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THE PHYSIOLOGICAL EFFECTS OF FLUSHING EWES

ON

OVULATION AND EMBRYO SURVIVAL.

CONTENTS.

1. INTRODUCTION

Prolificacy in sheep, under most types of flock management, may exert an overwhelming influence on profitability. Three major classes of sheep farming systems are found in New Zealand, namely

Extensive farming, on high country and droughty areas, where wool is the chief product,

Store sheep farming, on harder hill country, where income is derived from sales of both wool and surplus stock, and

Fat lamb farming, in the easier and improved areas, where sales of fat stock almost exclusively dictate the size of the income.

In all three types, ewe fertility is of paramount importance.

Under the extensive system of management, low lambing percentages coupled with high losses due to accident or disease, and wastage due to the culling programs necessary to maintain wool quality, may disastrously affect the flock by reducing the number of young stock available for replacement purposes. The breeds of sheep more commonly used in this type of farming, namely the Merino and ½ bred in New Zealand, are inherently lower in fertility than the improved mutton breeds, possibly because of conscious or unconscious selection away from this characteristic.

In store sheep farming, which in this country provides the breeding stock used in the fat lamb industry, low levels of fertility may so reduce the numbers of both young and old ewes available for sale as to cause penury among store sheep men and disaster to the finely-balanced organisation of the fat lamb industry. On such farms, the majority of the replacements for New Zealand's flock of 21 million breeding ewes is bred, and when it is remembered that the thirteen million lamb carcases and six million ewe carcases exported annually, together with wool, account for one half of the National Income, it is evident that ewe fertility problems on these farms become problems of National significance.

For the fat lamb farmer, the size of whose income depends mainly on the number of lambs born and reared per ewe, the maintenance of fertility in his own flock assumes primary importance while only in times of abnormally high wool prices is this not also true under the more extensive types of sheep farming.

As an aspect for study in animal physiology, fertility again takes pride of place for both interest and importance. Its nutritional, genetic and environmental relationships have only lately received the closer attention of scientific study, while many early observers have bequeathed to us a vast literary fund of fact, opinion and conjecture on the subject. With the more recent, and sometimes spectacular, advances in the fields of endocrinology, biochemistry, microscopy and experimental technique, some of this early work has been invalidated, whereas on the other hand many hypotheses and phenomena have been confirmed and elucidated.

The fundamentals having been established in any subject, research tends toward the practical aspects. Just such a trend is evidenced in contemporary studies of fertility augmentation using photoperiodic, nutritional, and hormonal effects, and in this respect it is noteworthy that the practice of flushing ewes is of much longer standing amongst shepherds than a cursory glance at the available literature on the subject would indicate. The modus operandi of flushing has never been accurately determined, but the general belief of most workers is that the improved nutrition, prior to and during the mating period, somehow acts as a dietary stimulant to pituitary activity, thereby causing a greater number of ova to be released from the ovaries at ovulation. Other incidental effects have been ascribed to the practice also, and although some have recently been discredited, many still remain unverified by experiment.

The following Review of Literature makes no attempt to point out possible solutions to the existing problems and is designed to introduce and clarify only those immediately concerned with this study.

2. REVIEW OF LITERATURE

Literature considered pertinent to this experiment is reviewed in this section, whereas further related material appears in the Discussion. The review is written under the following headings:-

A. Flushing, lambing percentage and related effects.

B. Ovulation rate and factors affecting it.

C. Ovum loss and Embryo mortality in early pregnancy.

D. Factors other than flushing which affect lambing percentage.

A. Flushing, lambing percentage and related effects:

To Aristotle, the ancient Greek philosopher, is accredited (cit. Marshall 1922) the observation that sheep fertility is increased in a favourable environment "where food is abundant." Thus it has long been known that nutrition can affect fertility, but it was not until the latter part of the 19th Century that this was closely studied.

Heape (1899), in a survey of fertility and abortion covering some 77,850 English sheep, found that poor ewes improving in condition at mating time gave better results (in the percentage of lambs born per 100 ewes) than ewes falling in condition at this time. He concluded that the condition of the ewe at tupping time had the greatest influence on the number and percentage of twin births.

Marshall (1903) after studying a large number of uteri from the Edinburgh abattoir concluded that ovulation rates closely approximated lambing percentages and that fertility was directly influenced by the number of Graffian follicles maturing at each heat. In 1904 Marshall concluded from this and from Heape's results, that flushing affects the ripening of follicles and the proportion of them undergoing degeneration, therefore the number available for ovulation at tupping time. Subsequently, 1908, from a survey of 79 flocks over three seasons, this worker showed that the percentage of twin births was higher, and of barren ewes lower, when ewes were flushed, thus raising the lambing percentage. These workers further believed that the practice of increasing the amount or quality of feed before and during the breeding season (i.e. flushing) brought ewes earlier to the tup.

Bell (1912) found, as had Heape before him, that twin-bearing ewes were those gaining weight in the breeding season while Marshall (1922) explained the action of flushing as follows, "Flushing acts by stimulating the generative tract by nourishment thereby hastening the onset of breeding and increasing fertility" and recommended improved feeding for three weeks before mating.

Experiments were conducted by Marshall & Potts (1924) to investigate these claimed advantages of flushing. The findings showed that flushed ewes gave an average increase of 18.7 lambs per 100 ewes over non-flushed. 350 ewes were involved in this trial, but no difference between groups was apparent with regard to the average date of onset of breeding. Breeds of higher fertility appeared to respond better to flushing in raising lamb yields and while no feeds appeared more advantageous than others, Marshall & Potts believed that a gain of at least 7 lbs. weight over the breeding season was necessary in flushing.

In the same year (1924) and later in 1926 and 1927, Nichols using survey methods found a close correlation between number and percentage of barren ewes and the number and percentage of non twin-bearing ewes in any flock. He concluded that the nutritional plane at breeding time affected both aspects of ewe fertility in the same way. He also demonstrated a 24.9% increase in the number of lambs born per 100 ewes as a result of flushing and that this did not occur when flocks were kept continually in high condition. Further facts emerging from the surveys of Nichols and Marshall were 1. that a high proportion of twin births occur early in the lambing season and 2. that farmers believed flushing ewes would give a more restricted lambing period.

While Asdell (1926) was unable to show any increase in lamb yields from flushed ewes, White and Roberts (1927), in a study of Welsh Mountain breeds under three types of management, concluded that the condition of the ewe at tupping time was chiefly responsible for the respective levels of fertility under these systems, and that feeding over this period especially affected the proportions of twin-bearing and barren ewes in the flock.

Darlow and Hawkins (1931 & 1932) using small numbers of ewes to investigate the effects of different feeds on oestrus behaviour, found little difference between the better quality rations and, as they obtained no twins from flushed ewes, concluded that flushing should commence before ewes enter the breeding season or that their feed differences were too small to have sufficient effect. They also found that the groups of ewes on green feed returned more often to the ram for repeat matings than did any of the other four groups. Possibly the small numbers of ewes in each group, i.e. nine, affected the result.

After observations over a five-year period involving annual comparisons of ewe groups ranging from 300 to 700, Smith (1933) established only a 7.4% difference in lamb births per 100 ewes in favour of the flushed groups. Believing a 10 lb. weight gain for the three weeks prior to mating was necessary, Smith concluded that he had "never obtained a true flushed state" in his ewes. The 568 flushed ewes gained only 0.9 lbs. while the 574 non-flushed ewes lost 3.6 lbs. per head. Smith was of the opinion that good fresh range was probably as effective as grain supplement for flushing. Whether the differential lambing percentage resulted from the good feeding of the flushed ewes or was merely an expression of the poor feeding of the non-flushed ewes is a debatable issue which raises the important point of whether live weight gain or improving condition is the best criterion of flushing. Clark (1934) who investigated the effect of flushing on ovulation rate (see later), found that large weight gains over the pre-mating period do not indicate an increased ovulatory rate, while to obtain the latter, ewes must be rising from a poor condition rather than maintained in good condition prior to mating.

Grant (1933) showed that the breeding season of sheep is often preceded by at least one ovulation unaccompanied by heat. He concluded that the effect of flushing in hastening the onset of breeding, was to convert such "silent heats" into normal heats at which mating instincts coincided with ovulation.

An experiment by Okulicev (1934) in which three of four groups of 115 ewes were fed flushing supplements gave the following results:-

Ewes fed.	Lambs/100 Ewes	% of Ewes bearing Twins	% Ewes barren
1. Pasture alone	103.5	27.5	19.5
2. Pasture + Barley	120.3	36.9	12.7
3. " + Oats	112.7	33.0	15.6
4. " + Millet	110.2	28.0	15.9

The conclusions reached were that:-

1. flushing increased multiple births and lambing percentage
2. " decreased the percentage of barren ewes
3. " " the % still-born and lamb mortality and
4. Barley appeared the best feed of those used with which to flush ewes.

From 1932 to 1934 Sidey et al., conducted a series of well-planned experiments to investigate the effects of flushing on lamb yields. From 126 flushed and 126 non-flushed ewes, annual differences of up to 26% of lambs born per 100 ewes mated were found in favour of the flushed group. No differences in the number of barren ewes in each group occurred, the increased number of lambs arising from an increase in the proportion of twin births which tended to be spread over the lambing season in the flushed group. The flushing feed used was a mixture of crushed wheat and peas and at no time were weight gains large for the flushed ewes. Poor condition ewes which were flushed gave 10% more lambs than either poor ewes not flushed or good ewes maintained in condition.

Polovceva, Zudovic and Okulicev (1938) split a mob of 100 ewes into three groups to study the effectiveness of concentrate feeding for a 20 day pre-mating period. The control group, fed pasture alone, and a group receiving an additional 100 grams of Oats and 10 grams of bone meal per head, had lambing percentages of 119, while the third group receiving twice the quantity of oats and 200 grams of sunflower cake per head in addition to pasture gave a 133% lambing rate. (This experiment will be further discussed in the next section of the review.)

A large scale experiment by Terrill and Stoehr (1939) where nearly 8,000 sheep were used, enabled these workers to apply statistical analysis to their data. A positive correlation between the gains in weight, which reflected feeding over the breeding season, and the lambing percentage of various groups was demonstrated while neither condition nor live weight changes at this time affected the proportion of ewes which eventually conceived. Ewes gaining in weight produced 6% more twins than those losing weight, whereas the length of time the rams were left with the ewes was directly and strongly correlated with the percentage of ewes conceiving. Thus flushing was shown to affect only the number of lambs produced by a fixed number of ewes and had no effect on barrenness.

The Australian workers, Underwood & Shier (1941) using two groups of 350 ewes, one of which was flushed, and the other not, after a pre-flushing starvation period to reduce their condition, showed a 20% difference in lambing percentage favouring the flushed group which was entirely due to increases in twin births among these ewes. Underwood & Shier concluded that quality was less important than quantity in the flushing feed although oat stubble and peas were used. More of the flushed ewes required repeat matings before conceiving although about equal proportions of barren ewes appeared in each group. Condition prior to flushing was considered important and the flushed ewes gained 15 lbs. while the controls lost 7 lbs. during the flushing period.

Hammond (1941) stated that flushing could improve fertility and prevent protracted lambings where rams were put out early to obtain early lambs. The same author (1942) advised getting ewes into a hard thrifty condition to obtain maximum benefits from flushing and explained the action of this practice on the basis of the increase of anterior pituitary hormone output.

Briggs et al. (1942) in a nine-year trial found flushing was neither able to increase the lambing rate nor speed the onset of the breeding season. However, only a small number of ewes, 6 or 7, was used each year in the three feeding groups and as the variation within groups was great, these results are of doubtful value.

Miller, Hart and Cole (1942) investigating the possible effect of flushing in overcoming dietary deficiencies found that diets low in vitamin A. and total nutrients gave poorer lambing rates than diets adequate in all nutrients. They concluded that low protein and phosphorus intakes during the breeding season had greater and more harmful effects than low Vitamin A and that ewe weight gains over the period were not essential for high fecundity if ewes were in good condition. This, they stated, does not mean that thin ewes, when flushed, may not produce more lambs than when left on poor feed. The number of sheep in any experiemntal group was limited to 20 or 25.

In a review of the existing knowledge of the advantages of flushing, Fraser (1949) concluded that this practice resulted in a higher proportion of twin births, and explained its failure to do so on some farms because (a) it can only make manifest the existing potential fertility level inherited from sire and dam and (b) on good land it is often difficult to lower condition sufficiently for flushing to have any effect.

The claims that flushing both hastened the onset of the breeding season and reduced the number of infertile matings, Fraser considered to have been adequately disproved by Clark (1931), Grant (1934), and Underwood & Shier (1941) and he quoted Heape, Marshall and Nichols together with his own experience to provide proof for the claim that flushing can reduce the proportion of barren ewes. Yeates (1949) cited Quinlan, Steyn & De Vas (1941) as showing that nutrition has no effect on the onset of the breeding season and in the same year demonstrated the photoperiodic stimulus involved. Fraser (1949), however, quoted Engela & Bonsma as stating that good nutrition may shorten anaestrum.

Recent Ruakura experiments on flushing (Wallace 1951) have demonstrated the following effects:-

1. Lambing percentage (lambs born/100 ewes conceiving) increase of from 10 to 20% which is chiefly due to an increase in the proportion of twin-bearing ewes in the flock.
2. More even spread of twin births throughout the lambing season under flushing as opposed to the restriction of twin births generally to the early part of this period.
3. With respect to the above phenomenon, flushing effects appear to be carried over for about a fortnight in stimulating multiple ovulations.
4. Flushing tends to hasten only slightly the onset of breeding season, but not to any noticeable extent.

5. Ewes flushed on pasture require more services per conception than those not flushed.
6. There is little difference in the proportion of barren ewes in flushed or non-flushed mobs at lambing.

These results were confirmed over two or three seasons with ewe mobs of 150 fed solely on grass and generally resemble the points established by earlier workers.

Till in an unpublished Thesis (1951) showed a similar difficulty in obtaining conception in ewes fed well on grass prior to mating, while Darroch et al. (1950) in grain-feeding trials using some 500 ewes in two feeding groups found that a 10% increase in births at lambing resulted from grain feeding over the 40 days prior to mating. Thin ewes without supplement tended to conceive earlier than good condition ewes although flushing lost the former this advantage. On closer analysis of the data it was further demonstrated that whereas ewes in good condition prior to mating outlambed those in poor condition by 11%, poor condition ewes which were flushed prior to mating achieved a 15.8% increase. The effects of flushing prior to mating were found to exert a much greater influence on lambing rates than did supplementary grain feeding at any other stage of pregnancy.

Finally, completing the cycle by referring to work done in the Mediterranean area where Aristotle made his original observations, an experiment by Vita (1951) showed that flushing could increase the lambing percentage at both summer and winter lambings. He demonstrated a greater than 30% increase at both lambings, from ewes flushed on hay and concentrates in winter and on rich alpine pasture in summer, and it was noticeable that the majority of twin births in the flushed group occurred in ewes which were, themselves, daughters of twin dams. The flushing period was of 20 days duration and there appeared to be no differences in the numbers of ewes conceiving in each group.

It is thus evident that wherever flushing has been used with ewes in poor condition, increases of lambing and twinning rate have resulted. Lack of uniformity within experimental material and small numbers of animals used, together with small feed differences, may account for the apparently negative results of Briggs et al. (1942) and Darlow & Hawkins (1931).

Hammond (1942) has indicated the means whereby flushing may act, and, in fact, the belief that flushing increases the ovulation rate is now widely held.

B. Ovulation rate and the factors affecting it:-

(i) Hormonal Control:- That ovulation and ovarian activity are controlled by the secretions of the anterior pituitary was first demonstrated by Smith and Engle (1927), and Zondek and Aschiem (1927), and that pituitary activity was governed by a change in the light to dark ratio by Yeates (1949). Yeates was able to induce breeding in anoestrus ewes by a change in seasonal photoperiodic stimuli received by the sheep.

In 1929, Bellerby showed that one of the effects of anterior pituitary extract was to cause more follicles to ripen while Hammond et al, (1942); Casida, Warwick & Meyer, (1944); and Robinson (1950 b) all demonstrated that superovulations may be induced in sheep using pregnant mares' serum and anterior pituitary extracts. The ova from such multiple ovulations were found highly fertilisable (Murphree et al, (1944); Casida et al, (1944), although Dowling (1949) and Robinson (1950 b) showed that with ovulation rates above 15, accelerated tubal transport may prevent a proportion of ova from becoming fertilised. Robinson (1950 b) has demonstrated a high interuterine mortality of embryos in superovulated ewes while Lopyrin (1938) and Zawadowskii (1941) showed that four to six lambs only may be born to treated ewes and usually less.

As no attempts were made in this experiment to exploit hormonal superovulation, this section of the review has been limited to an introduction to the mechanism of hypophyseal control and artificial augmentation of the ovulation rate. In recent years a vast amount of work has been done in this subject to determine dosage rates and times of injections which will produce optimal increases of ovulation rates and lambing rates (N.Z.D. of Ag. Annual Rept. 1951-52). The work done up to 1951 is admirably reviewed by Robinson (1951).

Thus there appears to be little doubt that varying levels of pituitary secretion of the gonadotrophins are responsible for the differing ovulation rates in ewes. It is now desirable to investigate the factors, other than the light status, which affect ovulation rate. Factors which affect lambing percentages are likely to do so e.g.

Breed
Age
Individuality
Environment and Season
Nutrition and flushing

while factors other than these which modify the secretion rate of the anterior pituitary are likely also to play some part. However, for the most part, direct values for ovulation rates are lacking, these being generally assumed from lambing performance (Heape, 1899; Carlyle & McConnell, 1902; Marshall & Potts, 1924; Nichols, 1926) perhaps in view of the early beliefs that lambing rates closely approximate ovulation rates due to the low interuterine mortality in sheep (Heape, 1899; Marshall, 1903, 1904; Hammond, 1914, 1921; Nichols, 1924, 1926; Clark, 1934; and others).

(ii) Breed:- As indicated above, ovulation rates for most breeds have been inferred from lambing percentages. Asdell (1946) has demonstrated considerable differences between breeds and that lambing percentages (and presumably ovulation rates) may vary from the Cheviot with 89% to the Romanov with 238%.

Although strict breed comparisons of ovulation rates are few, perhaps because the most easily obtainable material is found in slaughter houses where individual ewe records are unavailable, the following data indicate the extent of the factual matter on this subject.

Marshall (1903) with 55 Scottish ewes found an ovulation rate of 1.25, while Hammond (1921) gave figures indicating 1.45 for some 80 sheep, also of undesignated breed.

Quinlan and Mare (1931) (cit Roux, 1936) found an ovulation rate in South African Merinos of 1.0, while Clark (1934) showed differences between Rambouillets and Shropshires in America, which had mean values of 1.2 and 1.6 respectively. Grant (1934) found, in 441 slaughter-house ewes, an ovulation rate of 1.87 which included 54 ewes with 3, 8 with 4, and 3 with 5 ovulations at the heat studied.

McKenzie & Terrill (1937) showed that mature Rambouillet ewes varied between 1.43 and 1.0 in ovulation rate, while mature Hampshire ewes, between 1.78 and 1.21, within the same season.

Further data may be obtained from Henning (1939) who studied the pre and post-natal sex ratios of sheep, and from Roux (1936) and Dutt (1951), while Till (1951) demonstrated a variation in ovulation rate, in two-tooth Romney ewes, of 1.67 to 1.11 due to feeding differences. Thus there exists between breed differences and within breed differences in ovulation rates which probably reflect the respective pituitary gonadotrophin levels of the animals concerned. That these levels, which Robinson (1951) and Hafez (1951 and 1952) indicate affect the breeding season of the various breeds, will affect breed ovulation rate differences is likely, but in what manner is not clear, in view of the fact that breeds of similar fertility levels appear at opposite extremities of Robinson's (1951) pituitary activity diagram.

(iii) Age:- Increasing age up to maturity is generally accompanied by increasing lambing percentages and Bowstead (1930) found that ewes first bred as lambs produced a greater number of lambs as two and three year olds than did ewes first bred as two-tooths. Barton (1947) showed that ewes breeding as two-tooths gave more lambs in subsequent years than did those not breeding as two-tooths.

McKenzie & Terrill (1937) showed that 24 Hampshire lambs had an ovulation rate approaching 1.27 while mature ewes of the same breed had 1.59 ovulations per ewe in the same season. Hammond (1944) is the only other worker producing direct evidence of increases in ovulation rate with increasing age, finding that ovulation rate tends to increase with age in lambs rather than to decline with approaching anoestrum.

(iv) Individuality:- Because ewes are not capable of shedding only fractions of an egg, as would be inferred from mean values, it is apparent that they must shed either one, two, three or more. Johannson (1932) and Kelley (1942) believed that the ability to shed more than one ovum at each heat is heritable and breed differences bear this out. Johannson (1932) from observations in 40 Swedish flocks concluded that, in highly fertile individuals, single and multiple births (and presumably ovulations) generally alternate, but this may be a seasonal effect.

(v) Environment or Season:- Effects from these causes have long been inferred from lambing rates. From McKenzie and Phillips (1932), Kelley (1937, 1939) and Yeates (1949) it appears that temperature, per se, plays little part in ovulation. (See below under Hormonal interactions.)

The stage of the breeding season appears to affect ovulation rate, from Grant (1934) who found that ewes ovulating prior to January 1st. did so at the rate of 1.99

ova per ewe while those ovulating after this date exhibited the reduced rate of 1.83.

McKenzie and Terrill (1937) in a study of oestrus and ovulation phenomena produced the following figures demonstrating seasonal variations in Hampshire ewes:-

Period	Number	Mean Ovulations per Ewe
Aug. 16 - Sept. 15	20	1.50
Sept. 16 - Oct. 15	32	1.78
Oct. 16 - Nov. 15	23	1.74
Nov. 16 - Dec. 15	29	1.45
Dec. 16 - Jan. 15	31	1.26
Jan. 16 - Feb. 15	14	1.21

Similar results for Rambouillet ewes indicates an early seasonal peak followed by a gradual but steady decline which these workers believedis due to a progressive reduction in pituitary secretory activity.

(vi) Nutritional and Flushing:- Although the early workers (Marshall, 1904; Hammond, 1914) believed that flushing had its effect through ovulation rate, it was not until 1933 that Clark (1934) obtained direct evidence for their assumptions. In a two year study of the effect of flushing on ovulation rate, Clark used a flushed and a control group of poor condition Merinos in the first year and a similar design with good condition Shropshire ewes in the second. In both years, significantly larger weight gains were made by the flushed ewes over the pre-mating period, but, while the 20 flushed Merinos shed 1.4 ova/ewe and the 20 controls shed 1.0 ova/ewe, the Shropshire ewes showed a reversal of this with the flushed ewes ovulating 1.5 ova/ewe and controls 1.7 ova/ewe. Clark's conclusion was that flushing benefited only ewes in poor condition.

Polovceva et al (1938) obtained data on flushing and ovulation rate by conducting laparotomies at mating heats. Of those ewes fed grass alone, 51.8 per cent had multiple ovulations while those fed additional oats and sunflower cake had multiple ovulations in 58.3 per cent of their number. There was a difference of similar nature, but smaller magnitude, in the lambing rates.

By feeding one group of 27 ewes from weaning to mating with grain and hay, and another similar group with hay alone, Darlow and Casida (1939) found 19 ewes in the first, and 24 in the second group, had single ovulations while 8 of the first, and 3, only, of the second, had double ovulations. These workers concluded that the (flushing) treatment would have increased the lambing percentage by increasing the proportion of twin births in the first group.

In a paper on flushing, Hammond (1942) stated that "whatever the breed of ewe, there is no doubt that the amount of pituitary gonadotrophin, and therefore the number of ova shed, can be greatly influenced by feeding and management."

Till (1951), with two-tooth Romney ewes under four feeding treatments, demonstrated the effects of nutrition on ovulation rate as follows:-

Plane of Nutrition		Number	Ovulation Rate
High	High	20	1.67
Low	High	20	1.50
High	Low	20	1.28
Low	Low	20	1.11

The change from Low to High plane was made 14 days prior to mating to resemble flushing.

Finally in this section, it is perhaps important to note that the high ovulation rates quoted by Grant (1934), of 1.87, and Hammond (1944), of 1.79 and 1.43 for lambs, were obtained from slaughterhouse material which presumably had been well fed prior to slaughter, thus resembling the effects of flushing. Thus in young stock and those in poor condition, flushing can undoubtedly increase the number of ova shed per ewe.

(vii) Hormonal interactions:- The cyclic reproductive phenomena in sheep are the result of the interactions of pituitary and gonadal hormones (Robson, 1947; Hammond, 1948). Hormones from the one source are known to affect the secretion status of the other source, thus, Robson (1938) and others have demonstrated that progesterone from the corpus luteum will inhibit heat and ovulation during pregnancy, while Loeb, (1911 & 1923), Hammond, (1928), and Hammond Jnr. & Battacharyar (1942) have shown that extirpation of the corpus luteum causes a sudden return to heat with possible multiple ovulation. These effects are considered to result respectively from a repression and release of pituitary luteinising hormone due to fluctuations in progesterone levels. Rising oestrogen levels, on the other hand, are known to repress the pituitary follicle-stimulating-hormone production and stimulate luteinising hormone output hence ovulation in the sheep.

Turner (1948) demonstrated further interactions of the various endocrine glands with the pituitary, and believed that many effects of exteroceptive factors may be due to differences in the general well-being or the metabolic rate of the animal. Thus, in males at least, where high temperatures repress spermatogenesis,(Kelley, 1937), the mode of this action is via the thyroid secretion (Bogart & Mayer, 1947; Oloufa, Bogart & McKenzie, 1948). Such infertility may be overcome in rams by feeding thyroxine or iodinated proteins (Black, 1950).

It has recently been shown (Chu and You, 1945; and Maqsood & Reineke, 1950) that hyperactivity of the thyroid causes a depression of pituitary F.S.H., and increases L.H. from the same source, while the reverse is true in hypothyroid animals. Maqsood (1950), examining this effect, believes that the thyroid hormone may facilitate the utilisation of hypophyseal gonadotrophins by "conditioning" the cells involved, while Chu and You (1945) believed that the thyroid gland mediated the effects of oestrogen.

This section, in reviewing some of the existing facts on ovulation rate and those factors influencing its magnitude in sheep, demonstrates the paucity of definite knowledge on the subject. Undoubtedly this situation has arisen due to the value of these animals and hence the economic difficulties involved in slaughtering sufficient numbers to provide worthwhile results.

C. Ovum loss and embryo mortality in early pregnancy:-

The early investigations of Heape, Hammond and Nichols led to the belief that interuterine mortality in sheep was low and, while Heape (1899) found abortion occurred in only 2.39% of ewes, he found that barrenness, largely traceable to nutritional sources, was much in excess of abortion. Hammond (1921) also, showed 7 foetuses dead, and 8 further missing, in a total of 116 ovulations in 80 ewes - a 12% mortality. Robinson (1950 & 1951) has decried the paucity of literature available on early embryonic loss, especially for the sheep; this, in view of the importance it has assumed in connection with hormonal augmentation of fertility. Perhaps the premature and somewhat debatable conclusions of the earlier authorities have led to this difficulty and it is quite evident that the causes of such loss are still imperfectly known.

(i) Fertilisation and abnormalities in ova:-

Hammond (1921) has shown that as the number of ova shed per ewe increased the survival rate of ova as embryos decreased. This was especially so where the ovulations were all in the one ovary. No data as to the stage of pregnancy at which losses occurred was given. Winters and Feuffell (1936) demonstrated that at least 8% of ova never became fertilised. Henning (1939) showed losses of increasing magnitude as the number of ova shed per ewe increased, so that from 772 corpora lutea, 16% of possible embryos were subsequently missing at different foetal stages, while 8% only were lost in ewes with one corpus and 42% were gone from ewes shedding 3 ova at the mating heat. McKenzie & Terrill (1937) and Polovceva et al (1938) gave a partial explanation of this phenomenon by demonstrating that multiple ovulations may be synchronous, or asynchronous over a period of 17 hours or more. That sheep ova are only fertilisable for about 24 hours after ovulation was indicated by Green & Winters (1935), and it is generally accepted that fertilisation is necessary to permit entry of nutrients (Venable, 1946). Thus the life span of unfertilised ova of many species including sheep is short (Pincus, 1936; Chang, 1947; Austin, 1949; Robinson, 1950; and others). Corner (1921 a & b) ascribed certain pre-and post-implantation losses to developmental aberrations in pig ova possibly due to inherited lethal factors while Winters (1947) described two types of abnormal ova in cows, due to the same cause.

Tanabe and Casida (1949) recorded 20% and 30% of infertile ova in groups of 20 Guernsey and Holstein cows respectively, at three days post coitum, and Casida et al (1944) and Murphree et al (1944) showed a similar figure of 70% of shed ova fertilised in the sheep, also at three days.

Hammond's 1944 study where lambs had ovulation rate well above the possible incidence of twinning rates at lambin from such stock, appears to indicate rather large losses in either ova or early embryonic stages.

Failures in conception in up to 80% of ewes grazing oestrogen-potent subterranean clovers in Australia (Bennetts, 1947) have been shown, in a recent C.S.I.R. pamphlet (1952) due to an inability of shed ova to become fertilised.

Dutt (1951) with 90 ewes slaughtered at 3 days post coitum, reported that only 55·1% of ova were fertilised, and that of these, 20·5% showed abnormal development at three days, while Till (1951), who compared the numbers of recovered foetuses with the corpora lutea found in 80 ewes, showed losses of 1/6 of ova shed and not including those causing ewes to return to the ram. Till ascribed these losses to failure of fertilisation or implantation, but these causes were not distinguished. Dowling (1949) found that, in ewes multiply-ovulating after P.M.S. injections, only 38% of ova were recoverable and of these only 38% were fertilised. Dowling (1949), Rowson (1951) and Robinson (1951) attributed this effect to accelerated tubal transport, while subsequent mortality, in utero, may be due to the inability of the uterus to support and contain more than a limited number of foetuses, (Casida et al., 1944; Robinson, 1950).

(ii) Post-implantation:- Corner's (1921) work explains some of the losses at this stage, and Brambell (1948) and Brambell and Mills (1948) outlined a method of examining implantation loss in rabbits, although this is not applicable to the sheep. Cloete (1939) found that, in studies of early pregnancy in the sheep, it was difficult to ensure that the ewes were pregnant at slaughter, implying losses in the early stages. Robinson (1950 a) drew attention to the 17 to 19 day period of pregnancy in the sheep as possibly the time of maximal embryonic mortality. At this stage, the yolk sac completes its degeneration with the development of the early allantoic placentation and is considered one of the crucial stages of embryo development in many other species (Parkes, (1943); Brambell and Mills, (1947 & 1948) in rabbits; Corner (1921b) in pigs; Ewart (1897) in horses; Hammond (1921) in pigs and sheep.)

Thus there are indications of quite considerable losses in both ova and embryos in early pregnancy which are further demonstrated by the fairly general necessity to mate some sheep in most seasons two and three times before they eventually conceive and Hartman (1952) has recently produced evidence that, in mice and oppossums at least, losses during this stage are due to the faulty germ plasm of ova rather than to the maternal uterine environment.

D. Factors, other than flushing, which affect the lambing percentage:- To summarise more than briefly the work done in this field would be both tedious and unnecessary in this review, especially in view of the extent of the preceding sections which all bear on the subject. Therefore only a few selected works will be outlined here.

(i) Age:- Terrill and Stoehr (1939), Kelley (1943) and Barton (1947) and Hart and Stevens (1951) have all demonstrated increases of lambing percentage with maturity. Thus while the Southdown, an early maturing breed, reaches a peak in fertility at 5 years of age, the Romney and Corriedale breeds are still increasing in fertility at 6 to 7 years, (Hart & Stevens, 1952). The peaks of fertility reached by these and other breeds in this study, vary from 2 lambs/ewe tupped for the Border Leicester to 1.5 lambs/ewe tupped for the Southdown. From Barton (1947) the rise appears to be accompanied in the Romney ewe by a fairly consistent fall in both early lamb losses and percentage of ewes barren at lambing although this is probably partly due to culling of non-breeders in the early years. Kelley (1943) showed that in the Australian Merino, the peak is maintained from seven to ten years of age and is followed by a gradual decline in the percentage of twin-bearing ewes.

(ii) Breed and Individuality:- There is no doubt that fertility is heritable and Johannson (1932) considered that the characteristic may be handed down to single-born, as well as twin-born, offspring of parents possessing it.

Breed differences are evident from Hart and Stevens (1952), vide supra, and Asdell (1946), as mentioned under Ovulation rates, has produced a comprehensive list of breeds and their average levels of fertility.

Individual differences may be marked as in the case of a Border Leicester ewe having 17 lambs in 7 seasons, (Stevens, 1952). It is likely that fertile strains within breeds are to be found and may be established by constant breeding and selection using twin parents (Hart & Stevens, 1952). Such differences are most likely to be due to differences in the individual's hormonal output and while many inherited lethal factors can cause death in utero, inherited or developmental abnormalities of the genital tract may preclude conception (Tanabe & Casida, 1949).

(iii) Environment and Season:- Environmental factors influencing lambing percentages include

Temperature - which Marshall (1942) believed may have an effect and Yeates (1949) and others showed did not.

Rainfall - de Clerk (1940) and Kelley (1939) and others have correlated the rainfall occurring in certain droughty areas over the pre-mating period, with the lambing percentage of the following lambing. A close and positive correlation appeared in such areas and it was believed that this was due to a "natural flushing."

Light - Hart and Stevens (1952) suggest a fertility differential may exist between environments of a differing photoperiodic nature, instancing contrasting fertility levels in two areas in which the same breed of sheep are run. Furthermore, Yeates (1949) and Hart (1950) have demonstrated the practicability of using light treatments to stimulate heat and ovulation in anoestrus ewes.

Abortion - Many environmental factors including unfavourable weather at lambing, nutrition during gestation (either unsuitable or insufficient food), prevalence of disease, and the nature of the country itself, have been demonstrated to cause abortion Heape (1899). This worker also demonstrated breed differences and breed-locality interactions which caused reduced lambing percentages due to abortion.

Seasonal differences acting through all the above factors may cause quite marked variations in lambing percentages from year to year as the study of any flock lambing records will show.

Hammond, (1921) (cit. Marshall, 1922) believed that foetal atrophy, which may reduce lamb yields, was possibly due to adiposity, inbreeding, or to genetic lethal factors such as had been demonstrated in homozygous yellow mice.

(iv) Nutrition:- While year to year differences in lambing percentage generally result from annual variations in the total food supply, within-year seasonal variations may cause deficiencies of specific dietary components, essential for reproductive efficiency. Both general inanition from low plane feeding, and the deficiency of specific nutrients are known to affect lamb yields in any season.

Plane of Nutrition - moderately low planes of well-balanced rations during early pregnancy appear to have little effect on lambing performance (Coop, 1950 & 1952) although ewes entering pregnancy in good condition will give more lambs than those in poor condition (Darroch et al., 1950) while better feeding over the last 4 to 6 weeks is also important (Wallace, 1948; Underwood & Shier, 1942). Hart & Miller, (1937), have raised the level of fertility in range ewes from a lambing percentage of 80 to 135 by constant high plane feeding over 6 years. High plane rearing of young stock, by hastening puberty, results in improved conception rates at the first mating at least, (N.Z.D. of Ag. Ann. Rept. 1951-2).

Carbohydrate and Fat - These seldom affect lambing percentage in sheep (Reid, 1949) although energy intake may become the limiting factor to fertility on rough or sparse native pastures. (Darling, 1950).

Protein - Webster (1932) showed that on excessive protein intakes, males may exhibit a high proportion of abnormal sperms in semen samples. Miller, Hart & Cole (1937) demonstrated that very low dietary levels of protein reduced lambing rates although this seldom occurs where concentrates are fed (Hart & Miller, 1937). On pasture, with very early lambings, protein supplements in the form of specially grown feeds may be necessary (Coop, 1952).

Vitamins - Ruminant requirements of the water-soluble B vitamins are small, due to ruminal synthesis (Kon & Porter, 1948). Of the fat-soluble vitamins, Vitamin D has little effect on fertility especially where hay is fed to sheep.

Vitamin A - Lambs may be born dead or die soon after birth when ewes are deprived of this vitamin to the state of night blindness (Hart & Guilbert, 1933; Hart & Miller, 1937), although the latter workers believed that this may have been due in part to inadequacies of minerals and protein. On pasture, Vitamin A levels seldom become dangerously low for sheep as liver storage of the vitamin is considerable.

Vitamin E - in spite of numerous recorded cases of Vitamin E overcoming sterility in cows (Vogt-Moller and Bay, 1931 and many others), goats and sheep reproduced normally on diets free of this vitamin (Wilson et al., 1935; Thomas et al., 1938) even after two generations have been raised on such feeds (Matill, 1938).

Minerals - Phosphorus - sheep resembled cattle in that fertility may be restricted on very deficient diets (Bekker, 1932; Thieler and Green, 1932) although this is unlikely in areas where rainfall is adequate for normal grass growth.

<u>Calcium</u> - <u>Reid</u> (1949) showed that there are no reports of calcium deficiencies affecting lambing rates.

<u>Trace elements</u> - most vitamin and mineral studies have been conducted using laboratory animals, but <u>Bowstead</u> (1942) found that he could grade a higher proportion of lambs as strong, after pregnancies in which ewes were supplemented with cobalt, than with cobalt deficient diets.

<u>Other substances in grass diets</u>:-

Ergotised grasses caused abortions in cattle (<u>Wallace</u>, 1912, cit. <u>Marshall</u>, 1922).

Hormone-like substances prevented conception occurring in sheep (<u>Bennetts</u>, 1947).

Goiterogens in certain feeds caused goiterous conditions and death of lambs in utero (<u>Hart</u>, 1951).

The above factors in different localities and in certain seasons may all be individually important enough to reduce markedly the flock lambing percentage, but in general, and in the average season, only isolated ewes suffer from these effects. Furthermore, the seasonal and environmental effects may, by affecting nutritional supplies, appear to play a part in influencing the lambing percentage and differences attributed to these causes are difficult to separate.

3.a. Purpose, Nature and Scope of this experiment.

It would appear from the above review that the role generally assigned to flushing in increasing fecundity in sheep is that of a dietary stimulant to pituitary hormone output, this in its turn effecting the release of a greater number of ova in subsequent heat periods. While this may undoubtedly be so (in view of Clark (1934) and Darlow & Casida (1939) is not seriously questioned, although it is also apparent that many claims in this direction have been made on a surprisingly small amount of evidence. Further, it is again emphasized that ovum loss and embryo mortality in early pregnancy is not inconsiderable (as the early workers would have us believe). In view of the conception delays often associated with flushing, it is surprising that so little work has been done in this field, while the possibility that such losses may be increased or decreased by this practice, thereby raising the lambing figures, has never been explored.

This investigation was therefore undertaken as a pilot attempt to demonstrate, with more accuracy, the source of, or reason for, the additional lambs which result from flushing ewes, in as far as this practice may increase both ovulation rate and subsequent mortality or merely reduce mortality in developing ova at some as yet undefined stage of early pregnancy.

The nature of the experiment was such that a study of the time-relationships of ovum loss and embryo mortality at various stages in early pregnancy could be made. Thus the matings of 225 ewes in two separate mobs were observed and slaughter dates were measured for individual ewes from mating times. The ewes were slaughtered at one of four stages of early pregnancy covering

(i) Ovulation & Fertilisation

(ii) Early Free existence and Implantation

(iii) & (iv) Early Allantoic placentation (see later).

By this means a considerable collection of both field and laboratory data was made available for a study of comparative individual and group reactions to the flushing treatment applied.

To achieve these aims, the experiment was designed as follows:-

<u>1st Slaughter Stage</u> - The intention was to slaughter 33 ewes from both a flushed and a non-flushed group two to three days after each ewe had been mated. At this time ova are recoverable from the fallopian tubes and should be in an actively dividing state if fertilised. Unfertilised and abnormal ova can be readily distinguished from those which have been fertilised by microscopic examination.

<u>2nd Slaughter Stage</u> - 33 ewes from each group at 10 to 13 days post coitum, at which time the fertilised and viable ova implant in the endometrium. It was hoped that study at this stage might have demonstrated the extent to which implantation fails, or is successful, under conditions of both flushing and non-flushing. Ova which were abnormal or unfertilised at the earlier stage, would not be found at this stage, having degenerated in utero at 8 or 9 days.

<u>3rd Slaughter Stage</u> - 33 ewes from each group at 19 to 21 days post coitum, by which time embryos, which had survived earlier fertilisation and implantation, may or may not be present and viable because of the loss associated with changes in nutritive status occurring at 17 to 19 days. This latter time marks the period when the yolk sac regresses, and embryonic nutrition is carried on by uterine milk and by the rapidly developing allantoic outgrowths which ultimately form the placentae in sheep.

From the few remaining ewes, and also from those not conceiving to the first mating at the 3rd stage and returning to the ram, it was hoped that more specific information regarding the time of loss after mating might be investigated, by slaughter at some time which the above results indicated to be important in this respect.

This design was found subsequently to be impractiable and the reasons why this was so are fully discussed in later sections of this paper. The basic pattern of the experiment, however, remained unchanged.

3. MATERIAL AND METHODS

A. INTRODUCTORY

B. MATERIALS

1. **Location**
   - (i) Farm
   - (ii) Weather
   - (iii) Water

2. **Stock**
   - (i) Ewes
   - (ii) Rams
   - (iii) Ashley Dene Records

3. **Feeds**
   - (i) Pastures
   - (ii) Supplements

C. METHODS

1. **Stock**
   - (i) Selection and Randomisation
   - (ii) Management:-
     - (a) Branding
     - (b) Weighing
     - (c) Feeding
     - (d) Mustering and Husbandry
     - (e) Mating
     - (f) Slaughter and Collection of Material

2. **Laboratory**
   - (i) Killing Stages and Recovery Techniques:-
     - (a) Stage I - 2 - 3 days post coitum
     - (b) Stage II - 10 - 18 " " "
     - (c) Stage III - 19 - 30 " " "
     - (d) Stage IV - More than 30 days post coitum
   - (ii) Evaluation and Recording:-
     - (a) Ovaries
     - (b) Thyroids
     - (c) Uteri
     - (d) Oviducts
     - (e) Recovered ova
     - (f) Stage of development of ova
     - (g) Embryos

A. INTRODUCTORY

In selecting materials for livestock investigations it is desirable to limit, as far as possible, the sources of variation, in order to simplify the interpretation of the results. This implies the use of

1. Stock of only one breed to obviate breed differences
2. Stock of a limited age group to overcome age differences, and
3. Stock having as nearly identical productive capacity as possible to ensure that results are not merely an artefact arising from an unconscious bias in constituting the treated and control groups.

This method of selection of stock is ideally exemplified when identical or monozygotic twins are used to compare treatments. However, Clark (1931) and Johannsen (1932) and others have shown that these are extremely rare in sheep and are unavailable as a source of experimental animals in this species. The question of the generality of application of results derived from the use of highly selected material also arises. In physiological studies, however, it is reasonable to assume that effects which appear marked between two uniform selected groups will be generally applicable to the population as a whole.

Likewise, in choosing methods and techniques for the experiment, care must be exercised. Techniques should be preferably,both widely accepted and practiced and of maximum technical efficiency compatible with the existing facilities an the nature of the study. This alone will ensure against inaccurate results and enables repetition by subsequent workers in the same field of research.

B. MATERIALS

1. Location - (i) Farm - The experimental flock was run on a 39 acre paddock on the Ashley Dene property of Lincoln College. The soil is one of the Eyre silt loams, in places sandy and, in others, stoney. This overlies deep shingle and the soil thus tends to dry out badly in dry spells or after periods of warm North-west winds. At the time of the experiment pasture existed mainly in the form of dead matter and weeds. Shelter in the form of *Pinus insignus* wind breaks was present on the South, East and North-west sides.

(ii) Weather - The period of the experiment, the late summer and early autumn of 1952, was characterised by warm dry conditions. The little rain that did fall was rendered ineffectual from the point of view of pasture growth by subsequent drying winds. Heavy dews and clear days were the rule. Such conditions were theoretically ideal for a flushing trial, as both quality and quantity of the feed available was strictly limited.

(iii) Water - The area is watered by water races originating at the Waimakariri river. A minor flood in this river resulted in the races being cut off during part of the experimental period. This was unavoidable and unforseeable and some of the ewes were without water for periods of up to eleven days. What effect this had on the reproductive functions of these ewes is not known. It will be discussed in the results section of the paper. Certainly, marked effects on ewe live weights were recorded at this time, but whether these were due to water or to feed shortage is a moot point.

2. Stock - (i) Ewes - A total of 225 ewes was run in the main experimental group after laboratory practice had been obtained from 47 ewes and 10 lambs prior to the experiment itself. The experimental ewes were all mature sheep which made up a line of cast-for-age ewes from the Ashley Dene flocks, the majority being Corriedales and the remainder being Merinos or Corriedale X Romneys. This "remainder" group was not sufficiently large in numbers to justify separate treatment and so no attempt has been made to express results on either a breed or an age basis. Instead, the "off-types" were equally distributed between the experimentally treated and the control groups. Considerable variability might have been anticipated from heterogeneous material of this nature, (teeth had suffered generally with age), especially where the reactions to differing feeding treatments were under study. However, age at least ensured that all ewes, Merinos excepted, were accustomed to eating silage, the flushing feed.

(ii) Rams - Four rams were used. Two were Ryelands and two Cheviots. All had been proved fertile in previous seasons, but a clinical inspection of testicles was made and microscopic examinations of semen were conducted as routine insurance against infertility before they were put with the ewes this season.

(iii) Ashley Dene Lambing records - Certain Ashley Dene records, notably earlier lambing records of the experimental-group ewes and the 1952 lambing records for ewes of a similar age group are presented to provide comparative data as the Dene flock in 1952 was flushed in the same way as the experimental flushed group.

3. Feeds - (i) Pastures:- Pasture analyses of the area used revealed the following information:-

Jan.	Feb.	Mar.	April	
38.5%	16.9%	No	44.0%	- Ryegrass
28.3%	67.4%	Record	56.0%	- Dead matter
33.2%	15.6%	-	-	- Weeds
Nil	Nil	Nil	Nil	- Sub. Clover

Weeds included (Cocksfoot - Isolated patches only.
(Yorkshire Fog)
(Hair grass) - General, but in small quantity
(Stork's bill)

Observation of grazed pasture showed a short-lived germination of sub. clover after one rain and by April the Cocksfoot and Yorkshire Fog had become dead matter.

The two mobs were confined on either side of a temporary dividing fence in a 39 acre paddock of uniform pasture composition. The flushed group was run where the greatest volume of feed was available. The other side of the paddock, having received a fairly heavy stocking prior to the experiment, was used for the control sheep.

(ii) Silage and Hay:- As from Feb. 25th, the start of the experimental feeding period, the group of ewes to be flushed was fed lucerne silage in addition to the available pasture. The silage was of high nutritive value and of good quality and was fed at the following rates:-

Feb. 25th to March 11th	- 5.0 lbs.	of Wet Matter /ewe/day
Mar. 12th to March 19th	- 6.0 "	" " " " "
Mar. 20th to April 13th	- 8.0 to 10.0 lbs.	" " " " "

The weights fed were measured periodically at the stack by checking the weight of a measured volume. Subsequent analysis of samples of the Silage yielded the following figures:-

Dry Matter = 27%
Crude Protein = 24% (on Dry Matter basis.)

Some hay was fed towards the end of the experiment owing to pasture deterioration. This was good quality hay having:-

Dry Matter = 88%
Crude Protein = 10.5% (on Dry Matter basis) and was fed at the rate of:-

40 lbs./day to the Control group and
65 lbs./day " " Flushed group, as from April 13th.

C. METHODS

1. Stock - (i) Selection and Randomisation:- In an attempt to enable the application of statistics to experimental data, all the sheep made available were used. This accounts for the somewhat "mixed bag" which eventually comprised the two groups.

Randomisation was done in the field at the first weighing on Feb. 25th. and ewes were allotted to one of two mobs without knowledge of their previous records and on a live weight basis. It was felt that no element of bias could enter into such a method in view of the pairing of sheep of all weights between the groups.

An eye-judgement of "condition" of each sheep was also made at this time and the Merinos were split evenly, as was the group of ewes culled for footrot, between the two groups. The group to be flushed was chosen by chance. The resulting "flocks" were comprised as follows:-

		Flushed	Control
Corrie & Romney X Corrie	(Culled on teeth)	86	90
" " " " "	(Culled Footrot)	17	17
Merinos, ewes	(Culled deformed udders)	8	7
	Total	111	114

N.B. No attempt to randomise the groups on the bases of age, ancestry, or previous breeding performance could legitimately be made, as complete records were unavailable for many of the ewes. Weight randomisation in this experiment is similar to that used at Ruakura (1949) in a flushing trial.

(ii) <u>Management</u>:-

(a) <u>Branding</u>:- For ease of recognition and to enable individual mating records to be made, the ewes of both groups were branded serially using nine inch figures on the left flank. Ear tag numbers were used to record individual weights and were recorded with the brand numbers. To prevent confusion between individual numbers and between groups, the Flushed ewes were numbered 1 to 99 and 001 to 011 and the Control ewes from 199 to 214.

(b) <u>Weighing</u>:- The two groups of ewes were weighed periodically and the variations in trends of the group mean weights were used as a guide in feeding. Corrections of these means were made for time the ewes spent standing in the yards prior to weighing on those days when they were mustered too early. The correction figure used was 2/3 lb. per ewe per hour and was provided by Dr. Coop.

Carcase weights were recorded in the butchery as cold weight less kidneys and kidney fat.

(c) <u>Feeding</u>:- From the time of the initial weighing on Feb. 25th., the mobs were grazed separately until April 21st., after which date they were run as one flock. The Control mob was depastured on the poorer half of the paddock and fed no supplementary feed until April 13th., when the pasture became so bare that hay, at $\frac{1}{3}$ to $\frac{1}{2}$ lb. per head per day, was fed as a maintenance diet.

The Flushed mob received silage as a flushing feed in addition to the available grass. The silage was fed out every two days or else daily from a horse-drawn dray at the rates given above so that there was a four weeks flushing period before the rams were put out on March 24th. From March 25th till April 13th, a further three weeks, the flushing treatment was continued and after April 13th hay was fed at ½ to 2/3 lbs. per head per day and silage feeding discontinued.

The object of these respective feeding practices was

(a) to maintain a steady body weight and condition in the Control group and

(b) to flush the "Flushed group". The only available guide as to the efficacy of the treatment in this respect, was an improvement in both visible condition and live weight in the premating period.

(d) <u>General husbandry</u>:- All ewes were ring-crutched to facilitate mating. Those with footrot were treated by the usual method of paring and immersion in copper sulphate solution. Mustering for raddling the rams, and weighing the ewes, was sometimes done with the help of the Ashley Dene shepherd, but the ewes soon learned to move into the specially constructed yard at the gateway in the dividing fence. On some occasions the ewes were mustered somewhat early for weighing and corrections to mean weights had to be made. A few of the flushed ewes developed infective "pink-eye" and were treated successfully with 4% zinc sulphate solution.

(e) Mating:- The rams, having been tested for fertility on March 24th., were then put with the ewes. One ram of each breed was run with either mob as it was found that this reduced fighting.

To enable recording of individual mating dates for each ewe, the four rams were "keeled" with a mixture of coloured raddle (powdered) and neatsfoot oil applied daily at first and at two day intervals subsequently. The raddle colour was changed after 16 days of service from blue to red, this indicating those ewes not conceiving to the first mating.

Every two days, the ram pairs were alternated between ewe mobs to prevent any possible fall in fertility in the pair running with the poorly fed control ewes.

Matings were recorded twice daily, at 8.30 a.m. and 5.30 p.m. approximately, the brand numbers of all raddled ewes being carefully noted. Consecutive daily tallies were used to check the records and these latter were found subsequently to be accurate within a range of ± 12 hours. Accurate mating records were essential as the slaughter dates for each ewe were timed from the supposed time of conception calculated from these records.

The rams were with the ewes continuously from March 24th. till the end of April.

(f) Slaughter and Collection of Material:- On the days when the College butcher needed meat, ewes which had reached any of the various killing stages (to be outlined in the following section) were cut out of the mustered mobs and carried to the butchery by tractor and trailer. Here they were slaughtered in the normal way and the thyroid glands and genitals removed, put in damp towels on trays, and taken to the laboratory. The time and date of slaughter of each ewe was recorded and carcases labelled for weighing.

As the rate of killing by this method was slower than the rate at which the ewes reached the desired killing stages, it was found necessary to slaughter sixty-one ewes at the Kaiapoi Freezing Works. Here it was impossible to recover the thyroids, but the genitals were all obtained, placed in labelled water-proof bags, and transported quickly to the laboratory at the College.

Accuracy in labelling the genitals was achieved by recording consecutive ear tags or brands as the ewes were placed on the chain. The genitalia were then placed in labelled bags in the serial order while a check was made at the skinning board to overcome possible changes in the order of the carcases occurring at intermediate points on the chain.

2. <u>Laboratory</u> - (i) <u>Killing Stages and Recovery Techniques</u>:- As mentioned above, all killings were timed from the assumed date of conception. The choice of the various stages of pregnancy at which slaughter was to occur was made with a view to both the efficacy of recovery techniques

available at different stages and the value of the data obtainable from the material. It was hoped that the killing stages so chosen would most clearly demonstrate the extent of ovum and embryonic loss in the early period of pregnancy studied.

Material from the butchery thus fell into one or other of the following categories and was treated in the manner described:-

(a) Stage I - 2 - 3 days post coitum.

At this time most normally-shed ova are found in the fallopian tubes in the sheep, and as fertilisation must occur within 24 hours of ovulation (Green & Winters, 1935) and cleavage begins 36 hours after ovulation (Clark, 1934 b; Green & Winters, 1945) the following data was obtainable:-

1. Number of ova shed per ewe
2. Number and percentage of ewes shedding abnormal ova and
3. Number and percentage of ova which were fertilised and which were dividing normally at the stage at which individual ewes were killed.

Comparative data for flushed and control ewes was thus obtained with respect to all these effects.

A good recovery technique for tubal ova, as used for pigs by Corner (1921) and described for sheep by Clark (1934), was applied. It consisted of carefully dissecting the fallopian tube, on the side of the ovulating ovary, from the enveloping mesosalpinx of the broad ligament. The tube was then severed at its fimbriated and uterine extremities and straightened by removing the adhering connective tissue. Care was exercised in this so that the tube was not crushed accidentally in the scissors, as this might either damage the ova or cause them to stick in the tube during the subsequent washing. The straightened tube was then flushed through from its fimbriated end with a hypodermic syringe containing about 3 to 5 c.c.s of physiological saline, this being caught in a watch glass. More than one of these small washings was sometimes necessary, as ova occasionally appeared to lodge in the tubal epithelium. The small volume of fluid was useful in facilitating location of the ovum or ova which was best done under a low-power binocular microscope. Gentle tapping of the watch glass caused the ova to roll to the centre of the glass itself and thus into the field of microscopic vision.

When located, ova were transferred to slides by means of a fine-tubed rubber-bulbed syringe and then studied under higher power magnifications. Some were then mounted for photography.

Practice in ovum recovery technique as outlined was gained on some 25 to 30 ewes and lambs during the early part of the feeding period.

When the full complement of ova shed was not recovered by repeated use of the above technique, the respective uterine horns were excised and flushed through from both the infundibular and tubal ends in an effort to recover the missing ova. Only one such ovum was recovered ex utero.

(b) Stage II - 10 to 17 days post coitum.

During this stage of pregnancy, the attachment of the blastocyst and rapid elongation of the trophoblast occur at 10 to 12 and 13 days respectively, (Clark 1934 b; Green & Winters 1945), while at 17 to 18 days the placenta formation begins with the increased allantoic and cotyledonary epithelial development (Robinson 1951). These two stages of development, namely implantation and the start of allantoic placentation are perhaps the most important in early embryonic life. For this reason an attempt to elucidate the possible effects of flushing on these stages of pregnancy was considered both desirable and essential. However, unfertilised ova and those not developing normally may never reach the implantation stage so that the absence of identifiable material in utero limits the effectiveness of data obtained in these killings. Data obtainable from these killings included:-

1. Number of ova shed per ewe. (A count of corpora lutea from the mating heat.)
2. Proportion of shed ova reaching the stage of development at which individual ewes were slaughtered i.e. early and post-implantation.

3. Proportion of shed ova which either degenerate or remain unfertilised and hence become lost before implantation.

Such data were obtained from both flushed and control ewes to enable comparison between groups.

A technique used, but not fully described, by Green & Winters (1945) was attempted for recovery of 10 to 12 day old blastocysts which should have become implanted. With four specimens of this type, the uterus was laid open under saline and the endometrium examined under the dissecting microscope and hand lens to locate the implanted blastocyst. Nothing was found, although this may be due to either the fact that this early attachment is weak and the embryos easily damaged, or the possibility that fertilisation had not occurred.

To overcome these recovery difficulties, a simpler and more accurate method was devised for this stage. Ewes were slaughtered after 13 days had elapsed since mating, by which time any implanted blastocysts would have undergone greater development and become visible to the naked eye.

By this method uteri were placed in a saline bath and cuts made along the entire length of both horns on their ventral surface. This revealed the greatly elongated trophoblasts lying on the endometrium and these were floated off and removed in petri dishes for closer inspection under the microscope. After 14 days some trophoblasts were found to be disintegrating which implied death after implantation, but no evidence was available as to the stage of loss before implantation if the ovulations were not matched at more than 13 days by developing or degenerating embryos.

(c) <u>Stage III</u> - <u>19 to 30 days post coitum</u>.

This stage was used to demonstrate the difference between the survival rate of embryos over the period of 17 to 19 days of pregnancy when, according to <u>Robinson</u> (1951) embryonic loss in sheep is maximal. Comparative figures for flushed and non-flushed ewes were obtained. Data available from this killing stage included:-

1. No. of ova shed per ewe (corpora lutea of mating heat counted),
2. No. of ewes returning to the ram which implied loss of embryos at an earlier stage (see later),
3. Proportion of shed ova reaching this stage of development,
4. Proportion of the latter developing normally and the proportion degenerating before and after 18 days where present.

(d) <u>Stage IV</u> - <u>More than 30 days post coitum</u>.

This stage was unavoidable because the rate of mating exceeded the rate of slaughter. It provided data similar to that of the previous stage.

The recovery technique for stages (c) and (d) were identical. Uteri were placed in the dissecting dish ventral side uppermost. Incisions were then made into the uterine tissue, which was pulled clear of the foetal membranes with a pair of forceps, by cutting from the cervical ends towards the extremities of the cornua. This procedure revealed the embryos in situ and in their undamaged membranes. After recording the apparent strength of attachment of the early cotyledons, the position of the embryos in relation to the ovary ovulating, the embryos were removed and stored in their membranes and under physiological saline in a refrigerator for photographing.

(ii) Evaluation and Recording:- For all genitalia studied in the laboratory, the following data was recorded with the brand number and experimental group of each ewe from which the material was obtained.

(a) Ovaries - (i) All Mating heat corpora lutea were recorded. This gave information on which group ovulation rates and survival rates were based. At the 2 - 3 day stage the ovulations were represented as distinct small bright red rosettes on the surface of the ovary. There was no evidence of follicles luteirising without ovulation, as judged by the recovery rate of ova, thus it was assumed that each corpus luteum appearing at this and the later killing stages corresponded to the release of one ovum at mating. In view of the possibility that two mature corpora may fuse (Winters & Feuffel 1937; Robinson 1951) many ovaries were sliced longitudinally with a scalpel and in several cases partially-fused double and triple ovulations were detected although outwardly appearing single.

(ii) Corpora lutea from previous heats were recorded if still visible. This furnished some data on silent heats and ovulation rate variations although at later stages such corpora were not still evident.

(iii) The active state of the ovaries as judged from their size and the size and number of contained graffian follicles was recorded. It was thought that the presence of the latter, especially if large, might act as a guide in distinguishing between corpora lutea of pregnancy and waning cyclic corpora. However, in most of the ewes killed at stages other than 2 - 3 days p.c., moderately large follicles of from 0.4 to 0.6 m.m. diameter were present, which is in accordance with the findings of Grant (1934) and Quinlan and Mare (1931 - 1932).

(b) Thyroids - Weights of all thyroid glands were taken after removal of adhering external fat and records for all ewes, except those killed at the freezing works, were made.

(c) Uterus - Vascularity, turgidity and size of uteri were recorded. No histological sections were made, and in general it appeared that, as recently stated by Hartman (1952) the uterine environment is seldom responsible for reproductive aberrations. Some cases of metritis were recorded which precluded fertilisation.

(d) Oviducts - All washings were classed into good or poor and blocked oviducts recorded. These latter, with metritis and a mucus-blocked cervix, were responsible for some of the ewes not conceiving prior to slaughter. One ewe was found at slaughter to have a cystic follicle and was apparently mated several times, while another, which was discarded when failing to take the ram after 60 days, had what appeared to be a persistent corpus luteum in each ovary.

Material recovered by the laboratory techniques was evaluated and recorded as follows:-

(e) Recovered ova - (i) Fertilised Ova:- Normal cleavage, i.e. the appearance of two or more equally sized blastomeres was considered proof of fertilisation (after Tanabe & Casida (1949) with cows' ova and Robinson (1950) with ova from P.M.S. treated ewes.) Austin (1949) has pointed out that for the rat at least, it is virtually impossible to distinguish between fragmenting (parthenogenetic division) and normally segmenting ova using ordinary binocular microscopy, but in view of the use of equal division as the criterion for fertilisation in higher animals by the workers mentioned, it was considered justifiable in this experiment.

(ii) Unfertilised Ova:- These fell into the following classes:-

1. Normal unfertilised - No division of the single blastomere at 2 or 3 days

2. Abnormal - ova with one blastomere only and this free in the vitellus and having a non-spherical nucleus. Some of this type were found in the practice ewes and also in the experimental groups and were regarded as incapable of fertilisation or development.

(iii) Damaged Ova - Corner's 1921 technique was found in both the practice and some of the experimental ewes to damage a proportion of the ova. This may have been due to the recommended manipulation of the tubes and, where fluid pressure alone was used, more ova were recovered and less were damaged. Damaged ova were recovered as empty or broken zona pellucidas or containing disintegrated blastomere or blastomeres if fertilised.

The stage of killing, i.e. 2 to 3 days, precluded the possibility that these damaged ova may have been disintegrating unfertilised ova which are found normally at about 7 to 8 days in the uterus. Damaged ova were not included in calculations involving fertilisation.

(f) Stage of development of ova was evaluated on the basis of the number of blastomeres present and according to Green & Winters (1945). Two ova, one of two and one of four cells, were found to be actively dividing when recovered although no attempt to culture ova was made.

In no case were there more ova found than corpora lutea (i.e. monozygotic twinning or multi-ovular follicles) although it was looked for.

(g) Embryos - these were examined closely for normal development after Green & Winters (1945) although none were sectioned. The general appearance of embryos in comparison with the photographs of these workers, from timed matings and slaughters, was used as the criterion of normality and viability. As indicated by Robinson (1950), embryos exhibiting retarded growth prior to 20 days while apparently dead, may be found to be living on histological section. These embryos do not exist beyond 20 days, (Robinson, 1950).

In some specimens, the foetal circulatory system was still active in the laboratory while in others of the early stage specimens the foetal membranes were found in an obvious state of degeneration. Thus, whereas the former were considered to be living, the latter were judged non viable and an endeavour was made as above to assess the stage of development at which death of the respective embryos had occurred.

Crown-rump measurements could not be taken as the majority of embryos were less than 30 days old, therefore the comparative system outlined was used. Advice on doubtful cases was also sought from the supervisor.

The number and position of the embryo was recorded for each uterus and abnormalities of development in membranes embryos and fluids were carefully noted.

Photographs of many specimens were kindly taken by Mr. R.C. Blackmore.

Thus, by the use of a slaughter policy, an investigation into the effects of a flushing treatment was made by securing and comparing data from a flushed and a non-flushed group of ewes. The data was classified into four stages of early pregnancy, which were specifically studied in their relation to the effects of the flushing treatment on ovulation rate and survival rate of ova and embryos.

4. RESULTS

A. Introductory

B. The Effects of the Flushing Treatment on the General Metabolism of the Ewes

(i) Changes in Visible Condition in the Ewes Prior to Mating

(ii) Changes in Live Weight in the Flushed and Control Ewes during the Flushing Period

(iii) Other Metabolic Differences Between the Flushed and Non-flushed Groups

C. The Effects of the Flushing Treatment on Breeding Behaviour of the Ewes

(i) The Effects of Flushing and Non-flushing on the Dioestrus Cycle Length of the Ewes

(ii) The Effects of Flushing on the Rate and Regularity of Service in the Two Groups

D. The Effects of Flushing on Ovarian Activity

(i) Ovulation Rates and the Incidence of Multiple Ovulations in Ewes of the Flushed and Non-flushed Groups

(ii) The Relation of Time to the Occurrence of Multiple Ovulations in the Flushed and Non-flushed Ewes
(a) Before and After April 3rd
(b) Before, During and After the Water Shortage

(iii) Ovarian Activity and the Ovary Ovulating in the Flushed and Non-flushed Groups.

E. <u>The Effects of Flushing on the Survival of Ova and Early Embryos</u>

(i) The Effects of Flushing on the Number of Ewes Conceiving at their First Mating
(a) Flushed and Non-flushed Ewes Returning to the Ram after One Mating
(b) The Proportion of the Flushed and Non-flushed Ewes Non-pregnant after the First Mating

(ii) The Effects of Flushing on Ovum and Embryo Losses at the Various Stages of Pregnancy Studied
(a) Stage I - 2 to 3 days after mating
(b) Stage II - 13 to 17 days after mating
(c) Stages III & IV - More than 19 days after mating
(d) Losses at All Stages of Pregnancy studied

(iii) Ashley Dene Lambing Records for 1952

4. RESULTS

A. Introductory:-

From the literature reviewed, it is evident that flushing implies a rising condition in ewes prior to and during the mating season. While some workers believe that relatively large live weight gains are necessary at this time, others have shown that these are neither essential in bringing about a flushed state, nor of themselves, indicative of higher ovulatory rates. However, scientific research implies accurate measurement, and whereas live weight is readily measurable, any judgements of the condition of ewes are subject to the varying evaluations of different assessors.

Ovulation rates and lambing percentages appear to be partly dependent on nutritional and environmental influences, but breed, age, and individuality differences may outweigh these effects in both cases. Flushing attempts to exploit the nutritional effect in raising lambing percentages, but in this experiment, all the ewes were slaughtered in early pregnancy to investigate the effects on ovulation rate, and ovum and embryo survival at defined stages.

Note: The volume of records for individual ewes is extensive, and for this reason, except in certain cases below, these are presented in Appendix B. which may be obtained for inspection from the Animal Husbandry Dept. of Lincoln College.

B. The Effects of the Flushing Treatment on the General Metabolism of The Ewes.

(i) Changes in Visible Condition in the Ewes prior to Mating.

In Table I a comparison, of the flushed and control groups, is made on the basis of changes in condition, assessed at the first and third weighings, for individual ewes. These judgements represent respectively, the condition of each ewe at the start of the experiment and the condition just prior to the date on which the rams were put with the ewes, and therefore constitute a group assessment of changes during the pre-mating flushing period.

Table I: Changes in Condition of the Ewes over the Pre-mating Flushing Period.

	FLUSHED			CONTROL		
Grading	25th Feb.	19th Mar.	Difference	25th Feb.	19th Mar.	Difference
Very Poor	1 ewe	2 ewes	+ 1	1 ewe	3 ewes	+ 2
Poor	16 "	10 "	- 6	16 "	12 "	- 4
Medium	53 "	37 "	-16	53 "	48 "	- 5
Good	34 "	55 "	+21	36 "	43 "	+ 7
Total Ewes	104	104		106	106	

Note: Both groups began with the same number of ewes in each grade. This Analysis excludes the merino ewes of both groups as no record of their condition was available for Feb. 25th. The judgements were made by the student in collaboration with

Dr. Coop for the first, and Messrs. Clark and Stevens for the second, to prevent any element of bias.

Thus of 104 ewes flushed on silage, 21 ewes improved in condition and one deteriorated, the remainder undergoing little visible change, while of 106 non-flushed ewes, only 7 ewes visibly improved over the same period, 2 deteriorated in condition and the rest did not alter noticeably.

It is considered that, although assessment of condition is largely indefinable, and dependent on visual impressions, the figures above give a fair indication of the changes in condition of the ewes of both groups made in the field, and that while the flushed group improved, the control ewes only maintained their initial condition during the period up to mating.

(ii) Changes in Live Weight in the Flushed and Control Ewes during the Flushing Period.

Individual ewe weights, as recorded at the five weighings, are presented in Appendix B. The following Table indicates the general group trends as expressed by mean weight values, and from Table II it is possible to compare both groups of ewes with respect to group mean weight changes at the five weighings which resulted from differential feeding of the 2 groups. The Table also demonstrates the effect of the water shortage on ewe live weights which affects the 4th and 5th weighings of both groups.

Table II: Changes in Mean Live Weights of the Flushed and Non-Flushed Groups.

	FLUSHED			CONTROL		
Date of Weighing	Number of Ewes	Mean Wt. (Arith.)	S.E. of Mean	Number of Ewes	Mean Wt. (Arith.)	S.E. of Mean
25th Feb.	103	120·6 lbs	± 1·78 lb	106	122·7 lbs	± 1·12 lb
11th Mar.	103	120·4 "	± 1·58 "	106	119·3 "	± 1·85 "
19th Mar.	103	124·7 "	± 1·46 "	106	120·8 "	± 1·80 "
2nd Apr.	93	122·2 "	± 2·74 "	101	111·2 "	± 1·67 "
9th Apr.	72	119·3 "	± 2·07 "	77	117·7 "	± 1·50 "

Note: Only ewes weighed at the initial weighing are recorded throughout the table. This excludes the merino ewes and one other flushed ewe which escaped this weighing. A light shower fell during this weighing.

Values for the 3rd, 4th and 5th weighings of the Flushed group and the 4th and 5th weighings of the Control group have been corrected for time spent standing in the yards prior to these weighings at 2/3 lb. per hour.

No correction for the water shortage has been made.

The table indicates that no marked weight increases occurred in the Flushed group as a result of feeding silage although, except for the initial weighing, this group did maintain a mean advantage over the Control group.

The Control group maintained weight as was desired over the period prior to mating and after this time the 9·6 lb. drop in the mean was probably due to lack of water as there is a noticeable increase between the 4th and 5th weighings, these ewes having received water again just prior to the latter. The 9·6 lb. drop is highly significant at the 5% level of significance by Student's t-test.

The 4·1 lb. rise in the mean weight of the Flushed group just failed to reach significance at the 5% level by the same test, but was close to the theoretical increase possible from the silage level fed, as can be seen from the following calculation:-

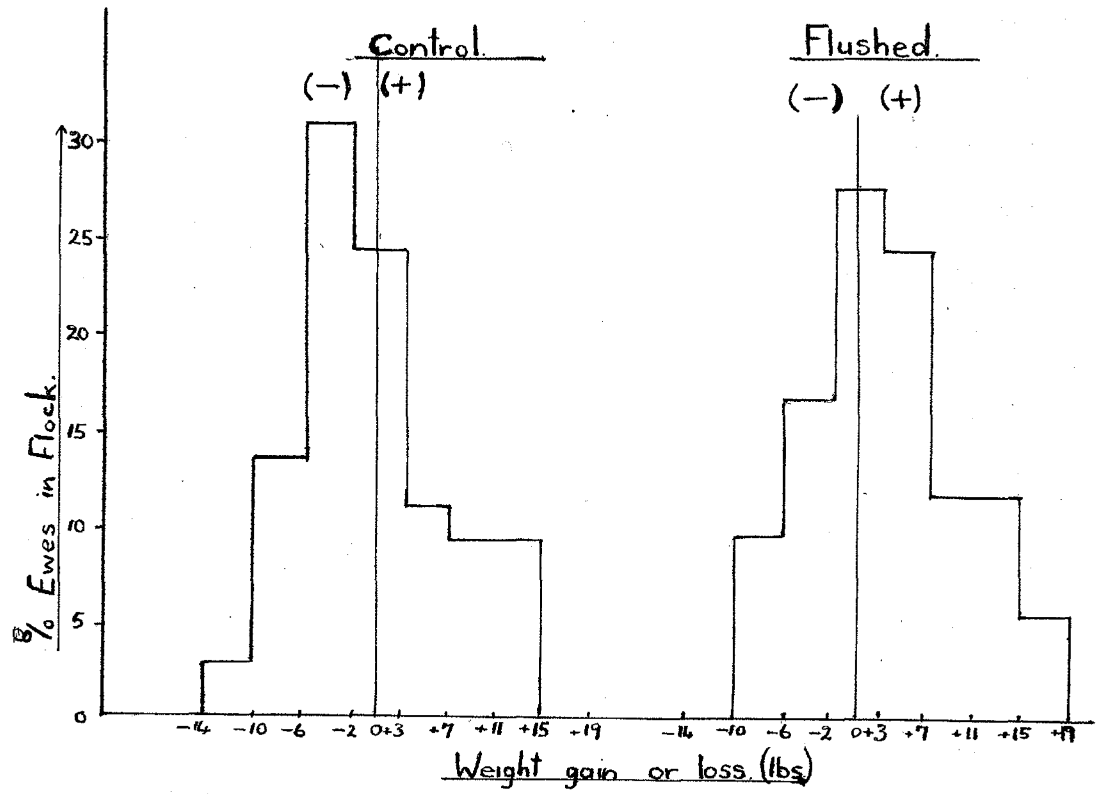
Silage intake/ewe from 25th Feb. to 19th Mar. = 33 lbs.
Assuming the silage had an S.E. value = 55 lbs.
Then the Starch Equivalent Intake = 18·3 lbs./ewe
Fattening value of this intake = +4·6 lbs./ewe (if fed above Maintenance)
Actual Weight Increase (Mean) = +4·1 lbs./ewe

The difference between theoretical gain and actual gain prior to mating can be accounted for by (a) wastage of silage at the start of flushing and poor utilisation later on, and (b) the pasture may not have supplied the full maintenance requirement to the flushed ewes.

After the 3rd weighing, which was made some five days prior to the commencement of mating, the silage ration was increased to 9 lbs. per head. This would have allowed a further possible weight gain of 1·5 lbs./head, making a theoretical total of 6·1 lbs./head in the Flushed group over the pre-mating flushing period from the silage.

While these gains were possible in the Flushed group, their complete achievement was prevented to some extent by two things, namely, the unavoidable water shortage, and the rapid deterioration of the pasture under a stocking of more than 6 ewes/acre in a poor growing season. A closer analysis of ewe weight changes in both groups, prior to mating, is demonstrated by the following diagrams.

Graph I Changes in Weight of the Flushed and Control ewes in the Pre-mating Flushing period.



These figures demonstrate that, for the period between the 1st and 3rd weighings,

63% of Flushed ewes gained from 1 to 20 lbs., and
37% " " " lost " 1 to 10 " , while
39% " Control ewes gained from 1 to 16 lbs., and
61% " " " lost " 1 to 14 "

Thus, in the Flushed group, both the proportion of ewes gaining weight and the extent of the weight gains made, were increased over the Control group, and the former numerical difference is statistically significant at the 5% level of probability, using a modified χ^2 test.

(iii) Other Metabolic Differences between the Flushed and Non-Flushed Groups.

In the course of the experiment, thyroid glands were removed from most of the sheep for study by another worker. The glands were all weighed and stored for subsequent iodine assay; a summary of the results of this latter technique kindly being made available by Dr.A.D. Care.

The following table briefly summarises the data available from these sources.

Table III: Weights and Iodine Content of Thyroid glands from the Flushed and Non-flushed Ewes.

	FLUSHED	CONTROL
Number of Samples	80 Ewes	81 Ewes
Mean Weight of Thyroids	7·3 ± 0·57 grms	6·8 ± 1·12 grms
Range (Maximum weight	46·8 grms	28·0 grms
(Minimum weight	2·6 grms	2·0 grms
Mean Th.Wt.of Ewes with more than 1 ovulation	8·4 ± 1·6 grms (35 ewes)	7·1 ± 3·9 grms (32 ewes)
Iodine Content of Medium sized Thyroids	0·50% (6 ewes)	0·58% (6 ewes)

Note: It must be mentioned that the accuracy of the Iodine analysis figures is doubtful after the first decimal place due to the limitations of the technique used.

From the table it is clear that there is no significance between any of the different mean weight values, while the apparent 16% difference in Iodine content cannot be regarded as demonstrating an increased thyroid secretory rate in the Flushed ewes. Such a difference may exist, but more accurate techniques are needed to demonstrate it.

C. <u>The Effects of the Flushing Treatment on Breeding Behaviour of the Ewes</u>.

(i) <u>The Effects of Flushing and Non-flushing on the Length of the Dioestrus Cycle</u>.

The cycle length of the Flushed and Non-flushed ewes was measurable for those ewes which returned to the ram after their first mating. As these figures are obtained from ewes suffering interruptions in early pregnancy, it is evident that some may be abnormally long where conception failed at later stages. However, all cycle lengths recorded were within the normal range and a summary of the data is presented in Table IV whereas a more detailed account of the mating dates of individual ewes is given in Table IX.

Table IV. Mean Cycle Lengths of Flushed and Non-flushed Ewes Returning to the Ram.

	FLUSHED	CONTROL
No. of Cycles	10	9
Mean Cycle Length	16·8 ± 0·24 days	17·5 ± ·41 days
Range	15·5 - 17·5 "	16·5 - 20·0 "

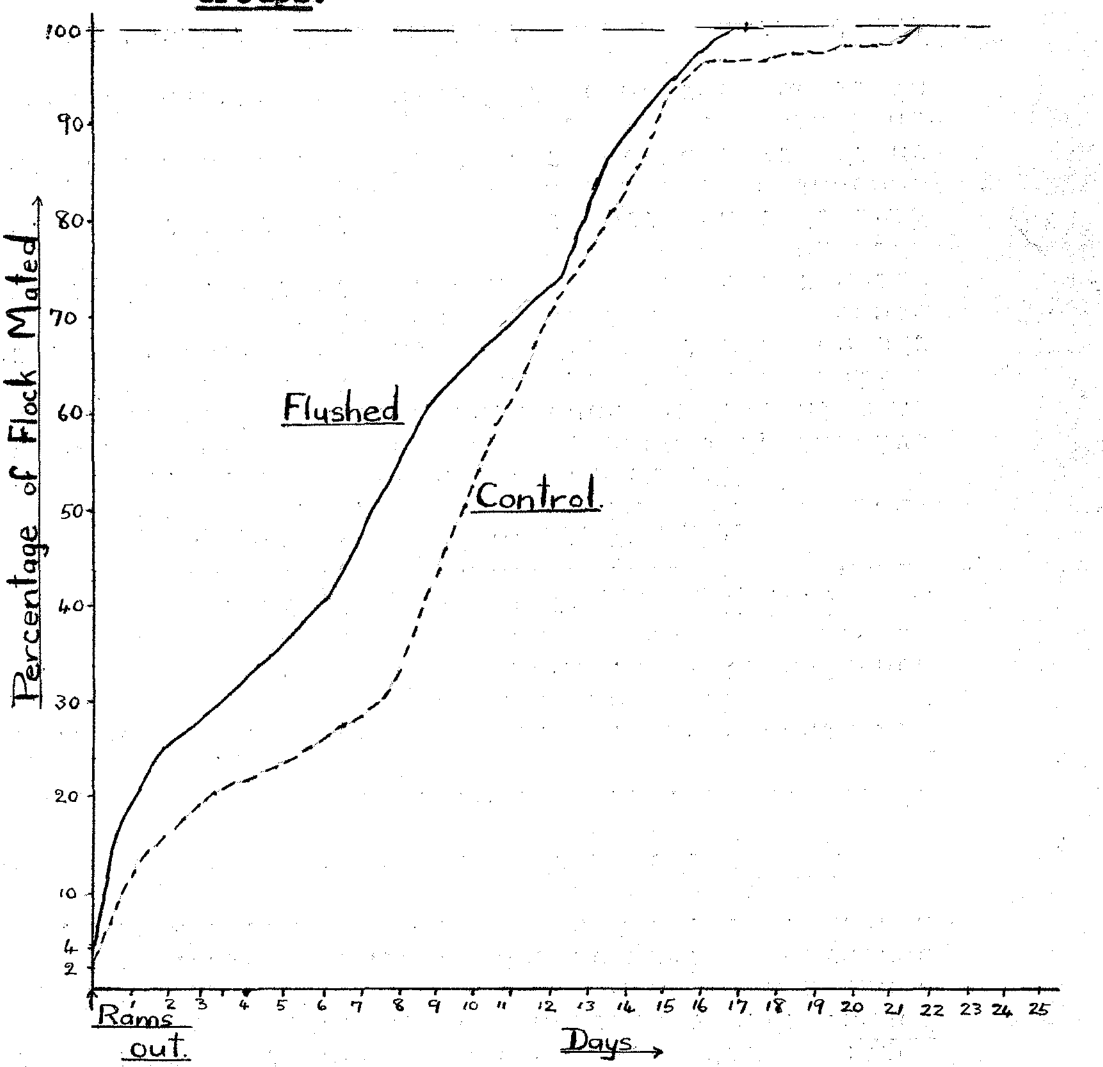
Note: With mating records accurate only to within ± 12 hours, this variation applies to each value given.
Cycle lengths were measured from the time of first observing mating at each oestrus period.
Length of the oestrus period was not observed.

Although the differences were not significant statistically, it appears that the Flushed ewes had shorter dioestrus cycles, before returning to the ram, than did the Non-flushed ewes, from the few cycles measured.

(ii) The Effect of Flushing on the Rate and Regularity of Service in the Two Groups.

The following graph demonstrates the rate at which ewes in either group were mated. This demonstrates merely the speed with which ewes in each group came into oestrus after the rams had been put with them, and it is emphasized here that no attempt was made to study the effect of flushing on the time of onset of the first seasonal oestrus cycle. A small proportion of the ewes were cycling before the flushing treatment began and still more had had cycles before tupping was begun.

Graph II. **Rates of Service in the Flushed and Non-flushed Groups.**



Note: Each point on the graph represents the cumulative total of ewes mated prior to the times and dates indicated. In this respect only does this Graph simulate those depicting the effect of flushing on time of onset of first oestrus. In none of the earlier matings were ewes covered which were not in heat, although this might have been anticipated with raddling on the first day of tupping.

It is obvious that the points on this graph are chiefly dependent on the number of ewes mated on the first day of tupping. This is largely a matter of chance depending mainly on the number of ewes which have just been in oestrus prior to the entry of the ram. However, in as far as the Graph demonstrates both more Flushed ewes in oestrus at the start of mating, and more regular service among these ewes during the first 17 days of tupping, it shows that either there were silent heats in the late-tupped Control ewes or that some of the Control ewes had abnormally long heats prior to their first mating.

Neither of these phenomena occurred in the Flushed ewes at this time and it is thus likely that Flushing ensures more regular and slightly shorter dioestrus cycles with full expression of heat at each oestrus than occurs under Non-flushing.

D. The Effects of Flushing on Ovarian Activity.

(i) Ovulation Rates and the Incidence of Multiple Ovulations in Ewes of the Flushed and Non-flushed Groups.

Macroscopic inspection of the ovaries of all ewes in both groups yielded the data for Table V, which summarises the group differences in numbers of ova shed per ewe expressed as the percentage in each group having single and multiple ovulations.

Table V. The Number and Percentage of Ewes in the Flushed and Non-flushed group with Single and Multiple Ovulations.

	FLUSHED		CONTROL	
	Number	Percentage	Number	Percentage
Single Ovulating Ewes	58	52·25%	71	62·28%
Multiple Ovulating Ewes	53	47·75%	43	37·72%
Total Ewes	111	100 %	114	100 %
Total Ovulations	167 Ovulations		160 Ovulations	
Mean Ovulation Rate	1·50 " /ewe		1·40 " /ewe	

Note: These Means are Arithmetic means. Standard Errors are not given as Ovulations do not follow normal Binomial distribution.

Thus Flushing increased the Ovulation rate, ova shed/ewe, and this was achieved through an increase in the proportion of the Flushed ewes which had multiple ovulations at the mating heat prior to slaughter.

The 10% increase in multiple-ovulating ewes in the Flushed group over those of the Control group was not statistically significant at the 5% level from this number of sheep.

The small extent of the differences may be related to the fact that the two groups were comprised of ewes of mixed age and breed, and which had, in the main, been culled for poor teeth. The latter fact would tend to reduce differences expected to arise from a feeding treatment, while the former could have a similar effect through an unconscious one-sided selection in making up the two groups.

(ii) The Relation of Time to the Occurrence of Multiple Ovulations in the Flushed and Non-flushed Ewes.

(a) Before and After April 3rd:- In Table VI, figures taken from slaughtered ewes, mated before and after April 3rd, are presented to demonstrate the changes in ovulation rate occurring with the advance of the breeding season in the Flushed and Non-flushed ewes. The date chosen was purely arbitrary, although intended to give nearly equal numbers of ewes before and after it.

Table VI. Changes in the Number and Percentage of Ewes having Multiple Ovulations, before and after April 3rd., and the Ovulation Rate in Flushed and Non-flushed Groups.

	FLUSHED	CONTROL
Before April 3rd.		
All Ewes ovulating	63 ewes	56 ewes
No. with Multiple Ovulations	26 "	23 "
% " " "	41·3%	41·0%
Ovulation Rate (all Ewes)	1·43 ova/ewe	1·45 ova/ewe
After April 3rd.		
All Ewes Ovulating	48 ewes	58 ewes
No. with Multiple Ovulations	27 "	20 "
% " " "	62·5%	34·5%
Ovulation Rate (all Ewes)	1·60 ova/ewe	1·35 ova/ewe

Thus, while the Flushed and Non-flushed ewes ovulating prior to April 3rd. had almost equal ovulation rates, Flushed ewes ovulating on and after this date did so at an increased rate, whereas the ovulating Control ewes did so at a reduced rate.

The increase in the percentage of multiple-ovulating ewes in the Flushed group after April 3rd is significant at the 5% level by the modified χ^2 test. The decrease within the Control group is not significant, but both the percentage of multiple-ovulating ewes, and the ovulation rate in the Flushed group ewes ovulating after April 3rd., are significantly higher than all values for the Control group by the same test.

(b) Before, During and After the Water Shortage:- Together with the somewhat arbitrary time division analysis above, an analysis of ovulation rates in those ewes which ovulated before, during, and after the period of the water shortage is presented. While more detailed figures for individual ewes appear in Appendix B, Table VII compares the Flushed and Non-flushed groups from the point of view of the numbers of ewes ovulating singly or multiply before the 29th. March, from this date until 10th. April (Flushed) and 8th. April (Control), during which period the water supply was cut off, and after the latter dates.

Table VII. **The Relation of the Water-supply to the Number of Ewes with Single and Multiple Ovulations and the Ovulation Rate, in both Groups.**

	FLUSHED	CONTROL
Total Ewes ovulating before 29th March	36	24
No. with Multiple Ovulations	18 ewes	13 ewes
% " " "	50%	54% -
Mean Ovulation Rate	1·50 ova/ewe	1·54 ova/ewe
Total Ewes ovulating between 29th March and	10th April - 65	8th April - 76
No. with Multiple Ovulations	31 ewes	23 ewes
% " " "	48%	30%
Mean Ovulation Rate	1·50 ova/ewe	1·34 ova/ewe
After	10th April	8th April
All Ewes Ovulating	10	14
No. with Multiple Ovulations	4 ewes	7 ewes
% " " "	40%	50%
Mean Ovulation Rate	1·50 ova/ewe	1·50 ova/ewe
Total Ewes	111 ewes	114 ewes
Group Mean O.R.	1·50	1·40 ova/ewe

Note: Ewe Numbers in the 3rd groups are small and thus liable to large sampling errors. Ewes with 2 or 3 ovulations are treated as multiple-ovulating in the tables.

As before, no Standard errors have been given with Ovulation rates. These probably follow the Pearson skewed distribution function which has been used for the modified χ^2 tests in this paper.

This table shows that pronounced increases in both the proportion of multiple-ovulating ewes, and the ovulation rate in the Non-flushed group, occurred after the re-establishment of the water supply. That this effect was not paralleled by the Flushed ewes, may have been due to the succulence of the silage, which only the latter group received.

Thus the water supply to the Control group, or its effect on the condition and weight of the ewes (vide supra), appears to have affected the ovulation rate of ewes within that group much in the same way as did the flushing treatment.

Note: Of 5 ewes ovulating after the return of the water, 4 had double ovulations, while of 9 ewes ovulating after additional hay was fed at a later date, only 3 had double ovulations. Thus, apparently, the water may have had a greater effect on the ovulation rate than did the feeding of small amounts of hay to similar ewes in the Control group.

(iii) Ovarian Activity and the Ovary Ovulating in the Flushed and Non-flushed Groups.

From the study of ovaries from the Flushed and Non-flushed ewes, slaughtered two and three days after mating, there appeared to be no follicles in either group, which could be considered of ovulating size, still present and unovulated. This means that flushing did not aid in bringing about ovulation in already ripe follicles, but rather stimulated more follicles to reach ovulating size in the Flushed ewes.

From earlier reports, it was anticipated that a higher proportion of ovulations would have occurred in the right ovaries. The following table shows the relative activity of left and right ovaries in the Flushed and Non-flushed ewes.

Table VIII. Ovulations in Left and Right Ovaries of the Flushed and Non-flushed Ewes.

	FLUSHED	CONTROL	TOTAL
Ovulations in Left Ovary	84	64	148
" " Right "	83	96	179
Total Ovulations (both ovaries)	167	160	327
% Ovulations from Right Ovary	49·7%	59·6%	54·6%

Although these differences are not statistically significant, it was found that under flushing, the left ovary was more active than it was under non-flushing.

E. The Effects of Flushing on the Survival of Ova and Early Embryos.

(i) The Effects of Flushing on the Number of Ewes Conceiving at Their First Mating.

(a) Flushed and Non-flushed Ewes Returning to The Ram after One Mating:-

In all, some 10 Flushed and 9 Non-flushed ewes actually returned to the ram for repeat matings at their second oestrus period. That this was not traceable to the individual rams is evident from the facts that equal numbers of ewes returned from both sets of rams, the rams were tested for fertility, they were regularly changed between the ewe groups, and finally, some ewes from both groups mated on the same days by different rams returned for second matings.

The reproductive performances of the ewes returning to the ram appear in the following table. They were all mated at two heats, except for Nos. 007 & 99 which were mated three and many times respectively, and for this reason are treated separately from the majority of ewes in either group which were mated at one heat period only.

Table IX. Reproductive Behaviour of the Flushed and Control Ewes Returning to the Ram.

Ewe No. Flushed	Date & Time of First Mating	Date & Time of Second Mating	Slaughter Stage & Days since Mating	Recovery Data & Remarks
30	25 Mar. 9 a.m.	11 Apr. 9 a.m.	III - 19 days	2 normal embryos
95	26 " 6 p.m.	12 " " "	II - 17 "	2 Embryos dead & with Sets Decomposed Membranes
007 (Merino)	27 " 9 a.m 3rd Mating	12 " " ") 29th" " ")	I - 2 "	1 Ovum Unfert. & Abnormal
63	28 Mar. 6 p.m	13 " " "	II - 16 "	Embryo dead with partly decomposed membranes
59	30 " 9 a.m.	16 " " "	II - 16 "	Nil in uterus
90	31 " 6 p.m.	18 " " "	II - 13 "	Embryo present & viable
42	1 Apr. 5 p.m.	19 " 10 "	IV - 33 "	2 Embryos present & viable
86	2 " 6 p.m.	19 " " "	I - 3 "	2 Fert. ova, 8 cell, R. Oviduct constricted
4	4 " 10 a.m.	20 " 11 "	I - 2 "	Metritis - inflamed uterus 1 Normal Unfert. Ovum.
99 (Merino)	9 " 9 a.m.	26 " 9 "	I - 3 "	Cystic G. Follicle in Left Ovary.

Ewe No. Control	Date & Time of First Mating	Date & Time of Second Mating	Slaughter Stage & Days since Mating	Recovery Data & Remarks
133	25 Mar. 9 a.m.	12 Apr. 9 a.m.	III - 21 days	2 Embryos both dead at 19 - 20 days
192	31 " " "	17 " 8 "	II - 15 "	Nil in Uterus. Thick mucus plug in cervix.
64	2 Apr. 6 p.m	19 " 10 "	IV - 40 "	2 Embryos (alive). Uterine muscle constricted in bands
173	3 " 9 a.m.	20 " 9 "	I - 2 "	1 Fert. 8 cell ovum
108 (Merino)	5 " 9 "	25 " 9 "	III - 27 "	2 Embryos - Live & Normal
214	9 " 8 a.m.	25 " 9 "	III - 25 "	Nil in Uterus, Persistent corpus Luteum, L. ovary.
166	9 " 8 "	26 " 9 "	I - 3 "	1 Unfert. Abnormal Ovum
210	9 " 6 p.m.	27 " 10 "	I - 2 "	1 Fert. 2 cell ovum
176	11 " 8 a.m.	28 " 11 "	I - 2 "	Right & Left oviducts occluded - Had never lambed in 4 seasons.

Note: Recovery was made after 2nd Mating

The following ewes, (Nos. 4 & 99 of the Flushed group, and (Nos.176, 192 & 214 of Control group were infertile due to genital abnormalities of a clinical nature.

Of the remaining ewes of this group,

(Nos. 95, 007, 63, & 59 (Flushed)
(Nos.133, & 166 (Control), were non-pregnant, after their second mating, at slaughter, but may have conceived at the 3rd mating although 007 did not.

(Nos. 30, 90, 42, & 86 (Flushed), and
(Nos.172, 108, 210, & 164 (Control) were still pregnant from their second mating at slaughter.

(b) <u>The Proportion of the Flushed and Non-flushed Ewes Non-pregnant after the First Mating</u>:-

Some ewes were found to be definitely non-pregnant at slaughter following the first mating. A summary of the stages at which pregnancy failed is given in Table X.

This table also demonstrates the partial embryo losses occurring in ewes which did remain pregnant from their first mating, and these losses are dealt with in the next section.

Table X. The Proportion of Ewes Found Non-pregnant after One Mating in Flushed and Control Groups and the Times of Ovum and Embryo Death.

FLUSHED					CONTROL				
Killing Stage	Total Ewes	Ova shed /ewe	No. Ewes Non.Preg.	Cause & Time of Loss	Killing Stage	Total Ewes	Ova shed /ewe	No. Ewes Non.Preg.	Cause & Time of Loss
I	17	1	4	(3 Abnormal Unfert. Ova (1 Fert. Ovum since died	I	18	1	4	(2 Abnormal Unfert. Ova (2 Normal Unfert. Ova
	5	2	Nil	-		4	2	Nil	-
		Also	(1 Ewe ovulated 1 ovum in butchery (unfert.) (1 Ewe had Normal unfert. Ovum						
Total	22	1·23	4	6 ova lost		22	1·18	4	4 ova lost.
II	5	1	3	(3 Embryos missing, 14 - 15 days	II	7	1	3	(2 Embryos missing at 13 & 15 days (1 Emb. degen. 15 days
	9	2	2	(Embryos both missing, 14 - 15 days in both ewes		6	2	1	(Embryos both missing, 13 days
						1	3	1	(All Embs. missing, 15 days
Total	14	1·64	5	7 Embryos		14	1·51	5	8 Embryos
III	17	1	2	(Embryos dead at 18 & 19 days	III	28	1	Nil	
	23	2	1	(Embryos both dead, 23 days		15	2	2	(Both Embs. dead or missing 20-23 days.
			Also	(4 Ewes, 1 of 2 dead 21 - 23 days (2 " ,1 of 2 missing 23 days				Also	(5 Ewes, 1 of 2 dead or missing, 20 - 26 days.
	2	3	Nil	(2 Embs. missing in 1 ewe, 24 days					
Total	42	1·64	3	12 Embryos		43	1·35	2	9 Embryos
IV	11	1	Nil	-	IV	9	1	Nil	-
	4	2	Nil	(2 Ewes, 1 of 2 missing, 53 days		6	2	Nil	(2 Ewes, 1 of 2 missing, 50 + days
	1	3	Nil	(2 of 3 embs. missing, 50 days in 1 ewe		3	3	Nil	(1 Ewe, 1 of 3 missing, 48 days (2 Ewes, 2 of 3 missing, 47 days
Total	16	1·38	Nil	4 foetuses		18	1·67	Nil	7 foetuses
Grand Total	94	1·50	12	29 Ova & Embryos	Grand Total	97	1·40	11	28 Ova & Embryos

Note: Ewes returning to the ram, or in which recovery was doubtful, are excluded from this table. The Ewes in Column four lost all ova or embryos and were thus Non-pregnant at Slaughter. Additional losses are also presented here for multiple-ovulating ewes still pregnant but suffering partial losses.

No ewes slaughtered in Stages III or IV were found to have had silent heats following the first mating.

Thus 12 ewes, or 12·9% of the Flushed Group, and
11 " , " 11·4% " " Control " , were
non-pregnant at slaughter after one mating.

Including the ewes which actually returned to the ram after one or two infertile matings,

22 ewes, or 23·4% of the 111 Flushed ewes, and
20 " , " 21·2% " " 114 Control " did
not conceive at their first mating.

(ii) The Effects of Flushing on Ovum and Embryo Losses at the Various Stages of Pregnancy Studied.

(a) Stage I - 2 to 3 days after mating:- From Table X it can be seen that, of the 27 ova recovered from the Flushed ewes at this stage,

3 were abnormal and infertile

1 died after fertilisation

1 ovulated so late after service and was thus not fertilised

1 other was normal but unfertilised. The latter two ova were found in double-ovulating ewes.

Thus 6 of 27 ova, or 22·2%, were infertile at 3 days from the 22 ewes.

(A further abnormal ovum was found in one of the Flushed ewes which had returned twice to the ram. This gives a total of 7 of 30 ova, or 20·3% of the fertilisable ova, non-viable at 3 days from 24 ewes.)

In the Control group ewes, from 26 recovered ova,

2 were abnormal and infertile

2 were normal but unfertilised so that <u>4 of 26, or 15·4%, were infertile at 3 days</u> from the 22 ewes slaughtered after their first mating.

(An additional abnormal ovum was found in a Control ewe returning to the ram giving a total of 5 of 29 or 17·4% of the fertilisable ova, non-viable at 3 days from 25 ewes.)

The 22·2% (Flushed) }
15·4% (Control) } values for infertile ova

are derived from unselected samples of the Flushed and Non-flushed ewes, in as far as all were slaughtered 2 or 3 days after their first mating.

It should be noted that ova damaged in recovery have not been included in these figures. Where genital abnormalities in the ewes precluded fertilisation these ewes have been discarded. The recovery rate of ova for the above figures, was 93·25%.

(b) <u>Stage II - 13 to 17 days after mating</u>:- From Table X it would appear that losses at this stage were much higher than at the previous stage. This is so because Stage I losses of up to 20% of ova probably took place before Stage II losses began, and where nothing was found in utero in Stage II, losses most likely occurred at or prior to implantation in these ewes.

True Stage II losses, i.e. for losses after three days and up to 17 days of pregnancy, can be inferred by difference of Stage II and Stage I percentages except where identifiable remains of embryos are found in the uteri, the latter being post-implantation losses.

Thus 7 of 23 embryos, or a total of 30·4% of those from 14 Flushed ewes, were missing at 14 to 15 days after first mating.

(A further 4 of 5 embryos were missing or decomposing in the Flushed ewes returning to the ram, and which were slaughtered at this stage, giving a total of 39·3% mortality in ova and embryos of 18 ewes up to 17 days pregnant.)

In the Control group, of 22 embryos, 8 were missing and 1 decomposing, or a total of 36·3% were non-viable in the 14 ewes slaughtered after 13 to 15 days of pregnancy (i.e. after implantation.)

By difference true Stage II losses become:-

Total at Stage II	30·4% (Flushed)	36·3% (Control)
Less Losses at Stage I	22·2% (")	15·4% (")
True Losses Stage II	8·2% (")	20·9% (")

Thus on the basis of ovum losses found in Stage I, the values for embryo loss subsequently, but prior to times when ewes would have returned to the ram, were considered to be 8·2% for 14 Flushed Ewes, and

20·9% " 14 Control " .

Note: The small number of ewes from which these figures are taken is due to the fact that the maximum rate at which ewes could be slaughtered (for meat) was 20 to 25 a week, whereas, to carry out the intended killing program, some ninety would have had to be killed each fortnight. There were no facilities available for storing carcases.

Values from such small numbers of ewes are liable to errors of sampling and thus should be carefully interpreted.

(c) Stages III & IV - More than 19 days after mating:- From Table X, it can be seen that, in the main, losses at Stages III & IV were small in relation to the number of ewes slaughtered, and that those of Stage IV were largely unidentified, thus these two Stages will be considered together in this section.

It is evident from Table IV that 19 days post coitum, the first day of this killing Stage, is outside the mean cycle lengths of both the Flushed and Control groups, although not outside the possible range for individual ewes. To study losses at 17 to 19 days of pregnancy, it was necessary to begin killings on the latter date to recover embryos dying in this period, even though some of these ewes would have returned to the ram after such loss. Thus 3 Flushed and 2 Control ewes were non-pregnant in Stage III, but none in Stage IV. Besides these, however, some of the multiple-ovulating ewes suffered partial loss although remaining pregnant.

These two slaughter stages demonstrate the importance of this investigation in as far as 16 embryos in the Flushed ewes and the same number in the Control ewes, a total of 16·0% (Flushed) and 18·1% (Control) of all embryos had been lost from the 58 Flushed and 61 Control ewes killed after 19 days had elapsed since their first mating.

Certain of these losses were identifiable as to the time of death. Table XI enumerates all losses in Stages III & IV in the Flushed and Control ewes more than 19 days pregnant at slaughter.

Table XI. Losses found in the Flushed & Control Ewes Slaughtered More than 19 days after their First Mating.

Description	FLUSHED	CONTROL
Embryos which died at 17 days	2	2
" " " " 18 "	3	-
" " " " 19 "	3	2
" " " " 20 "	-	2
Embryos missing at 23 to 55 days	8	10
Total	16 of 102 Embryos	16 of 88 Embryos

Note: None of the ewes in either group, slaughtered after 19 days had elapsed since mating, were found to have had a recent ovulation. Such ovulations, without coincident matings intervening between the first mating and slaughter, could be considered as silent heats, but none occurred.

That losses in these stages are smaller than those at previous stages is due to the fact that ewes having total embryonic loss of all ova prior to 19 days would have returned to the ram or been slaughtered at earlier stages, while ewes with only partial loss would still be pregnant.

Thus, from Tables X and XI, it can be seen that 8 of 102 embryos, or 8% of those from the 58 Flushed ewes slaughtered at these stages, could be identified as having died between the 17th and 20th day of pregnancy. A further 8·0% were unidentifiable as to the time at which loss occurred.

At the same stage of pregnancy, i.e. 17 to 20 days, 6 of 88 embryos, or 6·8% of those from the 61 Control ewes were found to have died, while a further 11·3% remained unidentified in Stage IV.

By excluding the unidentified Stage IV losses, some of which may have been due to mortality in Stage III, the possibility exists that the magnitude of the 17 to 20 day losses may be underestimated in these figures.

(d) Losses at All the Stages of Pregnancy Studied:-

The above sections and Table X have enumerated the losses which occurred at the individual stages of pregnancy which were specifically studied.

It is likely, however, that total losses, i.e. losses in all embryos / ewe conceiving, will tend to be greater in single ovulating ewes, while partial loss of the ewes' complement of embryos will tend to be greater in multiple ovulating ewes.

Table XII sets out the losses occurring in all ewes of both groups as they are affected by the number of ova shed/ewe, while a detailed account of losses by killing stages and by ovulations/ewe is given in Appendix A.

Table XII. The Losses of Potential Lambs in the Flushed and Non-flushed ewes as Related to the Number of Ova Shed/ewe.

	FLUSHED			Mean Values	CONTROL			Mean Values
Ovulations per ewe	1	2	3	1·50	1	2	3	1·40
Total Ovulations	50	82	9	141	62	62	12	136
Losses, No.	9	16	4	29	7	13	8	28
Losses, %	18·0%	19·5%	44·4%	20·6%	11·3%	20·9%	66·6%	20·6%

Note: This table includes only those ewes slaughtered after one mating. Losses are calculated from ewes in which ovulations were not matched by viable ova or embryos.

Thus from Table XII it is clear that, in both Flushed and Non-flushed ewes, as the number of ovulations/ewe increases, the mortality in ova and embryos increases also. However, no differentiation as to the stage at which these losses occurred is apparent in this Table. By reference to Table X the following facts can be obtained:-

In Single ovulating ewes of the Flushed group 77·7% of the losses occurred in those ewes killed at Stages I & II i.e. before 15 days P.C.

In the Multiple ovulating Flushed ewes, only 33·3% of the losses were found in Stage I & II ewes.

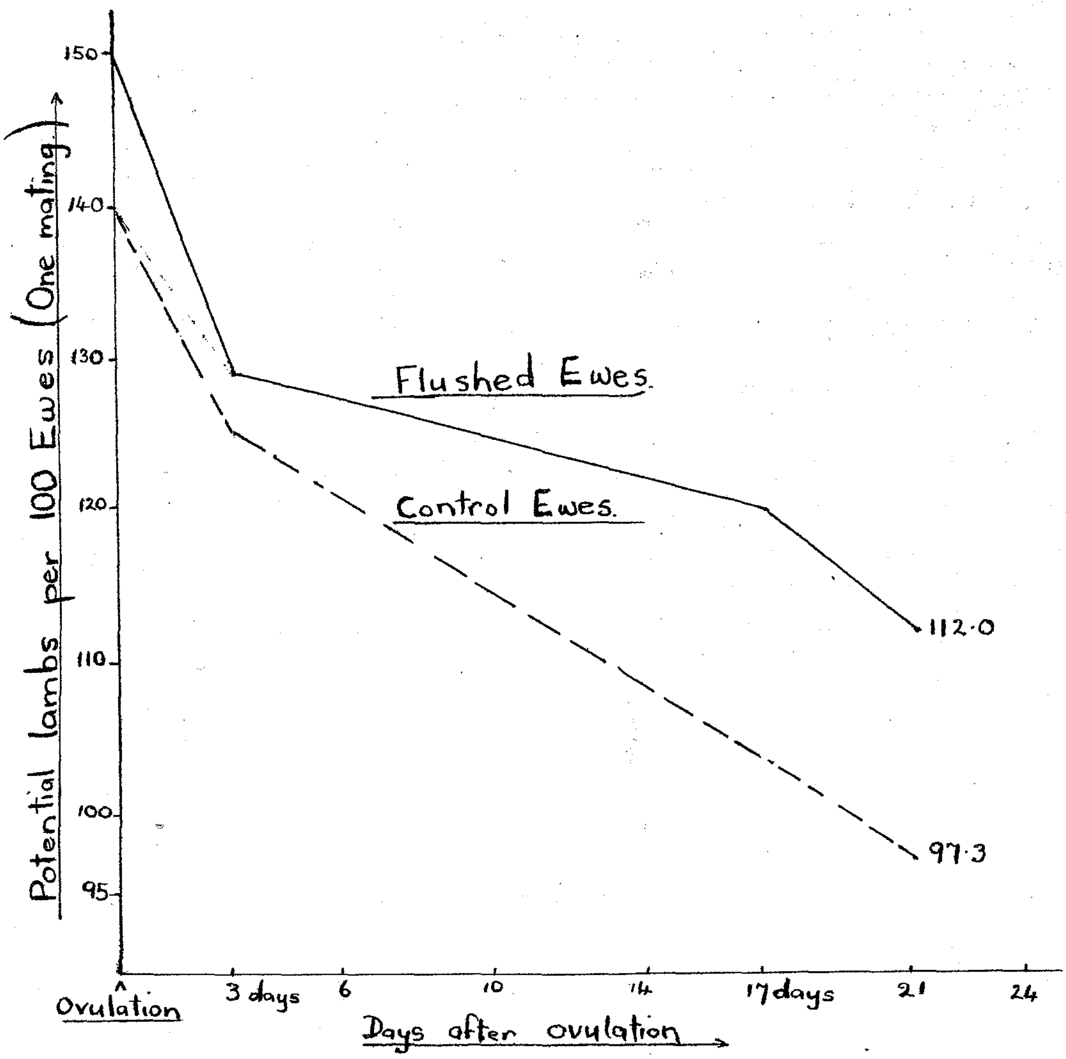
In the Single ovulating Control ewes all the losses were found in ewes killed at Stages I & II.

In Multiple ovulating ewes of the Control group only 23·7% were found in Stage I & II ewes.

Thus, it appears that losses in single ovulating ewes are more frequent prior to 15 days, and in multiple ovulating ewes, subsequent to 15 days.

A summary of the ovum and embryo losses in parts (a), (b) and (c) of this section is clearly demonstrated by the following Graph and related to the group ovulation rates.

<u>Graph III.</u> <u>Decrease in Potential Lambs from the Flushed and Control Ewes with Time.</u>



<u>Note</u>: Ewes returning to the ram are not included in the figures of the graph. The graph does not include the unidentified losses of Stage II and Stages III & IV which are considered to be expressed in the figures given for stages prior to these. See Appendix A for calculations.

The 11·3% unidentified losses at Stages III & IV for the Control group reflect the heavier embryonic loss in this group when compared with the 8·0% loss in the Flushed group at the same stage.

The "cumulative true total loss" of ova and embryos are thus:-

38·4% from the 94 Flushed ewes, and

43·1% from the 97 Control ewes as measured at three stages of early pregnancy and assuming unidentified losses to be contained in these figures.

Losses were thus highest at the ovum stage and the 17 to 19 day stage of pregnancy in the Flushed ewes, while, for the Control ewes, losses were highest at implantation and up to 17 days post coitum.

Ewes at the three stages of pregnancy at which losses were recorded in the above graph, were free of any genital abnormalities that could have precluded fertilisation. Although they were slaughtered after only one mating, similar reproductive behaviour was seen in the ewes which did return to the ram after conception failures.

(iii) Ashley Dene Lambing Records for 1952:- 435 full-mouthed ewes, from a line of which the Experimental sheep had been culled for failing mouths and footrot, were flushed in the same way as the Experimental Flushed group and lambed in the same year. Of these 4 died in pregnancy and 14 were empty at lambing.

The 431 ewes lambing gave 510 lambs alive at tailing, a lambing percentage of 118·1% as lambs tailed per ewes mated, less ewe deaths.

By subtracting the "Cumulative true total loss" from the ovulation rate in the Flushed group, as in Graph III, a possible lambing percentage of 112% is obtained. Thus a close agreement between the Ashley Dene ewes and the Flushed group is evident when it is remembered that many of the Flushed ewes would have eventually conceived under repeated matings.

The percentage of barren ewes in the Ashley Dene flock was 3·5%, and in the Flushed group, 5·4%.

The similarity of the above sets of figures indicates a low mortality rate after the 20th day of pregnancy.

It is believed that similar confirmatory results would have been obtained from a similar flock of non-flushed ewes for the Control group, as were obtained for the Flushed ewes in this experiment.

5. DISCUSSION.

A. The Metabolic Effects of Flushing.

Results have been presented demonstrating that increases in the proportion of ewes gaining weight and improving in visible condition were more common in a Flushed, than in a Non-flushed group of ewes prior to mating. These are the externally observable criteria of flushing, but, as shown by Clark (1934), and Miller, Hart & Cole (1942), weight gains are not sufficient, of themselves, to indicate higher ovulation rates in individual ewes. For this reason, a large number of animals must be used in flushing experiments and it is important that the unreliability of individual measurements of the two criteria be overcome by expressing only marked changes within such groups.

In general, the weight changes in this experiment are similar to, though smaller in magnitude, than those in the experiments of other workers. However, none of these workers record interruptions in the water supply of their ewes, nor does silage appear to be common among the feeds used. Being of lower food value than the pasture, grains and concentrates more generally used as flushing feeds, silage is likely to return smaller weight increases.

Furthermore, no attempt was made to reduce condition or liveweight of the ewes prior to flushing, as was done in the experiment of Underwood & Shier (1941), and this, too, would help prevent large weight gains from being made. That the live weight responses were small is, therefore, attributable to the low quality of the feed used and perhaps to the limited quantity fed in the early stages. This agrees with the conclusions of Marshall & Potts (1924), Terrill and Stoehr (1939) and Darlow and Hawkins (1932) who found that greater effects followed greater weight gains from higher feed levels in the groups studied. However, effective flushing did not appear to require large live weight increases, per se, but rather an improving condition prior to mating, and perhaps a poor condition prior to flushing. In this respect this experiment agrees with that of Clark (1934), although for individual ewes this was not so in that little difference was evident between distribution curves constructed from the relationship of weight changes before mating to single and multiple-ovulations of individual ewes within the two groups. Equal numbers of ewes losing and gaining weight in the Flushed group were found in the single and multiple-ovulating categories. Further to this, it was evident that individual ewes gaining in liveweight were not necessarily those which improved in condition, and vice versa, which demonstrates again the limited accuracy of both live weight measurements and condition assessments, as applied to individual sheep or to small groups. In the Non-flushed group, however, there were slightly more ewes which improved in condition among those which had multiple ovulations, while the higher-ovulating Flushed ewes were generally superior to the Control ewes after the pre-mating flushing, as demonstrated in Table I above.

This result agrees with that of Nichols (1924), Clark (1934 a), and Fraser (1949), although Smith believed that successful flushing required definite minimal weight gains in the ewes prior to mating to have any effect. Weight increases of the magnitude of most other workers were not made by the Flushed ewes in this experiment.

Nevertheless it appears likely from this, and earlier experiments, that the overcoming of relatively poor condition in ewes, and an increase in live weight prior to mating, both traceable to nutritional effects, may bring about a flushed state. Some individual ewes may lose weight, and others gain or maintain weight under flushing, and only a consideration of the trends exhibited by moderately large groups can be regarded as a safe guide to the effectiveness of the feeding standards used. That this is so is shown by the following figures which relate weight gains and losses of the individual ewes and the groups themselves over the 3 weeks pre-mating period, to the ovulation rates attained by the ewes at mating in this experiment.

Table XIV. The Relation of Weight Changes to the Number of Ova Shed per Ewe.

	FLUSHED	CONTROL
No. Ewes Gaining Wt.	68 ewes	43 ewes
No. " with 2 ov^ns	32 "	16 "
% " " " "	47·1%	37·2%
No. Ewes Losing Wt.	43 ewes	69 ewes
No. " with 2 ov^ns	21 "	27 "
% " " " "	48·8%	39·1%
Total % " " "	47·7%	37·7%

It is evident, from within-group comparisons, that ewes which lose weight, as well as those which gain weight, are equally likely to shed two ova rather than one, although the flushing treatment caused 10% more Flushed ewes to do so than did so under non-flushing. This was accompanied by an increase in the group Mean weight of the Flushed group and a general improvement in condition of the ewes of this group and the groups were moderately large.

The diet of the Control ewes in this experiment appeared to be low in volume rather than specifically deficient in any essential nutrients, as in the case of most other reported trials except, for example, that of Miller Hart & Cole (1942). The fact that many workers, including Smith (1933), Till (1951), Wallace (1951), and Vita (1951), used pasture alone for both their flushed and non-flushed ewes, while additional grain supplements gave better lambing results than did pasture alone for Okulicev (1934), and Polovceva et al (1938), would suggest that it is normal to expect lambing percent increases from both quantity and quality variations of the ration used. Underwood & Shier (1941), concluded that quantity was as important as quality in flushing feeds.

Metabolic effects other than changes in live weight and condition, due to feed differences employed, were not apparent from a study of thyroid status in the animals of either experimental group. Small differences between groups, and great individual variation within groups, were found in thyroid gland weights, but these may be more readily traceable to a variety of factors other than the Flushing treatment itself. The differences in thyroid iodine content between the groups may point to an increased secretion rate in glands of Flushed ewes Schultze & Turner (1945), and this may be of importance in relation to pituitary activity, and its relation to ovarian activity and secretion.

The failure and re-establishment of the water supply had marked effects on ewe weights, and while the latter can be correlated with an apparent flushing response in ovulation rate, this does not establish a causal relationship. Reduced water intakes may, in fact, decrease feed dry matter intake with dry feeds or roughages, but with a succulent like the silage used here, the reverse is probably true, which would agree with Bonsma (1938).

B. The Effects of Flushing on Ovarian Activity and Breeding Behaviour.

Many of the earlier investigators of flushing have been led to the belief that this practice can increase the blood concentration of follicle stimulating hormone from the anterior pituitary (Hammond, 1941; Wallace, 1951; and many others). The shortening of the dioestrus cycle and the more rapid and regular rate of service, together with the increased ovulation rate and proportion of ovulations occurring in the left ovary observed in this experiment, could lead to a similar conclusion, even though none of these increases are statistically significant (at $P \geq \cdot 05$) when considered individually.

Shortened dioestrus cycle lengths were observed by Briggs et al (1942) to result from flushing, as well as in this experiment, and the earlier and more regular breeding of flushed ewes is confirmed in the works of Wallace (1951) and Lewis (1951), and Clark (1934 a) and Grant (1934), respectively.

The absence of evidence of silent heats in the Flushed ewes, and the possible occurrence of three cases in the Control ewes, resembles the data of Grant (1934), who postulated the conversion of silent to normal heats as the means whereby flushing might hasten forward the onset of breeding. These three cases were ewes which were not mated until they had been with the ram for some 21 to 23 days, but while all had old corpora lutea in their ovaries, the exact age of these were indeterminate. A further Control ewe, No. 180, was slaughtered after not having been mated over 60 days, and had 2 generations of corpora lutea in her ovaries, i.e. at least 2 silent heats before slaughter.

Statements of Kelley (1942), and Reid (1949), bear out the possibility that flushing in this experiment increased ovarian secretion of oestrogen, which would result from the effects of the additional F.S.H., and account for the regular and fully expressed heats. These workers stated that the effects of a low supply of nutrients on fertility are manifested in irregular heat periods and failures to conceive, while Roux (1936) showed that a diet sufficient in protein gave more regular heats in ewes than did poorer diets.

It is still a matter for conjecture as to how the flushing procedure stimulates pituitary activity. Rinaldino (1949), with rats, and Hafez (1952 b), with sheep, have shown that refeeding after temporary inanition, and injection of pregnant mares' serum containing F.S.H., have similar effects in increasing ovarian activity. Loeb (1917) cit. Lewis (1951), Mason (1939), and recently Hafez (1952), with sheep, have shown that underfeeding adversely affects ovarian activity, and Hammond (1921), that it increases follicular atresia. Rinaldino (1949) has shown, with parabiosed rats, that the pituitary can store its secretions when the animal is starved, and that the eventual release of these stored hormones may be achieved by refeeding (cf. - flushing) or simulated by injections of the specific pituitary hormones themselves.

Even allowing for the additional gonadotrophin which might circulate after such a procedure, it is difficult to explain the relatively greater activity of the left ovary under flushing as observed in this experiment. The more equal occurrence of ovulations in left and right ovaries found in the Flushed group, is perhaps paralleled by a similar observation by Grant (1934) in fattened (?) slaughterhouse ewes. The preponderance of right ovary ovulations in the Control ewes is similar to, and of the same magnitude as, those recorded by Clark (1936) in cows, and McKenzie & Terrill (1937) and Henning (1939) in sheep.

The theory of Rinaldino, while possibly accounting for the increased ovulation rate at the heat following the start of flushing, is of less assistance in explaining the persistence of this effect or the overcoming of the seasonal decline in ovulation rate recorded in non-flushed ewes. Both Robinson (1951) and Hafez (1951) have postulated a seasonal rise and decline in pituitary activity as in the Control group, while evidence of a similar nature is given by McKenzie & Terrill (1937) and Grant (1934) for the seasonal decline in ovulation rate. This decline was not observed in the Flushed ewes, nor in the lambing data of other workers for flushed ewes, and it would appear that under this practice, the pituitary secretion rate does not fall so soon due to some nutritional stimulus.

Although external stimuli such as flushing, and possibly as the water fluctuations discussed above, are generally believed to have little effect in modifying the light-established cyclic phenomena (Lewis, 1951), Turner, (1948) believes that it is possible by affecting the general welfare and metabolism of the animal.

A direct study of the activity of the pituitary under flushing is hampered by the inability to recover the small amounts of gonadotrophins in the blood at this time, and the limited value of the cytological evidence apparent from histological examination of the secreting gland.

C. The Effects of Flushing on Survival of Ova and Early Embryos.

In a study of this nature, where slaughter intervenes in early pregnancy, the effective number of ewes available for appraisal may be far below the original number in the experimental flock. Some of the reasons for this state of affairs are traceable to the selected stages of pregnancy at which slaughters are done and to the individual variation which exists within any line of ewes.

Firstly, if slaughter is not made at stages when ova or embryos are recoverable, either alive or dead, a high proportion of losses may not be accurately traceable to any specific time or cause. Secondly, slaughter just prior to the date on which ewes not conceiving to a first mating, would return for a second, may show nothing if loss was early enough, and there is a marked individual variation in this time. Thirdly, ewes may be non-pregnant from such causes as ovum losses, ram infertility and genital abnormalities, and may not return to the ram at normal times owing to silent heats. In any of these cases, nothing is found at slaughter, even after sufficient time appears to have elapsed to allow remating. Finally, embryonic loss may have occurred after the stage of pregnancy at which slaughter intervened, and such losses would be unpredictable.

(i) Ewes failing to Conceive to one mating.

In all, 23·4% of the Flushed ewes failed to conceive at their first mating, while 21·2% of the Controls did likewise. The figure for the Flushed ewes is essentially the same as that given by Wallace (1951), as a mean from flushing experiments over two years, but that for the Control ewes is higher than Wallace's 9·5% mean for controls in the same seasons, although the variation between years was great. If, as is done in the next section, two Control ewes, non-pregnant due to early removal from the ram, are considered able to have conceived, the proportion non-pregnant at Slaughter becomes 19·1% which more closely resembles Wallace's first season figure of 14% in non-flushed ewes. That these misconceptions were the only ones traceable directly to the rams has been demonstrated above, although it is of interest to note that, of the matings which failed, 50% took place on or near the dates when all the ewes were mustered for weighing or for drafting for slaughter. In this respect, Hammond (1941) has pointed out the necessity of quiet treatment for ewes at mating time, while Beach (1947) has stated that in spontaneously ovulating species like the sheep, interruption of the full sex-play of mates may affect the establishment of synchronisation of desire and ovulation in the female.

A few of the Control ewes failing to conceive, did so in the period following the return of their water supply. The possible flushing effect of this incident has been mentioned already and this may account for these misconceptions.

The time and cause of losses in ewes failing to conceive becomes important when evolving methods of prevention, or in determining when the ewe will return to the ram, but this may be concealed by various means. The time of loss itself, and the time required for resorption will affect the interval between consecutive matings, thus in the Flushed ewes where the dioestrus cycles were shorter, ovum losses may have been the cause, while in the Control ewes where cycles were longer, post-implantation loss may have been greater. In the longer cycles it is likely that reactions between embryo and uterus are set up which delay the regression of the corpus luteum. Moore and Nalbandov (1952) demonstrated a nervous reaction of this nature which they considered affected the pituitary, but Hammond & Robson (1951) believe that oestrogens and not pituitary hormones maintain the corpus. Moreover, it has been amply demonstrated by Robson (1938 & 1947) that the secretion of ovarian oestrogen, at least, is unaffected by nervous stimuli arriving at the ovary.

Nevertheless, in ewes which return to the ram at normal cycle lengths, it can be confidently assumed that embryo mortalities occurred prior to 12 or 14 days, i.e. before or shortly after implantation. This assumption was borne out by 2 Flushed and 3 Control ewes which, among the ewes not returning to the ram by 20 days post coitum, for second matings, had suffered complete loss of embryos, and 2 Flushed ewes which had not returned to the ram by 16 and 17 days and still contained traces of degenerate embryos in utero. Corner (1921 b) and Hartman (1952), both showed that such apparent post-implantation losses may be due to faults in pre-implantation stages of developing ova in other species. Whether this is so for sheep is not known.

Abnormalities in genitalia which prevented conception were found in 5·4% of the Flushed ewes and in 2·6% of the Control ewes included in the above tables. Further ewes not included in the tables were one of each group which had unilateral tubal obstructions on their ovulating side, and two Control ewes having unfertilised ova in their fallopian tubes together with an inflamed condition of their uteri. These figures resemble closely those given by Tanabe & Casida (1949), from 104 cows showing no gross evidence of abnormality, but being of low fertility. Thus, it appears that of ewes failing to conceive to one mating, about 6% are incapable of ever conceiving, while more may be so if abnormal ova are considered to be genital abnormalities after Winters (1947) and Corner (1921 b), although it is not known whether such ova are consistently shed by individual ewes.

Thus the role of flushing in preventing conceptions at the first mating appears to be restricted to that of causing higher ovum or early embryo mortality in some individual ewes, especially in the early stages, and perhaps by altering the dioestrus cycle length.

(ii) Losses at the Ovum Stage.

A total of 28 Flushed and 32 Control ewes were slaughtered at 2 to 3 days post coitum, and 93·25% of the ova were recovered from these.

Of this number, however, 4 Flushed and 4 Control ewes were among those returning to the ram (see Table IX) and in 3 ewes of the Control group, some ova were damaged in recovery and all these ewes had to be discarded from Table X, reducing thereby the effective number which could be considered in the study of fertilisation.

In the remaining ewes, 22·2%, and 15·4%, of the ova from Flushed and Control ewes respectively, were infertil at 3 days. Dutt (1951) gives somewhat higher figures than those from this experiment, but these were from artificially inseminated ewes and included, in those considered abnormal at 3 days, those of which only fragments were recovered and those having broken zona pellucidas, which ova may have been damaged in recovery. These latter accounted for 20% of Dutt's abnormals, while only 55% of all ova were considered fertilised.

In respect to the observed ovum mortality effect, apparently due to flushing in this experiment, it is unfortunate that neither Clark (1934 a) nor Darlow & Casida (1939) gave similar figures from the ova recovered in their experiments.

To explain the greater losses in ova from the Flushed ewes, several possibilities exist, but no definite facts are available. Firstly, McKenzie & Terrill (1937), and Polovceva et al (1938) both observed that in multiple ovulating ewes, ovulation may be asynchronous with a possible time lag of up to 17 hours between the shedding of the first and last ovum. This is a likely explanation for the normal but unfertilised ova in ewes No. 7 and 47 of the Flushed group. On the other hand, it is likely that ewes 149 & 160 of the Control group, which had unfertilised normal ova, were taken from the ram too soon after the onset of heat

(1st. observed mating), and that no viable sperms reached these ova.* Both were single ovulating ewes. If these two ova are considered to be fertilised, ovum losses in Control ewes become 7·7% rather than 15·4%. Similar figures are given by Winters & Feuffel (1936), i.e. 8%.

* The reason for these ewes being removed from the ram was that the tractor, which was used to transport the sheep from the paddock to the butchery six miles away, was, at most times, not available and, at others, only at night. Thus the sheep had to be drafted for slaughter some time before they were actually killed. Subsequent to this accident, a ram was transported and left with the ewes till slaughter if they were to be killed in Stage I killings and had to be transported a day ahead of time.

In considering the means whereby flushing might have affected the proportion of abnormal ova shed, it is interesting to conjecture on the possibilities brought to light by Vial (1952), who found a high potency of oestrogen-like substances in lucerne silage. Bennetts (1946) has shown that ova from ewes grazing oestrogen-potent subterranean clover are difficult to fertilise. Further, if the effect of flushing on the length of the dioestrus cycle is valid, it is conceivable that immature ova may be shed at the advanced oestrus periods or that oestrus and ovulation may no longer be coincident. Corner (1921 b) and Winters (1947) believe abnormal ova to be due to inherited lethal factors, or, Corner, to traumatic effects.

Losses in the ovum stage were more prevalent in the single ovulating ewes of both groups, and no losses occurred in multiple ovulating Control ewes. These single ovulating ewes would all have returned to the ram for further services and it would be interesting to know whether some of them would have again shed abnormal ova. From the data of the ewes actually returning to the ram it appears as if some ewes might have a hereditary tendency to shed such ova.

Only a study which included a greater number of multiple ovulating ewes than there were in this case would show whether culling of ewes returning to the ram would be beneficial in raising flock fertility by eliminating such single-ovulating ewes and those consistently shedding abnormals as might be inferred from the above figures and from Barton (1947) and his non-breeding two-tooths.

(iii) Losses in Embryos after the Ovum stage.

The free uterine existence of ova was not directly studied, and the implantation stage was examined only subjectively in this experiment, in small numbers of ewes. Technical difficulties and the inability to carry out the desired slaughter policy were responsible for this as outlined in an earlier section. Thus losses ascribed below to implantation failure are open to criticism and liable to high experimental error.

In an earlier section, it has been shown that the total losses recorded in Stage II ewes (i.e. the early post-implantation killings), were 30·4% and 36·3% for the Flushed and Non-flushed groups respectively. These figures would, of course, include the losses which had occurred at the ovum stage in ewes of both groups, but it is clear that Stage II totals show an increase in loss over Stage I total losses for the Flushed and Control ewes. Part of these increases will be due to true ovum loss after 3 days, and part are possibly due to the fact that there were higher proportions of multiply ovulating ewes in Stage II killings than in Stage I killings, and among these ewes losses were very high (see Table X.) For example, Control ewe No. 158 had 3 ovulations but no embryos in Stage II, while there was no such triple ovulating Flushed ewe killed.

However, by subtracting the percentage loss in ova (from Stage I killings) from the percentage loss at the first post-implantation stage killings (Stage II), a reasonable figure is obtained for percentage loss between 3 and 17 days. Thus the Flushed group had 8·2% differential loss, and the Control group had 20·9%, by this method. If, however, the 2 Control ewes in Stage I, taken too early from the ram, and No. 158 (see above) are excluded, the figures become:-

		<u>Flushed</u>		<u>Control</u>
Total Stage II losses	=	30·4% of Ova	&	26·3% of Ova
True " " "	=	8·2%	&	18·6%

These figures may give a more accurate basis for comparison of the extent of losses in both groups, as this excludes two doubtful cases, and one abnormal case from one of the two compared groups, and allows for the increased loss which Hammond (1921) and Henning (1939) have shown to occur with increased ovulations in any ewe, and which is demonstrated above.

However, losses are higher in the Control group at Stage II, whichever way they are calculated, and while this may be affected by the small number of ewes considered at this stage, it appears that flushing may reduce losses between 3 and 17 days after mating. This aspect of flushing does not appear to have been investigated prior to this experiment, and thus no confirmatory data is yet available. Brambell (1948) and Brambell & Mills (1947) have shown that, in rabbits, embryonic loss prior to implantation declines with increasing body weight, and, further, that the greater the number implanting, the smaller will be the chance of subsequent loss. Hartman (1952) with rats and opossums, found that the 10% of ova which failed to implant contained faulty germ plasm rather than suffered from a faulty uterine environment. Hertig & Rock (1946) found similar results in infertile human subjects, as did Corner (1921 b) in sows. However, in the ewe, Huggett (1941) has demonstrated the importance of uterine milk in embryo nutrition, and it is possible that flushing may have its effect in reducing losses at this stage of pregnancy by this medium. The only other possibility, namely that flushing affects the progestational phase of the endometrial changes which Robson (1947), believes is essential for proper implantation. This could be achieved by the additional oestrogen which results from the increased ovarian activity, as the uterine changes due to progesterone can only follow sensitization of the endometrium by oestrogen (Robson, 1947), while the presence of multiple corpora lutea, rather than one, should increase the blood progesterone content.

Study of embryo losses from 17 to 20 days in larger numbers of ewes showed that their identifiable magnitude was 8·0%, and 6·8%, for the Flushed and Control ewes respectively. The remaining 8·0% (Flushed) and 11·3% (Control) embryo losses, unidentified as to stage of loss, from ewes killed later than 23 days after mating, may have been due, in some measure, to losses at 17 to 20 days which became obscured by subsequent resorption. Some of these unidentified losses in multiple ovulating ewes may have occurred at any stage after ovulation, and give little evidence in the particular study of times at which losses occurred.

The cause of the 17 to 20 day losses is probably partly nutritional in origin, as this is the period of regression of the embryonic yolk sac and change to the primitive allantoic placenta as the source of food for the embryo. At this time it is considered likely that uterine milk will provide most of the food used by the ungulate embryo, although Robinson (1950 & 1951) cites the work of Brambell (1942 & 1948), Brambell & Mills (1947 & 1948) and

Parkes (1943) on rabbits, Hammond (1921) and Corner (1921) with pigs, and Hammond (1921) and Robinson (1950 a & b) in ewes, all demonstrating the importance of this phase of embryo development in its relation to mortality. However, in the sheep, this 17 to 20 day stage of embryonic development also coincides with the time at which heat and ovulation would normally have occurred had not conception taken place. At such times, and at the 14th day, when,(Grant, 1934), a slight regression of the corpus luteum verum normally occurs, blood oestrogen levels probably rise temporarily (and perhaps more so under flushing) and may affect the gravid uterus through both the oestrogen-progesterone antagonism and the sensitisation of the myometrium to undergo involuntary contractions, both of which might weaken the attachments of the embryo. Hammond (1921) believed that early foetal atrophy was neither nutritional nor bacterial in origin, and Robinson (1950 b) has suggested that the size of the uterus and presumably the extent of post-implantation hypertrophy is a breed and individual function depending largely on the fertility of the ewe. This may set the limit to the number of embryos/ewe which can be maintained in utero after 20 days of pregnancy, especially where hormonal superfoetation is attempted.

Of multiple ovulating ewes, those which suffered only partial or nil losses of embryos before or at 20 days of pregnancy, can remain pregnant. Thus in the still-pregnant, multiple-ovulating ewes examined at more than 20 days post coitum, losses were

19·3% of embryos in the Flushed ewes, and

23·7% " " " " Control ewes.

These losses were unidentified as to their time of occurrence, but their magnitude, and the apparent difference between them, adequately demonstrate the inadvisability of assuming ovulation rates from lambing figures; this because these are the minimal losses in the more fertile ewes which have conceived to one mating and remained pregnant. If these losses are expressed as the percentage embryo loss in <u>all</u> ewes pregnant after 20 days (i.e. including single ovulating ewes with foetuses), the figures become 13·2% (Flushed) and 13·6% (Control) which resemble the 16% figure of <u>Hammond</u> (1921), <u>Henning</u> (1939) and <u>Till</u> (1951). The higher proportion of still-pregnant single-ovulating ewes in the Control group at this stage accounts for the reduction in magnitude of the difference between the above sets of figures.

It was considered possible, and found true, that some ova had migrated to the side of the uterus farthest from the ovulating ovary. Reports, by <u>Curson</u> (1934 a & b) and <u>Curson and Quinlan</u> (1934), on ewes, and <u>Dowling</u> (1949) on cows, confirm this. The phenomenon may account for the difficulties experienced by <u>Pincus</u> (1936) in recovering unfertilised ova ex utero, and, according to <u>Dowling</u> (1949), occurs within 12 to 24 hours after ova enter the uterus of the cow. This point is of importance in that the whole uterus must be searched for embryos in their early uterine existence.

(iv) All Ovum and Embryo Losses in Early Pregnancy.

Apparently many factors have tended to increase or decrease embryonic losses, and, while flushing, at least at the ovum stage, was associated with an increased rate of loss, the eventual total loss was less under this practice. (See Graph III & Appendix A). The former point explains the increases in ewes returning to the ram under flushing (observed also by Wallace (1951)), and the latter is in agreement with the data of Polovceva et al (1938), occurring in spite of the increased proportion of ewes having multiple ovulations. Doubtless this may explain, in part, the lambing rate improvements which have been recorded as due to flushing in the past, (Marshall, 1908; and Nichols, 1924, and many others.)

In actual ewe flocks, total losses of embryos which occur up to the equivalent of Stage III of this experiment, would be concealed in those ewes which eventually conceived to subsequent matings, while the apparent magnitude of loss in potential lambs from unlimited matings is reflected in the figures for partial losses in ewes still pregnant at 20 days post coitum. Real losses of ova and embryos, considering both the partial losses at Stage IV and the total losses for each required mating, are therefore considerably higher than is generally apparent from a study of mere lambing records, and there are few references of a descriptive nature in the literature on sheep reproduction which throw light on this subject.

That mating at more than one heat period may be required for highest flock fertility is evident from the above figures for the extent of loss which occurred in this flock after only one mating for each ewe, and Terrill & Stoehr (1939), have produced statistical evidence for the belief that the length of the breeding season (i.e. the time the rams are left out) has the greatest effect on the proportion of any flock which is barren at lambing. As flushing cannot affect this time period, and is unlikely to overcome genital abnormalities of a clinical or hereditary nature, it is unwise to infer that this practice, per se, can reduce barrenness in ewe flocks as has been done by Nichols (1924) and others.

6. GENERAL CONCLUSIONS.

1. Flushing only succeeds in raising lamb yields when ewes are in hard store condition prior to the feeding treatment. A general and readily apparent improvement in condition, and /or an increase in mean weight gains of the flock, may affect the endocrinological status of the ewes with varying individual results. Certain ewes may react by shedding more ova than they would otherwise have done, especially if ovulating later in the breeding season. This depends on the genetic potentialities of the individuals concerned.

2. Breeding behaviour, as expressed by the rate and regularity of service and the full expression of desire at oestrum, is governed by the secretion status of the ovaries and the anterior pituitary, and possibly other endocrine glands, and is benefited by high plane feeding in poor condition ewes prior to breeding, i.e. flushing. Excessive feeding of already fat ewes will not have these effects and may reverse them.

3. While successful flushing is generally accompanied by an increase in the proportion of ewes having multiple ovulations, and a general increase in the activity of both ovaries throughout the breeding season, there is also an increase in the proportion of conception failures in early pregnancy, and thus more flushed ewes return to the ram for

further matings.

4. Total or partial ovum losses up to 3 days post coitum appear to be higher under flushing than non-flushing.

5. Implantation losses are possibly reduced by flushing to some extent, although the subjective measurements used in this experiment are liable to considerable inaccuracies.

6. The proportion of ova which survive as embryos after 20 days of pregnancy, appears to be greater in flushed than in non-flushed ewes.

7. Great variation exists within any line of ewes, especially with regard to the reproductive efficiencies, and the causes of conception failure in individuals; flushing, while modifying these inherent effects in certain individuals, may not do so in others.

8. Ewes lambing singles, are not necessarily those which shed only one ovum at mating, as embryonic mortality of the order of 20% occurrs in multiple ovulating ewes which do conceive. This may be lower in flushed ewes and higher in non-flushed ewes.

9. Ewes returning repeatedly to the ram, after apparently fertile matings, are less fertile than those conceiving to one mating. These ewes are more likely to be single ovulating ewes, especially in flushed flocks.

10. Slaughter techniques following mating, offer a useful means of studying embryonic mortality, only if

slaughter is restricted to those times when losses are identifiable as to the stage at which mortality actually occurs. It is suggested that mortality is most prevalent in the ovum stage, and at the 17 - 20 day stage, although losses at implantation, which are difficult to distinguish from those occurring earlier, and sometimes losses much later in pregnancy, may be high from a variety of causes.

11. Barrenness in ewe flocks is dependent, chiefly, on the number of heats at which each ewe can be mated by fertile rams. Thus the length of time the rams are left with the ewes is important, and especially so under flushing. A few ewes will be barren at lambing due to genital abnormalities which preclude fertilisation and conception, and the only way in which flushing may decrease barrenness is by overcoming irregularities in the breeding behaviour of ewes during the breeding season itself.

12. Ewes can be successfully flushed on silage and grass, but greater differences between flushed and non-flushed ewes have been obtained using other feeds.

13. Losses of up to 40% of all ova shed normally occur after mating at various stages in embryonic development.

7. SUMMARY

1. An experiment has been described in which a study of ovulation rate and embryonic mortality was made on one group of 111 ewes flushed on lucerne silage, and on another similar group of 114 ewes fed only on bare pasture. The latter were used as controls.

2. Weight and condition changes were recorded for all ewes prior to mating, and after the rams were put out accurate twice-daily mating records were kept for each ewe.

3. Flushing was carried on for four weeks prior to the start of breeding, and for three weeks following this time.

4. All the ewes of each group were slaughtered in one of four killing stages, timed from their mating dates, and their genitalia inspected in the laboratory. Data pertaining to ovulation and embryo survival were recorded.

5. The results, and the techniques by which these were achieved, are given and fully described, and a discussion of these is also presented.

6. A survey of the results shows that,

(a) the number of ova shed/ewe was independent of weight changes in individual ewes in the pre-mating flushing period, but affected by group improvement in weight or condition in this period.

(b) Losses in ova by 3 days post coitum were higher in 22 Flushed ewes than in 22 Controls.

(c) Losses of embryos, in ewes killed between 13 and 17 days post coitum, were largely unidentifiable as to the time at which mortality occurred. Losses up to this period were greater in 14 Control ewes than in 14 Flushed ewes.

(d) Embryo losses occurring between 17 and 20 days of pregnancy were identifiable only up to 24 days. Such mortalities were similar in magnitude for the ewes of both groups slaughtered between 19 and 24 days post coitum. In ewes slaughtered later than 24 days post coitum, losses were higher in 43 Control ewes than in 42 Flushed ewes, although, being unidentifiable, they may have occurred at any stage after ovulation. It is very likely that some of these latter losses may have occurred at 17 - 20 days p.c., but were not traceable to this stage due to subsequent resorption.

(e) After one mating only, more Flushed ewes than Control ewes were non-pregnant at slaughter or returned to the ram at their next heat period(s).

(f) After one mating only, and in ewes remaining pregnant longer than 20 days, embryonic loss was greater for the Controls than for the Flushed ewes, and occurred only in the multiple ovulating ewes.

(g) Embryo losses were higher in ewes shedding two or three ova (i.e. partial losses) than in ewes shedding only one (i.e. total loss). While the former type of loss was more common in early embryo stages, the latter was more common at the ovum stage.

(h) Ovulation occurred with almost equal frequency in right and left ovaries in Flushed ewes, but somewhat more commonly in the right ovary in the Control ewes.

(i) Ewes which returned to the ram were found less fertile than those which did not. The causes of infertility have been outlined.

(j) An ovum abnormality, which precluded fertilisation and was found to be fairly common, has been described and illustrated.

(k) No case of monozygotic twinning was observed, but some ova were found, as embryos, on the opposite side of the uterus to that on which ovulation had occurred.

7. These results have been discussed in relation to the circumstances under which they were obtained and in the light of the existing knowledge of reproductive physiology and of similar data from other sources.

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PHOTOGRAPHS.

Fig. 1. An abnormal infertile ovum recovered from Flushed Ewe No. 91 at 50 hours post coitum. Note that the non-spherical, uncleaved blastomere possesses a concave depression (top right) and was lying free in an apparently degenerate vitellus. Note also the indistinct junction of the vitellus and zona pellucida and compare Figs. 2,3 & 4. Similar ova were recovered from some 12 ewes, both mated and unmated, flushed and non-flushed, in a total of ca. 300.

This type of uniconcave loose-nucleated ovum is abnormal and incapable of division.

Diagram 1. The same ovum, (see above), illustrating the points mentioned in connection with its abnormalities.

Figure 1.

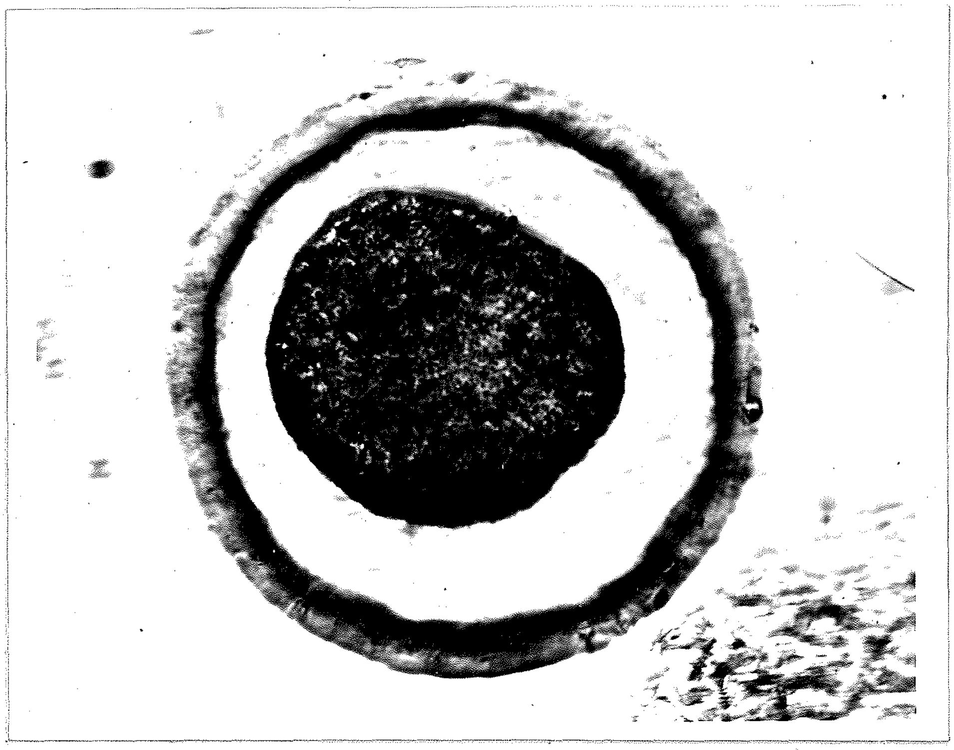
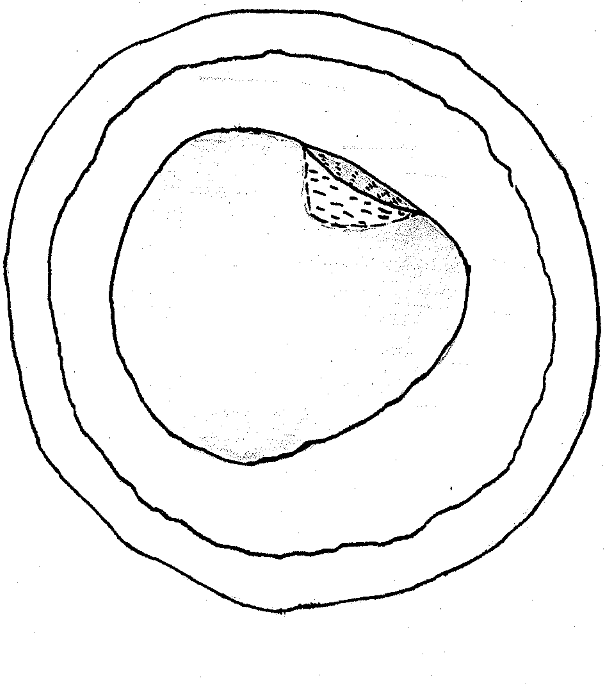


Diagram 1.



'ig. 2. A normal two-cell ovum recovered from Control Ewe No. 137 at 50 hours post coitum. The apparent inequality in blastomere size is due to distortion from pressure of the cover slip. Note the granular nature of the vitellus and nuclear protein.

'ig. 3. A normal uterine ovum recovered from a corriedale ewe at $5\frac{1}{2}$ days post coitum. The inequality of blastomere size is due, here, to the specialisation of embryo (small) and yolk (larger) cells. Most of the embryo cells are obscured in this photograph. Note the big yolk granules in larger cells.

Figure 2.

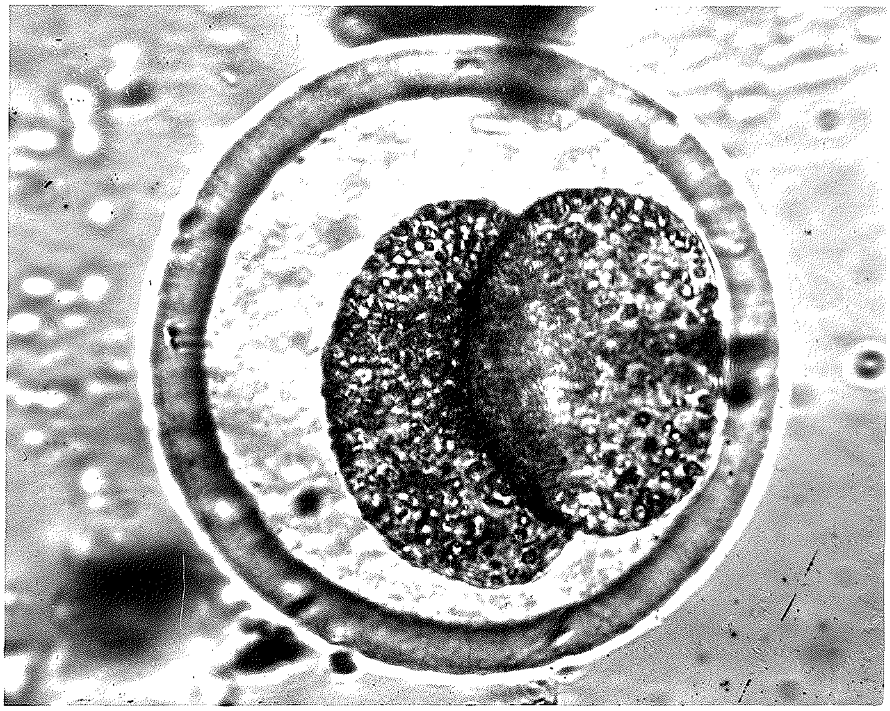
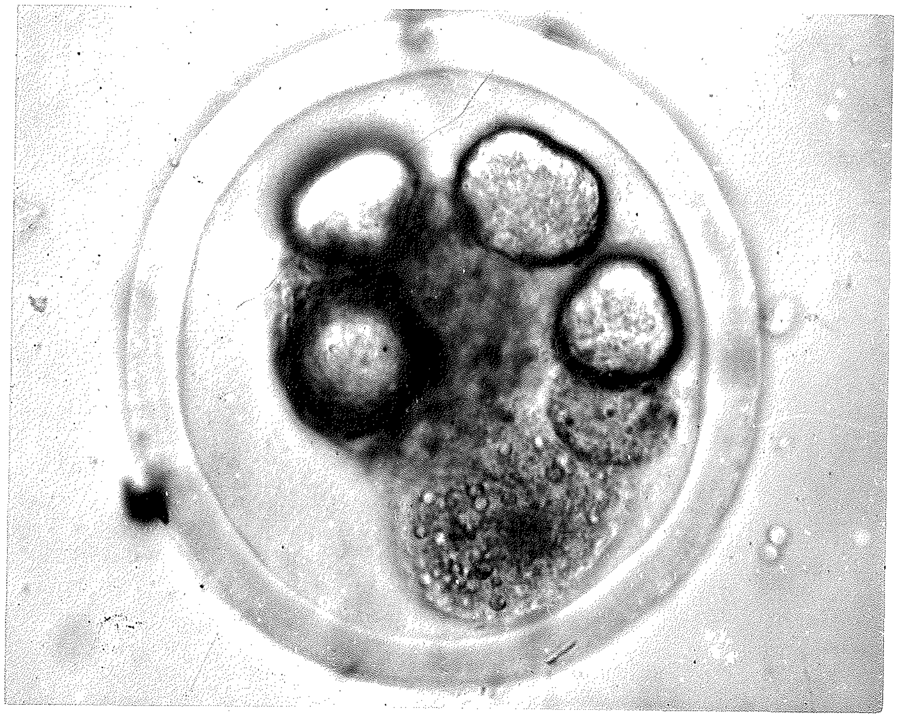


Figure 3.



<u>Fig. 4.</u> An early blastula stage ovum, recovered ex utero from a southdown ewe at 7 days post coitum, which has begun to degenerate. Note the presence of cellular detritus within the ovum itself, probably originating from the ruptured blastocoele wall. Note also the condensation and shrinkage of cytoplasm of the zona pellucida preparatory to its rupture. (Compare Figs. 2 & 3.)

<u>Fig. 5.</u> A dead blastocyst recovered at $9\frac{1}{2}$ days post coitum from a corriedale ewe. Note absence of the zona pellucida and evident signs of degeneration, i.e. cells "flaking" and decaying. Smaller cells of embryonic disc are obscured.

Figure 4.

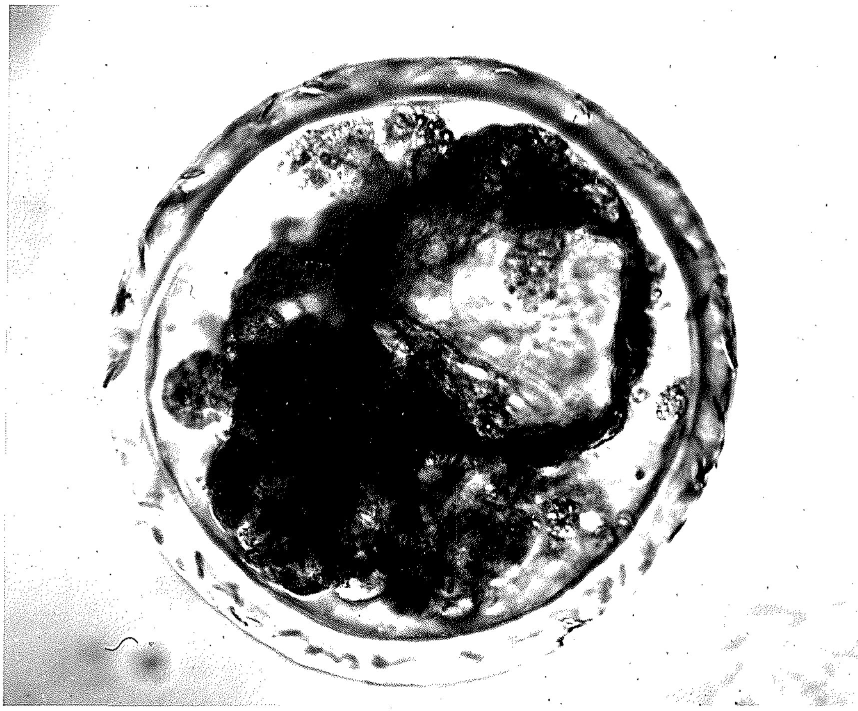


Figure 5.

Fig. 6. A normal and viable embryo recovered at 20 days p.c. from Flushed Ewe No. 25. Note large allantoic growth and its blood vessels (at top of photograph). Note also segmentation in hind region.

Fig. 7. A 23 day old embryo, recovered viable from Control Ewe No. 200. Note flexure of embryo not apparent in Fig. 6. and presence of amnion and defined vascular umbilicus. Note also the presence of limb buds (faint whitish spots below "shoulder"). Primitive heart observed still beating 2 hours after slaughter.

Photographs, by permission, by Mr. R.C. Blackmore.

Figure 6.

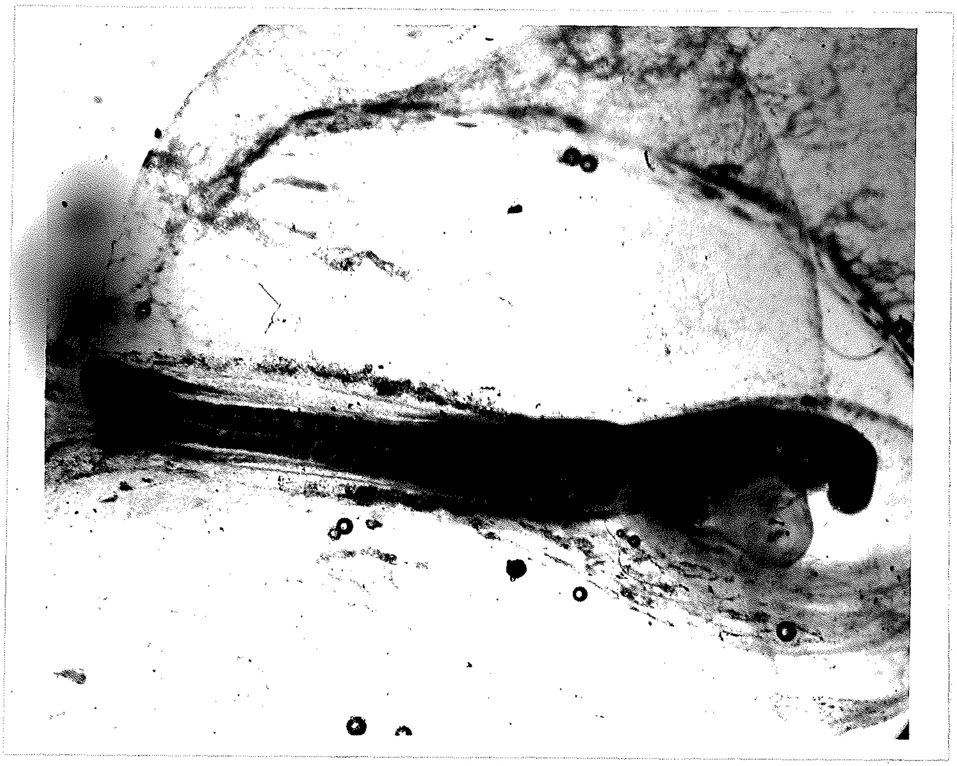
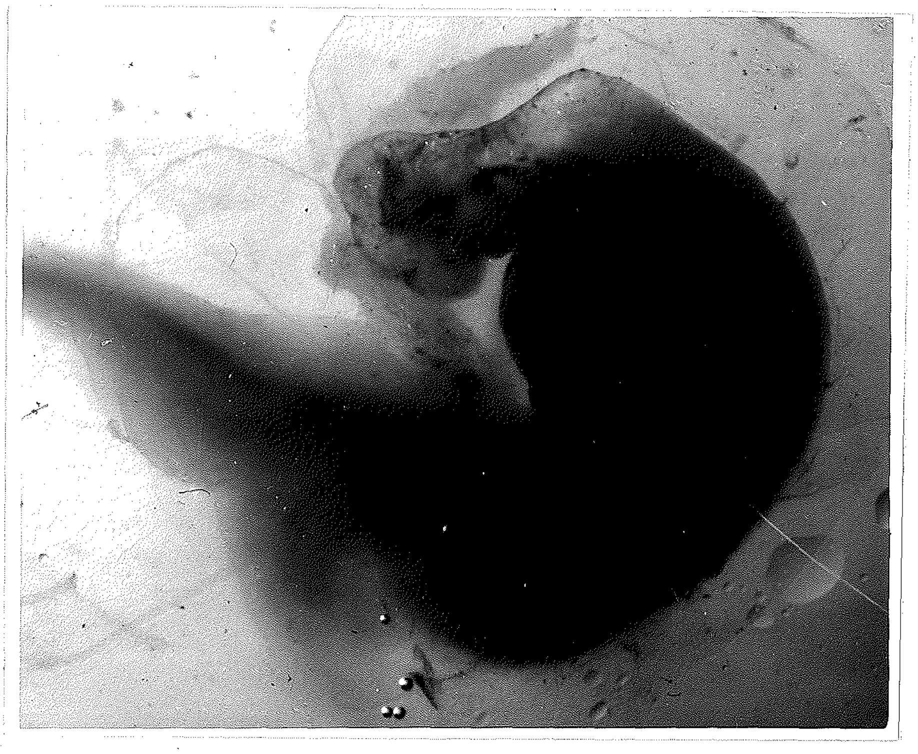


Figure 7.



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APPENDIX A

(a) Table I. % Losses by Stages and Ovulations.

Stage	Ovulations	Flushed	Control	Total
I	1	23·5%	11·1%	18·1%
	2	20·0	Nil	11·1
II	1	60·0	43·0	50·0
	2	22·2	33·3	22·2
	3	–	100·0	100·0
III	1	11·7	Nil	4·2
	2	16·8	23·3	19·8
	3	66·6	–	66·6
IV	1	Nil	Nil	Nil
	2	25·0	33·3	28·6
	3	66·6	55·5	66·6

Note: Nil loss means all ovulations accounted for as ova or embryos.
Dash means no ewes of the type considered in that particular killing.
This Table is based solely on ewes in Table X.

(b) **Table II.** **% Losses in both Groups, Observed and and Calculated at Various Killing Stages following One Mating.**

Description	Flushed	Control
Stage I (Identified)	22·2%	15·4%
Stage II (Total Observed	30·4	36·3
(" Calculated	8·2	20·9
Stage III (Identified)	8·0	6·8
Stages III & IV (Unidentified)	8·0	11·3
Later than Stage III (All pregnant)	13·2	13·6
" " " " (Multiple Ovulating) Still pregnant	19·3	23·7
Cumulative True Total Loss	38·4%	43·1%

Note: Losses underlined, being "identified", constitute Cumulative True Total Loss from ewes having one Mating. The Actual Total Losses cannot be accurately calculated as these include the unrecordable first ovulations of the ewes returning to the ram for second matings.

It is believed that the so-called Law of Cancelling Errors may apply in the method of arriving at the Cumulative True Total in as far as Stage I & II losses may be too high and Stage III losses too low for the Control ewes.

Stage II losses were only partly identified.

(c) Formula used to test Statistical significance by Student's t-test:-

$$'t' = \frac{\text{Mean F} - \text{Mean C}}{\sqrt{(S.E._F)^2 + (S.E._C)^2}}$$

Means being Arithmetic and S.E. values calculated from the following:-

$$S.E. = \sqrt{\frac{(\Sigma X)^2 - \frac{\Sigma(X^2)}{n}}{n}} \times \frac{1}{\sqrt{n}}$$

(d) The Modified (Pearson's) χ^2 test used was based on the following calculation:-

	I	II	
Flushed	a	b	a + b
Control	c	d	c + d
	a + c	b + d	a + b + c + d

$$\chi^2 = \frac{(ad - bc)^2 (a + b + c + d)}{(a + b)(c + d)(a + c)(b + d)}$$

Values of above compared with "t" & "χ^2" tables in Fisher's "Statistical Methods for Research Workers.