	11-12 day	4-5 day	11-12 day
Average	6.4 mm	10.3 mm	6 mm	11 mm
Range	4 - 8 mm	8 - 15 mm	5 - 7 mm	10 - 14 mm

3. The graafian follicles

It has been pointed out that follicles less than 2.5 mm diameter were not included in the follicle count. Table 10 describes fully the follicle numbers in each individual ovary which was recovered at slaughter, together with the diameters of the large follicles. Table 11 describes the group averages in which only ewes where both ovaries follicles were recovered at slaughter were taken into account. Consequently any ewe in which either one or both ovaries were lost at slaughter is not included in this table.

a. Large follicles

It can be seen that the number of large follicles (diameter greater than 5 mm) is remarkably similar from group to group, irrespective of treatment or date of

TABLE 10

Small and large follicles - all ewes

Group	Tag Number	Ovary 1 L	Ovary 1 S	Ovary 2 L	Ovary 2 S	Diam. of large follicles 5-6mm	7-8mm	9-10mm
1	44 H	2	5	0	9	2		
	101.49	2	1	2	2	4		
	223.49	1	2	1	3	2		
	474.49	1	8	-	-	1		
	520.49	2	5	1	6	3		
2 a i	88 H	1	4	0	5		1	
	97 H	3	1	-	-	3		
	405.47	3	2	0	4	3		
	190.48	2	2	-	-	2		
	91.49	3	9	1	10	3	1	
2 a ii	8 AG	1	11	1	7	2		
	12 Ag	1	1	2	6	3		
	71 H	1	7	2	11	3		
	132 H	0	9	1	10	1		
	258.49	3	5	0	6	3		
2 b i	2 AG	1	7	1	5	2		
	128 H	0	10	1	7	1		
	126 H	1	4	2	3	3		
	156 H	2	2	1	5	3		
	411.49	2	11	1	4	3		
2 b ii	57 H	2	4	0	11	2		
	168 H	1	13	0	11	1		
	8.48	1	5	1	2	2		
	169.49	2	10	0	17	2		
	517.49	3	3	-	-	3		
2 c i	42 H	0	12	2	7	2		
	57.49	2	5	1	6	3		
	204.49	2	4	1	8	3		
	306.49	1	4	0	6	1		
	359.49	2	11	1	4	3		
2 c ii	134 H	0	13	-	-			
	81.47	2	6	3	13	5		
	254.49	2	9	3	10	5		
	269.49	1	8	1	14	2		
	42.52	1	6	2	8	3		

TABLE 10 (cont.)

Group	Tag Number	Ovary 1 L	Ovary 1 S	Ovary 2 L	Ovary 2 S	Diam. of large follicles 5-6mm	7-8mm	9-10mm
3 a i	11 AG	1	6	0	6		1	
	2 H	0	5	1	6	1		
	76 H	1	1	1	3	2		
	83.49	1	15	1	4	2		
	383.49	2	3	-	-	2		
3 a ii	6 AG	0	6	1	6	1		
	14 H	1	8	1	10	2		
	133 H	2	3	0	10	2		
	149 H	2	4	2	9	4		
	145.50	0	4	1	7	1		
3 b i	4 AG	1	4	3	3	4		
	7 AG	2	2	2	0	3	1	
	49 H	3	2	1	3	4		
	104.48	2	2	0	4	2		
	458.49	1	15	2	5	3		
3 b ii	9 AG	0	10	2	7	2		
	15 AG	1	10	1	5	2		
	151 H	2	8	0	18	2		
	159 H	0	14	1	12	1		
	160 H	0	9	0	12			
3 c i	14 AG	1	5	2	4	3		
	18 H	0	8	2	7	2		
	62 H	0	2	-	-			
	107.49	1	8	1	5	2		
	152.51	0	5	-	-			
3 c ii	5 AG	3	4	0	3	3		
	137 H	0	7	2	10	2		
	439.49	3	12	2	14	5		
	446.49	0	13	1	13	1		
	464.49	1	11	1	14	2		

TABLE 10 (cont.)

Group	Tag Number	Ovary 1 L	Ovary 1 S	Ovary 2 L	Ovary 2 S	Diam. of large follicles 5-6 mm	7-8mm	9-10mm
4 a i	115 H	1	5	0	6	1		
	121 H	2	2	2	3	3		1
	150 H	0	11	-	-			
	329.49	0	4	2	1	2		
	13 H	-	-	-	-			
4 a ii	1 AG	1	4	1	6	2		
	9 H	1	4	0	5	1		
	55 H	2	2	3	3	5		
	256.49	0	2	2	4	2		
	279.49	1	15	0	10	1		
4 b i	19 H	2	1	0	9	1		1
	122H	2	10	0	11	2		
	110.49	0	8	0	7			
	168.49	0	5	0	9			
	280.49	-	-	-	-			
4 b ii	3 AG	3	9	0	17	3		
	48 H	2	5	-	-	2		
	56.47	2	11	0	16	2		
	229.49	1	7	2	7	3		
	385.49	1	19	0	6	1		
5 a i	13 AG	1	9	0	12	1		
	40 H	1	9	1	8	2		
	88 H	1	7	1	7		2	
	163 H	1	10	0	10	1		
	415.49	0	8	2	11	2		
5 a ii	16 H	1	8	0	15	1		
	58 H	0	18	2	9	2		
	136 H	0	20	1	13	1		
	88.49	0	10	1	7	1		
	521.49	-	-	-	-			
5 b i	10 AG	0	2	3	3	3		
	56 H	1	6	0	6	1		
	65 H	1	2	2	3	3		
	120 H	1	4	-	-	1		
	11.49	2	7	1	11	3		
5 b ii	4 H	2	13	1	5	3		
	50 H	1	6	1	11	2		
	109 H	0	13	1	5	1		
	230.49	2	13	0	7	2		
	356.49	1	11	1	11	2		

TABLE 11

Small and large follicles - group averages on a per ewe basis

Group	Number of ewes	Average L	Average S	Group	Number of ewes	Average L	Average S
1	4	2.7	8.25				
2 a i	3	2.7	11.33	2 a ii	5	2.4	14.6
2 b i	5	2.5	11.6	2 b ii	4	1.75	18.5
2 c i	4	2.2	14.0	2 c ii	4	3.75	18.5
3 a i	4	1.5	11.5	3 a ii	5	2.0	13.4
3 b i	5	3.4	8.0	3 b ii	5	1.4	21.0
3 c i	3	2.33	12.33	3 c ii	5	2.6	20.2
4 a i	3	2.33	7.0	4 a ii	5	2.2	11.0
4 b i	4	1.0	15.0	4 b ii	4	2.25	23.0
5 a i	5	1.6	18.2	5 a ii	4	1.25	25.0
5 b i	4	2.25	10.0	5 b ii	4	2.0	19.5

slaughter. The individual diameters of these large follicles have also been listed in Table 10. In only a small proportion of cases were there follicles greater than 6 mm. Of these only the two 10 mm follicles in group 4 a i and 4 b i were considered mature.

b. Small follicles

The numbers of small follicles varied from group to group, but this variation was not sufficient to mask an overall difference between dates of slaughter. That this difference between dates is significant isshown in the following analyses.

i. Analysis of Variance

Dates of slaughter - control ewes (excl. 50 H)

Source	df	SS	MS	F
Total	16	756.47		
Between dates of slaughter	1	250.74	250.74	7.44 *
Between sheep	15	505.73	33.72	

Note: * Significant at 5% level ($p < 0.05$)

Nine control ewes killed on 10th and 11th January had significantly fewer ($p < 0.05$) small follicles than ewes killed 8 days later.

ii. Analysis of Variance

Dates of slaughter - groups 2 and 3 (excl. 56.49)

Source	df	SS	MS	F
Total	51	2164.67		
Between dates of slaughter	1	521.28	521.28	16.51 **
Between sub-groups within dates	10	315.85	31.58	0.95 NS
Between sheep	40	1327.54	33.19	

Note: ** Significant at 1% level ($p < 0.01$)
NS Non-significant ($p > 0.05$)

Thus there was no difference between the several treatments of groups 2 and 3 either at the first or second killing; there was, however, a highly significant difference ($p < 0.01$) between the dates of slaughter when the groups were combined.

iii. Analysis of Variance

Datesof slaughter - groups 2, 3 and 5 (excl. 50 H and 56.49)

Source	df	SS	MS	F
Total	68	3074.96		
Between dates of slaughter	1	724.05	724.05	13.93 **
Between subgroups within dates	14	727.57	51.97	1.69 NS
Between sheep	53	1623.34	30.63	

The combination of these groups gives a highly significant difference ($p < 0.01$) between the dates of slaughter. It is important to note also that within dates of slaughter the control ewes do not differ from the treated ewes, in these groups.

Reference to Table 11 indicates that the factor(s)

causing the increase in number of small follicles is manifested in groups 4 a and 4 b, notwithstanding the implications arising from the presence of corpora lutea.

The possible causes of this difference, which will be discussed later, are:-

i. That the difference in the controls is fortuitous and the treated ewes responded to the progesterone by a decrease in the number of small follicles which was evident at the first killing, while the second killing would represent the normal picture at that time of the year.

ii. That the treatments were without obvious effect on the number of small follicles and that the increased numbers observed at the second killing were due to either (a) the ewes being closer to the normal breeding season, or (b) the presence of the rams; in either case the effect being a response to increased pituitary gland activity.

It will be seen that there is a further difference between groups 4 a and 4 b. Thus group 4 b contains at least twice as many small follicles per sheep as group 4 a, at each killing. This indicates that the duration of progesterone levels in the blood must be taken into account in the discussion.

C. Histological Changes in the Reproductive Tract

1. Fallopian tubes (oviducts)

a. Controls

i. Height of epithelium and width of stroma. An examination of the data on the control ewes gives an indication of the oviduct epithelium during the anoestrous period.

Tables 12, 13 and 14 include the information on the height of the epithelium in the three portions, fimbriated end (fimbria), mid-tube (~~isthmus~~ ampulla) and uterine end (~~ampulla~~ isthmus), together with the width of the stroma in the upper two regions.

While the height of the epithelium in the fimbriated and mid-tube portions appears to be similar, the uterine end of the oviduct has significantly shorter epithelium. (This may be compared with the average height of the uterine epithelium which was 13.3 μ .)

Analysis of Variance

Height of epithelium - uterine portion and fimbriated end - controls

Source	df	SS	MS	F
Total	167	999.9		
Between groups	1	216.06	216.06	6.47 *
Sheep within groups	12	400.48	33.37	13.4 **
Within sheep	154	383.36	2.49	

The following analysis indicates that the stroma in the fimbriated portion is significantly wider than in the mid-tube.

Analysis of Variance

Width of stroma - fimbriated end and mid-tube - controls

Source	df	SS	MS	F
Total	155	774.46		
Between groups	1	144.20	144.2	6.23 *
Sheep within groups	11	254.54	23.14	8.8 **
Within sheep	143	375.72	2.63	

TABLE 12

Group means and standard deviations of measurements of the Fallopian tube - fimbriated portion

Group	Number of ewes	Epithelium in μ	Stroma in μ
1	4	21.0 ± 2.15	12.1 ± 2.69
2 a i	3	19.7 ± 3.33	9.2 ± 1.97
2 c i	3	19.2 ± 2.59	10.4 ± 2.35
2 c ii	4	18.8 ± 3.17	8.6 ± 4.32
3 c ii	5	23.3 ± 5.33	7.7 ± 2.03
4 a i	2	20.0 ± 2.19	11.0 ± 4.0
4 a ii	4	19.5 ± 1.95	7.5 ± 2.19
4 b i	2	24.5 ± 2.47	9.5 ± 1.97
5	6	19.0 ± 2.55	8.3 ± 2.1

TABLE 13

Group means and standard deviations of measurements of the fallopian tube - mid-tube portion

Group	Number of ewes	Epithelium in μ	Stroma in μ
1	3	20.9 ± 1.76	6.1 ± 1.9
2 c ii	4	24.9 ± 3.52	6.1 ± 2.12
3 c ii	4	24.7 ± 5.59	5.5 ± 2.21
4 a i	4	23.9 ± 3.01	5.7 ± 1.78
4 a ii	3	22.2 ± 3.85	4.5 ± 1.7
4 b i	2	28.0 ± 2.55	5.1 ± 1.2
5	7	20.5 ± 1.98	6.4 ± 1.96

TABLE 14

Group means and standard deviations of measurements of the fallopian tube - uterine portion

Group	Number of ewes	Epithelium in μ
1	3	19.6 ± 1.44
2 a i	3	18.9 ± 2.85
2 c i	3	15.9 ± 1.3
3 c i	4	16.3 ± 1.45
2 c ii	4	17.1 ± 2.7
3 c ii	5	16.1 ± 4.11
4 a i	4	22.8 ± 3.7
4 a ii	4	20.0 ± 3.31
4 b i	2	27.0 ± 3.5
4 b ii	3	17.5 ± 2.13
5	8	16.7 ± 1.84

ii. Description. While the height of the epithelium was fairly regular the nature of the epithelial and stroma cells in the fimbria and ~~isthmus~~ ampulla was not very easy to define. In the uterine end, where the villi were reduced to slight folds, the epithelial cells were similar in shape and size and hence presented a very uniform, clearly defined columnar type of epithelium. The complexity of the folds, however, increased markedly in the upper portions of the tube, but in many cases the villi so formed were of shrunken appearance and did not occupy a very large amount of the lumen.

The cell outlines were not easily discernible, particularly the free ends from which there were cytoplasmic and nuclear extrusions, which at times were quite extensive. The nuclei varied from darkly staining, elongated and shrunken to more plump and lighter stained, the latter being in the minority.

Cilia were not generally obvious.

b. Comparison of controls with other groups

i. Uterine portion of the oviduct. It can be seen that the ewes examined in groups 4 a i, 4 a ii, and 4 b i, (all of which had ovulated) and in group 1 (which had received the highest level of progesterone) had higher epithelium than the controls. It is to be noted that the 3 ewes examined in group 4 b ii had not ovulated.

ii. Mid-tube and fimbriated portion of oviduct. There appears to have been some increase in height of epithelium in some groups, but the increase in variability between sheep in the same groups renders the interpretation of the data in Tables 12, 13 and 14 very difficult. This apparently marked

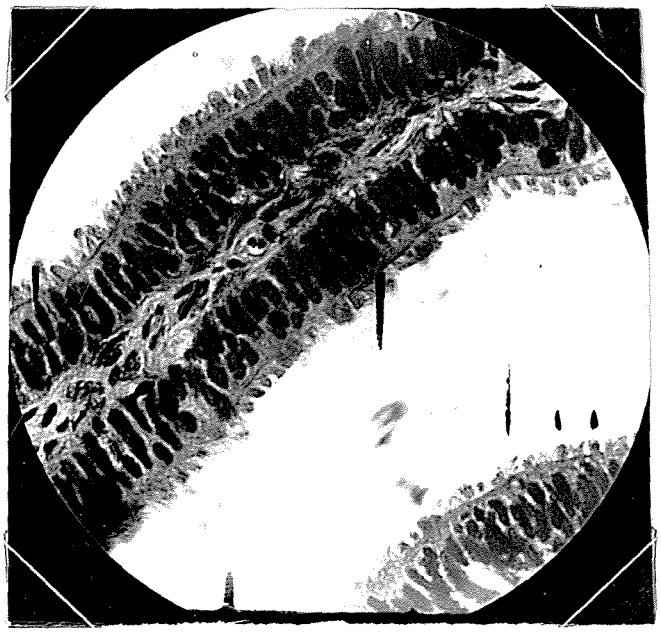


Figure 1. Ewe 356.49. Mid-tube portion of oviduct x500. Note cytoplasmic projections; elongated, darkly staining, crowded nuclei in both epithelium and stroma.

increase in variability may be due to the treatments or it may be a result of the small numbers examined in all groups except the controls.

2. Uterus

a. Measurement of intercotyledonary uterine epithelium

Listed in Table 15 are the group averages and standard deviations of the measurements of the height of the intercotyledonary uterine epithelium. An examination of the data which is summarised in the table led to the following results.

i. The average height of epithelium of all control ewes was 13.25 μ and there was no difference between the dates of slaughter. It will be noted however, that there was a highly significant difference between sheep. This variation between sheep is characteristic of all groups and accounts to some extent for the relatively large group standard deviations.

A further point is that the variation within sheep is relatively small; this is characteristic of all groups.

Analysis of Variance

Dates of slaughter - controls (excl. 50 H)

Source	df	SS	MS	F
Total	203	1055.23		
Between dates of slaughter	1	2.35	2.35	0.07 NS
Between sheep within dates	15	507.96	33.86	11.64 **
Within sheep	187	544.92	2.91	

ii. All groups had higher epithelium than the controls at the first killing. For example we include here the comparison

TABLE 15

Height of uterine epithelium

Group	First slaughter date (i)		Second slaughter date (ii)	
	Number of ewes examined	Height of epithelium and S. D. in μ	Number of ewes examined	Height of epithelium and S. D. in μ
1	5	21.5 ± 4.17		
2 a	5	23.8 ± 4.3	2	14.7 ± 2.65
2 b	5	26.1 ± 4.45	5	17.1 ± 3.46
2 c	4	22.6 ± 4.57	5	14.0 ± 1.8
3 a	5	22.8 ± 3.35	5	14.7 ± 2.17
3 b	5	22.3 ± 5.07	5	14.5 ± 2.4
3 c	5	18.6 ± 2.48	5	14.9 ± 3.71
4 a	4	24.9 ± 4.94	5	25.3 ± 3.44
4 b	5	29.6 ± 3.27	4	16.2 ± 1.87
5 a	4	13.4 ± 2.3	4	14.0 ± 2.15
5 b	5	13.3 ± 2.45	4	12.3 ± 1.9

57.49 (2 c i) 19.5 ± 3.42 μ

229.49 (4 b ii) 30.9 ± 3.13 μ

50H (5 b ii) 27.1 ± 2.2 μ

between group 3 c i and the controls.

Analysis of Variance

Comparison of group 3 c i and the controls (5 a i and 5 b i combined)

Source	df	SS	MS	F
Total	167	2010.85		
Between groups	1	965.19	965.19	22.24 **
Between sheep within groups	12	520.74	43.39	12.72 **
Within sheep	154	524.92	3.41	

iii. All the ewes which had corpora lutea at the time of the second killing (group 4 a ii, plus 229.49 and 50 H) had uterine epithelium approximately twice as high as the control ewes. The following analysis of variance shows that all other ewes at the second killing had significantly higher epithelium ($p < 0.05$) than the controls.

Analysis of Variance

Comparison of groups 2 a, 2 b, 2 c, 3 a, 3 b, 3 c, 4 b (combined) and the controls at the second killing

Source	df	SS	MS	F
Total	467	3837.72		
Between groups	1	314.12	314.12	5.98 *
Between sheep within groups	37	1943.1	52.52	14.27 **
Within sheep	429	1580.5	3.68	

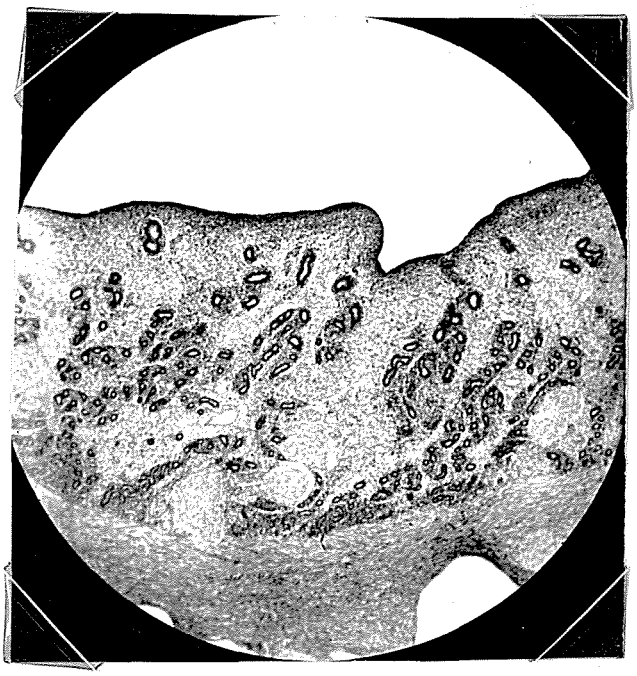


Figure 2. Ewe 109H (control). Intercotyledonary endometrium x50. Note low epithelium; poor gland development; extensive duct system; densely packed nuclei in stroma.

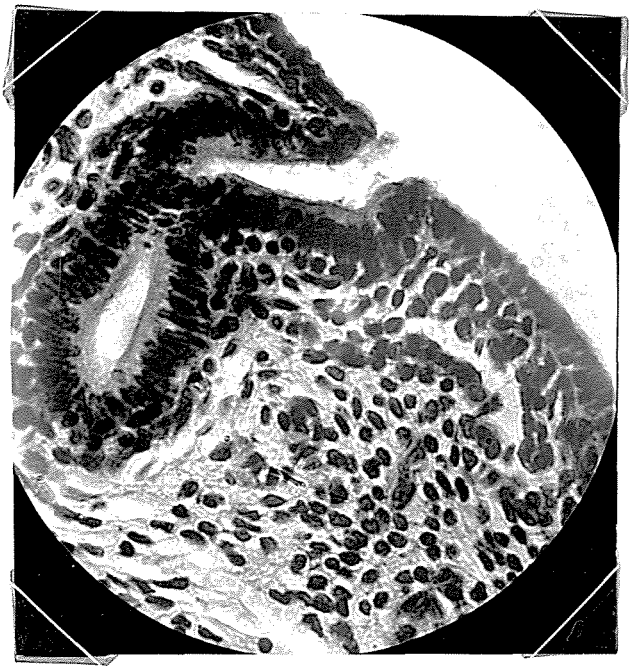


Figure 3. Ewe 109H (control). Intercotyledonary epithelium x500. Note low columnar epithelium; densely packed nuclei in stroma.

iv. There was a highly significant difference ($p < 0.01$) between the two dates of slaughter in groups 2 a, 2 b, 2 c, 3 a, 3 b, 3 c, 4 b (Combined.)

Analysis of Variance

Dates of slaughter - groups 2 a, 2 b, 2 c, 3 a, 3 b, 3 c, 4 b, (combined)

Source	df	SS	MS	F
Total	659	25118.1		
Between dates of slaughter	1	14414.3	14414.3	109.03 **
Between sheep within dates	53	7007.37	132.21	21.64 **
Within sheep	605	3696.42	6.11	

b. General description of uterine sections

i. Control ewes. The following is a general description of the uterine sections of the control ewes as examined under 8.75 magnifications as well as 140x and 840x.

The endometrium was approximately 1 mm in thickness. Although the uterine glands were poorly developed, the ducts were numerous and easily seen. The uterine epithelium was regular and the lumen was simply defined. The stroma consisted of densely packed nuclei, with occasional large cells containing pigment mainly in the sub-epithelial region and in the very dense stroma of the cotyledons. Numerous lymphocytes were present and were most frequent near the basal layers of the glandular and surface epithelium. The uterine glands under low power were seen to be shrunken and their basal nuclei were small, elongated and crowded. The numerous blood vessels contained few red blood cells, indicating, presumably, an ischemic condition.

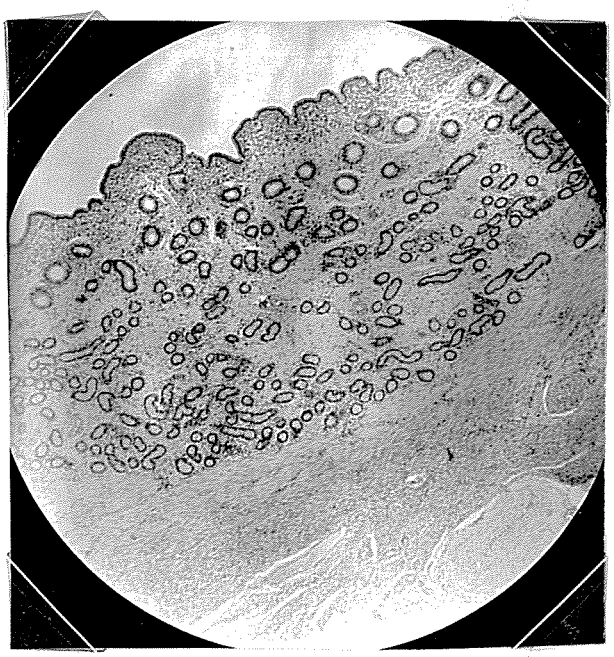


Figure 4. Ewe 256.49 (group 4 a ii). Intercotyledonary endometrium x50. Note folding of epithelium; oedematous stroma; glandular development.

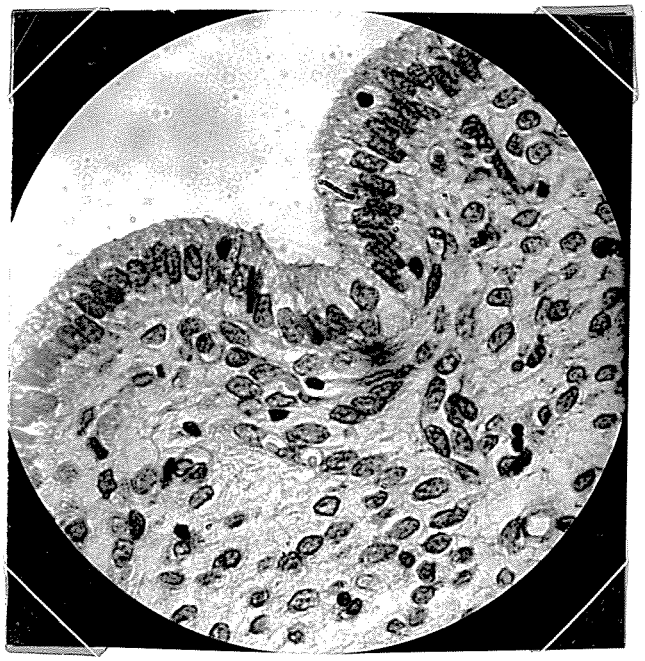


Figure 5. Ewe 256.49 (group 4 a ii(. Intercotyledonary epithelium x500. Note high, folded epithelium; rounded nuclei; oedematous stroma.

The epithelium of the cotyledons appeared to be indistinguisable from that of the surface intercotyledonary epithelium. The surface epithelium was almost cuboidal and the crowded nuclei occupied practically the entire cell.

ii. Group 4 a ii. The uteri of this group represent the extreme in treated animals. These ewes had ovulated and the corpora lutea had been present and presumably active for at least 10 days. The most striking differences were branching and folding of the intercotyledonary epithelium, greatly increased glandular development, oedematous stroma and higher glandular and surface epithelium.

The surface and glandular columnar epithelium was characterised by a marked increase in the cell diameters as well as the increase in height. The nuclei were plump and situated near the basal membrane and occupied approximately one half to one third of the cell. The glandular epithelium was similar to the uterine epithelium and the great increase in size in many cases led to the lumen of the glands being largely filled by the cell extremities.

Not only was the stroma oedematous, as judged by the greatly reduced number of nuclei per field, but the blood vessels were quite often engorged with red blood cells. An interesting point is that the number of leucocytes (mainly lymphocytes) seemed to have decreased, in some cases quite markedly.

iii. The intermediate stage was characteristic of the groups which received various levels of progesterone alone. The important observation made with respect to the various levels of progesterone treatments is that of a uniform response, as

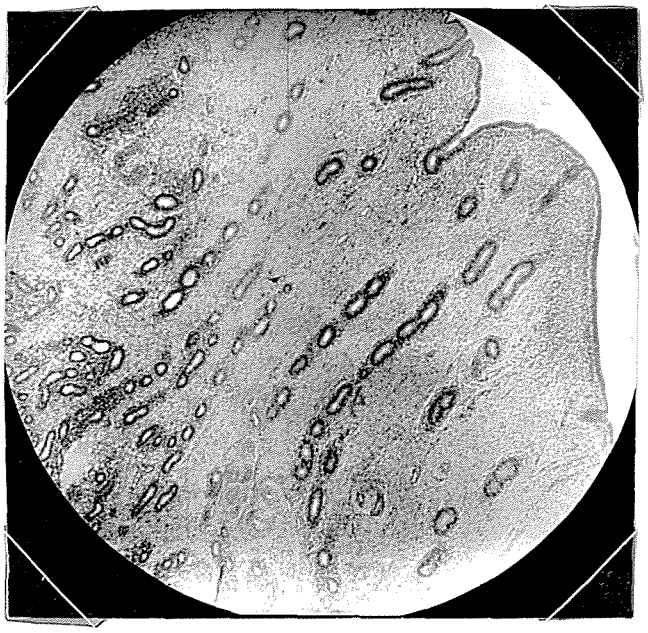


Figure 6. Ewe 4AG (group 3 b i). Intercotyledonary endometrium x50. Note columnar epithelium, showing signs of folding; some glandular development; oedema of stroma. Compare with figs. 2 and 4.

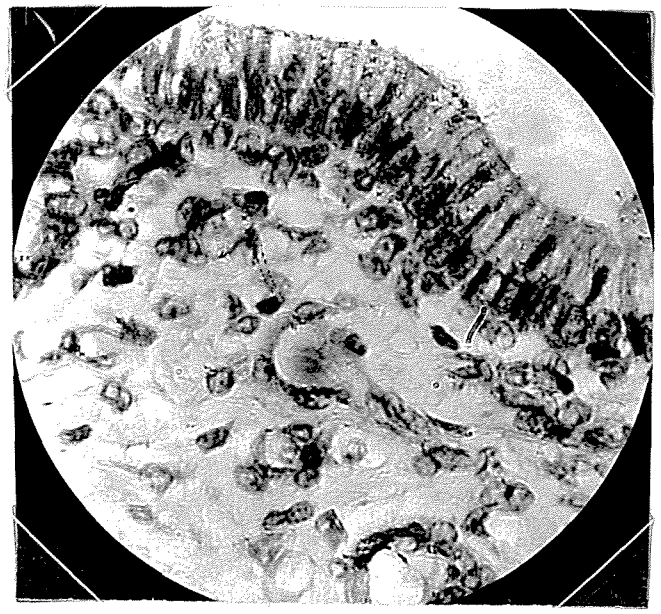


Figure 7. Ewe 4AG (group 3 b i). Intercotyledonary epithelium x 500. Note crowded nuclei; oedematous stroma.

judged 6 days after the last injection, to the progesterone, irrespective of the dosage.

3. Cervix

It has been noted that the only staining technique used was the haematoxylin and eosin combination. It is generally considered wise to use a mucin stain, such as muci-carmine, for cervical sections, in view of the well recognised mucus secreting action of the epithelium. In this study, however, this was not done and consequently the results are limited. Nevertheless it will be seen that a reasonably clear picture may be presented.

a. Control ewes

Therewere no obvious differences between the two dates of slaughter.

i. Epithelium. The epithelium lining the villi was of a psuedostratified columnar type of average height 18 - 20 μ. The nuclei, which were elongated, occupied the greater portion of the cells.

ii. Stroma. The connective tissue of the stroma contained few leucocytes and the nuclei of the cells were generally shrunken. The blood vessels appeared to be free of red blood cells.

iii. Base of crypts. The bulbous invagination at the base of the crypts was lined by two or three layers of cells, in which storage of mucus was apparently taking place.

b. Group 4 a ii.

Quite marked changes had taken place in the sections examined from this group. The average height of the epithelium

lining the villi was 25 μ, but variation was considerable. The basal nuclei had greatly increased in numbers and the epithelium presented a psuedostratified appearence. A few surface cells were distended. The villi had become somewhat wider and flattened as a result of oedema.

The stromal nuclei were rounded and the blood vessels contained many red blood cells. There were a few lymphocytes present.

The base of the crypts had taken on a glandular appearance; the cells were distended and the nuclei were pressed against the basal membrane.

c. Group 4 a i

The average height of the epithelium was 23 - 25 μ. The nuclei were plump and occupied the basal half of the cells. The epithelium was generally simple columnar and the portions of the cells not occupied by nuclei contained fibrillar cytoplasm. The cells were not obviously distended and crowding was negligible.

A large proportion of the stromal nuclei were rounded and there were more lymphocytes seen in this group than any other. The blood vessels were engorged.

The base of the crypts was of a complex glandular character and the cells were very distended.

d. Other groups

The groups which received the lower levels of progesterone (2 c and 3 c), showed slight differences at the first killing.

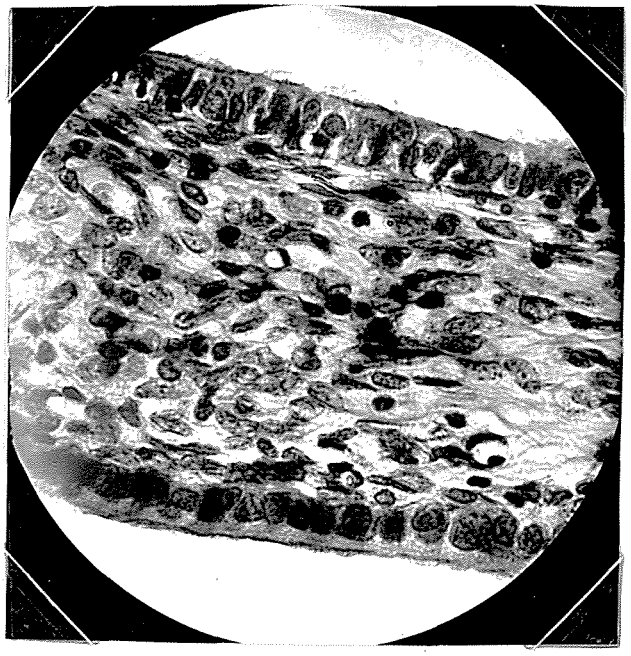


Figure 8. Ewe 120H (group control). Portion of cervical villus x500. Note pseudostratified epithelium; densly packed nuclei in stroma.

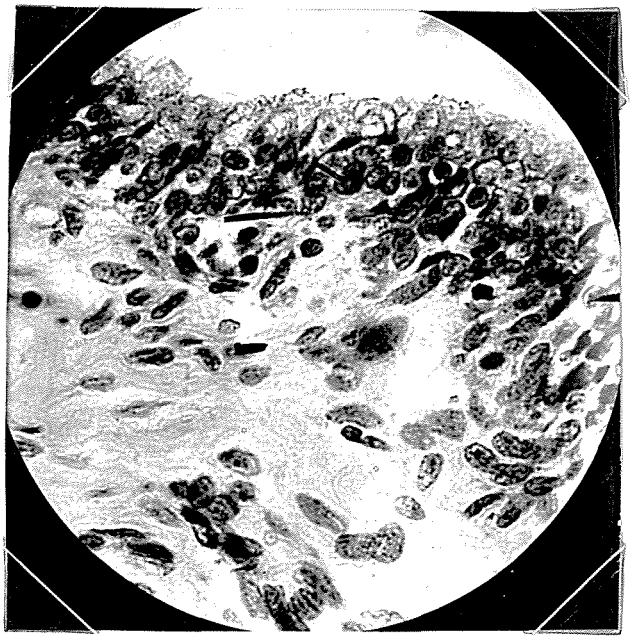


Figure 9. Ewe 55H (group 4 a ii). Portion of cervical villus x500. Note oedematous stroma; stratified epithelium; mucus on free surface.

These changes consisted of distension of blood vessels and the nuclei of the epithelium, the cells of which were slightly larger, were rounded.

The other groups at the first killing had higher epithelia containing rounded nuclei as well as increased vascularity of the stroma, in which in some cases the nuclei were larger.

All the sheep at the second date of slaughter, except those which contained corpora lutea, were similar to the controls, although there was occasionally a greater amount of stratification in the epithelium.

The effect of a corpus luteum on the villi was well demonstrated by No. 57.49 (group 2 c i). This ewe had ovulated 3-5 days prior to killing. The epithelium was folded and the large cells contained plump nuclei. The stromal nuclei were well rounded and the blood vessels were congested. It should be pointed out that the epithelial cells were not distended being merely regular high columnar epithelium.

f. General

The examination of the cervix suggests that the following changes have taken place as a result of the treatments.

As a consequence of increased blood supply there has been a development of the epithelium and an oedema of the stroma. The epithelium appears to have responded by a change in size and shape of the nuclei which has caused them to fill the cell, followed by an increase in height and width and the development of a fibrillar cytoplasm. This appears to have taken place

over a period of about 8 days under the influence of progesterone. The effect of the cell enlargement and the formation of the uniform high columnar epithelium has been to cause an increase in folding of the epithelium lining the villi. The stromal nuclei have become rounded and definitely stain more clearly. The oedema of the stroma is quite marked and the number of lymphocytes is greatest, as is the development of the base of the crypts at the end of this period.

The next stage probably includes secretion and storage, but there were no sheep killed for a further 8 days. What is probably the third stage, however - that of regression to the anoestrous state - is apparently seen in the ewes of group 4 a ii. The villi had become less well defined and in some cases the stromal nuclei had become shrunken. The most striking change was that of the epithelium, which, though still fairly high, had taken on a psuedostratified appearance. The presence of occasional distended cells in the epithelium lining the villi is taken as evidence of a previous secretory phase.

4. Vagina

In Table 16 are presented the data concerning the vaginas in the control groups - 5 a and 5 b. Table 17 contains the findings in groups 4 a and 4 b, whilst Table 18 comprises the remainder. It is intended that a full description be given of the picture as represented by the control ewes and that this will serve as a basis for comparison between all groups.

a. Detailed study of the results in Table 16

The most striking feature in the anoestrous ewe is the variability between most sheep, in the majority of characteristics examined. There was also noticed a considerable lack of

T A B L E 16

Vaginal characteristics - control ewes

Group	Ewe	Leucocytes	Crypts	Definition of cell membranes	Cellular cytoplasm	Basal nuclei	Vacuolar degeneration	Cornification	Height of Epithelium	
									Average μ	Range μ
5 a i	13 AG	++	DSS	D	G	0	+	+	100	70-120
	40 H	++	S	D	G	+	++	0	40	25-70
	88 H	++	S	D	G	+	0	0	115	70-190
	163 H	++	DS	D	G	+	0	+	60	35-85
	415.49	+++	DDSS	D-I	GF	0	0	0	50	25-70
5 a ii	16 H	++	DDSS	D-I	GF	+	+	0	60	35-70
	58 H	+	None	I	F	0	+++	0	30	25-35
	136 H	++	S	D	G	0	+	0	30-	25-50
	88.49	+	DSS	D	G	+	+	+	50	25-70
5 b i	56 H	+	SS	D-I	GF	+	+	0	40	35-60
	65 H	++	DSS	D-I	GF	0	+	+	70	50-120
	120 H	+	DS	D-I	GF	0	+	0	40	25-50
	11.49	+	None	I	F	0	+++	0	85	60-140
5 b ii	4 H	++	DS	D	G	+	+	0	80	50-120
	50 H	++	DDSS	D	G	0	+	+	60	50-85
	109 H	+++	DDSS	D	G	+	+	+	35	25-50
	230.49	+++	DD	D	G	+	0	0	75	50-95
	356.49	++	DS	D	GF	+	++	+	60	50-95

TABLE 17

Vaginal characteristics - group 4

Group	Ewe	Leucocytes	Crypts	Definition of cell membranes	Cellular cytoplasm	Basal nuclei	Vacuolar degeneration	Cornification	Height of Epithelium Average μ	Height of Epithelium Range μ
4 a i	115 H	+±	S	D-I	GF	O	++	O	30	20-35
	121 H	++	DDSS	D-I	GF	O	++	+	55	35-70
	150 H	++	DDSS	D-I	GF	O-	++	++	80	35-120
	329.49	++	DSS	D-I	GF	O-	++	+++	60	35-140
4 a ii	1 AG	+++	DDSS	D	G	O+	+	+	50	20-80
	9 H	++	DS	D-I	GF	O	++	O	50	35-80
	55 H	++	DS	D	~~DG~~	O	+	O	55	20-75
	256.49	++	DDSS	D	G	O	+	O	50	30-80
4 b i	19 H	+++	DDSS	D-I	GF	+	+	+	50	35-95
	122 H	+++	SS	I	F	O-	++	+	50	35-60
	110.49	+++	DDSS	D-I	GF	O	++	+	80	45-120
	168.49	++	DDSS	D-I	GF	O-	+	++	120	85-140
	280.49	+++	DSS	D-I	GF	O	++	++	80	35-120
4 b ii	3 AG	++	DSS	D-I	GF	O+	+	+	60	35-95
	48 H	+++	DSS	D-I	GF	O+	+	O	50	35-60
	56.47	++	DDSS	D-I	G	O+	+	+	60	20-95
	229.49	++	DDSS	D-I	GF	O+	+	+	5	70-140
	385.49	++	DS	D-I	GF	O+	+	O	45	20-50

TABLE 18

Vaginal characteristics - groups 1, 2.

Group	Ewe	Leucocytes	Crypts	Definition of cell membranes	Cellular cytoplasm	Basal nuclei	Vacuolar degeneration	Cornification	Height of Epithelium Average μ	Height of Epithelium Range μ
1	44 H	++	S	D	G	+	0	0	60	35-80
	101.49	++	DDSS	D	G	0	0	0	70	50-95
	223.49	++	None	I	F	0	++	0	60	35-95
	474.49	+++	D	D	G	+	+	0	55	35-70
	520.49	+++	None	D	G	+	+	0	50	35-70
2 a i	8 H	++	S	D-I	GF	+	+	0	85	60-120
	97 H	+++	SS	D-I	GF	0	+	0	50	35-70
	190.48	+++	DS	D-I	GF	+	+	0	70	50-95
	405.47	++	DS	D-I	GF	0	+	0	85	60-130
2 a ii	12 AG	++	DDSS	D	G	0	+	0	40	20-94
	132 H	++	S	D	G	+	+	0	35	20-50
2 b i	2 AG	++	DDSS	D	GF	+	+	+	35	20-60
	126 H	++	DDSS	D	F	+	+	0	60	35-70
	156 H	+++	DS	D	G	+	+	0	40	20-60
	411.49	++	D	D	G	+	+	0	70	50-105
2 b ii	168H	++	DS	D	G	0	+	0	50	35-70
	169.49	++	DDSS	D-I	G	+	+	0	45	20-95
	517.49	+	None	D-I	GF	0	++	0	35	20-55
	8.48	+	DS	D-I	G	0	+	0	60	35-80
2 c i	42 H	++	DS	D	G	+	++	0	60	20-95
	57.49	++	S	D	G	+	+	0	70	50-95
	204.49	++	DDSS	D	G	0	+	0	30	20-50
	306.49	++	SS	D	G	+	+	+	60	20-95
	359.49	++	SS	D	GF	0	+	0	25	20-50
2 c ii	134M	++	S	D-I	GF	0	++	0	50	35-70
	81.47	++	DDSS	D	G	+	+	0	70	50-95
	42.52	+	None	I	F	0	++	0	35	20-50
	254.49	+	S	D	G	+	+	0	70	50-85
	269.49	++	S	I	F	0	++	0	40	20-50

TABLE 18 (cont.)

Vaginal characteristics - group 3

Group	Ewe	Leucocytes	Crypts	Definition of cell membranes	Cellular cytoplasm	Basal nuclei	Vacuolar degeneration	Cornification	Height of Epithelium Average μ	Height of Epithelium Range μ
3 a i	11 AG	+++	DS	D-I	GF	0	+	0	65	35-95
	2 H	++	DSS	D-I	GF	+	+	0	40	20-70
	76 H	++	DS	D-I	GF	0	++	0	55	35-70
	83.49	+++	SS	D-I	GF	0	+	0	85	50-120
	383.49	+++	DSS	D	G	+	+	0	40	20-85
3 a ii	6 AG	++	DS	D	G	0	+	0	55	35-70
	14 H	++	DDSS	D-I	G	+	+	0	55	35-70
	133 H	++	None	I	F	0	+	0	30	20-50
	149 H	++	DS	D-I	GF	+	+	0	40	20-70
	145.50	++	DSS	D	G	+	+	0	65	35-80
3 b i	4 AG	+++	DS	D-I	GF	0	+	+	45	20-60
	7 AG	++	SS	D	GF	0	+	0	45	20-70
	49 H	++	DS	D	G	+	+	0	60	35-85
	104.48	++	S	D	G	+	+	0	50	20-70
	458.49	++	DS	D-I	G	+	+	0	30	20-35
3 b ii	9 AG	++	SS	D-I	G	+	+	0	55	35-70
	151 H	++	S	I	F	0	++	0	35	20-50
	159 H	++	DDSS	D	GF	0	+	0	70	50-95
	160 H	++	S	D-I	GF	0	+	0	30	20-50
3 c i	14 AG	++	DS	D	G	+	+	0	45	20-70
	18 H	++	DDSS	D	G	+	+	0	40	20-50
	62 H	++	DS	D-I	GF	0	++	0	55	20-85
	107.49	++	DS	D	G	0	+	0	40	25-60
	152.51	++	DDSS	D	G	+	+	0	60	25-70
3 c ii	5 AG	++	DDSS	D	G	+	+	0	30	20-50
	137 H	++	None	I	F	0	++	0	25	20-35
	439.49	++	S	D-I	GF	0	+	0	40	20-60
	446.49	++	DDSS	D-I	GF	0	+	0	60	35-70
	464.49	++	DDSS	D	G	0	+	+	60	35-70

uniformity within sheep, thereby leading to extreme difficulty in making a grading. Thus in 415.49 the cell membranes were easily seen in some fields and in others were quite indistinct; again in 50H there was a great variation in the nature of the nuclei in the basal layer - in some fields there were clearly defined, plump nuclei and in others there was a certain amount of crowding resulting in poorly-stained, elongated nuclei.

i. Leucocytes. The majority of sections contained 40-60 leucocytes per field; the exceptions, which contained about one hundred cells, were 415.49, 230.49 and 109 H. They consisted, in the main, of polymorphs (polymorphonuclear neutrophilic leucocytes) and were situated in the stroma just beneath the basal layer with a few odd ones scattered amongst the basal cells. (fig. 10).

ii. Crypts. For the purpose of this paper the term crypts is used to describe the epithelial invaginations between stromal papillae. Thus crypts are synonymous with epithelial papillae (Grant, 1934) and epithelial buds (Casida & McKenzie, 1932; Bell et al., 1941 a). No crypts were seen in 58 H (fig. 11) and 11.49, while 109 H (fig. 10) represents the other extreme, where both shallow and deep crypts were observed in large numbers - so much so that a measure of the height of the epithelium between crypts was most difficult. Judging from the results, the presence or absence of crypts and the nature of these crypts is a purely individual characteristic.

iii. Definition of epithelial cell membranes. The cell membranes were fairly distinct in the majority of ewes. There

was, however, some variation within sheep, which is indicated by the D - I grading.

iv. Nature of the cellular cytoplasm. It was only possible to detect extreme differences with certainty. There was no great variation within sheep, consequently the GF gradings are to be interpreted as indicating doubt, and not variability within sheep. It will be seen that a GF or F grading is invariably associated with a D - I or I grading.

v. Nuclei in the basal layer. There were considerable differences between sheep and within sheep in the degree of crowding and hence clarity of the basal nuclei. The O classification means that a few fields showed poorly defined basal nuclei. Generally, crowding and consequent poor definition as distinct from the plump, clearly seen nuclei, was more common in the deeper portions of the crypts.

iv. Vacuolar degeneration of epithelial cell cytoplasm. In marked cases of vacuole formation in the epithelial layers the nuclei were quite appreciably shrunken. It is noteworthy that the variation within sheep in this characteristic was small; however, as can be seen, variation between sheep was extensive. Ewes 58 H and 11.49 each showed an unusually high degree of vacuolar degeneration. This was accompanied by indifinite cell membranes and markedly fibrillar cytoplasm.

vii. Cornified epithelial cells. Where present, cornification was very slight, affecting usually only one or two of the outer cell layers. ~~This can be seen in fig 4~~. Note that cornification refers to the presence of flattened keratinised cells with or without nuclei. The most usual type of cell in

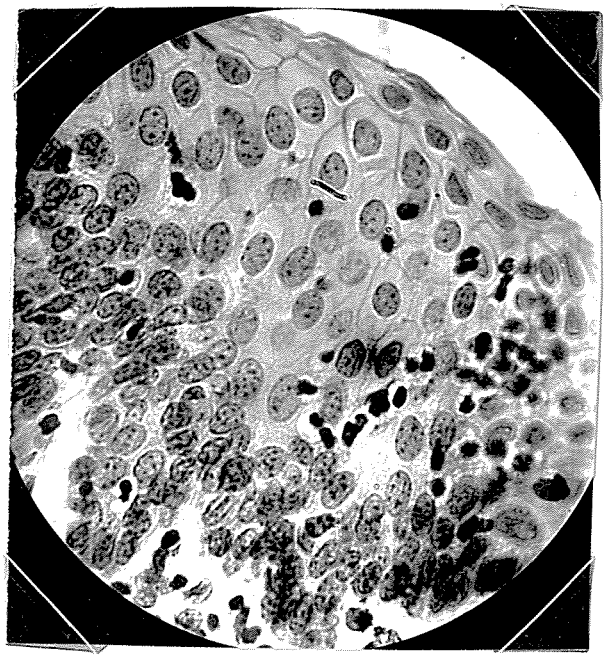


Figure 10. Ewe 109H (controls). Vaginal epithelium x500. Note clearly defined cell membranes; globular cytoplasm; plump nuclei; leucocytes; polyhedral outer layer of cells.

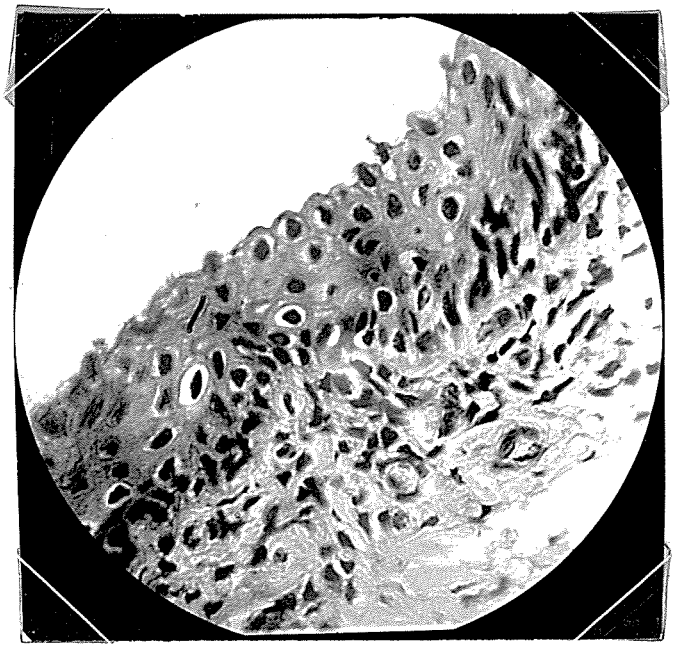


Figure 11. Ewe 58H (controls). Vaginal epithelium x500. Note fibrillar cytoplasm; vacuolar degeneration; crowded, poorly defined basal nuclei.

the outer layers of the epithelium was a polyhedral cell containing a clearly staining nucleus. Some sections of the epithelium in all control ewes showed this undifferentiated type of apithelium. The intermediate stage, which was characterised by the cells in the outer layers having lost their polyhedral shape and their nuclei showing various stages of decomposition, was not uncommon [redacted].

viii. Height of epithelium. The variation within sheep was very marked. Nevertheless it was noted that some individuals appeared to possess a relatively thin epithelium while others were relatively thick.

The general picture arising from an examination of the control group of ewes was as follows. The number of leucocytes varied from about 40 to 120 per field, and the numbers and sizes of the crypts were not at all constant. While the epithelial cell membranes were generally clearly defined and the cytoplasm mainly globular, there was some evidence of vacuolar degeneration in all cases. The basal nuclei were predominately plump and clear. In some ewes there was a slight amount of cornification, but desquamation was not obvious; it was most common to find the epithelium, the height of which averaged 65 μ with a range of 25 - 190 μ, composed of fairly regular cell layers, the outer one of which was of polyhedral cells with well-defined nuclei.

b. Comparison of treated ewes with controls

First slaughter

i. Group 1. The number of leucocytes appeared to have increased slightly and cornification was completely absent; also

the fact that only one sheep (101.49) showed any marked number of crypts may be significant.

ii. Groups 2 a, 2 b, 2 c, 3 a, 3 b, 3 c. Because of the differences between individuals it was impossible to decide whether any characteristics were consistently different from the controls.

iii. Group 4 a. The amount of cornification varied from none to very marked. The four ewes in this group presented a considerable amount of vacuolar degeneration as well as poorly-defined basal nuclei which were in many cases definitely crowded. The grading of cell membranes and cell cytoplasm was difficult to determine because of its intermediate type. The number of leucocytes, if anything, were less per field than the controls.

iv. Group 4 b. This group gave a fairly uniform picture characterised by more leucocytes, many crypts, ill-defined basal nuclei with correspondingly poorly-defined cell membranes and fibrillar cytoplasm, numerous vacuolated cells and variable amounts of cornification.

Second slaughter

There were no consistent differences between any of the groups and the controls.

D. Examination of the Pituitary Glands

1. Weights of the dried glands

The weights of the acetone dried pituitary glands varied

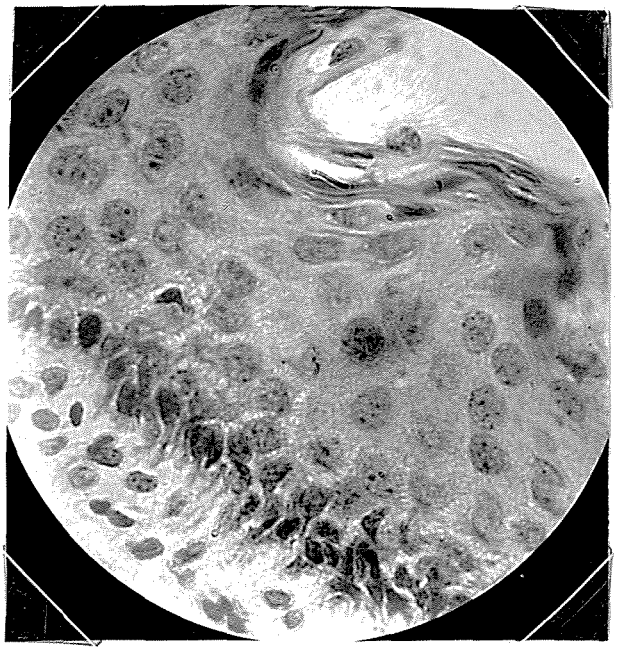


Figure 12. Ewe 329.49 (group 4 a 1). Vaginal epithelium x500. Note cornification; crowded, poorly defined basal nuclei.

from 159 mgms (306.49) to 497 mgms (136 H) with an average of 274 mgms. These figures, however, include estimated losses of 10 to 100 mgms per pituitary which occurred when the glands were later reground and passed through an 80-mesh sieve. Unfortunately the powder was not weighed after this second grinding and consequently it can only be stated that the final dried powder weights ranged from approximately 120 mgms to 400 mgms.

Whereas the majority of the pituitary glands were composed of a fine white powder which easily passed through the sieve, a few, e.g. 2 AG, 411.49, 7 AG, yielded a gritty, reddish brown substance. This was particularly difficult to keep in suspension and was not easy to inject.

2. Study of the control chicks

During the 6 weeks, July 26th to August 30th, a total of 84 control chicks were examined. The average for all chick testes weights was 14.5 mgms (Table 19). There was great variation within series (S.D. 4.12 mgms), as indicated in the analysis of variance accompanying Table 19. Coupled with this large variation the significant difference between series indicates a source of error in the biological material which may be expected to have a profound influence on the interpretation of the results.

T A B L E 19

Testes weights - control chicks

Series	n	$\bar{x}$	s
1	9	12.64	3.7
1 a	35	16.21	4.58
2	10	12.23	3.01
3	10	13.03	4.27
4	10	14.69	3.26
5	10	13.76	4.24
Total -	84	14.5	4.31

where n is the number of chicks
$\bar{x}$ is the mean
s is the standard deviation within series

Analysis of Variance

Source	df	SS	MS	F
Total	83	1539.35		
Between series	5	211.8	42.36	2.49 *
Within series	78	1327.55	17.02	

It was not possible to do more than make a cursory check on the variability of the chicks at the beginning of the injections. On Monday, August 15th, 13 chicks were killed. The testes weights averaged 7.28 mgms (standard deviation 1.63 mgms The control chicks of the same batch (Series 3 - Table 19) had an average testes weight, 6 days later, of 13.03 mgms S. D. 4.27 mgms). The effect of this apparently large increase in variance on the reliability of the chick assay will be discussed later.

3. Distilled water injections

The injection of distilled water alone into 20 chicks did not appear to have any effect on testes weights. This

can be seen in Table 20, where these are compared with the controls of the same batch (Series 2).

TABLE 20

Effect of distilled water injections on testes weights

Treatment	Number of chicks	Testes weights mg
Distilled water 2 ml in 8 injections	19	12.96 ± 2.63
Control - Series 2	10	12.23 ± 1.0

4. Comparison of intraperitoneal (I/P) and subcutaneous (S/C) routes of administration

Reference to Table 23 indicates that the Series 1 chicks suffered high mortality. In Series 1a there was included a comparison of I/P and S/C injections using pooled pituitary powder, left over from the Series 1 injections. These results are presented below in Table 21.

TABLE 21

Comparison of I/P and S/C administration of pooled pituitary powder - Series 1a

Treatment	Number of chicks Aug. 2nd	Aug. 6th	Body weight gm	Testes weight mg
15 mg pituitary powder, I/P	20	15	54.7 ± 4.24	12.12 ± 2.1
15 mg pituitary powder, S/C	20	20	64.6 ± 6.42	17.02 ± 5.56
Control	35	35	60.2 ± 5.65	16.21 ± 4.58

It can be seen that the effects of the intraperitoneal route were:-

i. 25% mortality
ii. decrease in body weights
iii. decrease in testes weights
iv. decrease in variance in testes weights

5. Effect of PMS injections on testes weights

It is apparent from the data presented in Table 22 that all levels of PMS greater than 10 iu per chick caused an increase in testes weights. It can be seen, however, that coincident with an increase in response there was an increase in variance. The data were tested for homogeneity of variance by Bartlett's Test for unequal sub-class numbers (Snedecor, page 251).

χ^2 = 11.3 for 4 df was significant ($p < 0.05$).

TABLE 22

Chick testes weight response to PMS - Series 1a

Amount of PMS per chick	Number of chicks	Body weight (gm)	Testes weight (mg)
10 iu	18	59	15.19 ± 3.04
15 iu	20	60	20.78 ± 4.68
20 iu	20	60	21.89 ± 5.45
30 iu	20	59	24.19 ± 6.68
40 iu	20	64	29.36 ± 6.14
Control	35	60	16.21 ± 4.58

The log transformation was tried and found to be an effective method for transforming the data. Thus, by Bartlett's test for homogeneity of variance

χ^2 = 1.67 for 4 df was not significant ($p = 0.8$).

The regression of log-testes weight on log-dose was found to be highly significant ($b = 0.4157$).

According to the requirements described by Emmens (1950) this dose-response line is satisfactory for quantitative assay. It is linear over a sufficient range, the variances are similar and it is relatively steep in relation to the variance.

It will be observed that it is unnecessary to correct for body weight differences. This situation also holds for the results of the assays of the pituitary glands for gonadotrophins.

6. Results of assays of the pituitary glands for gonadotrophins

The 100 pituitary glands of ewes from groups 2, 3, 4 and 5 were assayed in five series and a summary of the results are presented in Table 23. Also listed in the table are the results of two levels of PMS (15 iu and 30 iu) for each series as well as the results of the control chicks.

The glands of groups 2 a and 3 a (Series 1) were assayed using the I/P method. It can be seen that the dose levels (15 mgms/chick) and the route of injection combined to give results which were most unsatisfactory. The death losses amounted to half the chicks and the testes weights of the remainder did not differ from the controls.

It will be observed that in Series 2 there was no difference between the four sub-groups. In Series 3 there was again no difference between sub-groups, the following analysis of variance indicating that the lower average in 3 c i is not significantly different from 3 c ii,

TABLE 23

Chick testes weights - assay of sheep pituitary gland gonadotrophin and PMS

Series	Group	Ewe pituitary glands				PMS				Controls (see Table)	
		1st date of slaughter		2nd date of slaughter		15 iu		30 iu			
		No. of chicks	Testes weights and S.D. in mg	No. of chicks	Testes weights and S.D. in mg	No. of chicks	Testes weights and S.D. in mg	No. of chicks	Testes weights and S.D. in mg	No. of chicks	T.W.
1	2 a	13	12.2 ± 3.84	13	12.6 ± 4.46	4	16.4 ± 5.2	4	22.3 ± 8.2	9	12.6
	3 a	16	11.4 ± 3.96	10	12.2 ± 3.39						
2	2 b	25	16.8 ± 4.51	24	16.7 ± 4.45	9	21.7 ± 3.4	10	24.9 ± 5.78	10	12.2
	3 b	23	16.4 ± 3.2	22	16.2 ± 3.82						
3	2 c	19	19.4 ± 4.36	25	19.8 ± 4.67	10	18.9 ± 4.47	10	23.9 ± 6.48	10	13.0
	3 c	24	17.5 ± 4.75	20	19.3 ± 4.73						
4	5	48	16.2 ± 4.21	44	18.9 ± 5.25	9	16.8 ± 2.47	9	28.3 ± 9.28	10	14.7
5	4 a	22	15.8 ± 3.39	24	16.7 ± 3.78	10	21.3 ± 5.82	10	26.8 ± 6.68	10	13.8
	4 b	25	14.6 ± 3.33	19	17.6 ± 4.39						

Note: The following three ewes are not included in the above Table.

57.49	(2 c i)	16.7 ± 1.58	(4 chicks)
50 H	(5 b ii)	15.6 ± 2.18	(5 chicks)
229.49	(4 b ii)	22.0 ± 6.87	(5 chicks)

the reason being largely because of the great variance between sheep.

Analysis of Variance//

Comparison of groups 3 c i and 3 c ii

Source	df	SS	MS	F
Total	43	0.5218		
Between groups	1	0.0192	0.0192	0.81 NS
Between sheep within groups	8	0.1904	0.0238	2.59 *
Within sheep	34	0.3122	0.0092	

// Log transformation used for homogeneity of variances

A comparison of the control sheep indicates a significant difference between the dates of slaughter. This is true inspite of the fact there is also a significant difference between sheep within dates of slaughter. These findings are shown in the following analysis of variance.

Analysis of Variance//

Comparison between dates of slaughter - controls (excl. 50 H)

Source	df	SS	MS	F
Total	91	1.3239		
Between dates of slaughter	1	0.1046	0.1046	4.67 *
Between sheep within dates	17	0.3806	0.0224	1.95 *
Within sheep	73	0.8387	0.0115	

// Log transformation used for homogeneity of variance

It should be pointed out that the loss of one degree of freedom in the "between sheep within dates" column is due to the fact that ewe 50 H had been neglected in the analysis because she had ovulated. There is evidence

(Nalbandov, 1953) that the presence of a corpus luteum influences the gonadotrophin content of the pituitary gland.

For the same reason ewe 229.49 has not been included in the following analysis of variance which compares groups 4 b i and 4 b ii. It has been noted earlier that by a fortuitous arrangement the five ewes in group 4 b i had ovulated whereas only one (229.49) in 4 b ii had ovulated.

Analysis of Variance//

Comparison of groups 4 b i and 4 b ii (excl. 229.49)

Source	df	SS	MS	F	
Total	43	0.5160			
Between groups	1	0.0621	0.0621	5.12	NS
Between sheep within groups	7	0.0850	0.0121	1.15	NS
Within sheep	35	0.3689	0.0105		

// Log transformation used for homogeneity of variance

It can be seen from the above that there is no significant difference between 4 b i and 4 b ii.

It is possible to summarise the above section by stating that, within each series, the only significant difference observed is between the two dates of slaughter in the control ewes.

In view of the variation in the control chick testes weights between series and the variable responses of each batch of chicks to identical levels of PMS (see Table 23) it is not possible to make comparisons between series. This is a serious limitation in the chick assay method as carried out under the conditions of this experiment, and there does not appear to be a way out of this problem except possibly by complicated statistical techniques.

V DISCUSSION

A Manifestation of Oestrus

The administration of 800 iu PMS 50 hours after the final progesterone injection and 800 iu PMS alone (groups 4 a and 4 b) gave results which agree closely with reported work. Ovulation unaccompanied by oestrus is the usual response to a single PMS injection during anoestrus. On the other hand, pretreatment with progesterone has the effect of causing synchronous ovulation and oestrus. That the duration of the progesterone pretreatment is the major limiting factor in the number of ewes responding has been adequately pointed out by Robinson (1955b). He found that an increase to 6 days in the duration of progesterone stimulation results in a reliable oestrus response.

An indication of the mechanism by which this phenomenon is brought about is provided in the finding by Robinson & Moore (cited Robinson, 1955b) that the median effective dose of oestradiol benzoate required to induce oestrus in the ovariectomised ewe could be reduced by increasing the duration of progesterone pretreatment.

It would be reasonable to expect, therefore, that the failure of four ewes in group 4 a to exhibit oestrus could be due to an inadequate duration of progesterone treatment prior to the PMS injection. That this may not be the full explanation however, is evident from the fact that Robinson (1955b) has observed breed and age differences in oestrous responses of Suffold cross-breds and maiden and mature Romney ewes to the injection of PMS following progesterone pretreatment commencing four days earlier. Because such differences do not appear to

occur when the duration of progesterone pretreatment is 6 days and because results with a single injection of progesterone prior to PMS are poor, it is thought that an optimum level and duration of dosage is required for the ewe, this optimum varying with breed and age, at least. It may be that the Romney ewes in this experiment would have given a better response if the level of progesterone had been higher as well as if the duration of pretreatment has been longer.

When this experiment was designed it was felt that the treatments given to the ewes in groups 2 b and 2 c (25 mgms progesterone per day for four days) would be sufficient to cause ovulation, and possible oestrus, in a proportion of the ewes. Consequently, the various treatments in groups 1, 2 a, 3 a, 3 b and 3 c, were designed to give some information of the quantitative aspects of progesterone-induced ovulation and heat. Apparently, however, none of the treatments were effective in altering the pituitary-ovarian relationships sufficiently to induce maturation of follicles and subsequent ovulation.

In view of the fact that the mechanism whereby progesterone injections alone cause ovulation and heat in the anoestrous ewe is obscure, it is difficult to account for this failure.

It has been suggested by Dutt (1953b) that prolonged administration of progesterone leads to a "damming up" of luteinising hormone which is released at the conclusion of the progesterone injections and which is sufficient to initiate maturation of the follicle. Such an argument presupposes that the cause of anoestrus in the ewe is a decrease in production of LH. There is no evidence, however, that this is actually the

case; on the basis that PMS, which has an FSH-like action, will cause ovulation, one could well argue that it is the FSH which is low. Until it is possible to separate the total pituitary gonadotrophin into its constituent fractions any discussion along the above lines is purely speculative.

Regardless of the particular mechanism of the progesterone action, it is very likely that it does affect the pituitary gland either directly or indirectly and consequently an optimum level and duration of dosage is probably required before the pituitary-ovarian interelationships are such as to permit maturation of a follicle.

B Macroscopic Examination of the Ovaries

While the number of follicles of 5 - 6 mm diameter is fairly constant in each ovary of the typical anoestrous Romney ewe (groups 5 a i and 5 b i), the number of small follicles varies from 2 to 12. On a per ewe basis the total number of follicles present may be as low as 8 or as high as 22. These figures are the only ones available on the New Zealand Romney but they are not very different from observations on other breeds.

There does not seem to be any clear agreement as to what happens to the follicles during anoestrus. It is generally supposed that they are continually growing, but that when they reach a certain size they regress. If this is so it is surprising that the numbers of the larger follicles is relatively constant. It may be that beyond a certain critical size, approximately 5 mm in the Romney, growth proceeds under the control of a different set of conditions to those controlling

earlier growth. That occasionally in anoestrus, ewes do ovulate and also at the beginning and end of anoestrus spontaneous ovulations occur more frequently, suggests (a) that this second set of factors is characterised by a progressive change to an optimum which results in maturation and subsequent ovulation, and (b) that the occurrence of this optimum is related to the season, the lowest expectancy being in mid-anoestrus.

We may conclude by stating that not only are the optimum requirements for final maturation and ovulation not normally reached in the anoestrous ewe, but also for some reason a large proportion of the small follicles either regress or remain stationary until their turn comes to develop into large follicles, after which they apparently regress.

The significant increase in the number of small follicles in all groups at the second killing is intriguing. The most likely explanation is based on the fact that the presence of the ram has been shown to act as an external stimulus capable of modifying pituitary-gonad relationships. It is not known how the stimulus operates but it has been suggested that it acts via the pituitary and hence affects the output of gonadotrophins. It may be that the increase in ovarian activity observed in this experiment indicates that the presence of the ram in anoestrus influences the FSH output of the pituitary which in turn stimulates growth of the follicles in the ovary.

There is evidence that the approach of the normal breeding season is associated with an increase in numbers of small follicles (Kammlade, Jnr. et al., 1952). Although the normal breeding season of the New Zealand Romney does not commence until the first week of March, that is, at least six weeks later than

the second date of slaughter, it could be that the earlier indication of its approach is not the presence of spontaneous ovulations (Robinson, 1951) but rather an increase in follicle numbers. If this is the case then the increase noted may be quite independant of the presence of the rams.

A further complication arises when we consider groups 4 a and 4 b. Although the evidence is fragmentary it would seem that the only variable which could account for the differences in numbers of small follicles in these groups is the duration of the influence of progesterone. Thus prolonged progesterone activity (4 a) may have resulted in a diminution of FSH output or a direct action on the follicles themselves and consequently a decrease in the number of developing follicles. That this effect of progesterone is manifest only after prolonged duration is evident from the non-significant differences between controls and treated sheep at the first date of slaughter (analysis of variance, page 54).

Considerable importance is attached to the observation that the experimental corpora lutea conform in size to the normal expected in the breeding season. Without chemical estimation of progesterone in the blood or histological examination of the corpora lutea, it is not possible to the sure that an experimental corpora is functional; nevertheless, for the want of a more complete method of determination, the size relationships have been considered sufficient evidence to assume that they are functional.

C Histological Changes in the Reproductive Tract

The description of the histological examination of the

uterus, cervix and vagina of the normal anoestrous ewe agrees closely with previous reports. It may be pointed out that whereas the uterus and cervix presented a uniform 'quiescent' picture, the vaginal sections were characterised by great variability, there being no two ewes exhibiting similar findings in all characteristics examined.

The description of the fallopian tubes in the anoestrous ewe has not been attemped before. In particular, the reduced height of epithelium, shrinkage of the villi and protoplasmic extrusions indicate a stage of regression not seen in the cycling ewe.

On the basis of the above findings it would be reasonable to agree with other workers that the reproductive tract of the anoestrous ewe is in a relatively inactive state.

That all levels of progesterone administered have caused changes in the uterus and cervix is clear from the results. The effect of the oviducts is less well defined and it would appear that the vaginas did not respond at all. Where, however, ovulation and oestrus occurred there was evidence of changes in the latter two portions and also the responses in the uterus and cervix were enhanced.

The progestational response to progesterone alone was practically identical at all levels. When a corpus luteum was present it was noted that the amount of folding of the epithelium and the development of the uterine glands had proceeded much further. This might indicate that the relatively low levels of progesterone are sufficient to cause a response, but for full expression the duration of the treatment is the limiting

factor. It should be remembered that the production of oestrogen by the maturing follicle is likely to influence the response to progesterone. That oestrogen could be important is indicated by the cornification in the vaginas of the ewes in group 4 a i.

The changes in the cervix are interesting in that this organ appears to have a higher threshold to progesterone than the uterus. Once this threshold was reached, whether by duration or level of dosage, it did not respond quite readily. This finding suggests that a possible way in which priming with progesterone prior to PMS administration brings about the production of mucus, typical of oestrus, is that it prepares the cervix to respond to the normal physiological levels of oestrogens.

Poor lambing results following progesterone and PMS during anoestrus have been reported. The findings of Wallace (1955), in which 81 of 96 Romney ewes treated in February became pregnant, indicate the importance of clearly differentiating between mid and late anoestrus. Judging by Lambourne's (1955) figures a large proportion of Romney ewes have spontaneous ovulations during February and it is likely that this ovarian activity would influence the outcome of treatments.

It might be possible to account for the poor results obtained by Robinson (1955b) on the basis that the duration of progesterone pretreatment required to produce synchronous oestrus and ovulation with PMS is much less than that required to fully prepare the tract for sperm and egg transport, fertilisation and implantation.

The results of this experiment, however, show that the uterine response to progesterone is fairly rapid, while the cervix responds less readily. On the other hand the oviducts and vagina do not

seem to change unless oestrogen is present. It is suggested, therefore, that poor lambing results are not due to the improper preparation of the lower tract but that attention should be directed to the fallopian tubes, the preparation of which by progesterone appears to have been inadequate and in which the qualitative and quantitative relationships of progesterone and oestrogen are poorly understood.

D The Assay of the Pituitary Glands

There is an immediate temptation to relate the significant difference in gonadotrophin activity in the control ewes with the changes in follicle numbers. When, however, we compare the findings of Warwick (1946) and Kammlade, Jnr., et al. (1952) with the above there is an obvious discrepancy. These workers could find no difference in total gonadotrophin content of the pituitary between anoestrous and cycling ewes, and Kammlade, Jnr., et al. (1952) did not observe any change in potency as the breeding season approached. The only major deviation from a constant level throughout the year occurred at each ovulation, when a decrease in potency took place.

If the increase in follicle numbers is related to the increase in gonadotrophin, it would seem that this is because of an increase in FSH. In either case it is difficult to reconcile these findings with those just quoted. The explanation could be either that the analysis of the results observed by Kammlade, Jnr., et al. (1952) did not detect the difference, or that the assay method used has limitations which render comparisons difficult. The following discussion would tend to suggest that the latter possibility is the more likely.

The use of day old cockerels for gonadotrophin assay is

based on the response of the testes by an increase in weight as a result of gonadotrophin injection. It is not known whether the stimulus is direct or whether the injected material affects the output of the chick's own pituitary.

While it is agreed that both FSH and ICSH affect the testes, it is difficult to know what is being measured when whole pituitary powder is administered. Nalbandov (1953) is of the opinion that the assay does in fact measure total gonadotrophin, the FSH and ICSH combining additively. But if we consider the action of the two gonadotrophins on the female gonad it is very likely that the ratio of FSH/ICSH is the important factor. An unknown factor which may be important, however, is that beyond a certain range the dominant fraction may exert an action out of proportion to its concentration.

Histological examination of the testes may help resolve the problem, since FSH has been shown to affect the tubules and ICSH acts mainly on the interstitial cells (Breneman, 1936). Information has been gathered in this laboratory which supports the above contention.

Nevertheless, until it is known not only whether we are measuring total gonadotrophin, but also how much of the constituent fractions, it is difficult to arrive at an adequate interpretation of the method.

A further point which must be taken into consideration is the possible loss of potency of the pituitary gland during storage. Whereas Kupperman et al. (1941) have shown that acetone-dried sheep pituitaries do lose potency on storage, when compared with fresh specimens, Naito (1953) found that

acetone-dried goat pituitary glands did not lose potency over a period of two years. If we are to assume some loss during storage we cannot make allowance until it is known whether the original potency of the gland has any effect on the loss during storage.

The more obvious limitations, arising from a consideration of the results, concerning the use of the chick assay are:-

(a) Not only are the weights of day old chick testes quite variable but at the end of the first week the variance has increased. This increase in variance can be only partly accounted for in the variability of body weights.

(b) The comparison of the weights of testes of control birds from different batches indicates a source of variation which is very difficult to understand. Bergman & Turner (1942) have shown the importance of keeping the environment constant between batches and in this experiment this factor was thought to be carefully controlled.

(c) Different batches gave variable responses to identical injections of PMS (see Tables 22 and 23).

(d) The increase in variance with increasing dosage introduces problems in the analyses with a consequent decrease in efficiency.

It can be seen, therefore, that the interpretation of the results of the assay of thepituitary glands is fraught with difficulty. There are, however, some points which may be discussed.

In groups 2 and 3 there is apparently no difference in

gonadotrophic potency between the two dates of slaughter. This would probably indicate that if progesterone has an effect on the gonadotrophins in the pituitary, this effect is not manifest in the anoestrous ewe, or alternately a longer duration of treatment is needed. The similar findings in group 4 a i and 4 a ii would tend to preclude the latter possibility. Further, the non-significant difference between 4 b i and 4 b ii would tend to substantiate the former possibility.

It should be pointed out that the results in group 4 may be considered not to disagree with the finding by Kammlade, Jnr. et al. (1952) that the only definite change in total gonadotrophin occurs at about the time of ovulation. None of the ewes in this experiment were killed at a time near enoughto ovulation to show this but it may be noted that the average testes weights in groups 4 a i and 4 b i were the lowest recorded.

VI SUMMARY AND CONCLUSIONS

1. An experiment involving 105 anoestrous Romney ewes, in which were observed the effect of progesterone and PMS on manifestation of oestrus, macroscopic changes in the ovary, histological changes in the reproductive tract and gonadotrophin content of the pituitary gland, has been described.

2. Ewes in the control groups were found to be in relatively deep anoestrus on January 10th and 11th. By January 18th - 19th however, an increase in ovarian activity, manifested by an increase in follicle numbers, had taken place. This was thought to be due to either (a) the ewes being closer to the normal breeding season or (b) the introduction of the rams.

3. This increase in number of follicles in the control ewes was

accompanied by an increase in the total gonadotrophin content of the pituitary gland as determined by the day-old male chick assay technique.

4. The ewes treated with various levels of progesterone alone did not come into season or ovulate; there was, however, a response in the reproductive tract. This consisted of an increase in height of uterine epithelium and increased uterine glandular development accompanied by an increase in activity of the cervical mucosa which was greatest with high dosage levels.

5. The administration of progesterone prior to PMS, as compared to PMS alone, led to a greater number of ewes exhibiting oestrus, a greater number of ewes ovulating and an increase in the number of ova shed.

6. The development of a corpus luteum was responsible for marked changes in the uterus and cervix and minor changes in the vagina and fallopian tubes.

7. In this experiment progesterone administration did not appear to have an effect on the gonadotrophin content of the pituitary glands of the anoestrous ewe.

8. The definite limitations of the assay of gonadotrophin potency of acetone-dried sheep pituitary glands, using day old cockerels, have been discussed.

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