A STUDY OF OVULATION AND EARLY PRENATAL MORTALITY IN THE NEW ZEALAND ROMNEY EWE

A thesis presented in partial fulfilment of the requirements for the Degree of Master of Agricultural Science

by

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October, 1965

ACKNOWLEDGEMENTS

The author is indebted to his tutor Dr. M.F. McDonald for the advice, guidance and assistance given so freely both day and night for the duration of the study. Special thanks are due to Dr. F. Cockrem for advice in statistical analysis and to Dr. R.E. Munford for help in the assay of gonadotrophins.

Acknowledgements are due to Mr. C. Muir for his invaluable technical assistance; to Mr. L. Hawthorne and the farm staff; to Mr. E. Ormsby; to Mr. B. Thatcher; to Miss M.G. Campbell and her library staff; to the staff of the local Freezing Works and Abattoir; to Miss D.J. Scott for much of the photographic work; to Miss K. Christensen; and to my wife who acted in capacities ranging from heading dog to proof reader.

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CHAPTER I

INTRODUCTION

The production of a lamb is the end result of a series of events from the formation of fertile ova and spermatozoa to the independent existence of the lamb.

The main essentials for high rates of reproduction in sheep are a high proportion of multiple ovulations, successful fertilization and gestation, and a high rate of survival of new born lambs. Fertility is a qualitative term denoting ability to produce young, the quantitative equivalent being fecundity which denotes the number of young produced per individual (Asdell, 1946).

The expression prenatal mortality is used to cover all loss between ovulation and parturition being the discrepancy between the number of ova ovulated and the number of young born alive. The subject of prenatal mortality has been reviewed with reference to farm animals by Laing (1952), Casida (1953, 1956), Robinson (1957), and Hanly (1961).

In 1948 Brambell stated that there is no satisfactory estimate of the extent of prenatal mortality from fertilization to birth for any single species of animal. Estimates of prenatal death in sheep have not been numerous. Hulet, Voigtlander, Pope and Casida (1956) found 20 per cent prenatal death up to eighteen days of pregnancy, 30 per cent up to forty days of pregnancy, and Dutt and Simpson (1957) found 48 per cent by full term. Brambell (1948) estimated that 40 per cent of the ova shed were lost before parturition. He based his figures on the assumption that there is equal probability for loss of one ova if the sheep originally sheds one, two or three ova, although Hammond (1921) and Henning (1939) had shown that mortality increases as the number of ovulations per ewe rises from one to three.

A number of factors are known to effect the fecundity of the ewe. They include the breed and age of the ewe and the ewe's environment. These factors must be studied in relation to the onset of oestrus and the duration of the breeding season, ovulation, and prenatal mortality.

Factors associated with the onset of oestrus in the ewe are reviewed by de Baca, Warnick, Hitchcock and Bogart (1954). The ewe has a definite breeding season of sexual cycles followed by anoestrus, a period of sexual quiescence (Hammond, Jr., 1944). However, the ovary is not always inactive during anoestrus for spontaneous ovulations have been recorded during this period (e.g. Hammond, Jr., 1944; Roux, 1936). Normal physiological oestrus can only occur in the presence of a waning corpus luteum (Robinson, 1950). The first ovulation of the breeding season is usually unaccompanied by overt oestrus (Grant, 1934; Robinson, 1950).

The fecundity of the ewe is limited by the number of ova shed at any one oestrus. The incidence of fertilization is normally high in sheep mated to fertile males (Averill, 1955; Dutt, Ellington and Carlton, 1959; Edgar, 1962) but there is evidence that failure of fertilization can contribute considerably to total loss of fertility even among ewes mated to fertile rams (Dutt, Ellington and Carlton, 1956; Alliston and Ulberg, 1961). Hancock (1962) has reviewed fertilization in farm animals.

BREED

The date of onset of the ovine breeding season varies for different breeds (Hafez, 1952) while the duration of the breeding season may range from only one oestrous cycle, per year, in Ovis poli (Kupfer, 1928) to almost continuous breeding in Merinos and Karakuls. The duration of the breeding season of crossbred sheep is intermediate between the duration of the two parent breeds (Walker, 1943; Hammond, Jr., 1944). The onset of the breeding season is variable for different strains within the same breed (Kelly and Shaw, 1943) but there appears to be no tendency for individual ewes to be consistently early or late with regard to the onset of the breeding season (Williams, Garrigus, Norton and Nalbandov, 1956).

Breed differences in the duration of oestrus have been reviewed by Hafez, (1952). Walker (1943) gives the mean duration of oestrus of North and South Islands' New Zealand Romneys as 1.47 and 1.41 days respectively. Inkster (1953); McKenzie and Terrill (1937) and other workers have shown that the duration of oestrus is also affected by the age of ewe, stage of the breeding season and the locality. The above mentioned workers have recorded mean durations of oestrus ranging from 3 to 77 hours. Further data on the duration of oestrus have been obtained for North Island Romneys by Goot and Till; Goot (1949) found 67 per cent and 27 per cent of oestrous periods lasting respectively one and two days and Till (1950) found 50 per cent and 24 per cent lasting 24 to 48 hours and 10 to 38 hours respectively.

Breed differences in ovulation rate have been inferred mainly from lambing percentages. Data are presented by Asdell (1946) while precise estimates of ovulation rates have been obtained by Robinson (1950, 1951a) and others. Wallace (1954) has reported ovulation rates of 1.17 to 1.33 in North Island Romney ewes while Averill (1964) reports ovulation rates as high as 1.86 from Otago.

Inkster (1953) showed that there is in New Zealand a higher ovulation rate in $1\frac{1}{2}$ year-old Romney-Cheviot ewes (1.45 ova) compared with $1\frac{1}{2}$ year-old Romney ewes (1.00 ova).

AGE

Wide breed variations in age of ewes at the onset of puberty exist (Roux, 1936). The Blackhead Persian matures at about 6 months while Merinos mature at 16 to 20 months of age. There are also variations in age of ewe at puberty within a breed. The early occurrence of puberty is associated with high rates of growth but although puberty is delayed in lambs reared on a moderate plane of nutrition, these animals exhibit first oestrus at a lower body weight than high plane lambs (Allen, 1959). In the Romney breed, early born lambs may show signs of oestrus in their first autumn, but late born lambs will not show overt oestrus until the following season (Ch'áng and Raeside, 1957; Lewis, 1959). Ewe lambs and two-tooth ewes (approximately 18-month-old ewes) have a restricted breeding season characterised by a later onset and an earlier cessation of the season.

There appears to be a stage of 'adolescent sterility' in the ewe (Watson and Gamble, 1961). Alexander (1960) found that maiden ewes are less fertile than mature ewes although reproductive performance declines again with age after about the seventh year (Kelley, 1939). Edgar (1962) found a higher proportion of 18-month-old than 5-year-old ewes returned to oestrus a second time. He found the proportion of ewes with uncleaved ova was about the same in the two groups thus suggesting the difference in fertility may be accounted for by the subsequent death of a greater proportion of dividing ova in the young ewes. This could be caused by earlier relaxation of the utero-tubal junction in young ewes due to their level of oestrogen secretion being lower than that of mature sheep. The shorter oestrus (Lambourne, 1956) and less intense libido (Inkster, 1957) of young ewes lend weight to this possibility. Relaxation of the utero-tubal junction is thought to allow the ova to pass into the uterus earlier than optimum for survival (Edgar and Asdell, 1960).

Twinning and lambing percentages increase with age of ewe (Hart and Stevens, 1952; Wallace, 1958). Inkster (1959) and Edgar (1961) attribute the lower incidence of multiple births in young ewes to their lower ovulation rate compared with mature sheep. Coop (196~~4~~2) attributes this lower potential lambing percentage to a reflection of body weight for as an animal ages its liveweight increases. The effect of liveweight on fecundity is discussed in a later section. Cockrem (1965) in discussing this work of Coop suggested that a more rigorous approach to the collection and analysis of data than that used by Coop was required if the importance of a possible relationship is to be assessed. Cockrem did suggest that relationships between the ewe's pre-mating body weight and its subsequent fertility may reflect differences in weight gain over earlier periods which are critical for subsequent fertility.

MATING AND FERTILIZATION

Schinckel (1954), Radford and Watson (1957) and Edgar and Bilkey (1963) found that if rams join the ewes when the ewes are approaching their breeding season but before they experience the 'silent' oestrus which usually precedes their first oestrus, many of the ewes are stimulated to ovulate within about 5 or 6 days and come into oestrus about 17 days later. Once the ewes have commenced their own breeding cycles the presence of the ram cannot affect them in this way. Watson and Radford (1959) have demonstrated that the smell and sound of rams was sufficient to provide the stimulating influence. Direct contact with rams was not necessary. Edgar and Bilkey (1963) consider vasectomized rams are less effective than entire rams, and sometimes have no effect, in stimulating ovulation in susceptible ewes. The reasons for this apparent difference are unknown.

There are marked differences between 'two-tooth' and older ewes in the number mated (Lambourne, 1956). Inkster (1956) showed that the size of the paddock in which they were mated markedly affected the lambing percentage of young but not old ewes. A high proportion of ewes in oestrus will be mated by a tethered ram (Inkster, 1957b) although Lindsay and Robinson (1961) showed ewes had no obvious preference for tethered rams of any particular breed. Hafez (1952) found marked breed differences in the intensity of oestrus and noted that some ewes with high sex drive actively seek out rams. The intensity of oestrous behaviour is extremely variable, but there appears to be no relationship between intensity of oestrus and length of oestrus (McKenzie and Terrill, 1937). Reviews of sex behaviour in ewes are given by Asdell (1946), Robinson (1951) and Hafez (1952).

Within the breeding season there is an initial rise and then fall in the mean number of ovulations per ewe (e.g. Averill, 1959, 1965; McDonald, 1958). Work by McDonald and Averill with New Zealand Romney ewes shows the highest rates of ovulation occurred in April and May.

Breed differences in the length of oestrous cycle have been reported by Roux (1936), Hafez (1952) and other workers, while other research has not detected such differences (e.g. McKenzie and Terrill, 1937). Goot (1949) and Inkster (1953) found that approximately 90 per cent of Romney cycles fell within the 'normal' limits of 14 to 19 days (McKenzie and Terrill, 1937). The great majority of 'abnormal' cycles, longer than 19 days or shorter than 14 days, occur early or late in the breeding season (Williams, Garrigus, Norton and Nalbandov, 1956). These workers recorded that ewes late to begin the oestrous period tended to have first cycles within the normal range of 14 to 19 days while the earlier a ewe terminated the breeding season the less likely she is to have terminal cycles of abnormal length. Hammond, Jr., (1944) demonstrated a gradual lengthening of the oestrous cycle with succeeding cycles of the breeding season. Inkster (1953) recorded no such lengthening in the Romney.

Beatty (1961) has reviewed the genetics of mammalian gametes, Rollinson (1955) has reviewed hereditary factors affecting fertility generally and Johansson (1960) has reviewed the genetic causes of defects in gametes. Reviews on defective gametes include those written by Salisbury and Van Demark (1961), Hancock (1962) and Braden (1965).

The fertile life-span of the ovum is not accurately known, for accurate determination of the time of ovulation in relation to any easily detected external manifestation is, at present, impossible. All available data show the viable life of the egg is relatively short - 15 to 24 hours for the sheep (Hancock, 1962). The time of ovulation in the sheep varies considerably. Robinson (1959) concludes that it occurs about the end of heat while Asdell (1946) suggests it occurs up to 40 hours after the onset of oestrus or from 11 hours before to 6 hours after the end of oestrus.

The time taken for spermatozoa to reach the site of fertilization has been reviewed by Dauzier (1958a) and Salisbury and Van Demark (1961). Estimates based on microscopic examination can be grouped into two main classes. Firstly, where spermatozoa were found in the upper half of the Fallopian tube within a few minutes of mating (e.g. Starke, 1949) and secondly, where spermatozoa were found in the upper half of the Fallopian tube only after several hours (e.g. Dauzier 1958a).

Slow progression of spermatozoa from the cervix to the Fallopian tube of the ewe may occur for 24 hours or more after coitus irrespective of the occurrence or failure of initial rapid spermatozoan transport (Mattner, 1963b). The estimated functional life of ram spermatozoa in the female tract ranges from 22 to 48 hours (e.g. Dauzier, 1958a). Dauzier judged by the persistence of motility that spermatozoa survive better in the cervix. Thus slow continuous transport of spermatozoa to the tubes is likely to be of considerable importance in maintaining a population of viable spermatozoa within the Fallopian tubes of the inseminated ewe over a prolonged period as spermatozoa disappear relatively rapidly from the uterus and Fallopian tubes (Edgar and Asdell, 1960; Mattner, 1963a).

The cervix and utero-tubal junction comprise formidable barriers to sperm penetration so that no more than a few hundred sperm reach the vicinity of the ovum (Braden and Austin, 1954; Dauzier 1958a). The nature of these barriers and their contribution to infertility remains to be elucidated. Dauzier considers that dead spermatozoa are not transported across the utero-tubal junction. Occluded Fallopian tubes are the most common cause of permanent infertility in Romney ewes (Edgar, 1958).

Dauzier and Thibault (1959) have shown that fertilization of sheep ova can be achieved _in vitro_ with spermatozoa recovered from the genital tract of mated ewes, but not with freshly ejaculated semen, indicating that ram spermatozoa undergo capacitation in the reproductive tract of the ewe. Mattner (1963) has demonstrated that the lower limit of the time required for capacitation of ram spermatozoa in the Fallopian tube is approximately 1.5 hours. Braden (1959) concluded that degeneration of the ova after ovulation as a result of delayed sperm penetration is probably not a common cause of prenatal death of the embryo in mammals. He also states that there is little support for the contention that prenatal losses are frequently the result of degeneration of the egg before ovulation.

PHYSIOLOGICAL FACTORS

Several physiological factors are genetically correlated with fertility. These include face wool covering (Terrill, 1949; Fail and Dun, 1956), skin wrinkles (Bell, 1959), serum B-globulin types (Ashton and McDougall, 1958), birth-coat type (Schinckel, 1955) and a recessive lethal gene (Morley, 1954b). In no case is the physiological mechanism known.

Reports by Terrill (1941) and Shelton and Carpenter (1957) have shown that an excessive covering of wool on the face of sheep may be detrimental to most efficient production. Terrill (1949) has shown with Rambouillets that open-faced ewes produce 10 per cent more lambs than covered-facedewes. Heritability estimates of face covering include 0.48 by half-sib correlation (Shelton, Miller, Magee and Hardy, 1954), 0.62 ± 0.05 by paternal half-sib correlation or 0.34 ± 0.08 by intra-sire regression (Watkins, 1954).

Barton (1954) found open-faced Romney ewes clipped 0.3 - 0.7 lb. less wool per ewe but produced 14 - 26 per cent more lambs, weighing 2 - 3 lbs. heavier at weaning than ewes classified as covered-faced. Inkster (1955) reported a 0.6 lb. decrease in wool, a 15 per cent increase in lambing percentage and a 4 lb. increase in weaning weight of lambs from open-faced ewes in comparison with covered-faced ewes. Inkster found the difference in lambing percentage to be due almost entirely to the low performance of the covered-faced ewes at the two-tooth stage. Coop (1955) found a reduction of 23 per cent in lamb production per ewe mated in covered-faced ewes compared to open faced-ewes.

Cockrem (1962) reported that woolly-faced sheep were less able to adjust to the particular environment resulting from shearing than open faced sheep. Cockrem (1965 pers. comm.) found a greater proportion of ewes returning to the ram, a greater proportion of barren ewes, a greater proportion of lambs dying by three weeks of age and a decrease in lambing percentage of about 25 per cent in wool blind and eye-wigged woolly covered-faced ewes than in open-faced ewes.

ENVIRONMENT

(a) Disease:

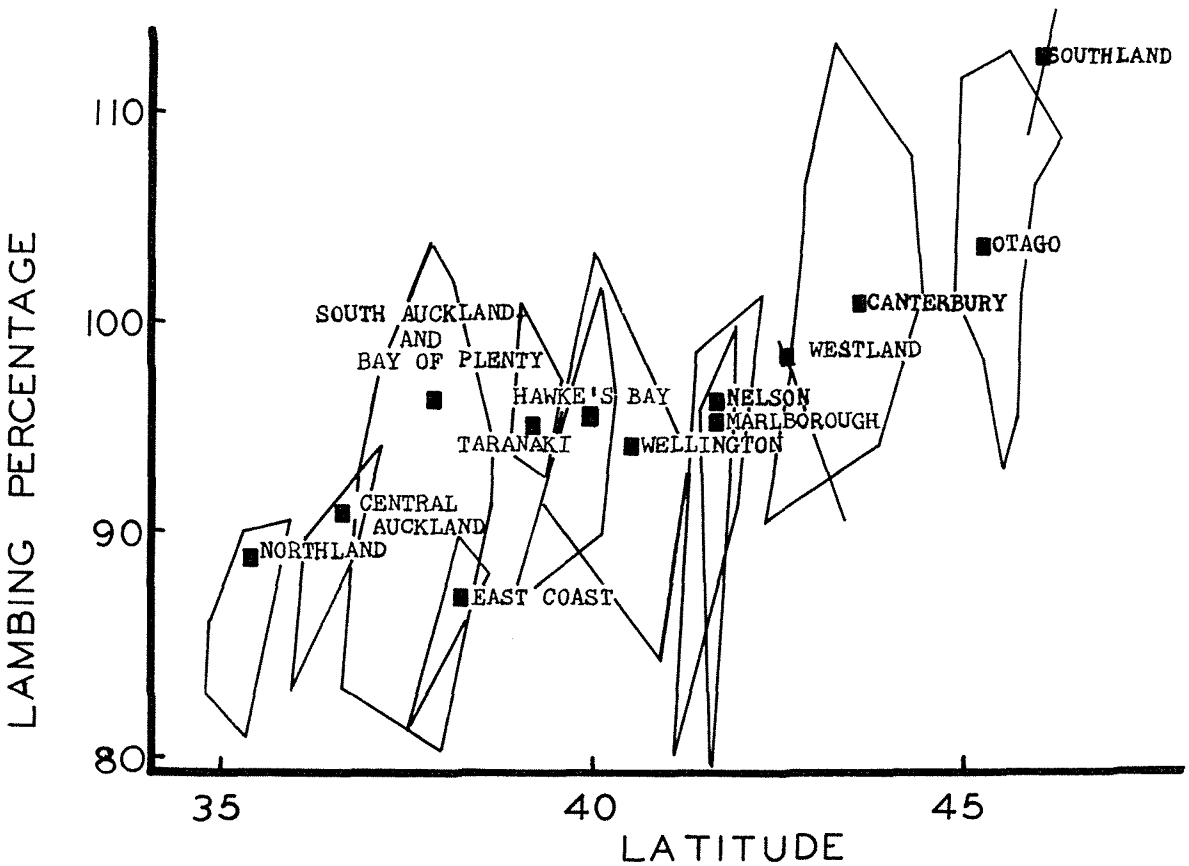
Infectious diseases that may cause embryonic loss and/or abortion include vibriosis (Seddon, 1953), listeriosis (Seddon, 1952), brucellosis (Hartley and Boyes, 1955), toxoplasmosis (Carne and Wickham, 1950), salmonellosis (Rae and Wall, 1952) and leptospirosis (Seddon, 1953). The part played by non-specific infections is not clear (e.g. Hawke, Kiddy, Wilson, Esposito and Winter, 1958). Observations made by Rowson, Lamming and Fry (1953) and Hignett (1959) suggest that variations in hormonal status or in nutritional factors might increase susceptibility to such organisms.

(b) Season and latitude:

There are three components of the environment known to play a major role in the sheep's reproduction. These components, rainfall, temperature and daylength, operate partly via direct effects on the animal and partly via

Figure 1 Estimates of lambing percentages within New Zealand statistical areas for the 1964 season compiled by the New Zealand Department of Agriculture.

■ represents the mean lambing percentage within a statistical area.


LAMBING PERCENTAGE
110
100
90
80
35
40
45
LATITUDE
SOUTHLAND
OTAGO
CANTERBURY
WESTLAND
SOUTH AUCKLAND
AND
BAY OF PLENTY
HAWKE'S BAY
NELSON
MARLBOROUGH
TARANAKI
WELLINGTON
CENTRAL
AUCKLAND
NORTHLAND
EAST COAST

plant growth thereby affecting the animal's nutrition.

Figure 1 presents the estimates of lambing percentages within New Zealand statistical areas for the 1964 season compiled by the New Zealand Department of Agriculture.

There are extreme variations present between counties within a statistical area in lambing percentages but yet there is in New Zealand a general lambing percentage increase with increasing latitude.

Reviews of the effect of latitude change on mammals have been written by Marshall (1937) and the Duke of Bedford and Marshall (1941). Marshall states that British sheep breeds when transported from the Northern to Southern hemispheres rapidly conform with the season of their new environment. This presents strong evidence that the seasonal nature of reproduction in the sheep is regulated to a large extent by the external environment. de Baca et al., (1954) cite that in Patagonia Romney ewes have a very restricted breeding season whereas ewes of the same breed in northern Argentina have a longer season. Yeates (1954) poses that control is by response to the light environment. Breeds for which a response to light has been demonstrated include the Suffolk (Yeates, 1949), Blackface Mountain (Hafez, 1952), Karakul (Eaton and Simmons, 1953) and Merino (Yeates, 1956). Hammond, Jr., (1954) points to two factors which may affect interpretation of light experiments. They are the influence of courtship, mating and paternal behaviour, and also the presumed existence of inherent rhythm. Radford (1960) also concludes that the question of photoperiodic control of sexual activity requires further clarification and suggests that factors other than lighting may play equally as important a role at least in some breeds.

Seasonal differences have been observed in the amounts of oestrogen which must be given to ovariectomized ewes following progesterone priming to induce oestrus (Raeside and McDonald, 1959; Reardon and Robinson, 1961). Lamond and Lambourne (1961) and Lamond and Bindon (1962) have shown that the interval of time after the last injection of progesterone, of a series of progesterone oestrous suppressive injections given during the breeding season, to the onset of oestrus varies with the time of year.

High temperatures may affect the percentage of abnormal ova shed but this effect is less severe when ewes are shorn before exposure to elevated temperatures (Dutt, Ellington and Carlton, 1959). In ewes exposed to high temperatures before breeding it is not known whether the deleterious effects occur before or after ovulation and fertilization (Dutt, 1963); Dutt showed that morphological abnormalities of ova can be induced by exposing ewes to high temperatures after the onset of oestrus and in some cases these abnormalities can be observed when the ewes are slaughtered three days later.

Embryonic loss and reduced foetal growth in the Romney and Merino are often attributed to high ambient temperatures (Yeates, 1956b), and this heat stress is aggravated by inadequate nutrition (Yeates, 1958). In New Zealand high humidity is often associated with elevated temperatures during the early breeding season. Stott and Williams (1962) working with dairy cows concluded that lower fertilization rate and high embryonic mortality are important factors in lowered breeding efficiency during periods of high temperature and humidity. Alliston, Egli and Ulberg (1961) by use of the ovum transfer technique have shown that apparently normal fertilized ova from ewes subjected to temperature stress may be incapable of developing in the uterus of a non stressed animal. Dutt (1963) concluded that the sheep zygote is most sensitive to the harmful effect of high ambient temperature during the initial stage of cleavage while in the oviduct.

(c) Psychological stress:

Recent work (Braden and Moule, 1964; Lang, 1964) has shown that stresses perhaps chiefly psychological in origin can induce ovulation, lead to the formation of cystic corpora lutea, and possibly cause loss of early embryos. Braden and Moule also found that during the breeding season severe stress sometimes lengthened the oestrous cycle by a few days.

(d) Nutrition:

Nutrition is an important factor affecting fecundity in the ewe. Lambing percentages are higher in flocks kept under good feeding conditions on lowland farms than in similar flocks kept under more rigorous conditions of hill or mountain land (White and Roberts, 1922). Clarke (1934) suggested that these differences are mainly due to changes in ovulation rate. Allen (1959) showed that on moderate planes of nutrition, ovulation rates were lower than in ewes maintained on a high plane of nutrition. Coop (1962) found that barrenness is relatively independent of liveweight above approximately 90 - 100 lb. but below this barrenness increases rapidly, that twinning increases approximately linearly with increasing liveweight and that lower breeding performance of two-tooth ewes in comparison with older ewes might be explained almost entirely in terms of liveweight at mating. The analysis and interpretation of data on body weight and fertility is discussed by Cockrem (1965). Nutritional aspects of fertility in the sheep have been reviewed by Schinckel (1963).

Flushing is a practice of feeding ewes an improved diet for a few weeks before mating so that they are in a rapidly rising condition when they are joined with the rams and mated. The feeding of an increased diet to ewes already in good condition and at high body weights does not constitute flushing. Many New Zealand farmers now follow a practice of feeding ewes a restricted diet after weaning in order to reduce their body weight. This reduction in body weight then allows the ewes to be flushed but this deliberate attempt to reduce the weight of the ewes is now considered a doubtful policy (Wallace, 1960, Coop, 1964).

Relatively short periods of improved feeding prior to breeding have been found to increase the proportion of ewes bearing twins (e.g. Wallace, 1953) while other workers (e.g. Briggs, Darlow, Hawkins, Wilham and Hauser, 1942) could show no consistent advantage from nutritional flushing. Hammond (1941), Underwood and Shier (1941) and Wallace (1953) all pronounced that the response to flushing is likely to be greatest when the ewes are at low body weights before flushing commences. Fertility differences have been recorded -

(1) when 'flushed' and 'control' groups have both been gaining weight but at different rates (El-Sheikh, Hulet, Pope and Casida, 1955)

(2) when 'flushed' and 'control' groups have both been losing weight but at different rates (Tribe and Seebeck, 1962), and

(3) when 'flushed' groups have gained and 'control' groups have maintained or lost weight (Underwood and Shier, 1941; McKenzie and Terrill, 1937; Allen and Lamming, 1961).

Observed differences in fecundity are due to differences in the frequency of multiple ovulation, rather than to a decrease in the number of barren ewes (e.g. Allen and Lamming, 1961; Tribe and Seebeck, 1962). Allen and Lamming suggest that ovulation rate is a function of the level of available nutrients in the diet and/or body reserves of stored energy. They found that with losses of liveweight there is a reduction of ovarian activity as evidenced by the reduction of follicle numbers and sizes. Allen and Lamming also showed that flushing did not lead to higher ovulation rates any more than by maintaining ewes in fat condition for several months. This conclusion is in agreement with Clark (1934) who showed that if ewes were in good condition ovulation rates were little affected by flushing.

If gonadotrophin is injected into ewes on submaintenance diets they show marked increases in ovulation rate (Wallace, 1954; Allen and Lamming, 1961). Wallace (1954) found the response to injections of pregnant mare serum measured by the number of corpora lutea was equal in ewes which were flushed and in ewes fed submaintenance diets while Allen and Lamming showed a greater, although non-significant increased response in the ewes fed submaintenance diets. Thus, it appears that the cause of reduced ovarian activity under conditions of low plane feeding is due to a lowered level of gonadotrophin reaching the ovaries. Normal release of gonadotrophin is under neuro-hormonal control (Harris, 1955), but the precise method by which nutrition affects the gonadotrophic release from the pituitary is still obscure.

The significance of oestrogens in pasture plants in relation to animal production is reviewed by Moule, Braden and Lamond (1963). High oestrogen levels in pasture or injections of stilboestrol may cause -

(1) in ewes infertility and maternal dystocia,

(2) in lambs increased post-natal mortality,

(3) in non-pregnant and virgin ewes uterine prolapse and lactation and

(4) in wethers may cause enlargement of the teats and lactation and enlargement of the accessory sex glands (e.g. Underwood, Shier, Davenport and

Bennetts, 1959). Ch'ang (1958, 1961) found red clover produced some symptoms of 'clover disease' in New Zealand Romney sheep. He has shown in $5\frac{1}{2}$-year-old ewes delayed onset of oestrus, late lambing as a result of delayed conception and drastic reduction in lambing percentage. Ch'ang looked for signs of dystocia, uterine prolapse, endometrial cysts and permanent sterility but no such signs were present. Cunningham and Hogan (1954) have reported lactation in virgin ewes grazing lucerne and lactation in wethers grazing pasture dominated by subterranean and red clovers. Coop and Clark (1960) reported delay in conception and reduced twinning percentages in ewes that grazed lucerne prior to and during the mating period. The factor responsible was not identified but was presumed to have been an oestrogen.

The possible effects of nutrition on the process of fertilization, implantation and early growth of the foetus are not known, but it is known that the later stages of pregnancy can be greatly influenced as the ewe becomes progressively more vulnerable to nutritional stress as the pregnancy advances (e.g. Wallace, 1948; Foote, Pope, Chapman and Casida, 1959). Especially under conditions of severe restriction of nutrient intake (Wallace, 1951; Papadopalous and Robinson, 1957) there may be decreased lambing percentages and an increased rate of ewe mortality as a consequence of pregnancy toxaemia (Reid, 1958, 1960). Important factors in this consideration include the body condition of the ewe, the availability and quality of feed, the foetal demand for nutrients, the number of foetuses, the relative competitive ability of foetal and maternal tissues for available nutrients, the ewe's capacity to mobilize reserves of fat and protein, the maternal appetite, the adreno-cortical response to nutritional stress and the presence or absence of other environmental and physiological stress (Thomson and Frazer, 1939; Underwood and Shier, 1942;

Underwood, Shier and Cariss, 1943; Wallace, 1948; Pálsson and Verges, 1952; Alexander, McCance and Watson, 1956).

Flushing has been found to advance the breeding season (Grant, 1934) but this has not been shown by other workers (e.g. Underwood and Shier, 1941; Till, 1950). Hafez (1952) and Allen and Lamming (1961) have shown that when ewes were subjected to chronic underfeeding, behavioural oestrus was suppressed but ovulation continued to occur. Quinlan and Maré (1931) and Roux (1936) working with the Merino in South Africa have shown that malnutrition in the Merino leads to a delay in the onset of the breeding season.

PURPOSE AND SCOPE OF INVESTIGATION

The purpose of this investigation was to examine further the relationships of body weight and face cover to ovulation and early prenatal mortality in the New Zealand Romney ewe. For this study the technique of ovum transfer was used whereby known numbers of fertilized ova, obtained from superovulated donor ewes, were transplanted into the reproductive tracts of synchronized experimental recipient ewes. Studies were made on the survival of transferred ova and development of embryos.

Results are presented concerning the synchronization of the oestrous cycles of donor and recipient ewes, the production of fertile ova in donor ewes, ovulation in recipient ewes and the factors which affect prenatal mortality.

CHAPTER II

MATERIALS AND METHODS

EXPERIMENTAL AREA

ANIMALS

EXPERIMENTAL PLAN

MANAGEMENT OF ANIMALS

HORMONES AND TREATMENT

RECOVERY OF OVA

TRANSFER OF OVA

FACE WOOL COVERING

(a) Face grading

(b) Wool sampling

SLAUGHTER OF EWES

OBSERVATIONS ON REPRODUCTIVE ORGANS

CHAPTER II

MATERIALS AND METHODS

EXPERIMENTAL AREA

The experiment was conducted on the 'Nutrition Block' of the Massey University of Manawatu Sheep Farm, Palmerston North. The experimental area was divided into one acre paddocks joined by a central race which ran to sheep yards. A building at the sheep yards contained the facilities for weighing sheep and for surgery.

ANIMALS

(a) Ewes

A flock of 330 Romney ewes was used in the study. One hundred and twenty-eight Romney ewes were purchased from a farm in the Wanganui hill country in February, 1964. They were culled for age (c.f.a.) ewes of 5 years of age, in store condition, some with poor teeth and others with signs of mastitis. On 3 February, 1964 these ewes were ear-tagged and weighed. The ewes were graded into 3 groups according to body weight and individually identified by branding with numbers on their sides (Table 1).

A further 30 c.f.a. ewes in good condition were added to the experimental donor flock on 20 February, 1964. These ewes were individually identified and the group was known as Donors 2 (1964 D_2). From 23 to 30 March, 1964 eleven additional ewes from another experimental Massey flock were added to be used as donors. This group of ewes, previously tagged and branded, was known as Donors 3 (1964 D_3).

T A B L E 1

GROUPS OF EXPERIMENTAL EWES USED IN THE STUDY DURING THE 1964 AND 1965 SEASONS GIVING THE RANGE OF LIVEWEIGHTS OF EWES

Season	Group	Origin	Liveweight* (lbs)	Number of ewes
1964	Heavy recipients (1964HR)	Wanganui	110 - 135 (1)	50
	Light recipients (1964LR)	Wanganui	85 - 103 (1)	50
	Donors 1 ($1964D_1$)	Wanganui	104 - 111 (1)	28
	Donors 2 ($1964D_2$)	Massey	113 - 159 (2)	30
	Donors 3 ($1964D_3$)	Massey	111 - 171 (3)	11
1965	Recipients-Tag 60 (1965T60)	Massey Face Cover flock, Tag 60.	99 - 175 (4)	51
	Recipients-Tag 61 (1965T61)	Massey Face Cover flock, Tag 61.	110 - 151 (4)	20
	Donors	Pahiatua	95 - 149 (4)	90

* Liveweights at first weighing

(1) Recorded on 3 February, 1964.

(2) Recorded on 20 February, 1964.

(3) Recorded on 30 March, 1964.

(4) Recorded on 23 February, 1965.

FIGURE 2 EXPERIMENTAL PLAN FOR 1964 SEASON

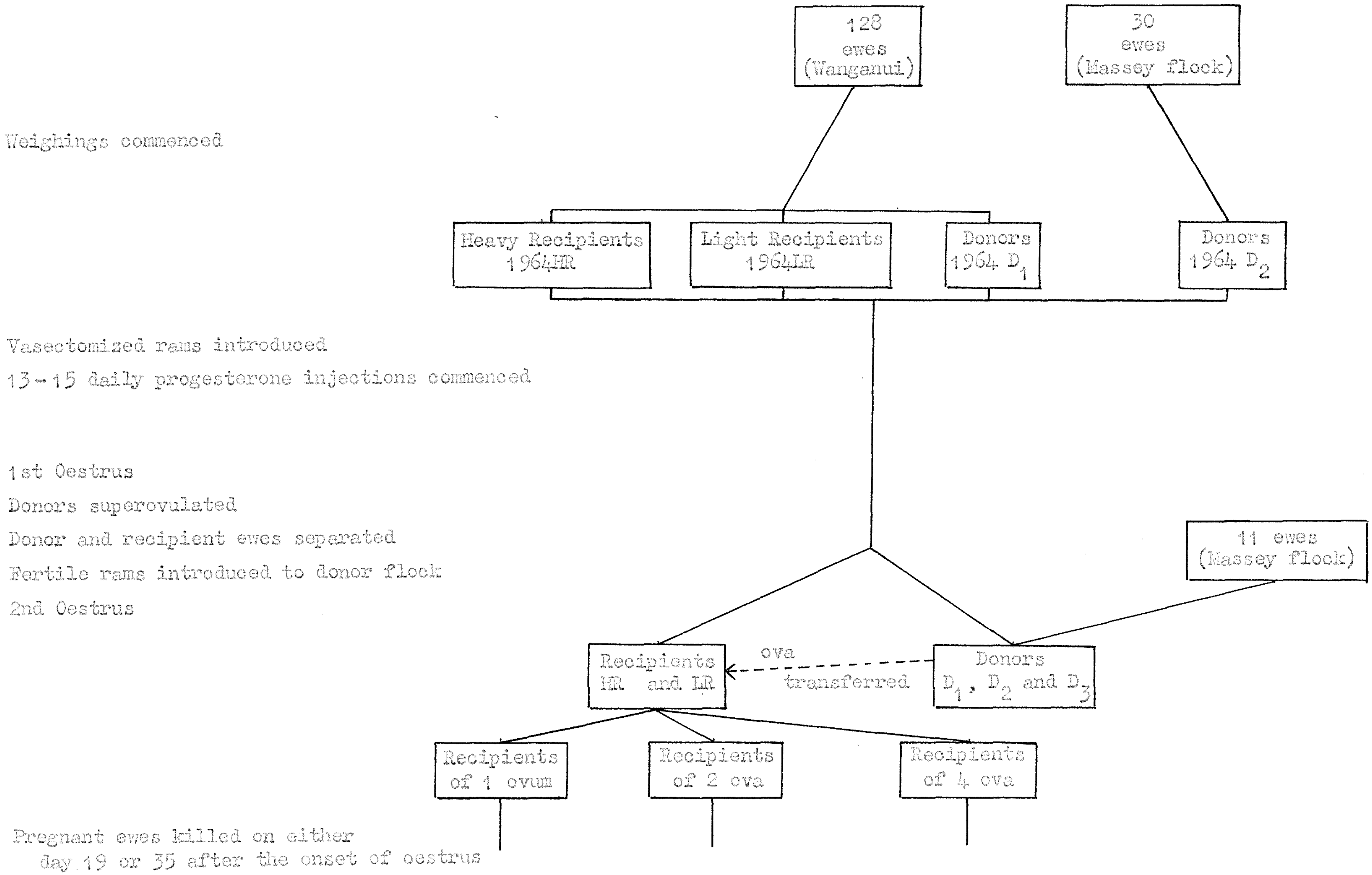
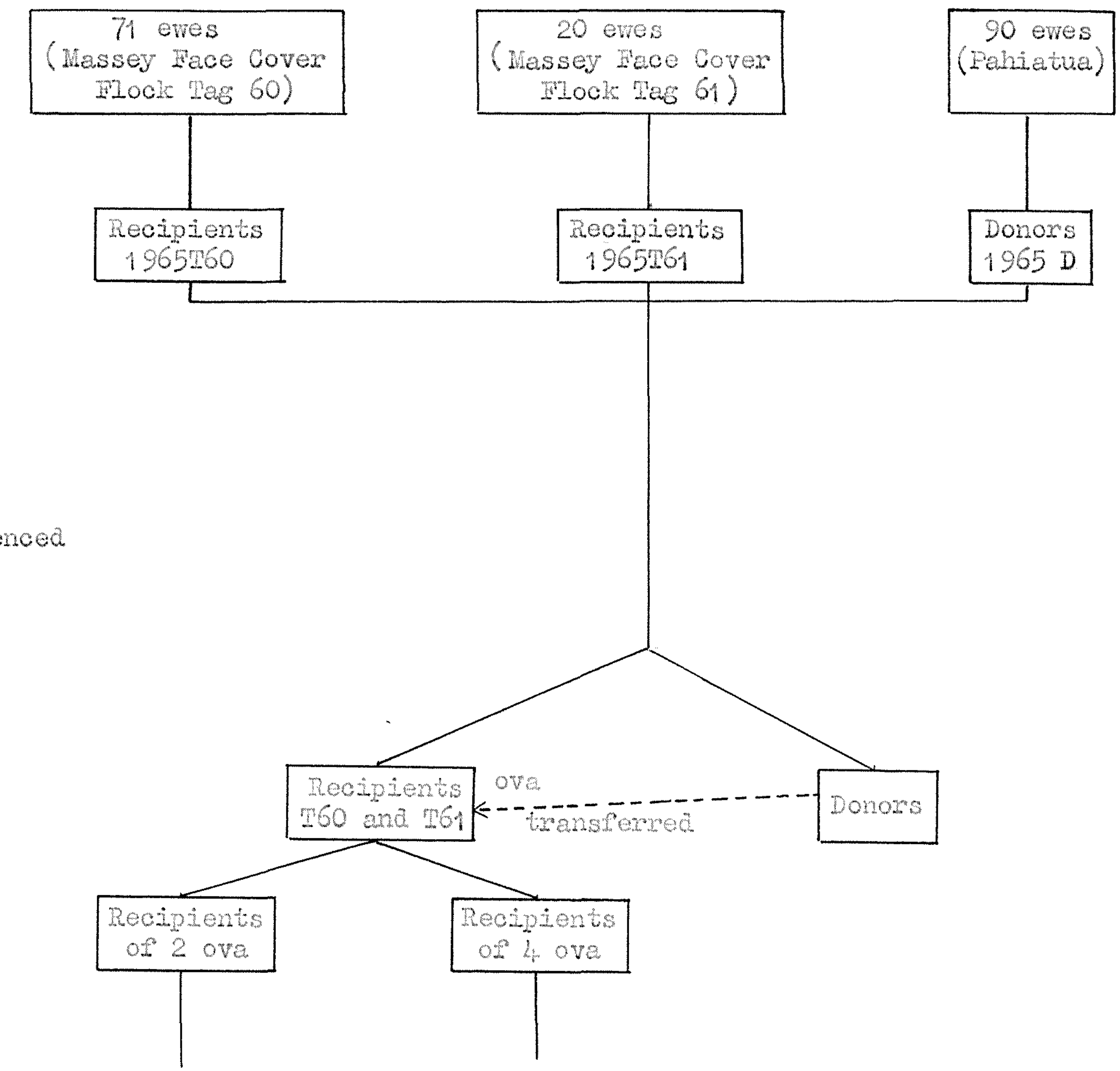


FIGURE 3 EXPERIMENTAL PLAN FOR 1965 SEASON



Weighings commenced

Vasectomized rams introduced

13 - 15 daily progesterone injections commenced

1st Oestrus
Donors superovulated
Donor and recipient ewes separated
Fertile rams introduced to donor flock
2nd Oestrus

Laparotomy

Pregnant ewes killed at day
19 after the onset of oestrus

Ninety Romney ewes were purchased from a farm in the Pahiatua hill country in February, 1965. They were c.f.a. five year old ewes in store condition, some with poor teeth and others with signs of mastitis. These ewes were ear-tagged and branded and were used as donors. Recipient ewes for the 1965 season, from the Massey Face Cover flock, were also similarly individually identified; of this flock 51 were born in 1960 and 20 in 1961.

(b) Rams

During the 1964 season four entire Romney rams of proven fertility and four vasectomized ('teaser') Romney x Cheviot rams, were used for mating the ewes. In 1965 three entire Southdown and two Romney rams all of proven fertility and four vasectomized Romney rams were used. Throughout the experimental period all rams were inspected at intervals and any requiring treatment were replaced and the affected ram treated and rested.

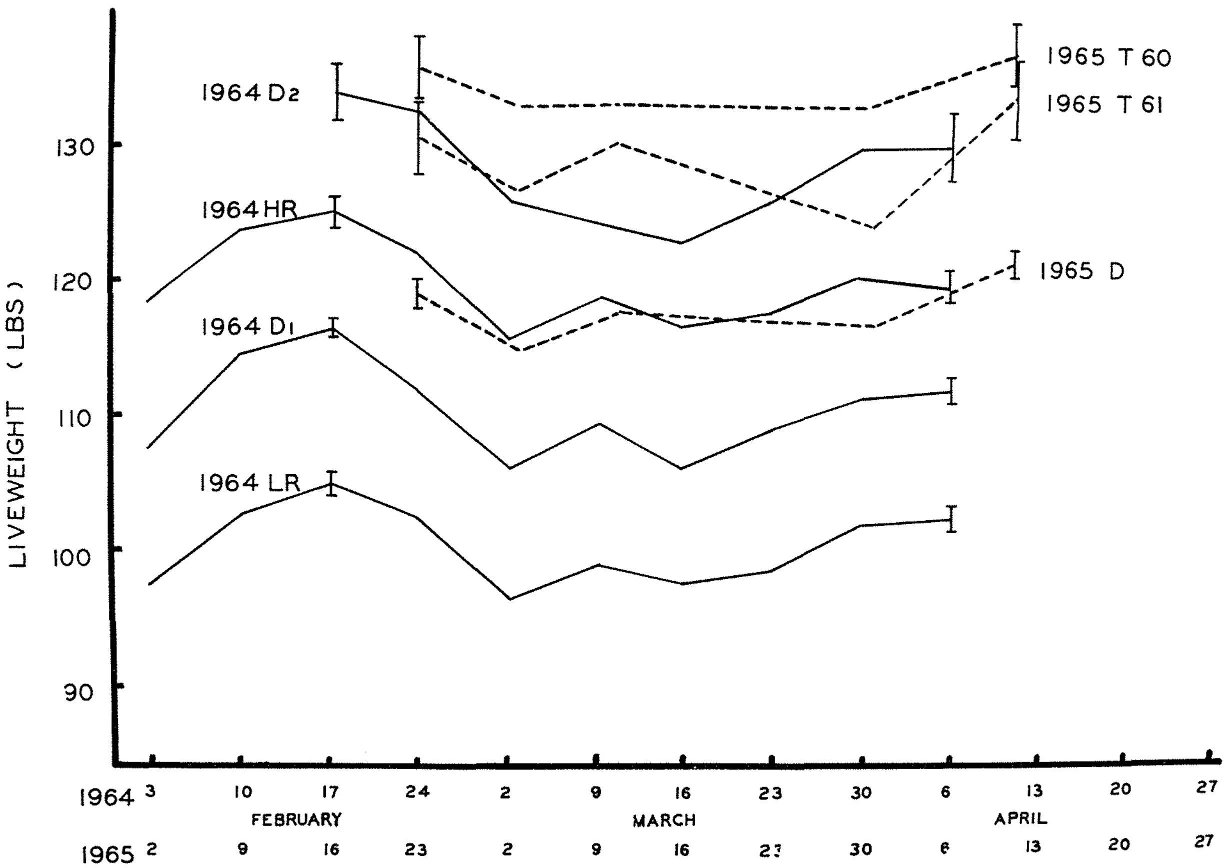
EXPERIMENTAL PLAN

The experimental plans are shown in Figures 2 and 3; further details are described in the following sections.

MANAGEMENT OF ANIMALS

All ewes were grazed on ryegrass-white clover pasture and managed to maintain constant body weight. Liveweights were recorded weekly at 9.0 a.m. in 1964 and less frequently at 9.0 a.m. in 1965. Sheep were always brought straight from the paddocks and immediately weighed. If the sheep were wet, weighing was delayed until the following day rather than waiting for the animals to dry and so weighing at a different time of the day. The liveweights of the groups of ewes during the experimental periods are given in Figure 4 and Appendix I.

Figure 4 Liveweights of groups of ewes in the 1964 and 1965 seasons showing the means and standard errors of means.



LIVEWEIGHT (LBS)
130
120
110
100
90
1964 D2
1964 HR
1964 D1
1964 LR
1965 T 60
1965 T 61
1965 D
1964 3 10 17 24 2 9 16 23 30 6 13 20 27
FEBRUARY
MARCH
APRIL
1965 2 9 16 23 2 9 16 23 30 6 13 20 27

The ewes were run with vasectomized rams and entire rams fitted with 'sire-sine' harnesses. Inspections for mating marks were made every 12 hours (8.0 a.m. and 8.0 p.m.) when only 'teaser' rams were present and every 8 hours (8.0 a.m., 4.0 p.m. and 12 midnight) when ewes were with entire rams. A ewe was recorded as showing oestrus if clearly marked over the rump and tail region. The colours of the crayons in the harnesses were changed at 12 day intervals and followed the series yellow, blue, red, green, blue, red. The yellow colour was unsatisfactory as it was difficult to see under the artificial light necessary for night observations. For this purpose the yards were illuminated by a 200 watt bulb. Each ewe was carefully examined around the rump region under strong torch light. Following oestrus excessive amounts of pigment on the rumps of the ewes were trimmed off with shears. Whenever ewes were yarded the rams were removed immediately to prevent 'rape' services.

All records were first entered in a notebook and then transferred to a record book in which the complete experimental history of any one ewe could be readily followed.

HORMONES AND TREATMENT

To synchronize the oestrous cycles of donor and recipient ewes progesterone injections were commenced over a twelve day period from 18 February, 1964 to 22 February, 1965. Ewes were given 13 to 15 daily intramuscular injections of 10 mg. of progesterone in 1 ml. of arachis oil. Each day a constant ratio (1964, 5 : 10; 1965, 9 : 7) of donor to recipient ewes was injected. Vasectomized rams were placed with all ewes from seven days prior to progesterone treatment until after oestrus had occurred or 'silent' oestrus was presumed to have occurred. Entire rams were then run with the donor ewes to allow fertile services at the following oestrus. Vasectomized rams continued to run with the recipient ewes.

In order to induce superovulation donor ewes were given a single subcutaneous injection of 500 to 1,500 i.u. of Pregnant Mares' Serum Gonadotrophin (P.M.S.)[1] on the 11, 12 or 13 day after first oestrus. For donor ewes which did not experience oestrus after progesterone treatment, an estimate of the time of ovulation was made; P.M.S. was administered approximately 12 days after this time.

RECOVERY OF OVA

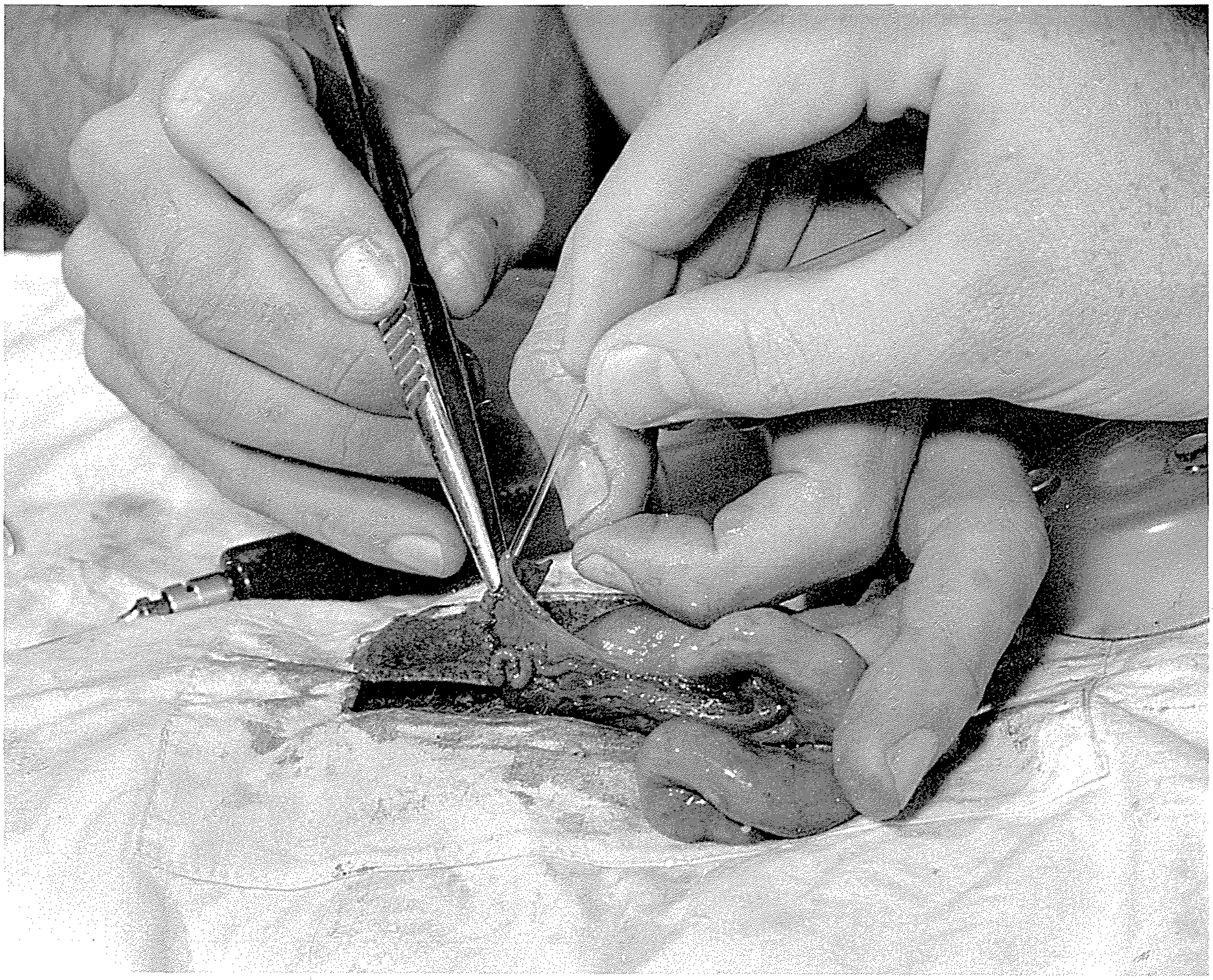
Laparotomies to collect ova from the reproductive tract were carried out 55 - 91 hours after ewes were first observed in oestrus. The animals were anaesthetized by intravenous administration of 'Nembutal'[2] alone or by a relatively smaller dose of 'Nembutal' following intravenous injection with 'Largatil'[3].

The reproductive tract of each ewe was exposed. Details and numbers of corpora lutea and Graafian follicles greater than 5 mm. in diameter on the ovaries were recorded. The number of recently formed corpora lutea was assumed to represent the number of ova shed as a result of P.M.S. injection.

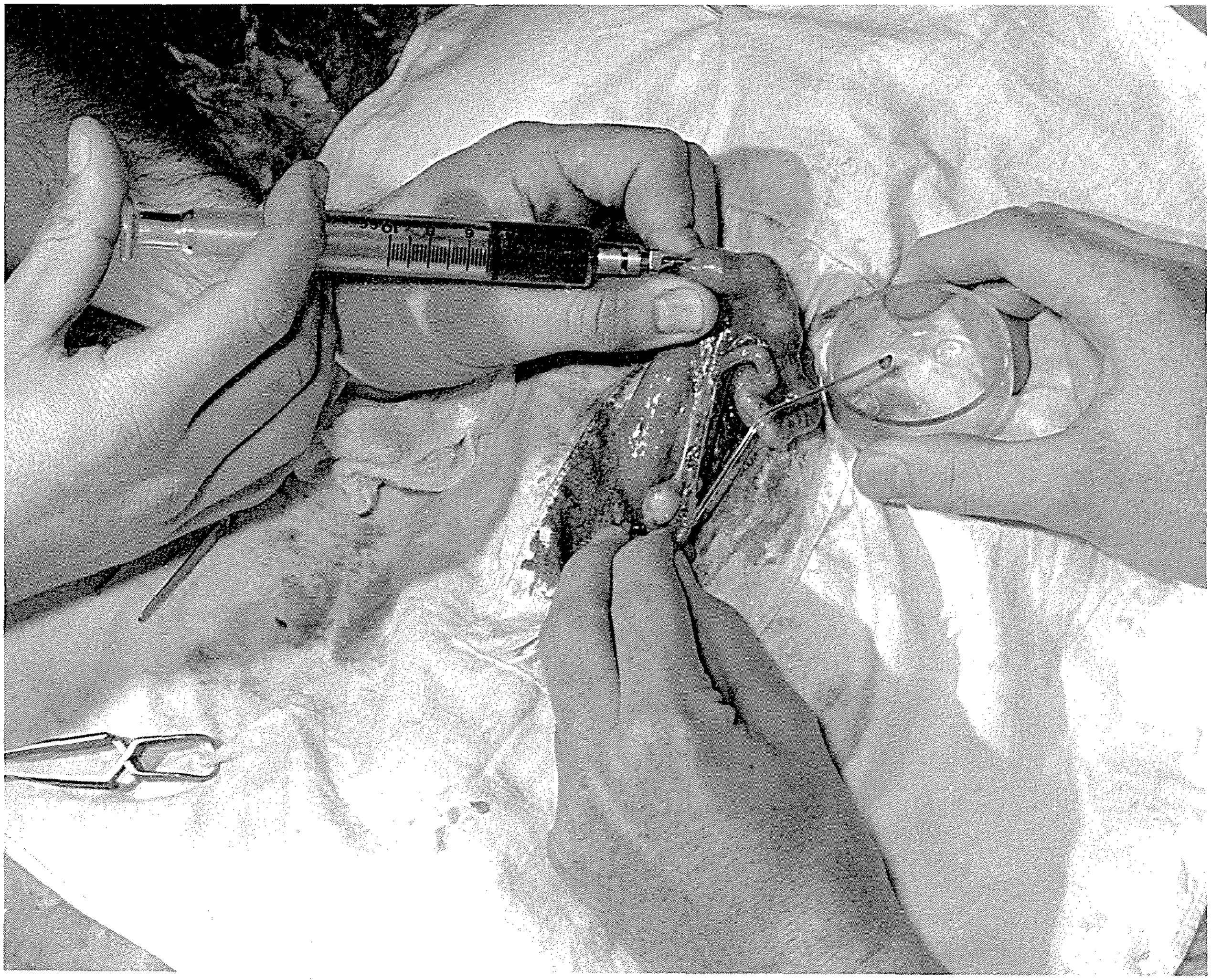
To obtain ova, sheep serum was used as the medium for flushing. Whole blood was collected from sheep at slaughter or from the jugular veins of live rams. After clotting, the serum was poured off and usually centrifuged. It was then frozen and stored. When required for ovum recovery the serum was thawed and warmed to 36°C. Unused serum was discarded.

1 Boots Pure Drug Company.
2 Pentobarbitone Sodium (Abbott Laboratories).
3 Chlorpromazine (May and Baker Limited).

Figure 5 Insertion of glass tube in Fimbria of Fallopian tube of donor ewe prior to attempting ovum recovery.



<u>Figure 6</u> Flushing serum through the upper portion of a uterine horn and adjacent Fallopian tube for the recovery of ova from a donor ewe.



Ova were recovered by flushing the Fallopian tubes and an upper portion of the uterine horns with sheep serum using essentially the same procedure as that described by Hunter, Adams and Rowson (1955). The method used is illustrated in Figures 5 and 6.

Immediately after collection the washings were examined under a binocular microscope at x35 magnification for the presence of ova. When no ova were found a further flushing of the tract was attempted.

Observations were recorded on the numbers of cleaved ova and their stages of cleavage, the numbers of uncleaved ova and the presence of spermatazoa attached to the zona pellucida and the numbers of ova that were abnormal in appearance.

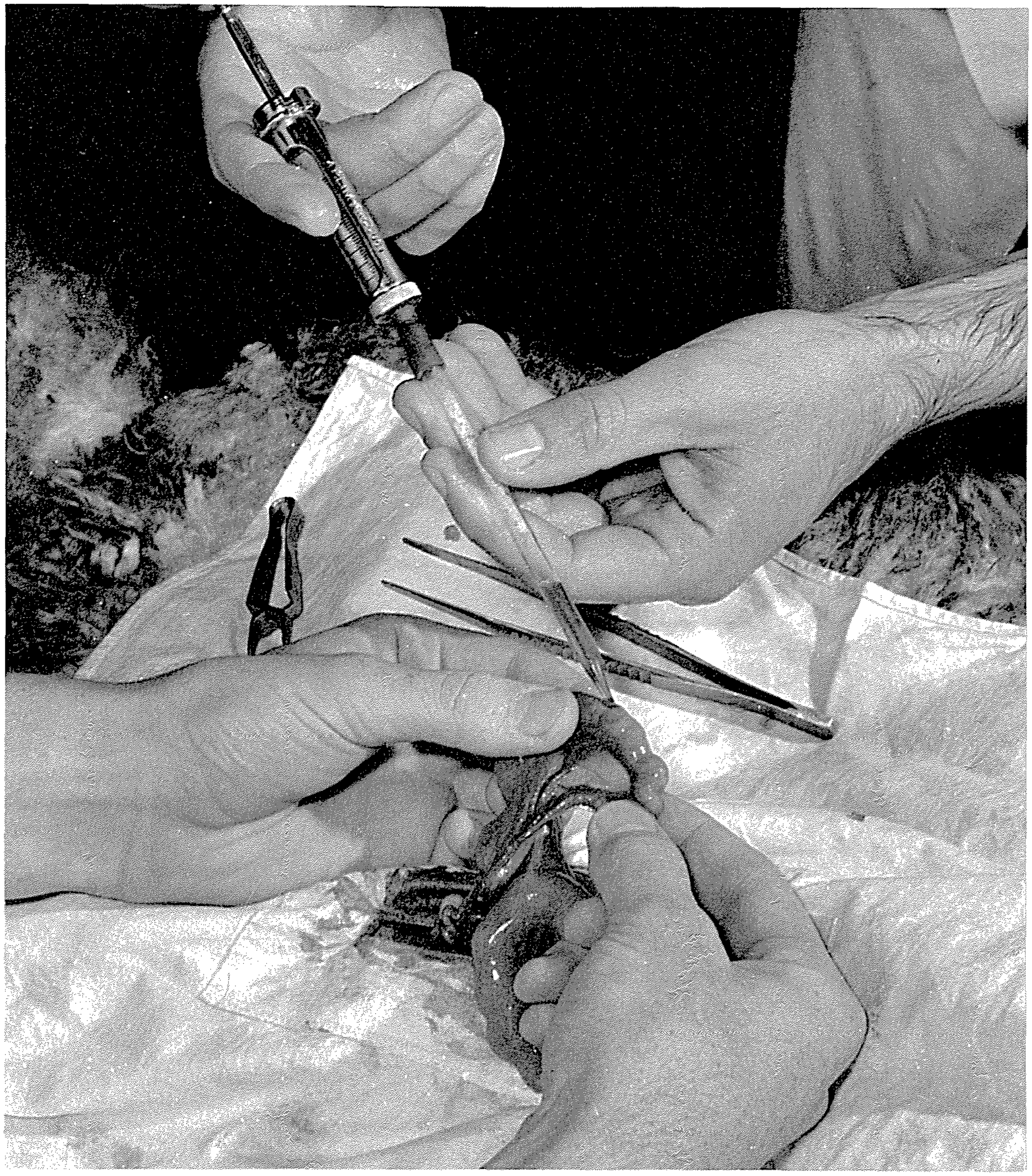
Ova were kept in glass dishes containing serum either at room temperature or in an incubator at 35°C.when ovum transfer was unlikely for at least one hour.

TRANSFER OF OVA

Laparotomies for the transfer of fertilized eggs to the reproductive tract of recipient ewes were carried out 55 - 99 hours after onset of oestrus.

The reproductive tracts of the ewes were exposed and details and numbers of corpora lutea and Graafian follicles greater than 5 mm. in diameter on the ovaries were recorded.

Ova intended for transfer were examined at x35 magnification under the binocular microscope to confirm the stage of cleavage. These were aspirated



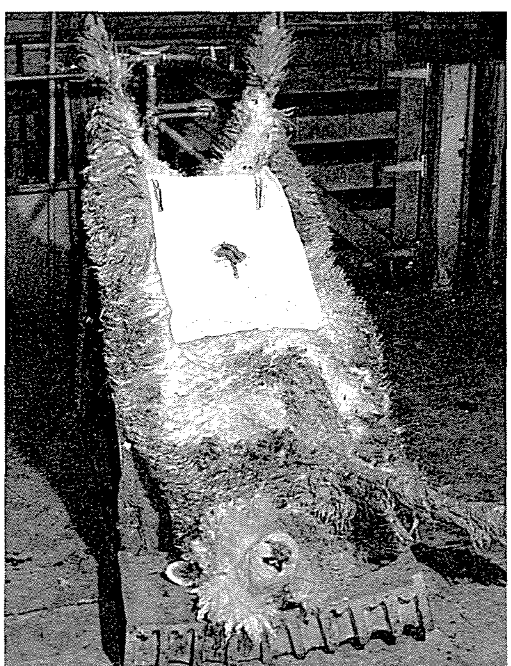


Figure 7 Transfer of ova into the lumen of a uterine horn of a recipient ewe.

Figure 8 Recipient ewe restrained in a laparotomy cradle immediately prior to transfer of ova.

into sterilized Pasteur pipettes with as little serum as possible, and then a small amount of air was sucked into the pipette to make certain that the ova remained in the pipette until the moment of transplantation.

The procedure described by Hunter et al., (1955) was then followed, (Figures 7 and 8) the ova being deposited by gentle evacuation of the syringe, either 2 - 3 cm. along the Fallopian tubes via the fimbria (2- and 4-cell eggs), or into the lumen of the uterine horns through punctures on the antimesometrial border (6- to 12-cell eggs). When two or four ova were to be transferred they were either introduced singly or two at a time. At no time were more than two ova placed in the same uterine horn or its adjacent Fallopian tube. Figures 7 and 8 illustrate the methods used in ovum transfer.

FACE WOOL COVERING

(a) Face grading

A system after Cockrem (pers. comm.) was used for visual grading of the area of the face covered with wool. A description of the face grades is as follows:-

1 maximum face covering of wool

1+

2 fairly wide face covering of wool

2+

3 moderate face covering of wool

3+

4 very open faced

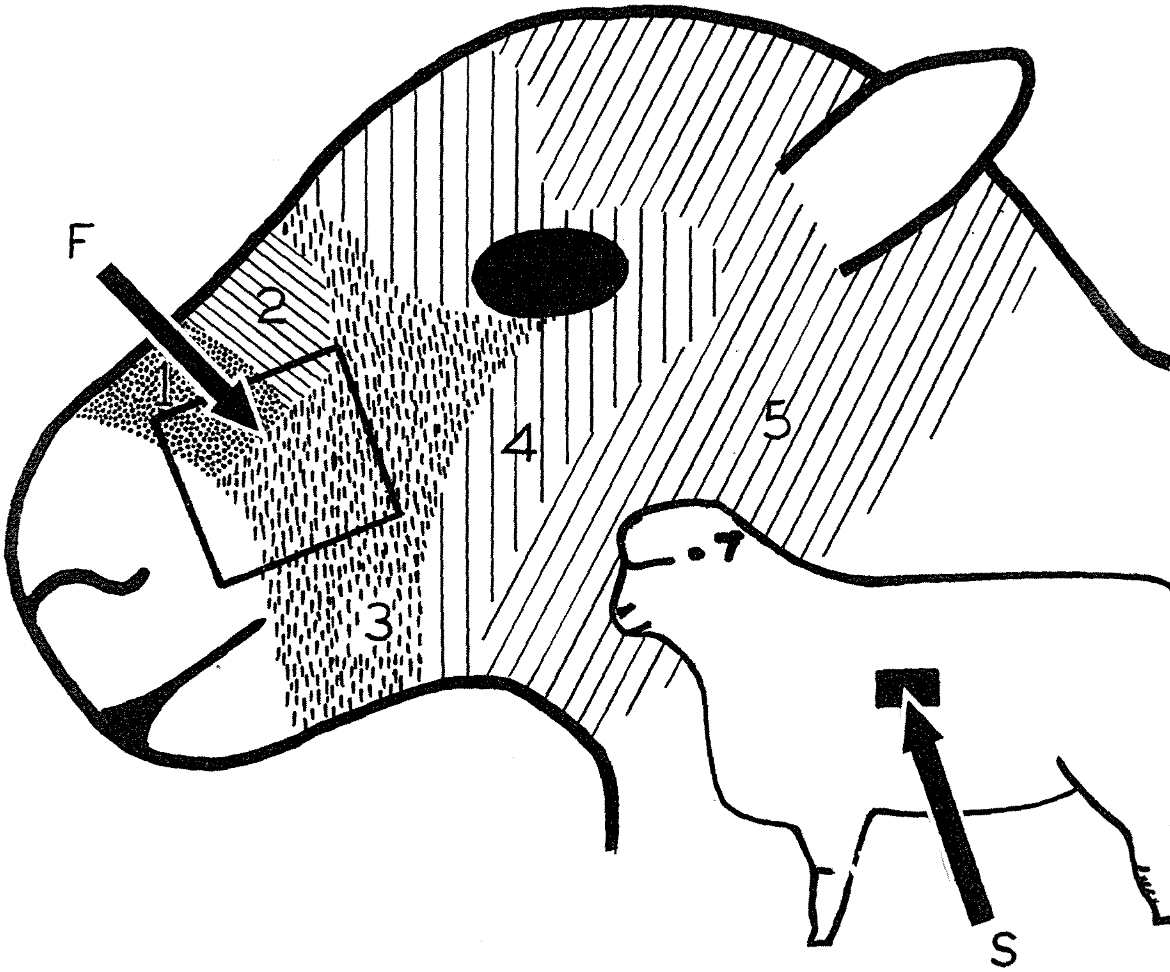
5 extremely open faced

Figures 9, 10, 11, 12 and 13 illustrate these gradings. Henceforth the visual grades will be referred to as face grades.

Figure 9 Diagrammatic representation of a sheep showing the face grade areas (1, 2, 3, 4 and 5).

F indicates the area of face from which the wool sample was taken.

S indicates the area from which the side body wool sample was taken.


F
2
1
4
5
3
S

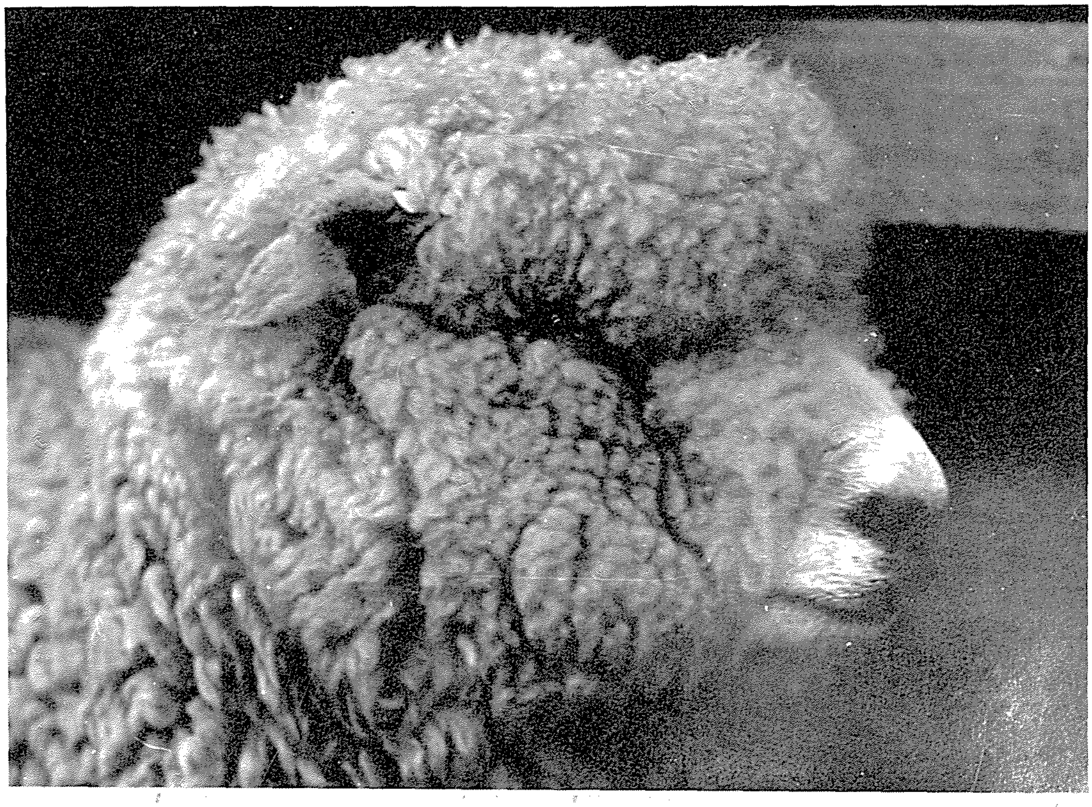


Figure 10 Example of face grade 1.

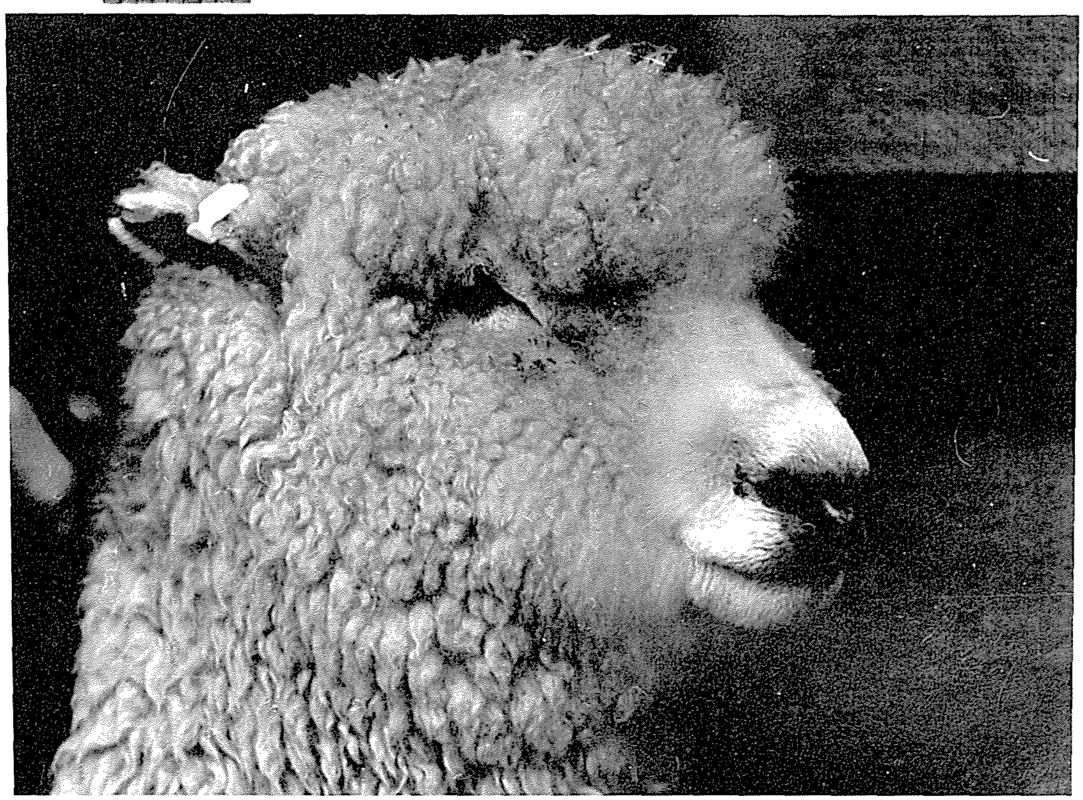


Figure 11 Example of face grade 2.

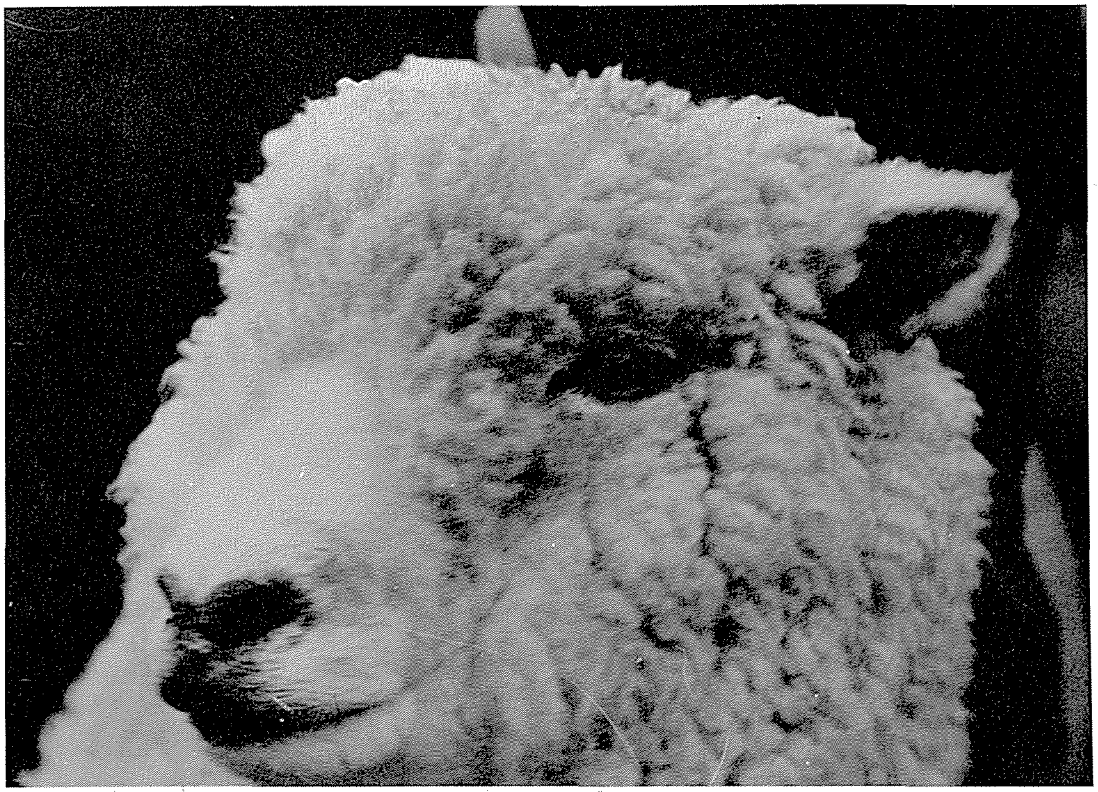


Figure 12   Example of face grade 2+.

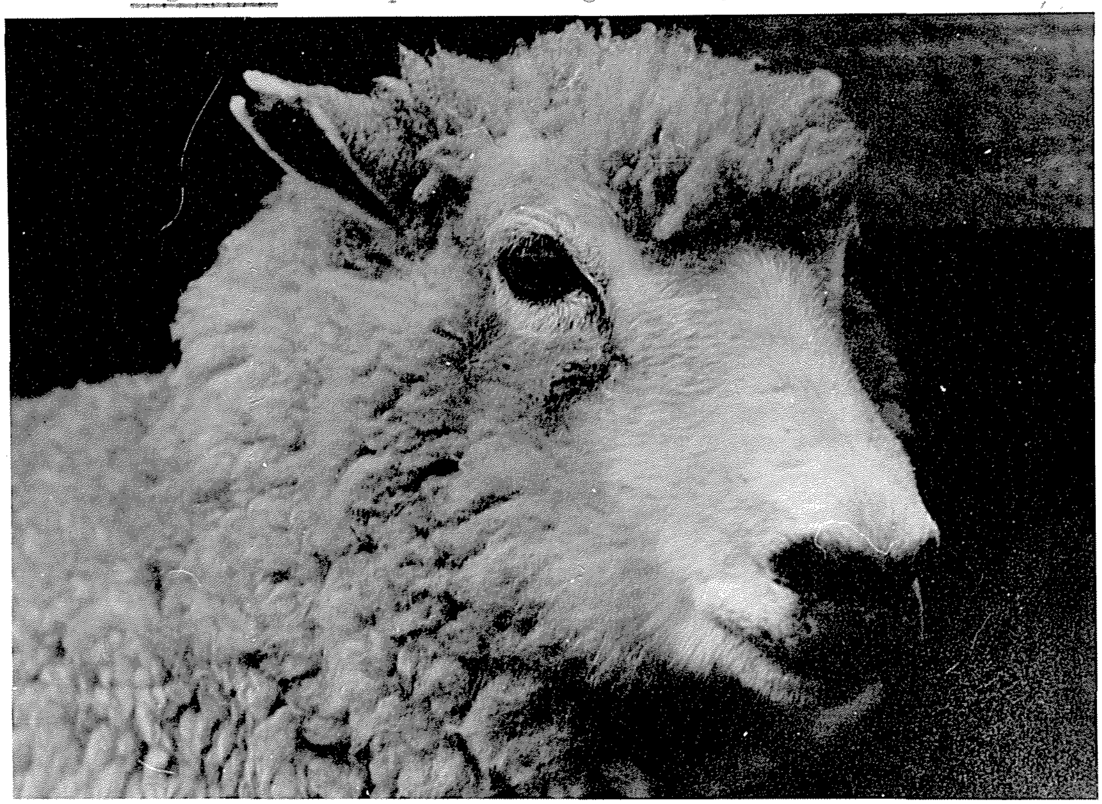


Figure 13   Example of face grade 3.

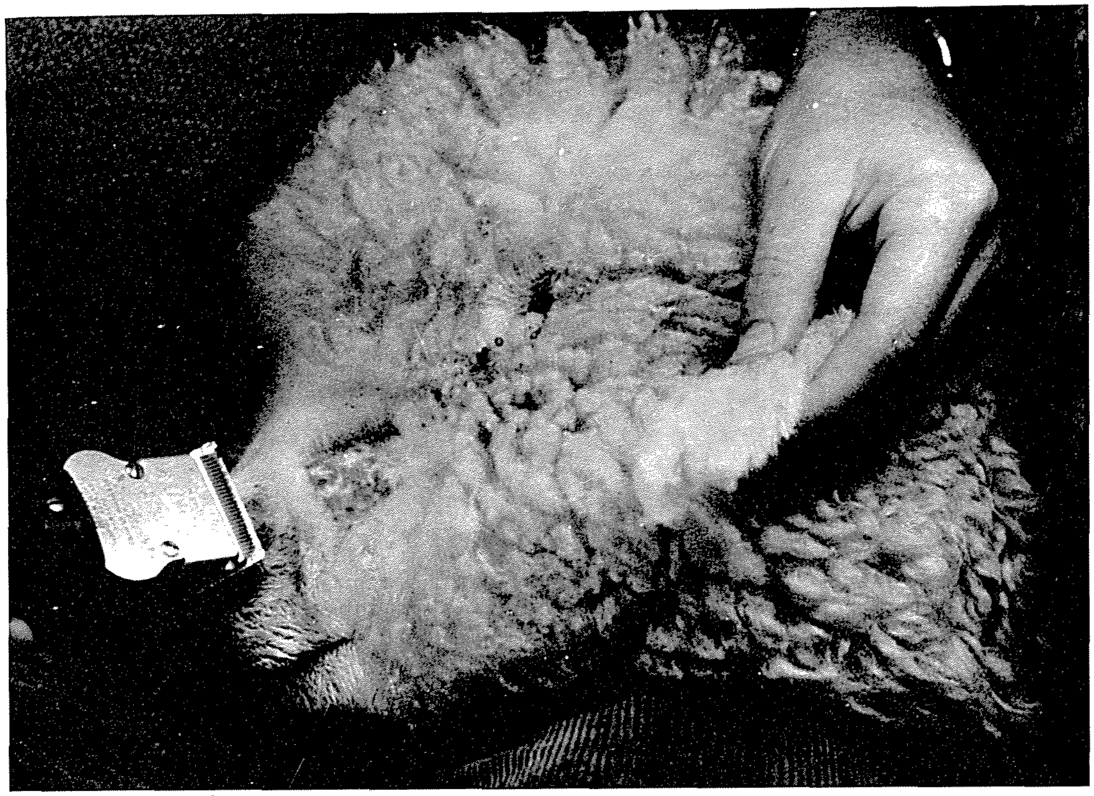


Figure 14 Removal of a face wool sample.

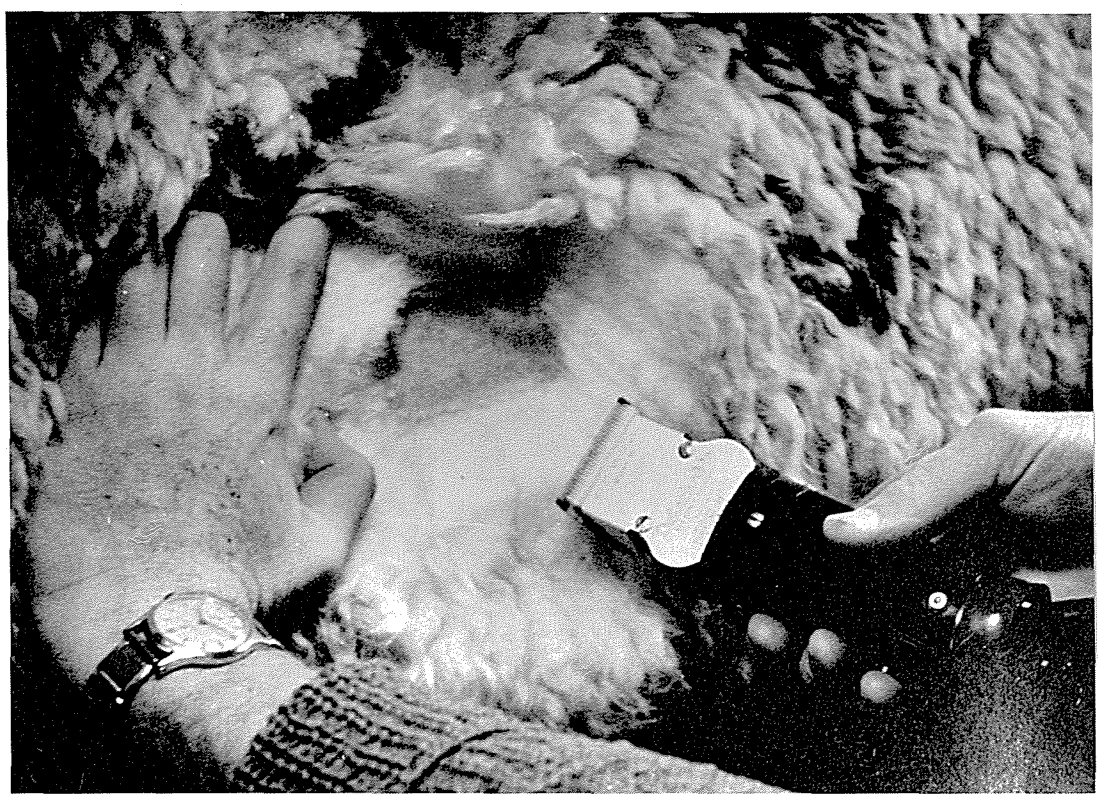


Figure 15 Removal of a side body wool sample.

(b) Wool sampling

Each sheep was held in a head stock while the operator sampled an area of wool on the face and an area of wool on the side of the sheep (Figures 9, 14 and 15).

The face sample was removed by shaving with an oster electric clipper (0000 blade) all the wool and kemp fibres from a rectangular area midway between the eye and tip of the nostril. All fibres cut were collected and placed in an envelope on which was recorded the sheep's brand, date of sampling, position of sampling and the lengths of the four sides of the area sampled. These lengths were measured with dividers care being taken not to stretch the skin.

The side body wool sample was taken by cutting a rectangular area on a mid-side level at the twelfth rib position. All fibres were collected and details of the sample recorded.

The samples were weighed to 0.0001 g. The side wool was scoured, for fifteen minutes in a solution of 'Teepol' and sodium hydroxide at about 115°F. The samples from the face were placed in muslin bags, and, with the minimum of agitation, scoured in benzol ether.

All samples were rinsed and held for 3 weeks in a controlled humidity room and then reweighed. Three calculations for each sheep were made as follows:-

Face cover = Weight of scoured wool per unit area from the area of the face sampled.

Side body wool = Weight of scoured wool per unit area from the area of the side sampled.

$$\text{Wool gradient} = \frac{\text{Weight per unit area of scoured face sample}}{\text{Weight per unit area of scoured side sample}} \times \frac{100}{1}$$

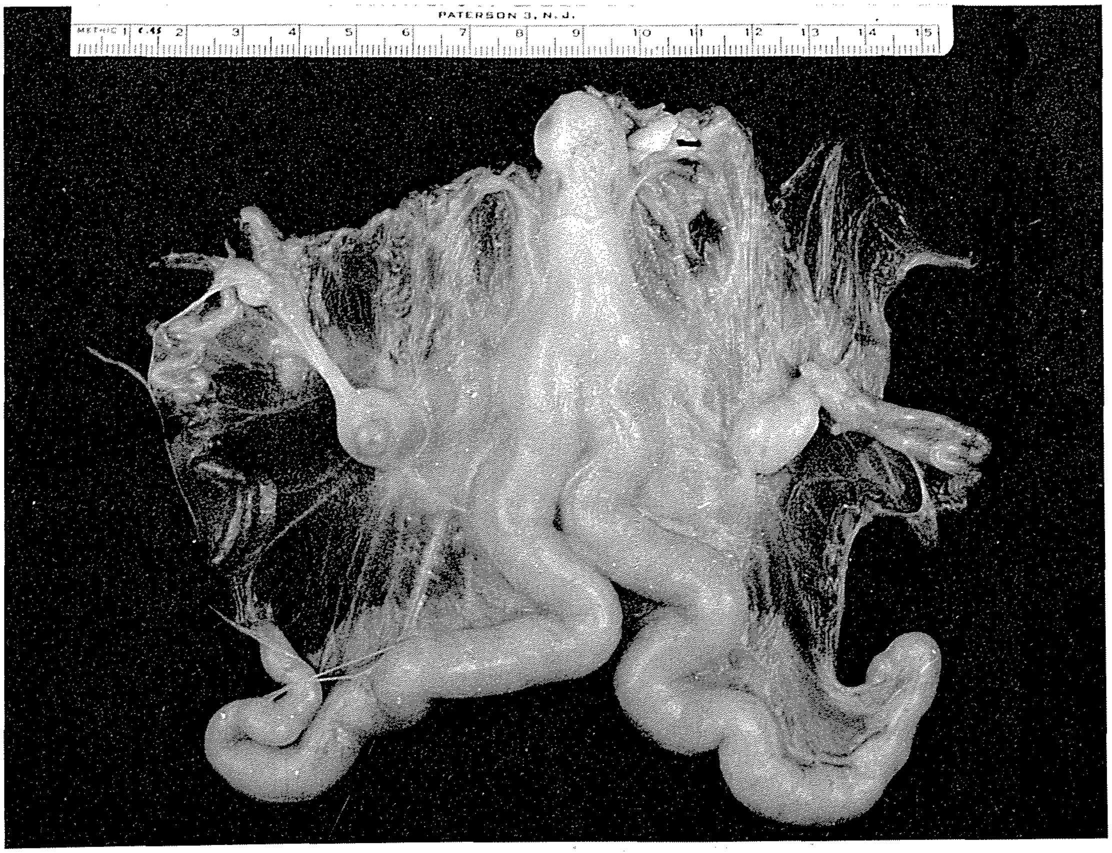


Figure 16 Genital tract of a pregnant ewe 19 days post oestrus.

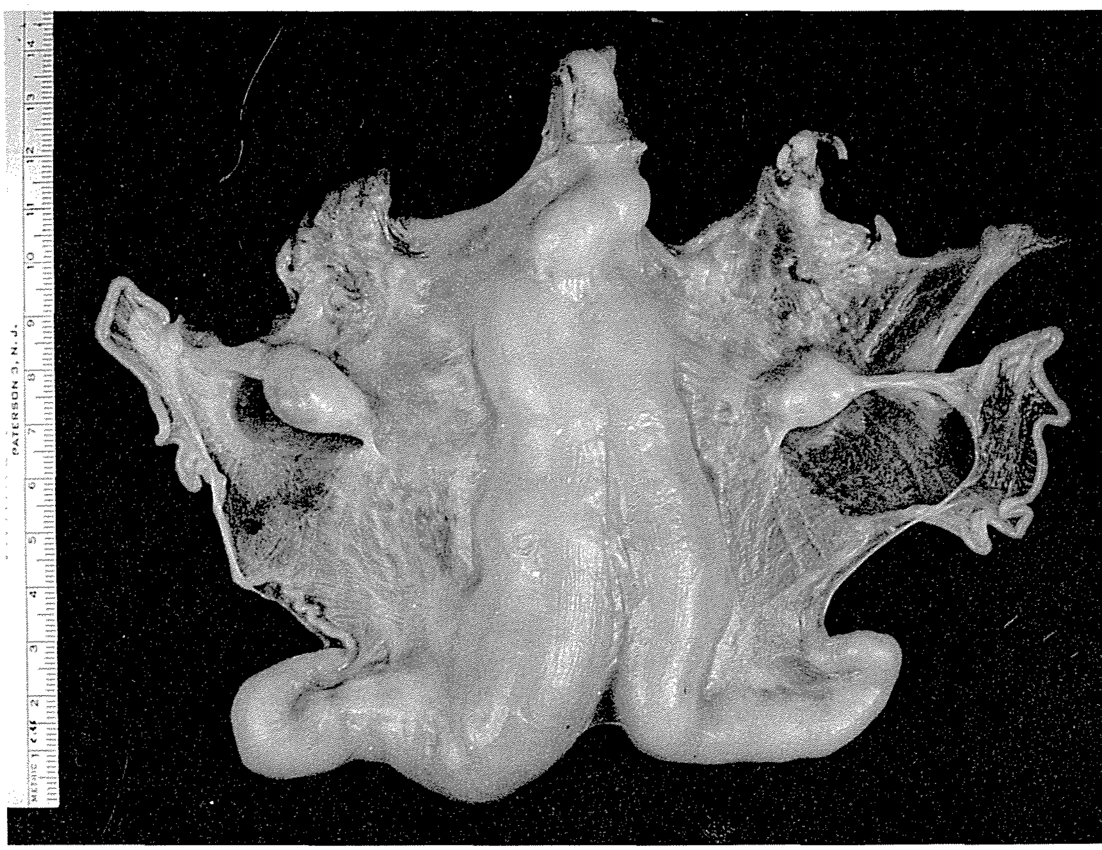


Figure 17 Genital tract of a pregnant ewe 35 days post oestrus.

Henceforth the terms face cover, side body wool and wool gradient as defined above will be used in this study. (Appendix II gives data on the relationship between face grades, face cover and wool gradient.)

SLAUGHTER OF EWES

Ewes were slaughtered at the Massey slaughter-house, the Municipal Abattoirs or a local Freezing Works. Ewes were starved for a minimum of 15 hours prior to killing. Within 15 minutes of slaughter the reproductive tracts were placed in plastic bags and individually identified. The weight of each carcase was recorded.

OBSERVATIONS ON REPRODUCTIVE ORGANS

Genital tracts were either at an age of 19 - 23 days *post oestrus* (Figure 16) or 34 - 38 days *post oestrus* at slaughter (Figure 17). All tracts were examined within six hours of slaughter.

1. Ewes 19 - 23 days after oestrus

The number of corpora lutea and large follicles were recorded. The tract was dissected to remove ovaries, vagina, external genital organs, mesenteries and suspensory ligaments.

The reproductive tract was placed on a shallow tray and the uterine horns opened with scissors. The number and position of the embryos and the relations of the embryonic membranes were observed. The embryos and membranes were carefully removed by flushing the tract with 0.85 per cent sodium chloride and then transferred to Petri dishes.

Observations were immediately made under a dissecting microscope to observe activity by the heart of the embryo. If the heart was not beating warm saline was placed about the embryo and usually this was sufficient stimulation to start heart movement. If heart movements were recorded the embryo was considered to be alive at slaughter. If no heart beat was observed the embryo could have been injured or could have suffered severe shock after slaughter before being observed. Thus a subjective assessment of the appearance of the blood in the embryo and allantois, the measurements of the embryo and its general appearance was made to decide if the embryo had been alive at slaughter.

The direct crown to rump length was measured on all embryos. The embryo was kept in its natural position without any attempt to straighten the curvature of the back. A travelling microscope was used so that measurements could be corrected to 0.1 mm.

The length of the allantois was measured with a steel caliper after having been separated from other foetal membranes.

The number of somites present was counted under a binocular microscope at x10 magnification. Embryos from four ewes were left for one day before attempting to count the somites. By this time it was impossible to count the number of somites accurately.

Any embryo without heart movement and with a crown to rump length less than 4.5 m.m. was considered to be dead.

The ovaries and embryos were stored in 5 per cent formal-saline solution in labelled bottles.

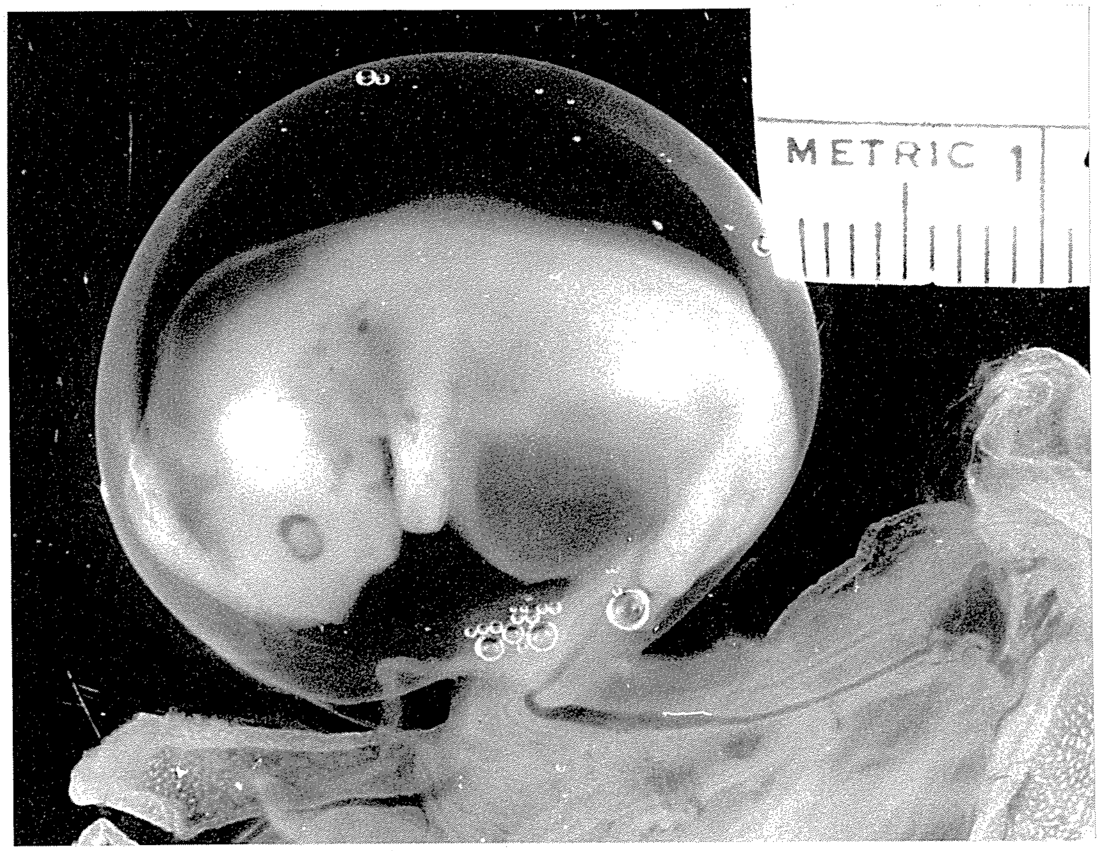


Figure 18 35 day embryo enclosed within amnion.

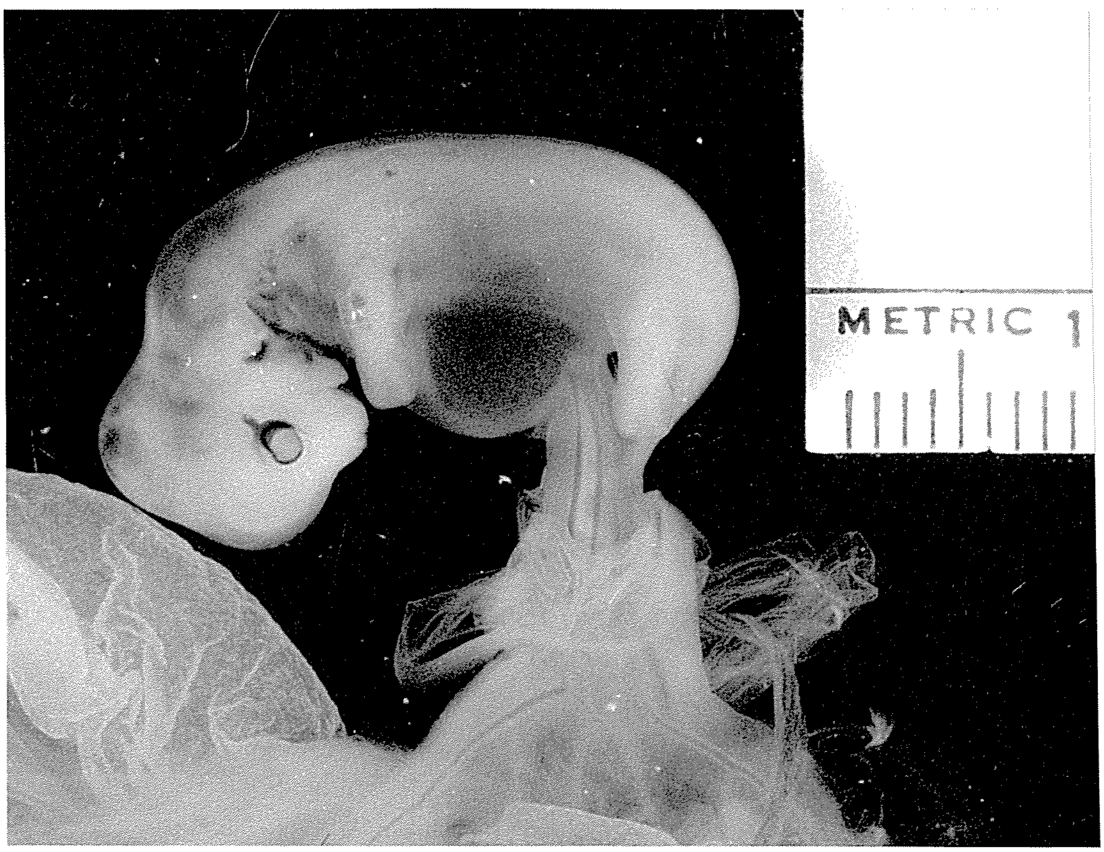


Figure 19 35 day embryo after dissection of amnion and before cutting the umbilical cord.

2. Ewes 34 - 38 days after oestrus

The number of corpora lutea and Graafian follicles were recorded. The ovaries were removed and the reproductive tract trimmed as already described. The tract was cut at the cervix 2 cm. from the uterus and at the utero-tubal junction. The uterus and its contents were weighed. After dissection the number and positions of the foetuses were recorded. All foetal contents of the uterus were then carefully transferred to Petri dishes (Figure 18). The uterus was then weighed.

The amnion was pierced (Figure 19) and the umbilical cord of the foetus severed 0.5 cm. from the navel. While in its natural position the direct crown - rump distance was measured with dividers. The foetus was weighed and its displacement measured by suspending the foetus by a piece of cotton in a 10 ml. graduated measuring cylinder half filled with normal saline solution.

The ovaries and foetuses were stored in 5 per cent formal-saline solution in labelled bottles.

CHAPTER III

STUDIES ON "SYNCHRONIZATION" OF EWES FOR OVUM TRANSFER

I. INDUCTION OF OESTRUS FOLLOWING PROGESTERONE "SYNCHRONIZATION".

II. INDUCTION OF SUPEROVULATION AND MATING OF DONOR EWES

1. Interval of time to onset of oestrus following P.M.S. injection
2. Ovarian response
3. Recovery of ova
   - (a) Number of corpora lutea
   - (b) Dose level of P.M.S.
   - (c) Time interval from onset of oestrus to laparotomy
4. Fertilization of ova
   - (a) Uncleaved ova
   - (b) Cleaved ova

C H A P T E R I I I

S T U D I E S O N " S Y N C H R O N I Z A T I O N " O F E W E S F O R O V U M T R A N S F E R

I. INDUCTION OF OESTRUS FOLLOWING PROGESTERONE "SYNCHORIZATION"

After the cessation of progesterone treatment some of the ewes did not experience oestrus. To confirm that ovulation had occurred in those ewes not experiencing oestrus laparotomies were conducted on two ewes estimated to be 5 days after 'silent' oestrus had occurred. Each ewe had one corpus luteum which from its appearance suggested it to be the result of an ovulation which had occurred approximately three days earlier. At subsequent laparotomy sheep experiencing 'silent' oestrus were found to have ovulated. This was recognised by the presence of regressing corpora lutea at a stage which appeared to be approximately 19 days after ovulation.

There was no significant difference between seasons in the proportion of ewes having a 'silent' oestrus (Table 2). There was a significant difference within the 1964 season ($P < 0.05$) between the 1964HR and 1964LR groups in the number of ewes experiencing oestrus (Table 3). After finding this significantly greater proportion of sheep showing oestrus in the group of lighter ewes (1964LR) an analysis of variance was carried out on the body weights of all ewes within the 1964 and 1965 seasons. Although the mean liveweight was greater in those ewes having a 'silent' oestrus than in ewes experiencing oestrus, this was not found to be statistically significant.

Following the last progesterone injection the interval of time until the observation of the onset of oestrus varied from 48 to 144 hours. The

TABLE 2

INCIDENCE OF OESTRUS IN EWES AFTER PROGESTERONE TREATMENT SHOWING THE MEAN BODY WEIGHT OF EWES WITHIN EACH GROUP

Season	Number of ewes	Oestrus or 'Silent' oestrus	Percentage of ewes treated within the season	Body Weight Mean $\pm$ S.E.	Body Weight Variance
1964	158	Oestrus	62.0	110.2 $\pm$ 0.12	133.2 (a)
		'Silent' oestrus	38.0	114.1 $\pm$ 0.22	179.2 (b)
1965	161	Oestrus	67.7	123.8 $\pm$ 0.13	210.6 (c)
		'Silent' oestrus	32.3	124.6 $\pm$ 0.34	311.2 (d)

Comparison of differences

Proportion of ewes in oestrus, 1964 v 1965 : Chi^2 = 1.1, Not significant.

Live weight differences within seasons (analysis of variance)

a v b : Not significant

c v d : Not significant

T A B L E 3

INCIDENCE OF OESTRUS IN DONOR EWES IN THE 1964 SEASON FOLLOWING PROGESTERONE TREATMENT

Group	Number of ewes	Oestrus or 'Silent' oestrus	Percentage of ewes treated within group
1964HR	50	Oestrus	48.0
		'Silent oestrus	52.0
1964LR	50	Oestrus	68.0
		'Silent' oestrus	32.0

Comparison of differences

Proportion of ewes in oestrus. 1964HR v 1964LR : $Chi^2 = 4.10$, $P < 0.05$

mean time of onset of oestrus was approximately 80 hours after cessation of progesterone treatment.

Table 4 shows, by means of a two way frequency table, the time of onset of oestrus in relation to the body weight of the ewe. A significant correlation coefficient ($P < 0.01$) for body weight and time to onset of oestrus after the final injection of progesterone was computed ($r = 0.38$).

T A B L E 4

INCIDENCE OF OESTRUS IN EWES FOLLOWING PROGESTERONE TREATMENT IN RELATION TO THE INTERVAL OF TIME TO ONSET OF OESTRUS AFTER THE FINAL PROGESTERONE INJECTION AND THE BODY WEIGHT OF EACH EWE

Body weight of ewe (lbs)	Time of observation of onset of oestrus after final progesterone injection (hours)									Total number of ewes	
	48	60	72	84	96	108	120	132	144	In oestrus	With 'Silent' oestrus
	Number of ewes in oestrus										
165 - 161								1		1	
160 - 156				1					1	2	1
155 - 151								1		1	1
150 - 146				1	2	2	2			7	6
145 - 141			2	1	1	1	1			6	7
140 - 136		1	1	1	3	1	1			8	3
135 - 131			6	2	2		1	2	1	14	10
130 - 126	1	1	6		8	2		1	1	20	9
125 - 121		1	5	2	5				1	14	7
120 - 116	1	3	7	6	5	5	2			29	14
115 - 111	3	1	10	6	9	1		1		31	13
110 - 106		3	13	4	1	4				25	15
105 - 101		5	9	4	3		1			22	16
100 - 96	1	3	5	5	2					16	6
95 - 91		3	1	4						8	2
90 - 86				2		1				3	2
Total number of ewes in oestrus	6	21	65	39	41	17	8	6	4		

$\hat{Y} = 62.51 + 0.1924X$

r = .38 ** Significant at 1% level.

II. INDUCTION OF SUPEROVULATION AND MATING OF DONOR EWES

1. Interval of time to onset of oestrus following P.M.S. injection

Sixty-seven ewes were injected on either day 12 or 13 of the oestrous cycle with 1,000 i.u. P.M.S. The lengths of the oestrous cycles of the ewes treated with this dose are shown in Table 5. An analysis of variance was applied to test the significance of the shorter mean cycle length of those ewes injected on day 12. As the results included data from the two seasons a test for an interaction was carried out, but was not found to be significant ($F = 1.1138$, $df = 63$ and 1, Not significant). The disproportion of numbers of ewes between each group was allowed for.

The length of oestrous cycles after injection of 1,200 i.u. and 1,500 i.u. P.M.S. on days 11, 12 and 13 of the cycle and the length of oestrous cycles after injection of 1,000 i.u. P.M.S. on day 11 were also examined. The numbers of animals in each of the about groups, in the two seasons were too few to allow meaningful conclusions to be made.

TABLE 5

OESTROUS CYCLE LENGTH FOLLOWING INJECTION OF 1,000 i.u. P.M.S. ON EITHER DAY 12 OR DAY 13 OF THE OESTROUS CYCLE

Season	Number of ewes	Day of oestrous cycle of treatment	Cycle length (days) Mean	±	S.E.
1964	14	12	15.5	±	0.28
	13	13	16.1	±	0.52
1965	37	12	15.7	±	0.12
	3	13	16.1	±	0.31

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Mean Square	F value	Significance of F value
Between days	1	2.471	59.73	$P < 0.01$
Between years	1	0.150	3.64	Not significant
Interactions	1	0.046		
Individuals	63	0.041		

2. Ovarian response

In the 1964 and 1965 seasons one and two ewes respectively were found to have cystic ovaries. The ovarian response of ewes to the various dose levels of P.M.S., dependent upon the ewes having ovulated, are shown in Table 6.

The increase in dose level of P.M.S. from 1,000 i.u. to 1,500 i.u. resulted in an increase in ovulation rate ($P < 0.01$). In the 1964 season the increase in dose level from 1,000 i.u. to 1,200 i.u. P.M.S. resulted in a decrease in ovulation rate ($P < 0.05$).

Whether the ewes had a 'silent' or an overt oestrus prior to P.M.S. injection had no significant effect on the ovarian response to the various dose levels of P.M.S. administered.

In the 1964 season a smaller proportion of the donor ewes were injected with P.M.S. on day 11 of the oestrous cycle. The ovarian response of these ewes was poor so in the 1965 season all ewes were injected on either day 12 or 13 of the oestrous cycle. Whether the P.M.S. was administered on day 12 or day 13 had no effect on the ovarian response.

3. Recovery of ova

Laparotomies were conducted on 147 ewes to allow ovum recovery to be carried out. Table 7 shows the number of ewes in which recovery of ova was attempted, the number of ewes which yielded ova, the number of corpora lutea in ewes in which ovum recovery was attempted and the percentage of ova recovered.

T A B L E 6

MEAN NUMBER OF CORPORA LUTEA FOLLOWING P.M.S. INJECTION RELATIVE TO THE DOSE LEVEL OF P.M.S. AND THE YEAR OF INJECTION

Season	Dose level of P.M.S. (i.u.)	"Previous" oestrus	Number of ewes with corpora lutea	Number of corpora lutea			
				Mean	±	S.E.	Variance
1964	1,000	'Silent' and Overt	43	3.58	±	0.36	5.52
	1,200	'Silent' and Overt	12	3.08	±	0.65	5.00
	1,500	'Silent' and Overt	3	9.00	±	5.71	97.69
1965	1,000	'Silent'	11	5.91	±	1.36	22.70
		Overt	39	4.51	±	0.48	8.90
	1,500	'Silent'	12	6.25	±	1.26	18.94
		Overt	15	10.20	±	1.52	34.93

TABLE 7

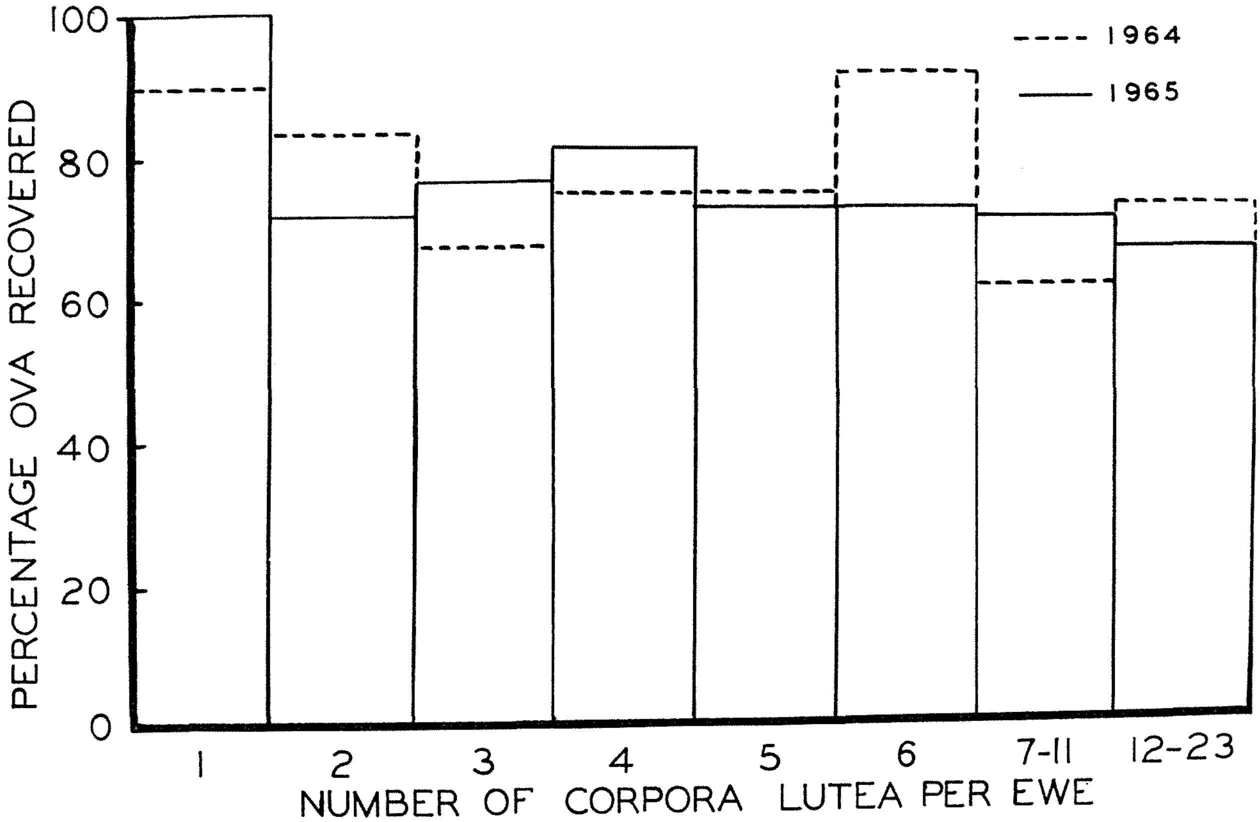
NUMBER OF EWES IN WHICH OVUM RECOVERY WAS ATTEMPTED, THE NUMBER OF EWES WHICH YIELDED OVA AND THE PERCENTAGE OF OVA RECOVERED

Season	Number of ewes subjected to laparotomy	Number of ewes in which ovum recovery was attempted	Number of ewes which yielded ova	Number of corpora lutea in ewes in which ovum recovery was attempted	Percentage of ova recovered
1964	63	61	59	217	76.0
1965	84	82	79	489	71.0
Total :	147	143	138	706	72.5

Besides the 3 ewes which had cystic ovaries at laparotomy there were 3 ewes with abnormal genital tracts. In 1964 one ewe had swollen Fallopian tubes while in 1965 one ewe had blocked utero - tubal junctions and one ewe had ovaries which could not be exposed to the exterior. A further ewe in 1964 was examined when the appearance of the corpora lutea indicated ovulation had occurred approximately 8 days prior to laparotomy; ovum recovery was not attempted.

(a) Number of corpora lutea

Table 8 and Figure 20 show the percentage of ova recovered of the total number of ova shed. The percentage of ova recovered was unaffected by the ovulation rate.



1964
1965
100
80
60
40
20
0
PERCENTAGE OVA RECOVERED
1
2
3
4
5
6
7-11
12-23
NUMBER OF CORPORA LUTEA PER EWE

T A B L E 8

RECOVERY OF OVA AND STAGE OF CLEAVAGE OF RECOVERED OVA AS INFLUENCED BY THE NUMBER OF CORPORA LUTEA PER EWE

Season	Corpora lutea per ewe	Number of ewes	Percentage of Ova shed		
			Recovered cleaved ova	Recovered uncleaved ova	Ova lost
1964	1	10	70.0	20.0	10.0
	2	18	58.3	25.0	16.7
	3	11	63.6	3.0	33.3
	4	3	50.0	25.0	25.0
	5	8	70.0	5.0	25.0
	6	4	54.2	37.5	8.3
	7-11	5	50.0	11.4	38.6
	22	1	72.7	0.0	27.3
1965	1	3	66.7	33.3	0.0
	2	14	60.7	10.7	28.6
	3	16	60.4	16.7	22.9
	4	8	68.7	12.5	18.7
	5	9	48.9	24.4	26.7
	6	5	60.0	13.3	26.7
	7-11	15	37.1	34.1	28.8
	12-23	11	25.6	41.1	33.3

Figure 20 Percentage of ova recovered relative to the number of corpora lutea per ewe.

(b) Dose level of P.M.S.

The dose level of P.M.S. administered was not found to influence the proportion of ova recovered (Table 9). Of note is the relatively low percentage of ova recovered from those ewes which were injected with 1,000 i.u. P.M.S. in the 1965 season compared to ewes on the same dose level in the 1964 season. This difference was found to be not statistically significant.

TABLE 9

RECOVERY OF OVA AS INFLUENCED BY DOSE LEVEL OF P.M.S. ADMINISTERED

Season	P.M.S. dose level (i.u.)	Number of ewes	Mean number of corpora lutea	Percentage of ova recovered
1964	1,000	43	3.58	78.5 (a)
	1,200	12	3.08	83.8
	1,500	3	9.00	74.1
1965	1,000	50	4.82	68.1 (b)
	1,500	27	8.44	74.7

Comparison of differences

Percentage of ova recovered, a v b : Chi^2 = 0.827, Not significant.

(c) Time interval from onset of oestrus to laparotomy

Operations on donor ewes were timed from 55 to 91 hours after the onset of oestrus. Operations were timed so that ova would still be in the Fallopian tubes and preferably at the 8-cell stage or older. Table 10 shows that there was no decrease in the percentages of ova shed which were recovered as the interval from the onset of oestrus to laparotomy increased.

TABLE 10

RECOVERY OF OVA AS INFLUENCED BY THE INTERVAL OF TIME TO LAPAROTOMY AFTER ONSET OF OESTRUS

Season		Interval of time to recovery of ova after onset of oestrus (hours)						
		55-60	61-66	67-72	73-78	79-84	85-90	91-96
1964	Number of ewes	12	17	13	17		1	
	Percentage ova recovered	73.1	72.6	79.7	72.6		83.3	
1965	Number of ewes	3	9	13	29	22	5	1
	Percentage ova recovered	70.4	88.3	78.8	58.3	74.4	70.6	100.0

4. Fertilization of ova

(a) Uncleaved ova

A large proportion of ova (34.4 per cent) obtained at laparotomy were uncleaved thus reducing the number of ova available for transfer to recipient ewes. Table 11 presents the numbers of cleaved and uncleaved ova recovered in the 1964 and 1965 seasons. A greater proportion of ova recovered in the 1965 season were abnormal or uncleaved ($P < 0.1$).

TABLE 11

NUMBERS OF UNCLEAVED AND CLEAVED OVA RECOVERED IN THE 1964 AND 1965 SEASONS

Season	Ova recovered	Cleaved ova recovered	Abnormal or uncleaved ova recovered
1964	165	134	31 (a)
1965	341	198	143 (b)
Total	506	332	172

Comparison of difference

Proportion of uncleaved ova or abnormal ova recovered, 1964 v 1965

a v b : $Chi^2 = 3.00$, $P < 0.1$.

Over the two seasons only 16 ewes gave both cleaved and uncleaved ova at recovery. From only 9 ewes were cleaved and uncleaved ova recovered from the same Fallopian tube. The uncleaved ova from the 4 ewes in 1964 giving both uncleaved and cleaved ova were observed under a dissecting microscope at x35 magnification. Spermatozoa were found attached to the zona pellucida of the ova from 2 of these ewes. No spermatozoa were found attached to the zona pellucida of the remaining uncleaved ova. A number of ova from two sheep were slightly concave in appearance. The corpora lutea of these ewes appeared to be of different ages. Several corpora lutea appeared very young indicating late ovulations, while other corpora lutea on the same ovaries appeared as of the expected age. The uterus of one of these ewes was very flaccid - not tense as is usual for the uterus 2 - 3 days after ovulation.

Table 12 shows the number of ewes which gave cleaved, uncleaved or cleaved and uncleaved ova as affected by the dose level of P.M.S. administered. This table also presents these results as percentages of the total number of ewes within a dose level group within a season.

Twenty-eight ewes gave only uncleaved ova. Except in those ewes treated with 1,500 i.u. P.M.S. in the 1965 season, the appearance of the corpora lutea of these ewes indicated that ovulation had occurred about 60 - 80 hours prior to laparotomy. The ewes on the high dose level of P.M.S. in 1965 frequently had corpora lutea on the one ovary which did not appear to be of the same age indicating a proportion of relatively late ovulations. The occurrence of these late ovulations could have been a significant factor preventing the fertilization of the ova shed perhaps by affecting transport of spermatozoa to the site of fertilization. Ova recovered from these ewes should have been at least at the 4-cell stage and most at a later stage of development. No uncleaved ova

TABLE 12

NUMBER OF EWES WHICH GAVE CLEAVED, UNCLEAVED OR CLEAVED AND UNCLEAVED OVA

AS AFFECTED BY THE DOSE LEVEL OF P.M.S. ADMINISTERED

Season	P.M.S. dose level (i.u.)	Number of ewes	Number and Percentages* of ewes yielding: No ova	Cleaved ova only	Uncleaved ova only	Both cleaved and uncleaved ova
1964	-	2		1	1	
	1,000	44	1 (2.3%)	33 (75.0%)	6 (13.6%)	3 (6.8%)
	1,200	12		8 (66.7%)	3 (25.0%)	1 (8.3%)
	1,500	3		3		
1965	-	3	1	2		
	500	1		1		
	900	1			1	
	1,000	50	2 (4.0%)	35 (70.0%)	5 (10.0%)	8 (16.0%)
	1,500	27		11 (40.7%)	12 (44.4%)	4 (14.8%)

* Percentages are of total ewes within a dose level group within a season.

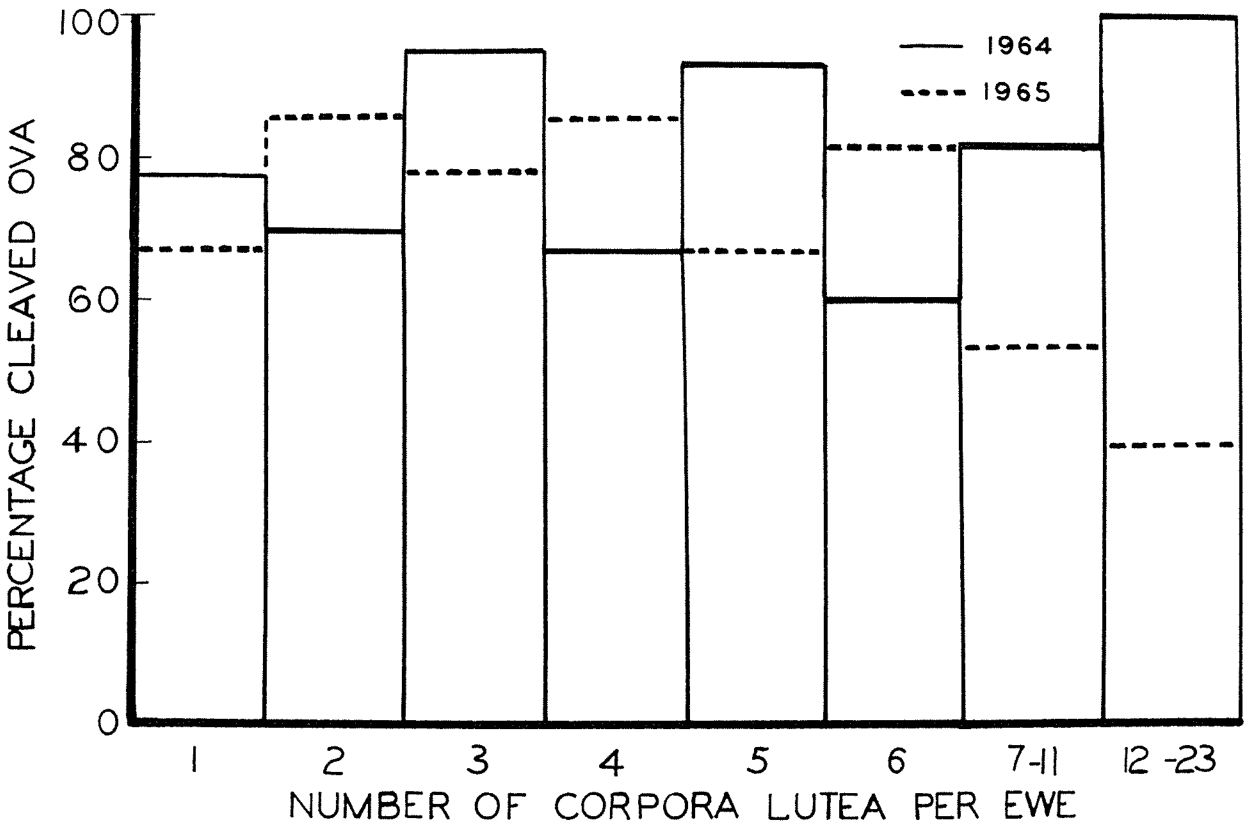
recovered from ewes administered with 1,500 i.u. P.M.S., had any spermatozoa attached to the zona pellucida.

In the 1965 season a significantly greater proportion of ewes injected with 1,500 i.u. P.M.S. than ewes injected with 1,000 i.u. P.M.S. gave only uncleaved ova ($Chi^2 = 11.166$, $P < 0.01$). The significance of this is accented since there is no significant difference in the proportion of sheep giving both cleaved and uncleaved ova between these two groups ($Chi^2 = 0.0137$, Not statistically significant).

(b) Cleaved ova

Table 13 and Figure 21 show that fertilization, (or cleavage of ova) was not related to the number of corpora lutea per ewe up to an ovarian response of about 10 or 11 corpora lutea after P.M.S. treatment. A decrease in the proportion of cleaved ova was shown in ewes which had between 12 and 22 corpora lutea. This was a reflection of the dose levels of P.M.S. administered, the higher dose levels of P.M.S. having given the higher ovarian responses. The effect of a large number of corpora lutea was one of either all or no ova being cleaved. In the two seasons 18 sheep responded to P.M.S. treatment with more than 10 corpora lutea per ewe and of these 18 sheep only one ewe gave both cleaved and uncleaved ova at recovery, 7 ewes gave only cleaved ova while 10 ewes gave only uncleaved ova.

Tables 14 and 15 and Figure 22 present data on the stage of cleavage of the ova recovered as affected by the interval of time between onset of oestrus and laparotomy.



100
80
60
40
20
0
PERCENTAGE CLEAVED OVA
1964
1965
1
2
3
4
5
6
7-11
12 -23
NUMBER OF CORPORA LUTEA PER EWE

TABLE 13

CLEAVAGE OF OVA RELATIVE TO THE NUMBER OF CORPORA LUTEA PER EWE

Season	Corpora lutea per ewe	Number of ewes	Percentage of ova recovered: Cleaved ova	Uncleaved or abnormal ova
	1	10	77.8	22.2
	2	18	70.0	30.0
	3	11	95.4	4.6
1964	4	3	66.7	33.3
	5	8	93.3	6.7
	6	4	58.9	41.1
	7 - 11	5	81.4	18.6
	22	1	100.0	0.0
	1	3	66.7	33.3
	2	14	85.0	15.0
	3	16	78.4	21.6
1965	4	8	84.6	15.4
	5	9	66.7	33.3
	6	5	81.8	18.2
	7 - 11	15	52.1	47.9
	12 - 23	11	38.4	61.6

Figure 21 Percentage of cleaved ova relative to the number of corpora lutea per ewe.

T A B L E   1 4

MEAN INTERVAL OF TIME AFTER ONSET OF OESTRUS TO RECOVERY OF CLEAVED OVA AT THE VARIOUS STAGES OF DEVELOPMENT

Stage of cleavage (Number of cells per ovum)	Interval of time after onset of oestrus to recovery of ova (hours)			
	Mean	±	S.E.	Variance
12	83	±	3.0	79.00
8 - 12	74	±	2.2	24.00
8	73	±	0.3	19.80
6 - 8	71	±	1.1	40.13
6	67	±	1.1	38.84
4 - 6	66	±	0.8	8.66
4	69	±	1.9	94.20
2	79	±	2.0	57.86

Figure 22   Interval of time after onset of oestrus to recovery of cleaved ova at the various stages of ovum development.

represents mean and standard deviation of mean.

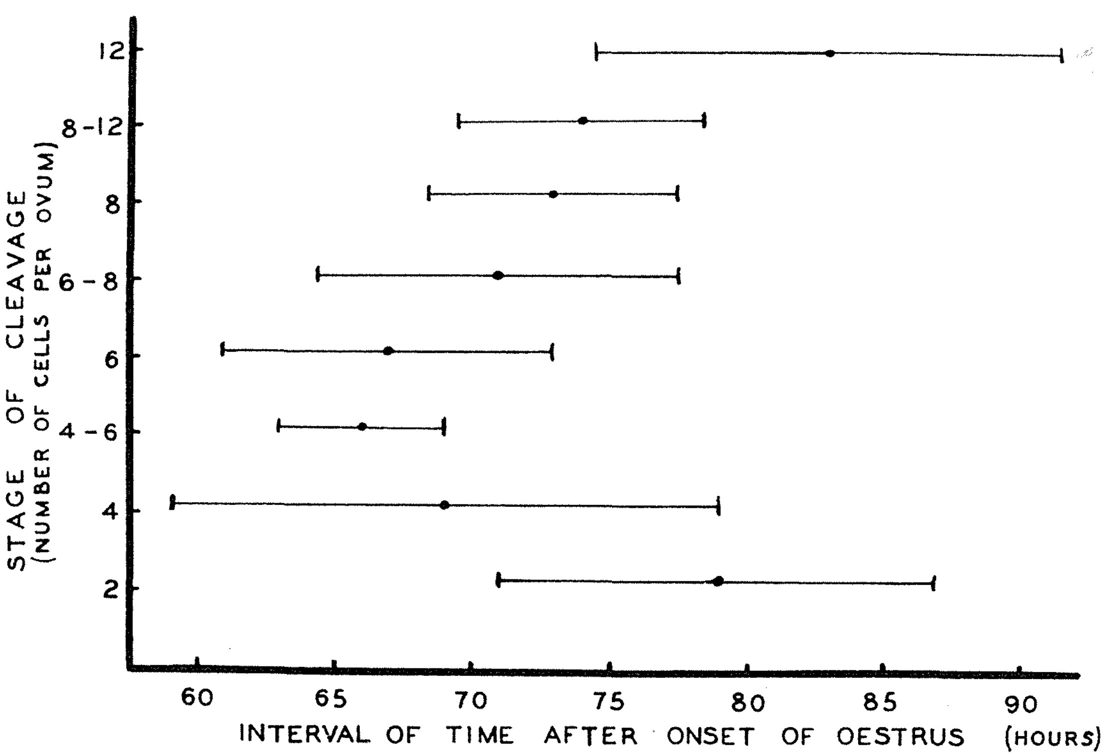


TABLE 15

NUMBER OF CLEAVED OVA RECOVERED AND THE SHORTEST INTERVAL OF TIME AFTER THE ONSET OF OESTRUS TO RECOVERY OF OVA AT VARIOUS STAGES OF CLEAVAGE

Stage of cleavage (number of cells per ovum)	Number of ova recovered	Earliest interval of time of recovery after onset of oestrus (hours)
12	9	66
8 - 12	5	72
8	195	58
6 - 8	31	63
6	33	55
4 - 6	13	60
4	25	55
2	15	64

In total 240 ova were at a more advanced stage of development than the 6-cell stage of cleavage. A significantly greater proportion of ova developed beyond the 6-cell stage were recovered from ewes which were examined after 68 hours after the onset of oestrus rather than from ewes which were operated on before this time. (Chi^2 = 3.876, $P < 0.05$). The finding of a number of 2-cell ova recovered at a relatively late stage after onset of oestrus supports the earlier conclusion that late ovulations relative to onset of oestrus had occurred.

C H A P T E R I V

S T U D I E S O N O V U L A T I O N I N R E C I P I E N T E W E S

I. LIVEWEIGHT

II. FACE WOOL COVERING

1. Face grading

2. Face cover, side body wool and wool gradient

CHAPTER IV

STUDIES ON OVULATION IN RECIPIENT EWES

The numbers of corpora lutea in the ovaries of 151 c.f.a. recipient ewes at the same stage of the breeding season were studied. The ewes were from four groups, 1964HR, 1964LR, 1965T60 and 1965T61, and the observations were recorded at laparotomy immediately prior to ovum transplantation. No hormone had been administered to the ewes after their first oestrus. At laparotomy three corpora lutea were found in one ewe. For purposes of analysis this sheep, in group 1965T60, was included with the ewes in which two corpora lutea were found.

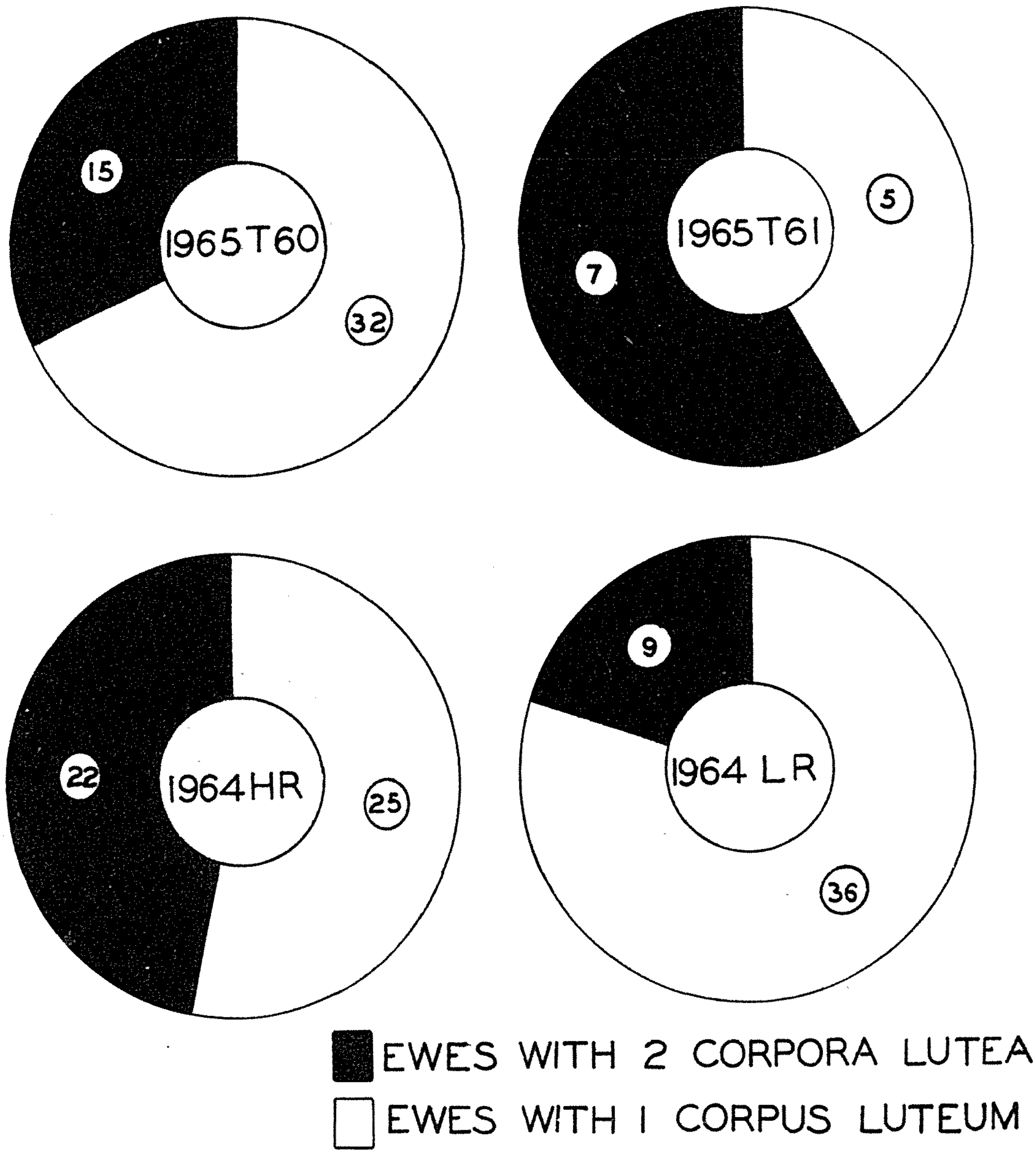
The liveweight of the ewes was negatively correlated with their face cover ($r = -0.272$, d.f. = 147, $P < 0.01$).

I. LIVEWEIGHT

The ovarian responses relative to the liveweights of the recipient ewes, recorded on 6 April, 1964 and 11 April, 1965, were examined and the results are presented in Table 16. Table 16 and Figure 23 show that there was a greater proportion of ewes with two corpora lutea in group 1964HR than in group 1964LR ($Chi^2 = 7.395$, $P < 0.01$). The data also shows that, within the four groups, ewes which had two corpora lutea had higher mean liveweights than ewes in which only one corpus luteum was present. Analysis of variance (Table 17) showed these liveweight differences to be significant ($P < 0.01$).

Figure 23 Diagrammatic representation of proportion of ewes with one or two corpora lutea within experimental recipient groups.

(The number of ewes are shown in small circles)


15
1965T60
32
5
1965T61
7
9
22
1964HR
25
1964 LR
36
EWES WITH 2 CORPORA LUTEA
EWES WITH 1 CORPUS LUTEUM

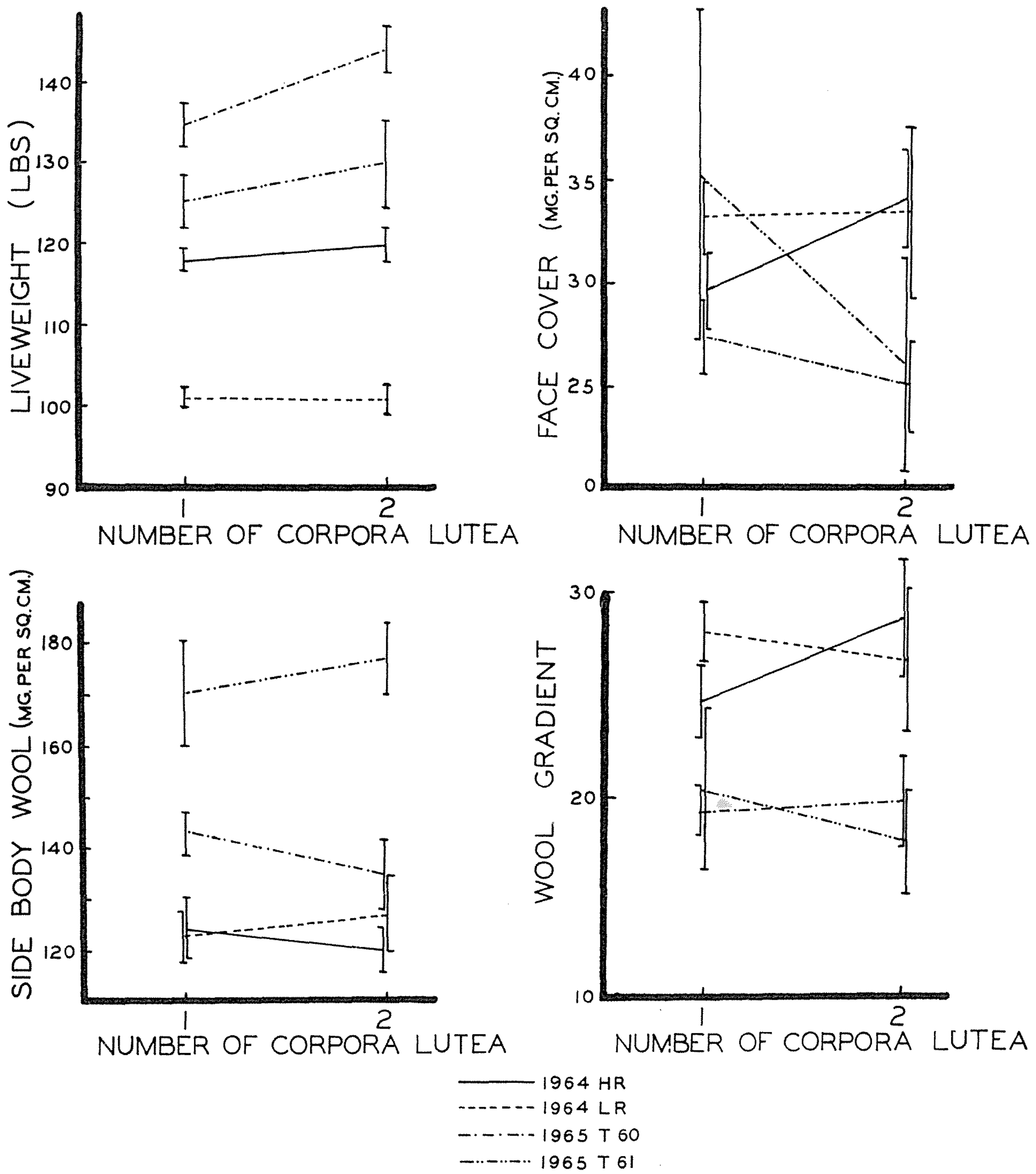

LIVEWEIGHT (LBS)
140
130
120
110
100
90
1
2
NUMBER OF CORPORA LUTEA
FACE COVER (MG. PER SQ. CM.)
40
35
30
25
0
1
2
NUMBER OF CORPORA LUTEA
SIDE BODY WOOL (MG. PER SQ. CM.)
180
160
140
120
1
2
NUMBER OF CORPORA LUTEA
WOOL GRADIENT
30
20
10
1
2
NUMBER OF CORPORA LUTEA
1964 HR
1964 LR
1965 T 60
1965 T 61

TABLE 16

LIVEWEIGHT, FACE COVER, SIDE BODY WOOL AND WOOL GRADIENT RELATIVE TO THE NUMBER OF CORPORA LUTEA IN RECIPIENT EWES

(a) LIVEWEIGHT (lbs)

Group	1 corpus luteum Number of ewes	1 corpus luteum Liveweight Mean	±	S.E.	2 corpora lutea Number of ewes	2 corpora lutea Liveweight Mean	±	S.E.
1964HR	25	118.1	±	1.42	22	120.0	±	2.16
1964LR	36	101.2	±	1.10	9	101.7	±	1.86
1965T60	32	134.7	±	2.97	15	143.9	±	3.49
1965T61	5	125.2	±	3.47	7	130.1	±	5.35

(b) FACE COVER (mg. per sq. cm.)

Group	1 corpus luteum Number of ewes	1 corpus luteum Face cover Mean	±	S.E.	2 corpora lutea Number of ewes	2 corpora lutea Face cover Mean	±	S.E.
1964HR	25	29.65	±	1.94	20	34.01	±	2.52
1964LR	35	33.51	±	1.82	9	33.52	±	4.48
1965T60	32	27.43	±	1.85	15	25.17	±	2.48
1965T61	5	35.27	±	8.74	7	26.26	±	5.22

Figure 24 Liveweight, face cover, side body wool and wool gradient relative to the number of corpora lutea in recipient ewes.

I represents mean and standard error of mean

TABLE 16 (Continued)

(c) SIDE BODY WOOL (mg. per sq. cm.)

Group	1 corpus luteum Number of ewes	1 corpus luteum Side body wool Mean	±	S.E.	2 corpora lutea Number of ewes	2 corpora lutea Side body wool Mean	±	S.E.
1964HR	25	124.0	±	6.02	20	119.8	±	4.56
1964IR	35	123.2	±	4.76	9	127.2	±	7.83
1965T60	32	143.4	±	4.55	15	134.8	±	7.28
1965T61	5	170.6	±	10.63	7	177.1	±	6.79

(d) WOOL GRADIENT (Percentage)

Group	1 corpus luteum Number of ewes	1 corpus luteum Wool gradient Mean	±	S.E.	2 corpora lutea Number of ewes	2 corpora lutea Wool gradient Mean	±	S.E.
1964HR	25	24.7	±	1.8	20	28.8	±	2.9
1964IR	35	28.1	±	9	9	26.7	±	3.6
1965T60	32	19.4	±	15	15	19.6	±	2.3
1965T61	5	20.1	±	7	7	15.1	±	2.6

The group of ewes with the highest mean liveweight was 1965T60. This group had a smaller proportion of ewes with two corpora lutea than the group 1965T61 ($Chi^2 = 2.85$; $0.05 < P < 0.1$) and the group 1964HR ($Chi^2 = 2.18$; Not significant).

The group of ewes with the lightest mean liveweight, 1964LR, had the lowest proportion of ewes with 2 corpora lutea. The groups 1964LR and 1964HR were from the same Wanganui flock thus demonstrating a significant within flock liveweight relationship to ovulation rate. This factor was also apparent from the higher mean liveweights within the four groups of those ewes with two corpora lutea compared to ewes with one corpus luteum.

TABLE 17

ANALYSIS OF VARIANCE OF LIVEWEIGHTS OF RECIPIENT EWES RELATIVE TO THE NUMBER OF CORPORA LUTEA IN THE OVARIES

Source of Variation	d.f.	Mean Square	F value	Significance of F value
Total	150			
Groups	3	9,267.0		
Ewes with one or two corpora lutea within groups	4	7,072.5	57.94	$P < 0.01$
Ewes in groups	143	122.1		

II. FACE WOOL COVERING

1. Face grading

Over the total flock of recipients sheep with two corpora lutea included a greater proportion of ewes graded 2+, 3 and 3+ (open-faced) than ewes graded 1, 1+ and 2 (closed-faced) (Chi^2 = 4.3, $P < 0.05$). Table 18 shows the number of animals classified according to face grade and mean number of corpora lutea per ewe within each grade.

TABLE 18

NUMBER OF EWES CLASSIFIED ACCORDING TO FACE GRADE WITH THE MEAN NUMBER OF CORPORA LUTEA PER EWE WITHIN EACH GRADE

Classification of face grade	Face grade	Number of ewes	Number of corpora lutea per ewe
Closed	1, 1+	24	1.42
	2	60	1.22
	Total closed	84	
Open	2+	30	1.47
	3, 3+	34	1.41
	Total open	64	

The data indicate that the ovulation rate in ewes graded 1 and 1+ was similar to that in ewes graded 3 and 3+. This result is probably a reflection of the small number of animals graded 1 and 1+. Of the 24 ewes graded 1 and 1+, seven were from group 1964HR and of these seven animals, five had two corpora lutea. This small group contributes markedly to the mean of 1.42 corpora lutea per ewe; if only ewes in groups 1964LR, 1965T60 and 1965T61 are considered the mean is reduced to 1.24 corpora lutea per ewe.

2. Face cover, side body wool and wool gradient

Table 16 and Figure 24 show the numbers of corpora lutea and the means of face cover, side body wool and wool gradient of recipient ewes within groups. There was no significant relationship between the number of corpora lutea and the face cover, side body wool or wool gradient measurements.

The data for ovulation rate and each of the various wool measurements were further examined, relative to variation in liveweight of the ewes, by analyses of covariance. Table 19 shows the results for analyses of body weight and face cover on number of corpora lutea within one group of recipient ewes. As these results show, and in all other analyses made within groups of recipient ewes, there were no significant differences in the slopes of the two regression lines of the sub-groups of ewes with either one or two corpora lutea. There were also no significant differences in the adjusted means of the two sub-groups.

T A B L E   1 2

ANALYSIS OF COVARIANCE FOR FACE COVER AND BODY WEIGHT ON NUMBER OF CORPORA LUTEA IN EWES OF GROUP 1965T60

Source of Variation	Regression Coefficient	Deviations from Regression d.f.	Deviations from Regression Mean Square
Sub-group with 1 corpus luteum	- .1145	30	109.09
Sub-group with 2 corpora lutea	- .2250	13	89.56
Within		43	103.19
Reg. Coefficient		1	24.15
Common	- .1394	44	101.39
Adj. Means		1	9.75
Total		45	99.36

CHAPTER V

STUDIES ON SURVIVAL OF TRANSFERRED OVA

I. MEASUREMENTS OF LIVING EMBRYOS

II. MEASUREMENTS OF DEAD EMBRYOS

III. TIME OF PRENATAL MORTALITY

IV. SURVIVAL OF TRANSFERRED OVA

1. Number of corpora lutea in recipient ewes
2. Comparative 'age' of genital tract and transferred ova
3. Interval of time from ovum recovery to transfer
4. Stage of cleavage of ova at transfer
5. Number of ova transferred
6. Liveweight of recipient ewes
7. Face cover of recipient ewes

CHAPTER V

STUDIES ON SURVIVAL OF TRANSFERRED OVA

Recipient ewes which had not returned to oestrus by day 19 after the onset of oestrus were slaughtered. During the 1964 season approximately half the slaughtered ewes were killed at about day 19 after onset of oestrus and the other half at about day 35. During the 1965 season all slaughtered ewes were killed about day 19. No recipient ewe was slaughtered if it had returned to oestrus.

Table 20 shows the number of ewes which underwent ovum transfer and the number of ewes which were either slaughtered or returned to oestrus by day 19 after the onset of oestrus. The data shows that thirteen ewes killed on day 19 were not pregnant. Of these thirteen ewes, nine ewes either contained a dead embryo or decaying embryonic membranes while in the other four ewes no embryonic tissue was found. Three ewes which each contained a single living embryo also contained the decaying embryonic membranes of another embryo.

T A B L E 2 0

DISTRIBUTION OF RECIPIENT EWES RELATIVE TO THE NUMBER AND SURVIVAL OF TRANSFERRED OVA

Experimental Group	Day of slaughter of ewe after onset of oestrus	Number of ewes relative to the number of ova transferred and either the number of embryos alive at slaughter of ewes or the number of ewes returning to oestrus												
		4 ova transferred						2 ova transferred				1 ovum transferred		
		Number of embryos alive at slaughter					Number of ewes returning to oestrus by day 19	Number of embryos alive at slaughter			Number of ewes returning to oestrus by day 19	Number of embryos alive at slaughter		Number of ewes returning to oestrus by day 19
		4	3	2	1	0		2	1	0		1	0	
1964HR	19 - 23		1				1	2	1	3(3)	7	3	3{3}	6
	34 - 38	1	1					3	3			6		
1964LR	19 - 23	1					1	2	2(1)		9	5	1[1]	7
	34 - 38		1					3				5		
1965T60	19 - 23	2	4	4	3(1)	/1/ 3(1) [1]	13	2	6(1)	{1} 3[2]	8			
1965T61	19 - 23							6	4		4			
ALL EWES		4	7	4	3	3	15	18	16	6	28	19	4	13

Number of ewes shown inside symbol:

{ } 1 dead embryo present
( ) 1 set of membranes present
/ / Thick decaying mass present
[ ] No embryonic tissue present

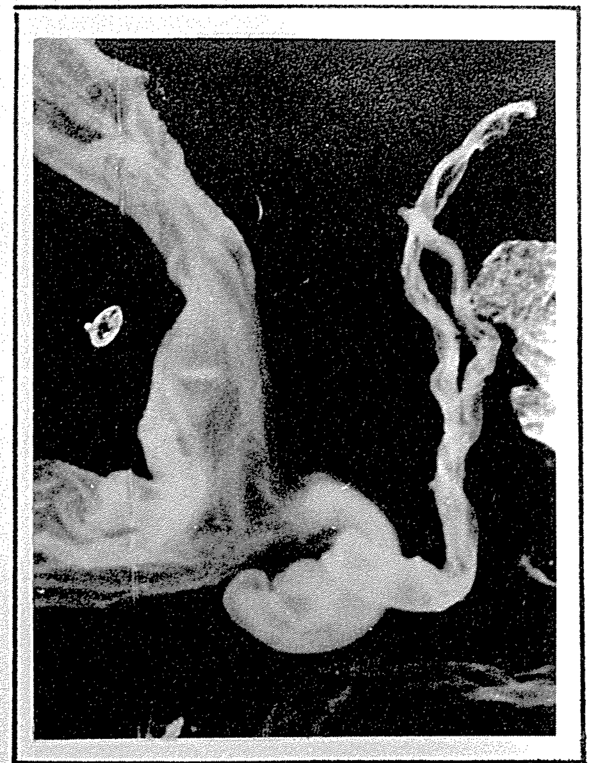
Figure 25 A 19 day embryos

B 20 day embryo (x 4.5)

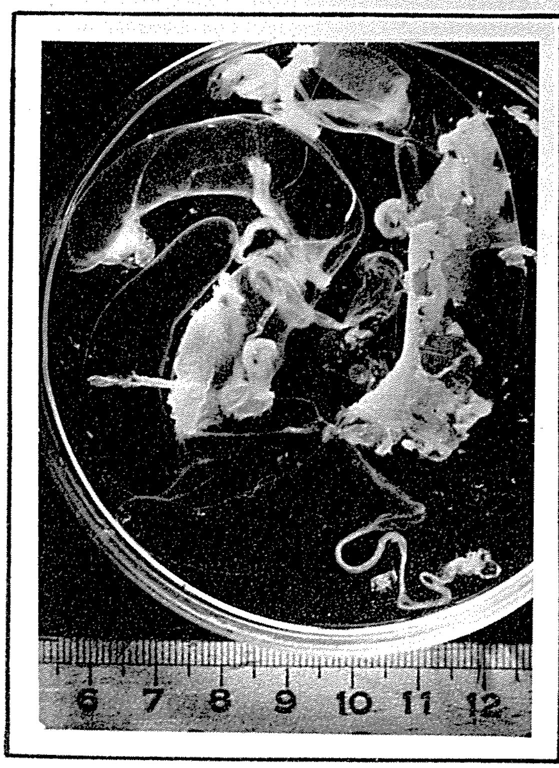
C 21 day embryo

D 22 day embryo

A



B



C

D

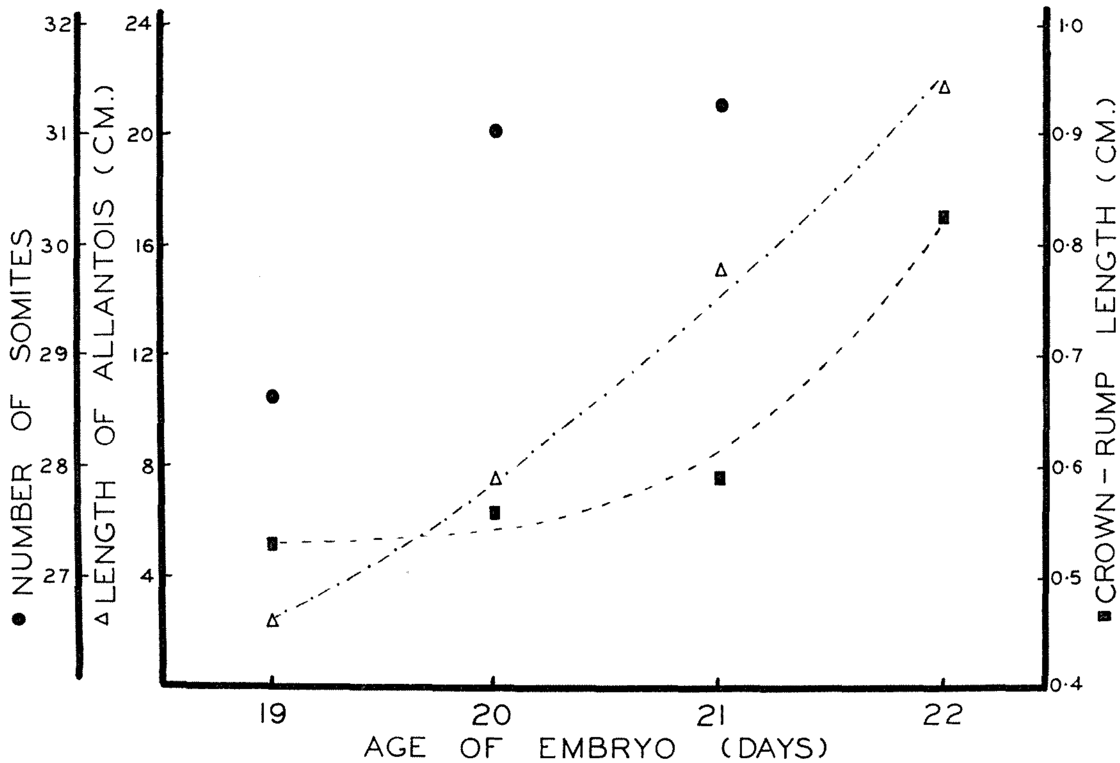


Figure 26 Measurements of embryos 19 - 22 days after oestrus.

TABLE 21

MEASUREMENTS OF EMBRYOS 19 - 22 DAYS AFTER OESTRUS

Age of embryo (days)	Crown-Rump Length (cm) Mean	±	S.E.	Number of embryos
19	0.528	±	0.009	48
20	0.558	±	0.005	13
21	0.589	±	0.014	18
22	0.827	±	0.004	8

Age of embryo (days)	Number of Somites Mean	±	S.E.	Number of embryos
19	28.6	±	0.30	41
20	31.0	±	0.56	10
21	31.27	±	0.43	11

Age of embryo (days)	Length of Allantois (cm.) Mean	±	S.E.	Number of embryos
19	2.13	±	0.23	47
20	7.49	±	0.88	13
21	11.00	±	0.96	15
22	21.60	±	1.43	5

I. MEASUREMENTS OF LIVING EMBRYOS

The "C" shape of the embryo described by Green and Winters (1945) was found to be fully developed by 20 - 21 days (Figure 25). Embryos of 19, 20 and 21 days of age were rather translucent and permitted observation of many internal structures. The heart chambers were easily seen and rhythmic contractions and relaxations of the heart occurred in some embryos. The optic region and upper limb buds were visible at the 19 day stage.

At 19 and 20 days of age the number of somites could be counted with relative ease but at 21 days of age the embryos were losing their translucence and accurate counting of somites became difficult. An accurate count of somites was impossible by the 22 day stage using the methods employed in this project. No estimate is presented for the number of somites in 22 day embryos.

Table 21 and Figure 26 present data on the three measurements for crown-rump length, number of somites and length of allantois attempted on all embryos at the 19-22 day stage. All embryos included in the table were classified as alive at the time of killing the ewe. Once dissected free of embryonic membranes almost immediate study was required for an accurate count of the number of somites to be made. Data on the number of somites from 17 embryos of 19 - 21 days of age are not included in Table 21. At the time of counting the number of somites these 17 embryos had lost much of their translucence and therefore made accurate counting impossible using the methods employed in this study.

The 35 day old embryos demonstrated the transitional stage between the embryonic and foetal periods. Measurements of the embryos at this age have given an indication of the larger size of single embryos compared to the size of multiple embryos (Table 22). This difference was found to be not significant at the 5 per cent level.

TABLE 22

MEASUREMENTS OF GENITAL TRACTS AND EMBRYOS AT THE 35 DAY STAGE FROM EWES WITH ONE OR MULTIPLE NUMBER OF EMBRYOS

From ewes with 1 embryo:	Mean	±	S.E.	
Weight of Uterus + Membranes + Foetus (g.)	177.1	±	8.60	
Weight of Uterus (g.)	85.9	±	2.67	
Weight of Foetus (g.)	2.002	±	0.073	(a^1)
Length from Crown to Rump (cm.)	2.91	±	0.050	(b^1)
Displacement (ml.)	2.05	±	0.078	(c^1)

From ewes with 2 or more embryos:	Mean	±	S.E.	
Weight of Uterus + Membranes + Foetuses (g.)	313.9	±	15.76	
Weight of Uterus (g.)	115.6	±	3.22	
Weight of Foetus (g.)	1.942	±	0.084	(a^2)
Length from Crown to Rump (cm.)	2.82	±	0.043	(b^2)
Displacement (ml.)	1.95	±	0.079	(c^2)

Comparison of differences (analysis of variance)

a^1 v a^2 : Not significant

b^1 v b^2 : Not significant

c^1 v c^2 : Not significant

II. MEASUREMENTS OF DEAD EMBRYOS

Table 20 shows the number of ewes which had a dead embryo present at slaughter. Some ewes had only a few membranes present indicating an earlier prenatal death while one ewe had its uterus filled with a thick creamy mass. Four ewes each contained one embryo which was classified as dead. In these embryos no heart movement was observed even after adding warm saline solution to the medium. As well, the blood in their embryonic vessels was paler than that typical of blood of a living embryo. The measurements of crown-rump length, number of somites and length of allantois also suggested an earlier death (Table 23).

TABLE 23

MEASUREMENTS OF FOUR EMBRYOS CLASSIFIED AS DEAD AT THE TIME OF EWE SLAUGHTER

Age of embryo at slaughter (days)	Crown-rump length of embryo (cm.)	Number of Somites	Length of allantois (cm.)
19	0.40	15	4.61
19	0.40	22	1.88
19	0.20	6	1.47
19		Degenerating mass of tissue	

III. TIME OF PRENATAL MORTALITY

The slaughter of recipient ewes which had not returned to oestrus by days 19 - 23 or by days 34 - 38 after onset of oestrus allowed a study of the approximate time of death of transferred ova.

Ewes killed on day 19 - 23 post oestrus were not all pregnant (Table 20). Sixty-one ewes were killed at this stage and 48 of these ewes were pregnant. Four ewes showed no signs of a recent embryonic death. Thus it was concluded that these four ewes had contained either embryos which died about days 15 - 17, this being a stage late enough in the oestrous cycle to delay the onset of the following oestrus, or blastocysts which had died before or at implantation and the ewe experienced an abnormally long oestrous cycle. Three of the pregnant ewes contained embryonic membranes indicating the final stages of the process of decay of an embryo. Nine non-pregnant ewes contained the remains of dead embryos and /or their embryonic membranes thus indicating recent embryonic death.

During the 1964 season a number of ewes were killed at approximately the 35 day stage from which was gained an indication of the extent of prenatal mortality occurring between days 19 and 35. All ewes killed about days 34 - 38 after onset of oestrus were pregnant. No ewe killed at this stage contained any degenerate embryonic tissue. No evidence of any early prenatal mortality occurring between days 19 and 38 after onset of oestrus was found. Combining all the evidence - that no one ewe returned to oestrus after day 19 of the oestrous cycle, that no one ewe contained a dead or dying embryo or any decaying membranes in the group of ewes killed at about day 35 and that a smaller proportion of living embryos to ova originally transferred was not present in either the 19 day or 35 day slaughter groups - it was concluded that in this study the transferred ova are either lost or early prenatal mortality occurred prior to day 19 after onset of oestrus.

Most prenatal mortality must thus have occurred at, or prior to, the time of implantation so allowing the ewes to return to oestrus by day 17 of the cycle. There was no evidence to negate the possibility that much of this loss was due to ovum loss at, or immediately after, transfer. From day 17 until about day 19 after onset of oestrus the rate of embryonic mortality was low. No evidence of prenatal mortality occurring between day 19 and day 35 was found.

IV. SURVIVAL OF TRANSFERRED OVA

1. Number of corpora lutea in recipient ewes

Table 24 shows the percentage survival of transferred ova in ewes of the four experimental recipient groups classified according to the number of ova transferred and the number of corpora lutea per recipient ewe. This table shows that the results of this study give no indication of the number of corpora lutea per recipient ewe affecting the survival of transferred ova.

2. Comparative 'age' of genital tract and transferred ova

At times it became necessary to transfer ova from different donors to the one recipient. This fact decreased the number of ova which provided results for this analysis of the affect of the comparative 'age' of genital tract and transferred ova to survival of ova. Data from recipient ewes was only included in the analysis if the ova were gained from tracts within 1.5 hr of each other in 'age'. 'Ages' of tracts and ova were measured in hours from onset of oestrus.

TABLE 24

SURVIVAL OF TRANSFERRED OVA IN RECIPIENT EWES WITH EITHER ONE OR TWO CORPORA LUTEA CLASSIFIED BY THE EXPERIMENTAL GROUP AND THE NUMBER OF OVA TRANSFERRED

Group	Number of corpora lutea per ewe	Number of ova transferred: 4 — Number of ewes	4 — Percentage survival of ova	2 — Number of ewes	2 — Percentage survival of ova	1 — Number of ewes	1 — Percentage survival of ova
1964HR	2	2	50	8	44	8	63
	1	2	75	11	32	10	40
1964LR	2			3	17	5	60
	1	3	58	13	42	13	54
1965T60	2	7	36	8	19		
	1	21	25	11	32		
1965T61	2			8	56		
	1			6	58		
Total	2	9	39 (a)	27	37 (c)	13	62 (e)
	1	26	33 (b)	41	39 (d)	23	48 (f)

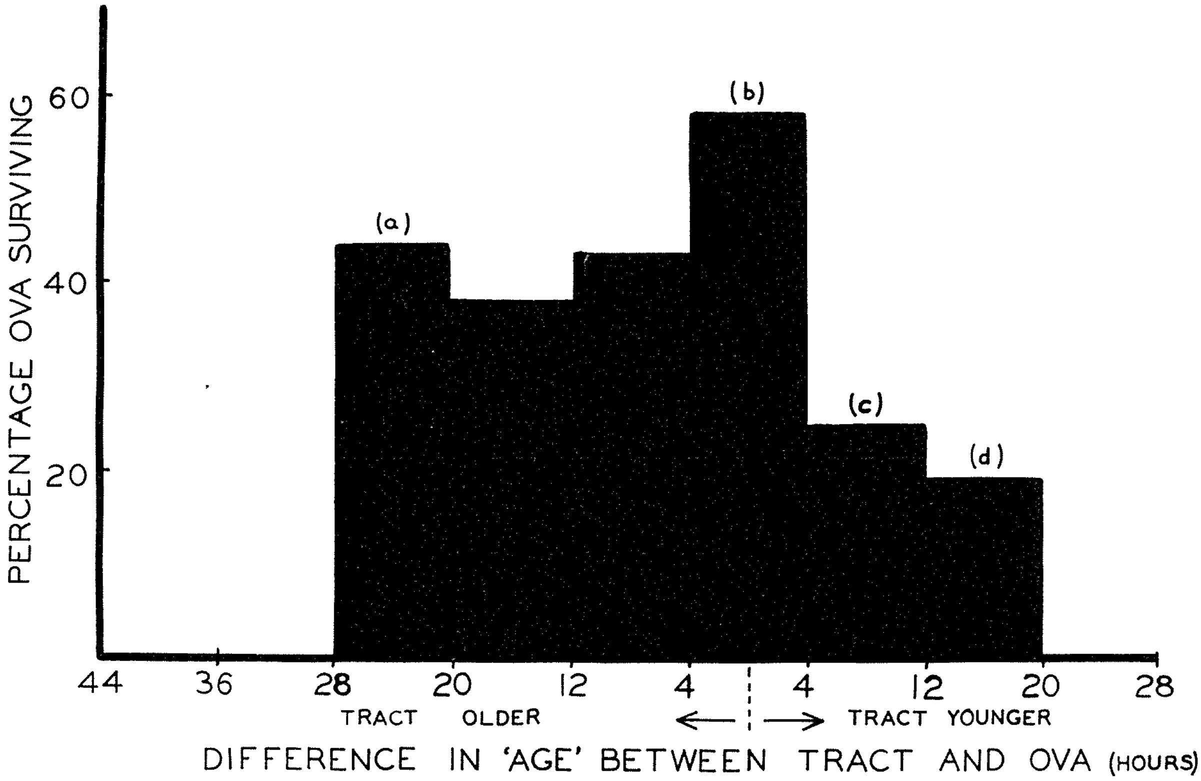
Comparison of differences

Proportion of ova surviving (Chi^2)

a v b : Not significant

c v d : Not significant

e v f : Not significant


PERCENTAGE OVA SURVIVING
60
40
20
(a)
(b)
(c)
(d)
44
36
28
20
12
4
4
12
20
28
TRACT OLDER
TRACT YOUNGER
DIFFERENCE IN 'AGE' BETWEEN TRACT AND OVA (HOURS)

T A B L E 25

SURVIVAL OF TRANSFERRED OVA RELATIVE TO THE COMPARATIVE 'AGE' OF GENITAL TRACT AND OVA

'Age of tract in relation to 'age' of ova transferred	Number of ova transferred	Number of ova developing	Percentage of ova developing
Tract 37 - 44 hr older	5	0	0
Tract 29 - 36 hr older	4	0	0
Tract 21 - 28 hr older	36	16	44 (a)
Tract 13 - 20 hr older	45	17	38
Tract 5 - 12 hr older	49	21	43
Tract 4 hr older to 3 hr younger	48	28	58 (b)
Tract 4 - 11 hr younger	64	16	25 (c)
Tract 12 - 19 hr younger	32	6	19 (d)
Tract 20 - 27 hr younger	4	0	0

Comparison of differences:

Percentage of ova surviving (Chi^2)

a v b : Not significant

b v c : $0.05 < P < 0.02$

b v d : $0.05 < P < 0.02$

a v d : $0.2 < P < 0.1$

Figure 27 Survival of transferred ova relative to the comparative 'age' of genital tract and ova.

The results are presented in Table 25 and Figure 27 which show that ova transferred were significantly affected by the difference in ages of tract and transferred ova ($P < 0.01$). There was a significant decrease in survival rate of those ova transferred to tracts which were 4 - 11 hr younger than the ova. No significant difference was found in the proportion of ova surviving between those groups of ewes in which the tract was 4 hr older to 3 hr younger than the ova and in which the tract was 21 - 28 hr older. These findings indicate a greater chance for ovum survival in older tracts although in this study it was not shown to be statistically significantly.

3. Interval of time from recovery to transfer

One ovum developed to the 19 day stage after 405 minutes between recovery and the time of transfer. Less than 10 per cent of ova were stored longer than 120 minutes before transfer. The results shows no decrease in the chance of survival of transferred ova by increasing the time from recovery to transfer within the interval of time used in this experiment.

4. Stage of cleavage of the ova at transfer

During the 1964 and 1965 seasons 44 ova at the 2-cell, 4-cell or 4-cell to 6-cell stage of cleavage were transfered. Only three of these ova definitely survived to the 19 day stage while a further ten ova could possibly have developed. Young ova were occasionally transferred together with older ova thereby making it impossible to determine which of the ova developed.

In considering likely reasons for the low percentage of 2- to 6-cell ova which survived after transfer it must be remembered that 2-cell and 4-cell ova were transferred to the Fallopian tubes while ova of a greater stage of cleavage were transferred to the uterine horns.

Figure 28 Diagrammatic representation of embryonic survival until slaughter in ewes to which one, two or four ova were transferred.

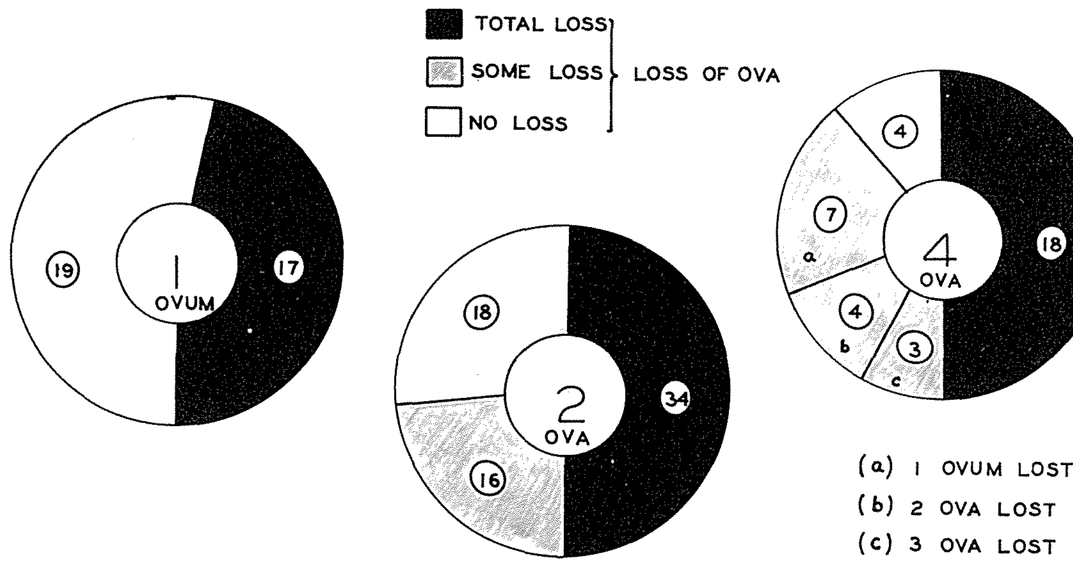
(Number of ewes shown in small circles)

Figure 29 Percentage of ova surviving to slaughter in ewes which received one, two or four ova.

Comparison of differences (Chi^2)

Proportion of ova surviving

a v c	:	$P < 0.05$
a v b	:	Not significant
b v c	:	Not significant


TOTAL LOSS
SOME LOSS
NO LOSS
LOSS OF OVA
19
1 OVUM
17
18
2 OVA
34
16
4
7
a
4
b
3
c
4 OVA
18
(a) 1 OVUM LOST
(b) 2 OVA LOST
(c) 3 OVA LOST

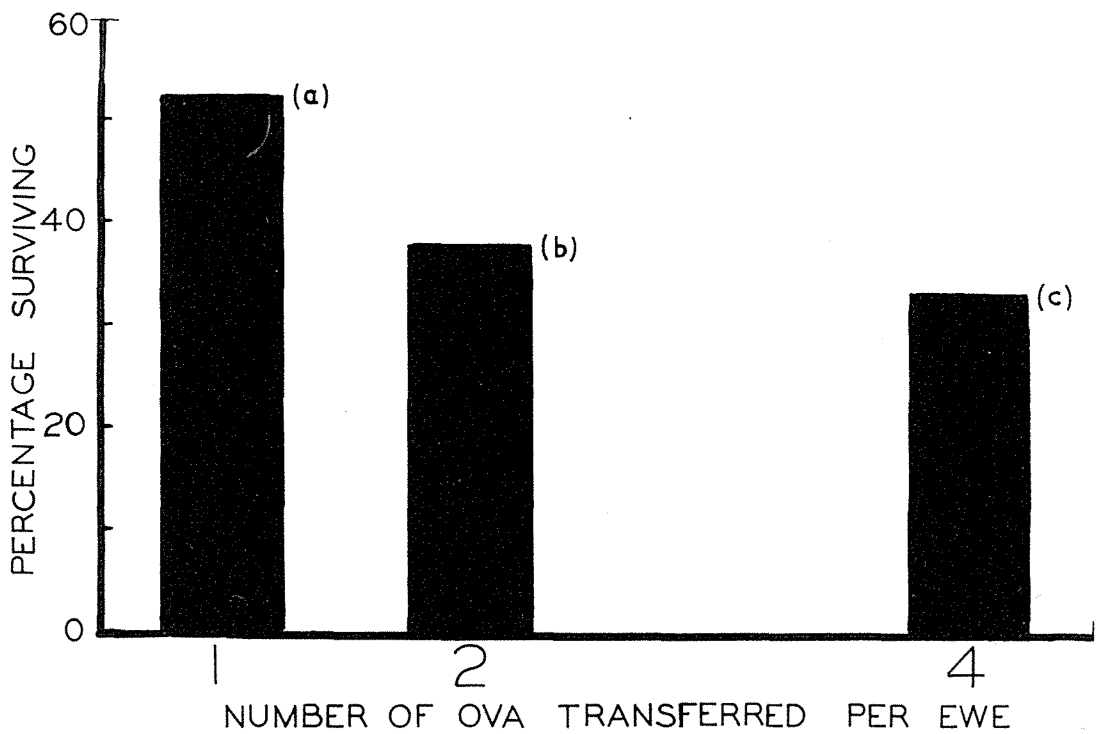
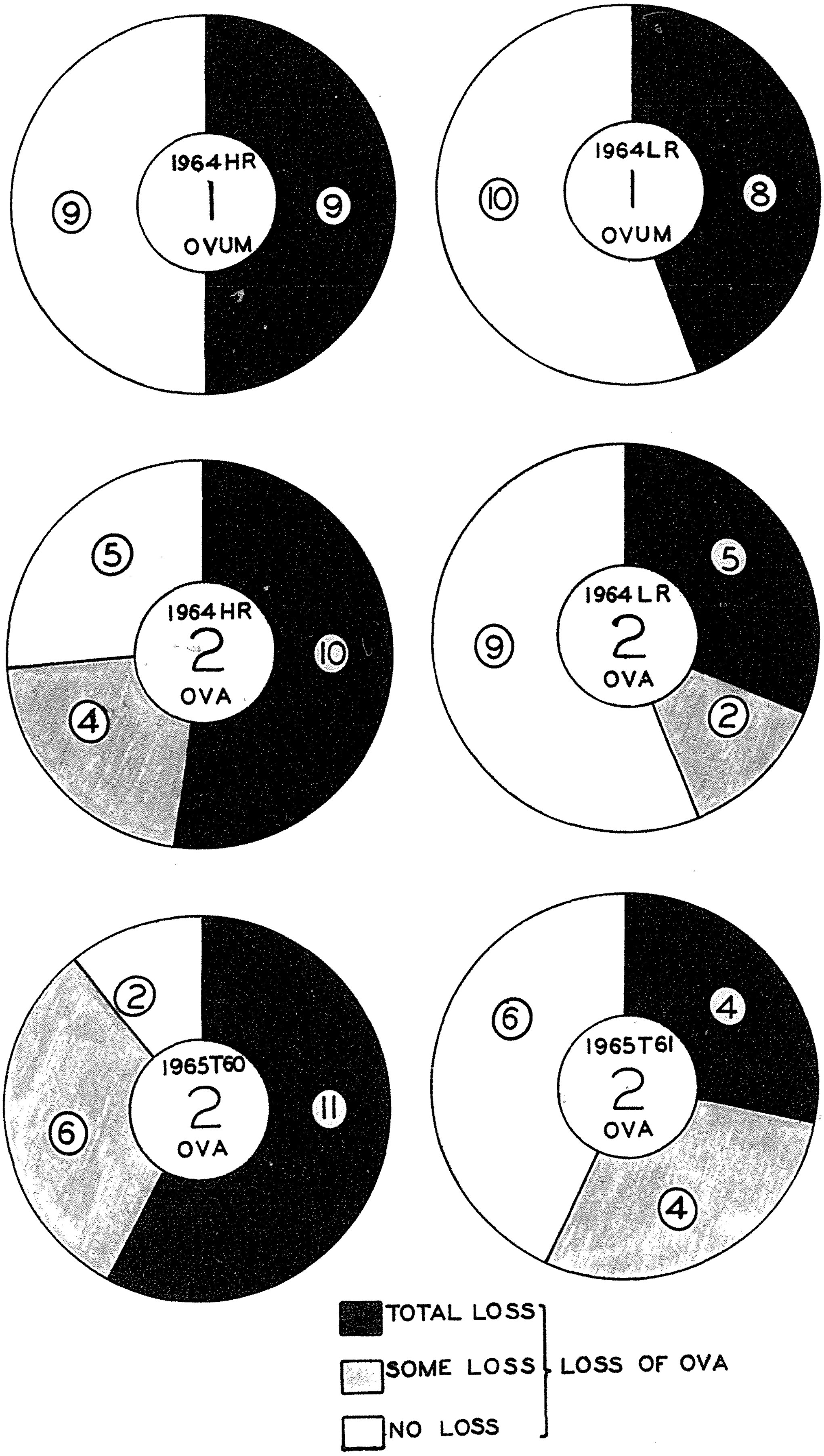

PERCENTAGE SURVIVING
60
40
20
0
(a)
(b)
(c)
1
2
4
NUMBER OF OVA TRANSFERRED PER EWE

Figure 30 Diagrammatic representation of embryonic survival until slaughter in ewes in the four experimental groups which received one or two ova.

(Number of ewes shown in small circles)


1964HR
1
OVUM
9
9
1964LR
1
OVUM
10
8
1964HR
2
OVA
5
4
10
1964LR
2
OVA
9
5
2
1965T60
2
OVA
2
6
11
1965T61
2
OVA
6
4
4
TOTAL LOSS
SOME LOSS
NO LOSS
LOSS OF OVA

5. Number of ova transferred

Figures 28 and 30 present diagrammatically the results of all transfers included in the present analysis of data for the 1964 and 1965 seasons. Of the sheep receiving one, two and four ova, 52.8 per cent, 50.0 per cent and 50.0 per cent respectively were pregnant at killing. The transfer of a greater number of ova had not increased the chance of a resulting pregnancy.

Figure 29 indicates a lower percentage of ova surviving to slaughter if two rather than one ovum is transferred. This figure also shows that an ovum had a greater chance of developing after it was transferred to a single ovum recipient ewe than to a four ova recipient. ($P < 0.05$).

The hypothesis that all ova transferred to recipient ewes in this experiment did not have an equal chance of survival was found to be true. The results of the analysis are presented in Table 26. The method of analysis was after Snedecor (1946) using the method of comparison of a sample distribution with the binomial.

TABLE 26

TEST OF HYPOTHESIS THAT ALL OVA DID NOT HAVE AN EQUAL CHANCE OF SURVIVAL

A. Considering all recipient ewes

Number of ova transferred	Number of living embryos: 4	3	2	1	0	*Significance of deviations from expected numbers
	Number of ewes					
4	4	7	4	3	18	$P < 0.01$
2			18	16	34	$P < 0.01$
1				17	19	Not significant

B. Considering all pregnant recipient ewes

Number of ova transferred	Number of living embryos: 4	3	2	1	*Significance of deviations from expected numbers
	Number of ewes				
4	4	7	4	3	Not significant
2			18	16	$P < 0.05$

* Probabilities from Chi^2

The hypothesis that all ova transferred to ewes which were pregnant at killing did not have an equal chance of survival was also found to be supported (Table 26). The lack of a significant Chi^2 value for those ewes receiving 4 ova and becoming pregnant was possibly a reflection of the small number of ewes included in the analysis.

6. Liveweight of the recipient ewes

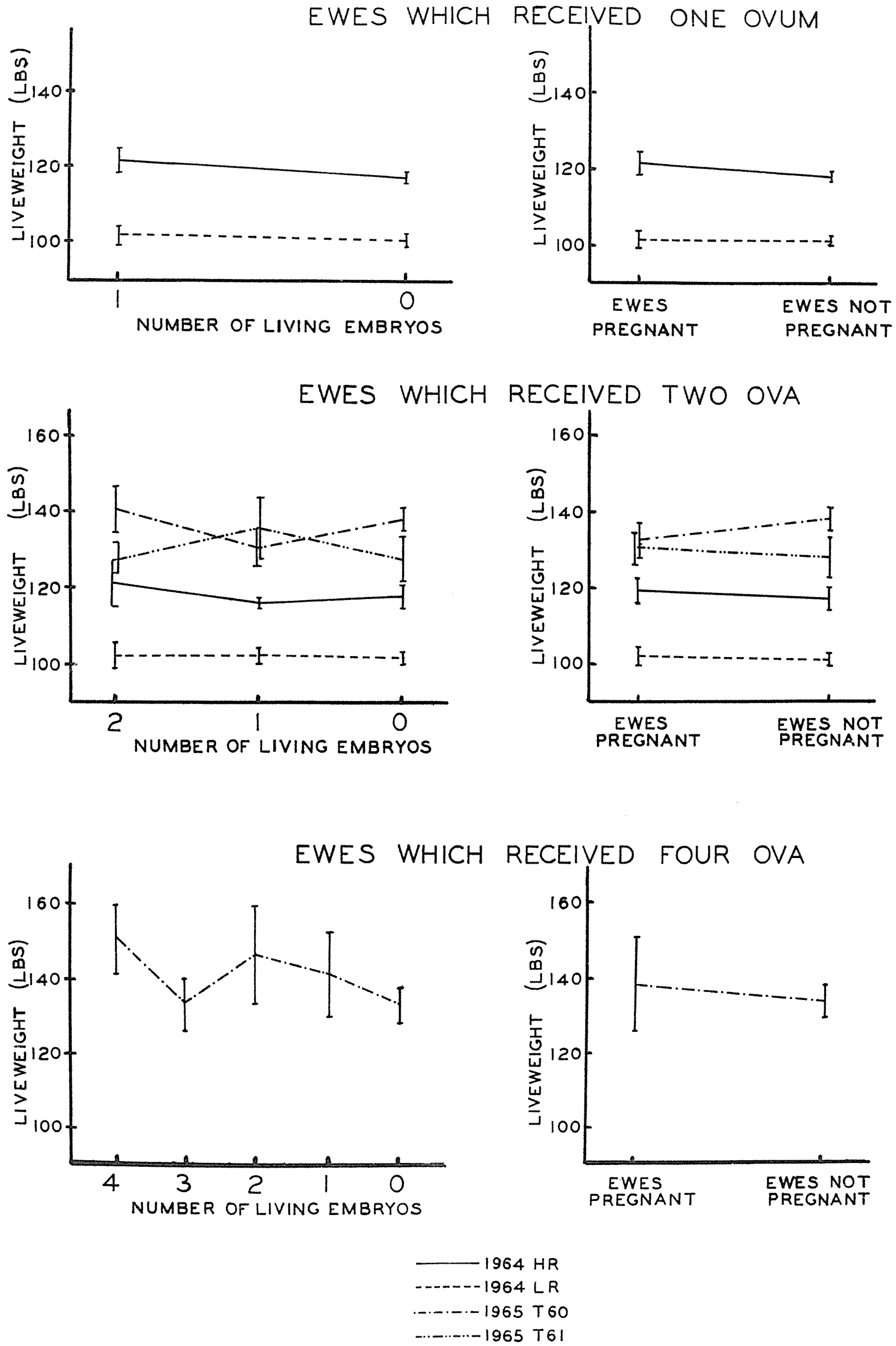
In the 1964 season 36 ewes received one ovum each. Half the ewes receiving one ovum were randomly selected from the group 1964HR and the other half of the ewes from the group 1964LR. At slaughter these ewes were classified as having either a living embryo or that the ovum had died subsequent to transfer.

TABLE 27

ANALYSIS OF VARIANCE OF LIVEWEIGHT OF EWES RECEIVING ONE OVUM OF WHICH 1 OR 0 EMBRYOS SURVIVED TO SLAUGHTER

Source of variation	d.f.	Mean Square	F value	Significance of F value
Total	35			
Groups	1	2,934		
Sub-groups within groups	2	26	0.605	Not significant
Ewes within sub-groups	32	43		

A significant different ($P < 0.01$) in the mean liveweights of ewes receiving one ovum between the groups 1964HR and 1964LR was found. No significant difference was found in the mean liveweight between those ewes in which the ovum produced a living embryo and those in which no living embryo was produced (Table 27). This result is also shown in Figure 31 and Table 29. There was also no significant difference between groups in the proportion of ewes producing a living embryo. This is shown in diagrammatic representation in Figure 28.



EWES WHICH RECEIVED ONE OVUM
LIVEWEIGHT (LBS)
100
120
140
1
0
NUMBER OF LIVING EMBRYOS
EWES PREGNANT
EWES NOT PREGNANT
EWES WHICH RECEIVED TWO OVA
LIVEWEIGHT (LBS)
100
120
140
160
2
1
0
NUMBER OF LIVING EMBRYOS
EWES PREGNANT
EWES NOT PREGNANT
EWES WHICH RECEIVED FOUR OVA
LIVEWEIGHT (LBS)
100
120
140
160
4
3
2
1
0
NUMBER OF LIVING EMBRYOS
EWES PREGNANT
EWES NOT PREGNANT
1964 HR
1964 LR
1965 T60
1965 T61

TABLE 28

BODY WEIGHT OF EWES CLASSIFIED BY THE NUMBER OF OVA TRANSFERRED AND THE NUMBER OF EMBRYOS ALIVE AT SLAUGHTER WITHIN THE FOUR EXPERIMENTAL GROUPS

Group	Experimental Sub-group: Number of ova received	Experimental Sub-group: Number of embryos alive at slaughter	Number of ewes	Body Weight (lbs.) Mean	±	S.E.
1964HR	1	1	9	121.2	±	2.90
	1	0	9	117.9	±	1.27
1964LR	1	1	10	101.9	±	2.40
	1	0	8	101.0	±	1.66
	2	2	5	121.0	±	6.52
1964HR	2	1	4	116.5	±	1.19
	2	0	10	118.3	±	3.12
	2	2	5	102.8	±	3.78
1964LR	2	1	2	104.0	±	2.00
	2	0	9	102.0	±	1.76
	2	2	2	140.5	±	6.52
1965T60	2	1	6	130.5	±	5.40
	2	0	11	138.7	±	3.23
	2	2	6	127.7	±	4.10
1965T61	2	1	4	135.7	±	8.58
	2	0	4	128.7	±	6.37
	4	4	2	150.5	±	9.51
	4	3	4	134.5	±	6.91
1965T60	4	2	4	147.2	±	13.83
	4	1	3	141.7	±	11.05
	4	0	16	134.0	±	4.23

Figure 31 Liveweight of ewes relative to the number of ova transferred and the number of embryos alive at slaughter within the four experimental groups.

I represents mean and standard error of mean

A total of 68 ewes received 2 ova at transfer over the two seasons. The results of these transfers are shown in Table 28 and Figures 28 and 31.

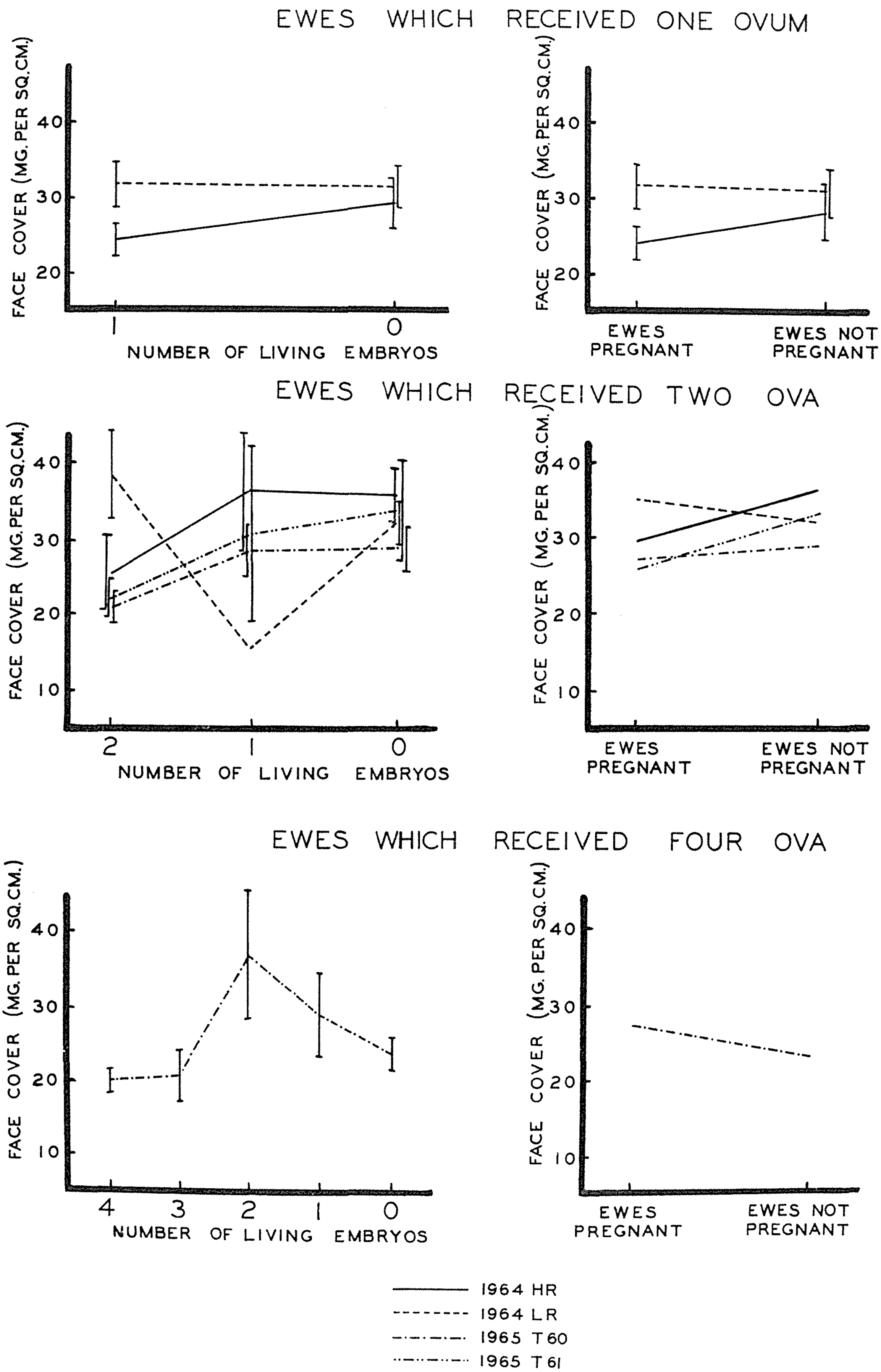
TABLE 29

ANALYSIS OF VARIANCE OF LIVEWEIGHT OF EWES RECEIVING TWO OVA OF WHICH 2, 1 OR 0 EMBRYOS SURVIVED TO SLAUGHTER

Source of variation	d.f.	Mean square	F value	Significance of Fvalue
Total	67			
Group	3	3,717		
Sub-groups within groups	8	65	0.504	Not significant
Ewes within sub-groups	56	129		

The analysis of variance of the liveweights of recipients with two ova (Table 29) showed no significant difference in body weight between ewes in which 2, 1 or 0 embryos survived. Figure 30 demonstrates the differing proportions of ewes which had either 2, 1 or 0 embryos alive within the 4 experimental groups. The group with the lightest mean liveweight 1964LR, had the greatest proportion of ewes with two living embryos while the group with the heaviest mean liveweight, 1961T60, had the greatest proportion of ewes with no living embryos.

The group with the greatest proportion of ewes with two living embryos, 1964LR, was the group with the smallest proportion of ewes with one living embryo. The group with the largest proportion of ewes with no living embryos, 1965T60, was also the group with the largest proportion of ewes with one living embryo and the smallest proportion of ewes with two living embryos.


EWES WHICH RECEIVED ONE OVUM
FACE COVER (MG. PER SQ.CM.)
40
30
20
1
0
NUMBER OF LIVING EMBRYOS
EWES PREGNANT
EWES NOT PREGNANT
EWES WHICH RECEIVED TWO OVA
10
2
EWES WHICH RECEIVED FOUR OVA
4
3
1964 HR
1964 LR
1965 T60
1965 T61

T A B L E 3 0

FACE COVER OF EWES CLASSIFIED BY THE NUMBER OF OVA TRANSFERRED AND THE NUMBER OF EMBRYOS ALIVE AT SLAUGHTER WITHIN THE FOUR EXPERIMENTAL GROUPS

Group	Experimental sub-group: Number of ova received	Experimental sub-group: Number of embryos alive at slaughter	Number of ewes	Face cover ($mg./cm^2.$) Mean	±	S.E.
1964HR	1	1	8	24.36	±	2.18
	1	0	9	28.30	±	3.35
1964LR	1	1	10	31.83	±	3.00
	1	0	8	31.21	±	2.95
1964HR	2	2	5	25.50	±	5.08
	2	1	3	36.29	±	7.83
	2	0	10	35.69	±	3.62
1964LR	2	2	5	38.82	±	5.11
	2	1	1	15.79	±	-
	2	0	9	32.25	±	2.81
1965T60	2	2	2	21.86	±	2.36
	2	1	6	28.45	±	3.68
	2	0	11	29.13	±	3.08
1965T61	2	2	6	22.31	±	2.55
	2	1	4	30.43	±	12.66
	2	0	4	33.78	±	6.95
1965T60	4	4	2	19.97	±	1.74
	4	3	4	20.83	±	3.67
	4	2	4	36.75	±	8.34
	4	1	3	29.05	±	5.56
	4	0	16	23.53	±	2.34

Figure 32 Face cover of ewes relative to the number of ova transferred and the number of embryos alive at slaughter within the four experimental groups.

I represents mean and standard error of mean

T A B L E 31

ANALYSES OF VARIANCE OF FACE COVER OF EWES RECEIVING EITHER (a) TWO OVA OF WHICH 2, 1 OR 0 OVA SURVIVED TO SLAUGHTER OR (b) ONE OVUM OF WHICH EITHER 1 OR 0 OVA SURVIVED TO SLAUGHTER

(a) Ewes receiving two ova

Source of Variation	d.f.	Mean square	F value	Significance of F value
Total	65			
Groups	3	142.54		
Sub-groups within groups	8	920.54	28.767	$P < 0.01$
Ewes within sub-groups	54	32.00		

(b) Ewes receiving one ovum

Source of Variation	d.f.	Mean square	F value	Significance of F value
Total	34			
Groups	1	228.52		
Sub-groups within groups	2	33.86	0.4415	Not significant
Ewes within sub-groups	31	76.69		

An insufficient number of 4 ova transfers were carried out in the 1964 season to allow any conclusions to be drawn from the data. Further data from 4 ova transfers to 29 ewes was gained from the group 1965T60.

Figure 31 presents the mean liveweights of ewes receiving two ova within groups dependent upon the number of embryos alive at slaughter and also dependent upon the ewes being either pregnant or non pregnant at slaughter. This figure shows little evidence of a relationship between liveweight and survival of ova being present within any one group. Only one group, 1965T60, has a higher mean liveweight for ewes which were not pregnant after having received two ova. The other three groups which received two ova per ewe had a lower mean liveweight for ewes which were not pregnant. This lower mean liveweight for non-pregnant ewes was also shown in both groups receiving one ovum and in the group, 1965T60, which received four ova. Analyses of variance showed no one of these differences in mean liveweight to be significant.

7. Face cover of recipient ewes

Table 30 and Figure 32 show results for face cover of recipient ewes dependent upon the number of ova transferred and the number of embryos alive at slaughter. The results of analyses of variance of these data for ewes which received two ova are given in Table 31.

The analyses of variance of data for ewes receiving two ova showed a significant difference in the mean face cover of the sub-groups of ewes within the four experimental groups. The results from the one ovum transfers, although not showing a significant difference in face cover between pregnant and non pregnant ewes, supports the significant result from the ewes which received two ova, for the mean face cover of recipient ewes was greater in non pregnant than in pregnant ewes. But, the result from the group 1965T60, which received four ova, does not show any increase in the mean face cover of non pregnant ewes.

CHAPTER VI

FURTHER INVESTIGATIONS OF OVULATION AND PRENATAL MORTALITY

I. EXPERIMENTAL PROCEDURE

II. RESULTS

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   (a) Liveweight
   (b) Face wool covering

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CHAPTER VI

FURTHER INVESTIGATIONS OF OVULATION AND PRENATAL MORTALITY

Opportunity arose to study ovulation and prenatal mortality in ewes which had been mated naturally. The ewes available for this study were those ewes not slaughtered during the experiment in 1964. This study will henceforth be referred to as 1964 Experiment 2.

I. EXPERIMENTAL PROCEDURE

The experimental ewes were those originally from the flock of 128 Romney ewes purchased from Wanganui. Altogether 61 ewes were available, comprising 18 from group 1964HR, 28 from group 1964D_1 and 22 from group 1964LR.

The rams used for fertile mating were the entire rams used in the main 1964 season experiment.

All ewes were grazed on ryegrass-white clover pasture and were managed to ensure that there would be a satisfactory feed supply on the experimental area over the winter months. Therefore no attempt was made to maintain the ewes at a constant body weight. Liveweights were recorded weekly at 9.0 a.m. using the same method as that employed previously. The mean weekly liveweights of ewes in the three groups are shown in Table 32.

T A B L E 32

MEAN WEEKLY LIVEWEIGHTS RECORDED IN 1964 EXPERIMENT 2

Date of weighing	Experimental Group		
	1964HR Liveweights (lbs)	1964D Liveweights (lbs)	1964IR Liveweights (lbs)
6 April	119.5	112.6	102.2
13 April	121.6	111.5	104.1
20 April	127.9	114.1	109.5
27 April	125.7	113.3	107.4
5 May	132.0	120.9	113.3
11 May	126.2	115.3	106.3
18 May	125.6	116.0	106.2
25 May	130.7	119.6	111.6

Liveweights used in subsequent analyses were those recorded on 25 May, 1964. Ewes were inspected daily at 4.0 p.m. for detection of oestrus. Following the main 1964 season experiment, ewes were run with vasectomized rams until 4 May, 1964. By this date all ewes had experienced, during the 1964 season, either four oestrous periods or one 'silent' oestrus and three oestrous periods. Entire rams of proven fertility were introduced on 4 May, 1964. All ewes were killed within 18 - 30 days of fertile mating. Observations were made on the genital tracts of sheep soon after slaughter using methods already described.

II. RESULTS

The data obtained in this experiment was analysed to gain further information on factors related to ovulation and early prenatal mortality.

1. OVULATION

(a) Liveweight

A greater proportion ($P < 0.02$) of ewes in the group 1964HR had two corpora lutea than in the group 1964LR (Table 33). There was also a greater proportion ($P < 0.05$) of ewes in the group 1964HR with two corpora lutea than in the group $1964D_1$. A slightly greater, although non-significant, proportion of ewes in group $1964D_1$ had two corpora lutea than in group 1964LR.

TABLE 33

NUMBERS OF EWES WITH EITHER ONE OR TWO CORPORA LUTEA

(1964 EXPERIMENT 2)

Group	Number of ewes with two corpora lutea	Number of ewes with one corpus luteum
1964HR	9	9
$1964D_1$	4	18
1964LR	3	18

Comparison of differences:

Proportion of ewes with 2 corpora lutea (Chi^2)

1964HR v $1964D_1$, $Chi^2 = 4.6$, $P < 0.05$
1964HR v 1964LR, $Chi^2 = 5.8$, $P < 0.02$

An analysis of variance (Table 34) was made of the body weights of all ewes included in the experiment. Reservation must be held to the level of significance of one per cent obtained between those ewes with two corpora lutea and those with one corpus luteum. This is because the groups 1964HR and 1964LR had been selected as being those ewes not pregnant after the main experiment.

TABLE 34

ANALYSIS OF VARIANCE OF BODY WEIGHTS OF EWES WITH ONE OR TWO CORPORA LUTEA (1964 EXPERIMENT 2)

Source of Variation	d.f.	Mean Square	F value	Significance of F value
Total	60			
Between groups with one or two corpora lutea	1	1,121	11.548	$P < 0.01$
Within groups	59	97.07		

(b) Face wool covering

Table 35 gives the number of ewes with either one or two corpora lutea within the various face grade groups. Ewes graded 1, 1+ and 2 were classed as being woolly-faced ewes while those graded 2, 3 and 3+ were classed as being open-faced. This table shows a greater proportion of open-faced sheep ($P < 0.02$) in those ewes with two corpora lutea than in those ewes with only one corpus luteum.

TABLE 35

FACE GRADES WITHIN GROUPS OF EWES WITH ONE OR TWO CORPORA LUTEA

(1964 EXPERIMENT 2)

Face grades	Ewes with one corpus luteum	Ewes with two corpora lutea
1 and 1+	7	2
2	23	3
Total woolly	30	5
2+	7	3
3 and 3+	8	8
Total open	15	11

Comparison of differences

Proportion of ewes with two corpora lutea (Chi^2)

Open-faced ewes v Woolly-faced ewes ; Chi^2 = 6.05, $P < 0.02$

The mean face cover measurement was lower for those ewes with two corpora lutea than for those ewes with one corpus luteum. This difference was not found to be significant (Table 36).

TABLE 36

ANALYSIS OF VARIANCE OF FACE COVER OF EWES WITH ONE OR TWO CORPORA LUTEA

(1964 EXPERIMENT 2)

Source of Variation	d.f.	Mean Square	F value	Significance of F value
Total	60			
Between groups with 1 or 2 corpora lutea	1	323.8	1.969	Not significant
Within groups	59	164.4		

2. SURVIVAL OF OVA

(a) Liveweight

Table 37 presents the mean body weights of ewes classified according to the number of corpora lutea per ewe and the number of embryos alive at slaughter. One ewe had one corpus luteum but had two embryos alive at slaughter. This probably arose because either the fertilized ovum divided into two ova in the early stages of cleavage or two ova were shed from the same follicle. This ewe was considered in the analysis together with the other ewes which had a single corpus luteum.

TABLE 37

BODY WEIGHTS OF EWES CLASSIFIED BY THE NUMBER OF CORPORA LUTEA AND THE NUMBER OF EMBRYOS ALIVE AT SLAUGHTER (1964 EXPERIMENT 2)

Number of corpora lutea	Number of embryos alive at slaughter	Number of ewes	Body weight (lbs.) Mean	±	S.E.
2	2	10	126.6	±	4.61
2	1	3	125.3	±	5.21
2	0	3	130.7	±	5.93
1	2	1	141		-
1	1	31	116.1	±	1.36
1	0	13	118.6	±	2.58

There was no significant relationship between liveweight and survival of ova. The mean liveweight of pregnant ewes with two corpora lutea was lower than that of the non-pregnant ewes. Similarly the mean liveweight of pregnant ewes with one corpus luteum was lower than that of the non-pregnant ewes with one corpus luteum.

(b) Face wool covering

Table 38 presents the mean face cover of ewes classified according to the number of corpora lutea per ewe and the number of embryos alive at slaughter. There was no significant relationships between face cover and ovum survival.

No relationship between face grade and survival of embryos was found.

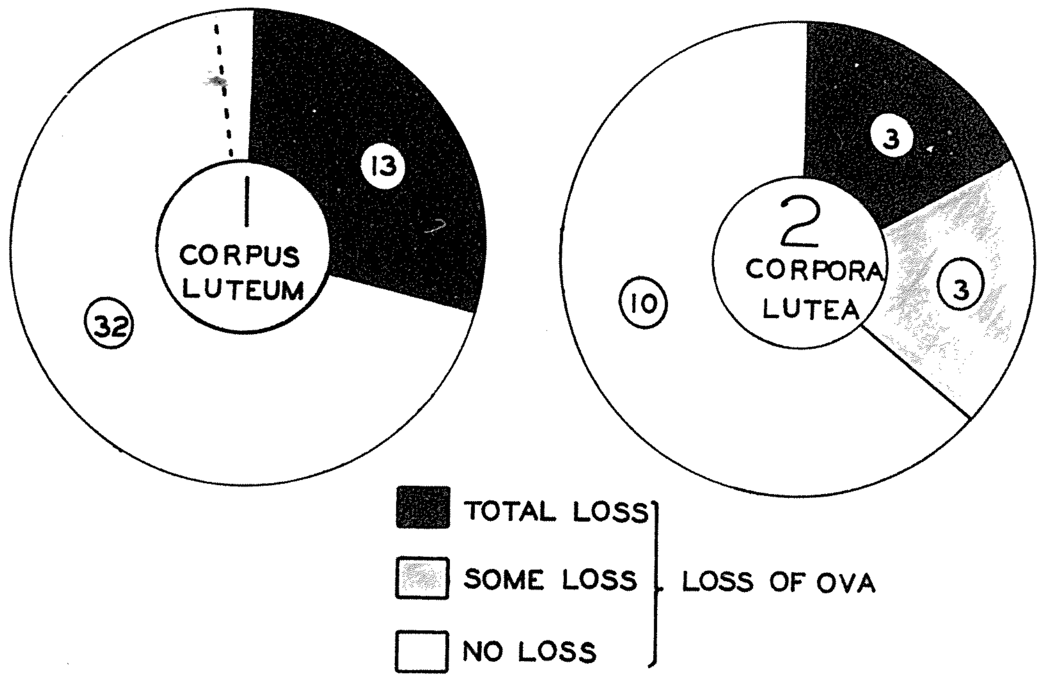

Figure 33 Diagrammatic representation of embryonic survival until slaughter in ewes with one or two corpora lutea. (The number of ewes shown in small circles)

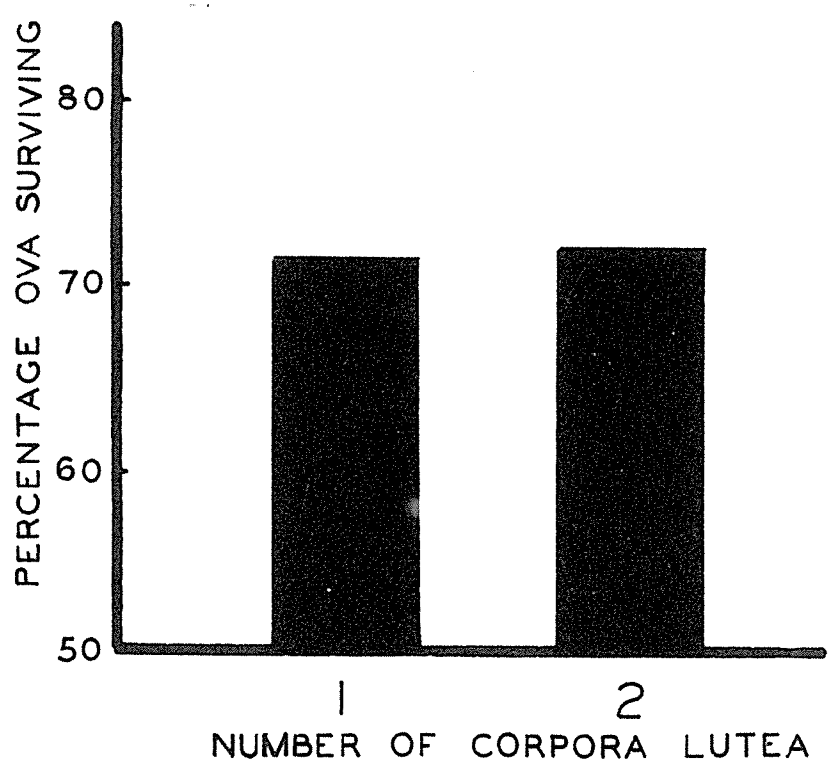

Figure 34 Percentage of ova surviving to slaughter of ewes relative to the number of corpora lutea.

TABLE 38

FACE COVER OF EWES CLASSIFIED ACCORDING TO THE NUMBER OF CORPORA LUTEA AND THE NUMBER OF EMBRYOS ALIVE AT SLAUGHTER

Number of corpora lutea	Number of embryos alive at slaughter	Number of ewes	Face cover (mg/cm.^{2}) Mean	±	S.E.
2	2	10	26.95	±	4.13
2	1	3	37.79	±	8.37
2	0	3	22.73	±	0.69
1	2	1	43.95		-
1	1	31	33.01	±	3.18
1	0	13	33.40	±	6.24

(c) Number of corpora lutea

Figure 33 illustrates for ewes with either one or two corpora lutea the proportion of animals in which prenatal mortality had occurred. A greater although non-significant proportion of ewes were pregnant at slaughter in the group of ewes with two corpora lutea than from the group of ewes with one corpus luteum.

The percentage of ova surviving to slaughter as related to the number of corpora lutea is shown in Figure 34. Approximately 70 per cent of the ova shed survived there being no relationship between the number of corpora lutea and the survival of ova.

C H A P T E R   V I I

D I S C U S S I O N

SYNCHRONIZATION OF OESTROUS CYCLES

PRODUCTION OF OVA FOLLOWING HORMONAL TREATMENT

OVULATION

SURVIVAL OF OVA

C H A P T E R   V I I

D I S C U S S I O N

SYNCHRONIZATION OF OESTROUS CYCLES

Ovum transfers to recipient New Zealand Romney ewes were carried out during the early stages of the breeding season. In order that the oestrous periods of donor and recipient ewes would be synchronized at the time of transfer, progesterone treatment was initiated during the anoestrous period. Infertility in the sheep following the use of progesterone and P.M.S. has been noted (Davies and Dun, 1957). It is possible that progesterone injections are responsible for impaired fertility (Davies, 1960). It was therefore decided that all ewes would have one oestrous cycle prior to ovum transfer without any progesterone administered. Hancock and Hovell (1961) carried out transfers at the first and second oestrus occurring after progesterone treatment and also to untreated ewes. They did not find any convincing evidence in favour of any one method, but recognized that the different treatments may have an effect on fertility.

Progesterone treatment was commenced prior to the time at which the 'first' ewes in the flock would be experiencing 'silent' oestrus. After the cessation of progesterone treatment approximately 65 per cent of the ewes exhibited oestrus while the remainder had a 'silent' oestrus. It was confirmed that the ewes experiencing 'silent' oestrus had ovulated. There was a significantly greater proportion of ewes in oestrus in the group 1964LR than in the group 1964HR, although, in the total flock the heavier ewes did not have a greater proportion of ewes showing oestrus than the light ewes.

All ewes commenced progesterone treatment within a twelve day period and therefore subsequently underwent laparotomy approximately at the same stage of the breeding season. A constant ratio of donor to recipient ewes were injected with progesterone daily so as to ensure a satisfactory supply of ova at the time of laparotomy. Any recipient ewe for which ova were not available did not undergo laparotomy. A significant correlation between body weight of the ewe and time to onset of oestrus after the final injection of progesterone was found. The greater the body weight of the ewe the later was the onset of oestrus. The interval of time to the onset of oestrus varied from 48 to 144 hours after the administration of the final progesterone injection. This, together with the fact that the following oestrous cycle was of variable length, decreased the chances of all recipient ewes being perfectly synchronized with donor ewes at laparotomy, and, on occasions this resulted in an insufficient number of donor ewes being available. Hunter (1954) pointed out that for synchronization as a preliminary to ovum transfer sufficient ewes to allow for some spread of the post-progesterone oestrus was advisable. Robinson (1956) showed that an injection of 500 i.u. P.M.S. twenty-four hours after the final progesterone injection, gave an earlier and more predictable time of onset of oestrus than did progesterone treatment alone.

PRODUCTION OF OVA FOLLOWING HORMONAL TREATMENT

Coincident oestrus and ovulation have been induced in New Zealand Romney ewes by the injection of progesterone and/or gonadotrophin during the breeding season (Wallace, 1954; Wallace, Lambourne and Sinclair, 1954) and anoestrus season (Lambourne, 1955; Raeside and Lamond, 1956; Edgar, 1958; McDonald, 1961). Considerable variation in ovulation response to gonadotrophin, particularly when high dose levels are administered, have been reported. As an example, Wallace (1954) reported that 1,000 i.u. P.M.S. when injected during the oestrous cycle had caused from one to thirteen ovulations per animal.

In this experiment three main dose levels, 1,000 i.u., 1,200 i.u. and 1,500 i.u. P.M.S., were administered on the twelfth or thirteenth day of the oestrous cycle. This treatment lead to marked increases in the incidence of multiple ovulations. To assess the day for injection of P.M.S. in those ewes which experienced 'silent' oestrus after progesterone treatment it was considered that ovulation had occurred at the same time as in ewes which exhibited oestrus. There was no difference in the response to P.M.S. between the two groups in either the interval of time to onset of oestrus after P.M.S. administration or in the ovarian response thus suggesting no difference in the time of ovulation after the cessation of progesterone treatment between the two groups.

Factors which determine the number of ova available for transfer are the number of potential donor ewes available, the ovulation rate in these potential donors and the number of fertilized ova recovered.

The administration of gonadotrophin during the follicular phase of the cycle hastens the onset of the following oestrus (e.g. Warwick and Casida, 1943; Wallace, 1953). Wallace found little difference between the cycle lengths of animals injected on the tenth, eleventh, twelfth, thirteenth or fourteenth days of the oestrous cycle. The results of this experiment have shown a significant decrease in the length of the oestrous cycle of ewes injected with 1,000 i.u. P.M.S. on day 12 rather than on day 13 of the oestrous cycle. The animals used in this experiment did not show any relationship between the dose level of P.M.S. administered and the oestrous cycle length as was recorded by Wallace, Lambourne and Sinclair (1954).

The length of oestrous cycle partially determines the number of donor ewes available to supply ova at transfer. Considering this and the significant relationship between liveweight and interval to onset of oestrus after progesterone there may be a reduction in the proportion of donor to recipient ewes available for laparotomy at a required time. Such was the case in the 1965 season when the mean body weight of donor ewes was less than that of the recipient ewes and this resulted in an insufficient number of donors being available on the final two days on which laparotomies were performed.

Robinson (1951b) reported significant increases in the number of ovulations per ewe following treatments with 500 - 2,000 i.u. P.M.S. The results obtained in this experiment showed no increase in the ovarian response with the increase in dose level from 1,000 i.u. to 1,200 i.u. although there was a significant increase when the dose level of 1,500 i.u. was administered. (The P.M.S. administered was tested by assay against an international standard and was found to be of the correct potency. The methods used in this study were not found to affect the potency of the P.M.S. used). The ovarian response to 1,200 i.u. P.M.S. is therefore a true reflection of the variability in response to the high levels of P.M.S. administered in this experiment.

In the 1964 season only three ewes were treated with 1,500 i.u. P.M.S. and these ewes showed a greater ovarian response than ewes treated with 1,000 i.u. or 1,200 i.u. P.M.S. with no evidence of an increase in the proportion of uncleaved ova. Thus it was decided that during the 1965 season a greater proportion of donor ewes would be injected with 1,500 i.u. P.M.S.

The number of ova shed in donor ewes ranged from one to twenty-three. There was no significant decrease in the percentage of ova recovered with increase in the number of ova shed. This finding is in agreement with the result of Hancock and Hovell (1961).

There was no decrease in the percentage of ova recovered with increase in the interval of time from onset of oestrus to laparotomy. All laparotomies were carried out on donor ewes within 96 hours of the onset of oestrus.

It was assumed that the uncleaved ova were unfertilized for laparotomies were carried out on donor ewes at the earliest 55 hours after the onset of oestrus. Care was taken in this experiment to distinguish normal cleavage from spontaneous cleavage which can occur in unfertilized ova. To avoid delay at transfer the ova were examined only briefly under the binocular microscope. Austin (1949) and Chang (1950) have shown that fragments approximately equal in size and resembling blastomeres may be found in degenerating ova, so that some of the ova which did not develop after transfer may have been fragmenting at the time of transfer.

Laparotomies were carried out on donor ewes so as to recover a maximum number of ova at the 8-cell stage (Hancock and Hovell, 1961). Averill and Rowson (1958) recommended that laparotomy should be done about 60 hours after the ewe had been found to be marked by the ram at morning inspection when the ewes are examined twice daily and when the ewes have free access to a fertile ram. Hancock and Hovell (1961) operated a full 72 hours after hand mating for fertile mating may have been as much as 16 hours after the onset of oestrus. These workers state that the procedure was effective in avoiding the recovery of ova younger than the 6-cell stage but may have caused an increase in the number of ova lost.

Twenty-seven ewes were injected with 1,500 i.u. P.M.S. in the 1965 season and of these about 45 per cent gave only uncleaved ova at recovery. A further 15 per cent gave both cleaved and uncleaved ova. The reason for such a decrease in the proportion of cleaved ova resulting from high level doses of P.M.S. is of considerable importance for it is apparent that although the high level doses of P.M.S. result in high ovulation rates a lower dose level may result in slightly lower ovulation rates but with greatly increased rates of fertilization.

Laffey and Hart (1959) state that spermatozoa are always seen in the zona pellucida of normally cleaved sheep ova. Spermatozoa were found attached to the zona pellucida of the uncleaved ova from only two ewes which were injected with 1,500 i.u. of P.M.S. A number of ova from two ewes on this dose level of P.M.S. were slightly concave in appearance. Averill (1958) found the proportion of abnormal ova from ewes treated with P.M.S. to not exceed the proportion from untreated ewes. It was thus concluded that most of the uncleaved ova recovered from those ewes on 1,500 i.u. P.M.S. were normal ova but unfertilized.

Robinson (1951b) found that in sheep where high dose levels of P.M.S. were used (1,000 - 2,000 i.u.) a large number of ova were released, accelerated tubal transport occurred and the proportion of ova fertilized was reduced. In this experiment there was no evidence of rapid transport of ova in the Fallopian tubes as there was no decrease in the recovery rate of ova up to 96 hours after the onset of oestrus. In this experiment it appeared that the spermatozoa were not reaching the normal site of fertilization. This could be due to conditions which either interfere with the transport of spermatozoa or create an unfavourable environment for spermatozoan survival. This conclusion agrees with that of Dauzier (1958a) in his findings on the transport and survival of spermatozoa in the genital tract of ewes at different stages of the oestrous cycle. The ewes on the high dose level of P.M.S. (1,500 i.u.) during the 1965 season regularly showed corpora lutea which appeared of different 'ages' on the one ovary. The presence of unruptured follicles could have been a contributing factor to the failure of spermatozoan transport and for the prevention of fertilization of the early shed ova. This conclusion that ovulation had been spread over a considerable period of time was supported by the fact that at times both 2- and 8-cell ova were recovered from the same uterine horn in those ewes in which fertilization had occurred after the administration of 1,500 i.u. P.M.S.

Previous work with fertilized ova recovered from donor ewes treated with P.M.S. has shown that these ova are fully viable (Hunter, Adams and Rowson, 1955; Averill and Rowson, 1958; Moore and Rowson, 1960; Hancock and Hovell, 1961b). No evidence was found in this experiment to suggest that ova shed after P.M.S. treatment were in any way different from ova shed from ewes to which no P.M.S. had been administered.

OVULATION

Factors related to ovulation were studied from the data obtained at the laparotomy of recipient ewes and at the slaughter of ewes in Experiment 2. All data obtained at laparotomy were from ewes at the same stage of the breeding season. This reduced the effect that date of oestrus has on the ovulation rate (Averill, 1959; McDonald, 1958). These workers have shown that there is a within breeding season rise and fall in the mean number of ovulations with the highest rates of ovulation occurring in April and May.

Relatively short periods of improved feeding prior to fertile mating have been found to increase the proportion of ewes producing twins (Wallace, 1953). In this experiment no nutritional flushing was carried out so as to study the relationship between liveweight and ovulation rate while the body weight was held constant.

In the 1964 season a significantly greater proportion of the ewes in group 1964HR had two corpora lutea than the ewes in the group 1964LR. These two groups were the heaviest 50 ewes and the lightest 50 ewes respectively originally from the same flock of ewes. A combined treatise of the results from the 1964 and 1965 seasons showed a significantly greater mean liveweight for ewes which had two rather than one corpus luteum.

Nevertheless it was found that the group with the highest mean liveweight did not have the highest proportion of ewes with two corpora lutea. This fact that the group 1965T60 had a smaller proportion of ewes with two corpora lutea than the groups 1965T61 and 1964HR emphasises that the relationship between liveweight and ovulation must be examined on a within flock basis (Cockrem, 1964). The group of ewes with the lowest proportion of ewes with two corpora lutea, 1964LR, was the group of ewes with the lightest mean liveweights.

The result obtained at laparotomy, that within a flock heavier ewes tend to have higher ovulation rates, was confirmed in Experiment 2. It was found that there was a significantly greater proportion of ewes with two corpora lutea in the heaviest group, 1964HR, than in the two lighter groups, 1964D$_1$ and 1964LR. Although there had been a certain amount of selection of which ewes would be included in this experiment, the data were used to study further the relationship between ovulation rate and liveweight.

The liveweight of the ewes was found to be negatively correlated with their face cover. It is believed that this is the first such case where a significant correlation between liveweight and face cover has been found for c.f.a. Romney ewes. The study was designed primarily to study the relationship between liveweight and fertility but opportunity was also taken to study the relationships between face cover and fertility.

Higher lambing percentages have been obtained from open-faced sheep than from closed-faced sheep (e.g. Coop, 1955; Cockrem, 1962). Over the total recipient flock there was a higher proportion of ewes graded with open faces within the group of ewes with two corpora lutea than within the group with one corpus luteum. Further analyses using face cover measurements and wool gradients gave no further significant result.

SURVIVAL OF OVA

Most studies of the prenatal development of the sheep have involved accurately aged and measured embryos and foetuses. Some of the measurements have been made on embryos and foetuses that had been fixed in formaldehyde (Cloete, 1939) but Green and Winters (1945) report that fixation alters some of the dimensions. As the results of this experiment also required the accurate measurement of specimens, all measurements were made prior to fixation in formal-saline. At dissection of the genital tracts the presence of heart movements was taken to be proof that the embryo was alive.

Measurements taken of 19 - 23 day embryos indicate that to assess the age of a viable embryo the most critical measurements would be the crown-rump and allantois lengths. The number of somites was found to give no indication of the age of the embryo. Measurements taken indicate the rapid growth of the embryo between day 19 and day 23 after onset of oestrus.

Moore, Rowson and Short (1960) found that the survival and development of transferred ova was unaffected either by superovulation or by the breed of ewe from which the transferred ova were obtained. They slaughtered ewes on day 17 or day 18 after the onset of oestrus. They considered that with the criteria they used for classification of embryos that it was very difficult to determine whether retarded embryos were alive or dead at the time of autopsy. Robinson (1951a) also found this difficulty with embryos recovered from ewes which were up to three weeks pregnant. He considered that retarded embryos were non-viable and that retardation represented the beginning of embryonic death.

At slaughter of ewes at day 19 after the onset of oestrus there was evidence of a small amount of recent embryonic mortality. A few ewes contained no embryonic membranes in their uteri although they had not returned to oestrus while a further small proportion of the ewes contained either a dead embryo or degenerating embryonic tissue. There was no evidence of any prenatal mortality having occurred between day 19 and day 35. Therefore it was concluded that the highest levels of prenatal mortality occurred in the first three weeks of pregnancy. Moore et al. (1960) found two peaks of embryonic mortality, one occurring before and the other after the attachment of embryos to the endometrium. Embryonic death accounted for almost all the prenatal mortality and the major portion of the loss occurred before attachment. They found that foetal mortality occurring later than the 17th or 18th day of pregnancy was negligible.

This study emphasizes the need for more detailed investigations at the time of implantation. No evidence of implantation having occurred is recognizable in the ewe after prenatal mortality as in such species as the rabbit (Allen, Brambell and Mills, 1947). Whether or not the extent of prenatal mortality that occurred in this experiment after transplantation of ova was partly due to the early rejection of the ova or later prenatal mortality is not known.

This experiment was designed so that ewes received one, two or four cleaved ova. It was considered for purposes of analysis that all cleaved transferred ova were alive at transfer. To assess the effect ovum transplantation had on ovum survival it was necessary to examine the methods used in the recovery and transfer of ova. Moore et al., (1960) considered that with the survival rates being obtained in such studies the use of ovum transfer can now be used to study factors which might influence the survival and development of fertilized sheep eggs.

Surgical procedures employed in this study presented no special difficulties and it is considered that the technical difficulties were not sufficient to explain the rates of prenatal mortality obtained. Hancock and Hovell (1961) considered a vulnerable part of the surgical procedure to be the actual transfer of ova. They found that great care was needed to ensure that the ova were deposited within the cavity of the uterus and not under the uterine mucosa. Care was at all times taken to avoid such an occurrence in this experiment.

It is felt that in this experiment the main danger lay in the transfer of ova to the Fallopian tubes. When the ova were exhausted from the pipette they were carried in a certain quantity of fluid. There was a tendency for this fluid to flow back towards the fimbria and this could well have carried transplanted ova with it.

Moore and Shelton (1964) found that ovum survival increased significantly with the age of ovum transferred. These workers were unable to determine whether younger eggs are more susceptible to the collection and transfer procedures, or to transfer itself. Limited evidence has been presented by Averill and Rowson (1958) that the uterus does not provide an environment suitable for the survival and development of eggs of 2- or 4-cells.

Moore and Shelton (1964) reported that tubal transfers were more successful than uterine transfers. Moore et al., (1960) and Moore and Shelton (1962) reported that the survival of eggs of less than 8-cells transferred to the tubes was significantly less than that of eggs of 8-cells or more transferred to the uterus. They suggested that the difference was due to the site of transfer. Moore and Shelton showed that the site of transfer and age of eggs were confounded and that both factors are important. Moore et al., (1960) advanced two explanations for the lower rates of survival following tubal transfer; firstly that sheep eggs at the early stages of cleavage may be particularly susceptible to conditions of storage and transfer; and secondly that accelerated or tubal transport may occur.

During the course of this experiment 44 ova at less than the 6-cell stage were transferred but only three of these ova definitely survived to the slaughter of the recipient ewes while a further ten could possibly have developed. The findings of Moore and Shelton (1964) suggest that this low rate of survival resulted mainly because younger ova were transferred and not because transfers were made to the tubes rather than to the uterus. The results of this experiment make it apparent that it is desirable to transfer only ova which are at least at the 8-cell stage of development. It is not known if the technique of transferring 8-cell ova to the Fallopian tubes would have increased the ovum survival rate for this method was not employed in this study All 8-cell ova were transferred to the uterine horns.

Averill and Rowson (1958) recommended the use of special dialysis tubes for storage of ova prior to transfer. Averill (1956) suggested that a change in osmotic concentration of the serum may affect the survival but Hancock and Hovell (1961) suggest that dialysis tubes give no advantage over storing ova in conventional tubes at least for up to five and a half hours. In this experiment ova were stored in small glass containers covered by watch-glasses. Less than 10 per cent of ova were held longer than 120 minutes before transfer after recovery. One ovum survived after 405 minutes storage prior to transfer. No effect on the chance of survival was found by increasing the time of storage. Hancock and Hovell (1961) achieved successful transfer with unheated serum. Thus it was concluded that any slight drop in temperature or slight change in osmotic pressure of the serum at the time of storage that may have occurred did not have any significant effect.

Hancock and Hovell (1961) state that for a team familiar with the technique, eight ewes form a convenient group for operations to be carried out upon during the course of one day. In excess of 15 ewes were often operated on in one day in this study and in 1965 a concurrent series of laparotomies were carried out by some members of the team at times increasing the daily number of laparotomies to more than twenty.

In reviewing the literature it became evident that the problem of carrying out a large number of laparotomies in one day has not been found by other workers for they have not attempted in any way to restrict the transfer of ova to a short period in the breeding season. In this experiment an attempt was made to equate the seasonal factor for all ewes. As all transfers were to be made at the same stage of the breeding season, even though the donor and recipient ewes were not at all times as closely synchronized as would be optimum, the transplantations were nevertheless carried out. To conduct egg transfers where close synchronization was desired a greatly increased number of donor ewes would be required or a greatly increased supply of cleaved ova from the available donors.

Few experiments have been reported that were specifically designed to study the relationship of ovular age to endometrial development. However, some information bearing on this problem is included in papers on ovum transfer.

Ovum transfers in rodents have shown the need for close synchronization of the stage of development of the transferred ova and the stage of luteal development in the ovary of the recipient animal. Chang (1950) recorded maximum survival rates in recipient rabbits which were exactly synchronized with their donors but did not comment upon the fact more ova survived when the tract was one or two days younger than when the tract was one or two days older than the ova. However, Dowling (1949) obtained excellent results in the same species where the tract was older. Results not in accord with one another have been found with work on the rat. Nicholas (1933) recorded higher rates of survival when ova were younger than the recipient tracts than when the ova were comparatively older. On the other hand, Noyes and Dickman (1960, 1961) recorded that rat eggs of the same age or one day older than the uterus were more likely to survive than those eggs one day younger.

In the sheep Hunter, Adams and Rowson (1955) found that higher rates of survival occurred when the development of the ova was ahead of the uterine development of the recipient animals. Hancock and Hovell (1961) and Moore and Shelton (1964) present no evidence that there is any difference in transferring relatively young or old ova to the uteri. They found that accurate synchronization gave the best results.

The results of this experiment demonstrated that the highest survival rates of transferred ova were obtained when there was accurate synchronization of donor and recipient ewes. There was no significant decrease in the survival rate for ova transferred to tracts up to 28 hours older than to exactly synchronized tracts. The results indicate a greater chance of survival in older rather than younger tracts. No ova survived in tracts which were either more than 20 hours younger or 28 hours older than the transferred ova. These results are indicative of rather specific lower and upper limits of ovular and endometrial development that will allow the development of transferred ova.

Averill and Rowson (1958) found a consistent but non-significant increase in the ability of ova to develop in the presence of two or more corpora lutea as opposed to a single corpus luteum in the ovaries of the recipient ewes. Moore et al., (1960) found that superovulation of recipients had little or no effect upon either the number of ewes which became pregnant or on the survival of transferred eggs. This study showed no relationship between the number of corpora lutea in recipient ewes and the survival of transferred ova.

Moore et al., (1960) found that the proportion of recipient ewes becoming pregnant was the same in groups which received either two or five ova although the proportion of ova surviving at all stages was greater when only two ova were transferred.

The results of this experiment showed that the transfer of one, two or four ova did not affect the proportion of ewes which were pregnant at slaughter. Ovum survival rates within the three classes of recipient ewes were 52.8 per cent for one ovum transfers, 38.2 per cent for two ova transfers and 33.3 per cent for four ova transfers. These results confirm those of Moore and Shelton (1964) who found that egg losses had a strong tendancy to fall on litters as a whole.

It appears that the success or failure of any transfer is largely dependent upon the inherent ability of the ewe to support a pregnancy than to the number of ova transferred. It is thought likely that this factor contributes largely to the fact that 20-30 per cent of ewes do not conceive at normal mating. This inability to maintain a pregnancy was shown to be transient for many of the non-pregnant ewes were successfully mated and found to be pregnant at a later date in the season during the course of Experiment 2.

The hypothesis that all ova transferred to recipient ewes did not have an equal chance of survival was found to be true. The hypothesis that such an equal survival chance did not exist in those ewes which were pregnant was also found to be correct. Moore and Shelton (1964) found that when the maternal environment was suitable egg losses are at random and not excessive.

When considering the results of this study it must be realised that there were a large number of groups and sub-groups. An attempt was made to decrease this number where possible. All single ovum transfers were carried out in the 1964 season while most four ova transfers were carried out to one group in the 1965 season. Transfers of two ova were made to all experimental groups. The number of sub-groups increased as the number of ova transferred increased. When four ova were transferred there were the five sub-groups where four, three, two or one or no ova survived.

From the results of this experiment it must be concluded that there is no significant relationship between the liveweight of ewes and the survival of transferred ova. Within the groups of ewes receiving two ova the group with the lowest mean liveweight had the greatest proportion of ewes with two living embryos at slaughter while the heaviest group of ewes had the largest proportion of ewes with no living embryos at slaughter. However, this trend was not maintained within the individual groups. In only one group, 1965T60 receiving two ova, was the mean liveweight of the pregnant ewes lower than that of the non-pregnant ewes.

Lambing percentages have been found to be higher in flocks kept under good feeding conditions on lowland farms than in similar flocks kept under more rigorous conditions of hill and mountain land (White and Roberts, 1922). Clarke (1934) suggested that such differences in lambing percentages were due mainly to the differences in the ovulation rates of the ewes. The results of this study support this suggestion in that there was a significant increase in ovulation rate with increase in liveweight, but there was no apparent increase in the survival chance of ova shed by or transferred to the heavier ewes.

Allen and Lamming (1961) suggest that ovulation rate in the ewe is a function of the level of available nutrients in the diet and/or the body reserves of stored energy. The results of this experiment demonstrate that these factors probably do not affect the prenatal mortality rate in the first month of pregnancy but do affect the ovulation rate.

In this study it was found that ewes which received two ova at transfer and were pregnant at slaughter had a significantly lower mean face cover than non-pregnant ewes in the same groups. Similarly, those ewes which were pregnant at slaughter after receiving one ovum had a lower mean face cover than non-pregnant ewes although this difference was not significant. This relationship was not found in the group which received four ova. No liveweight difference was found between pregnant and non-pregnant ewes in these same groups even though there was a correlation between the face cover and liveweight of the ewes. To date all evidence has pointed to the effects of face cover and liveweight acting in the same manner or at least producing similar results. This study then indicates that face cover may at times act independently of liveweight.

In the 1964 Experiment 2 further results were obtained on the survival of ova shed. Robinson (1951) and Averill (1956) found that 90 per cent of ova shed are fertilized. Liveweight and face cover were not related to ovum survival in this experiment. There was no relationship between ovum survival and the number of ova shed and there was a small, although non-significant, increase in the proportion of ewes pregnant in the group of ewes with two corpora lutea. The fact that no significant relationships were found between ovum survival and other factors is probably a reflection of the small number of ewes available for this study.

CHAPTER VIII

SUMMARY

SYNCHRONIZATION AND SUPEROVULATION

FERTILIZATION IN DONOR EWES

OVULATION IN RECIPIENT EWES

PRENATAL DEVELOPMENT

CHAPTER VIII

SUMMARY

In the course of the early portion of two breeding seasons 330 New Zealand Romney ewes were used to study the relationships of liveweight and face cover to ovulation and early prenatal mortality. In this study one, two or four cleaved ova were transferred to the genital tracts of recipient ewes. Such a technique avoided the problem of fertilization failure in recipient ewes and allowed study of prenatal mortality in ewes which had a known number of cleaved ova.

The reproductive tracts of donor and recipient ewes were synchronized prior to transfer of ova by the administration of 13 to 15 daily intramuscular injections of 10 mg. of progesterone. Progesterone treatment was commenced for all ewes within a twelve day period commencing from 18 February, 1964 and 22 February, 1965. All ewes experienced either two oestrous periods or one 'silent' oestrus and one overt oestrus prior to ovum transfer.

Ova were obtained from 147 donor ewes superovulated by the administration of from 500 i.u. to 1,500 i.u. P.M.S. on days 11, 12 or 13 of the oestrous cycle. Recipient ewes received no hormonal treatment in the cycle prior to that in which transfers were carried out. Observation of genital tracts of recipient ewes at the time of laparotomy allowed a study of ovulation.

Studies were made on the survival and development of the transferred ova.

In a further experiment 61 ewes were mated to fertile rams and further study of factors related to ovulation and early prenatal mortality made.

The results may be summarized as follows:

SYNCHRONIZATION AND SUPEROVULATION

1. Following progesterone treatment all ewes ovulated although 'silent' oestrus occurred in some animals. The proportion of ewes exhibiting oestrus was constant in both seasons. There was some evidence that oestrus occurred in a greater proportion of ewes with low liveweights.

2. The mean interval of time to onset of oestrus after the final injection of progesterone was approximately 80 hours with a range of 48 to 144 hours.

3. The liveweight of the ewe and the time to the onset of oestrus after the cessation of progesterone treatment were significantly correlated ($r = 0.38$).

4. The injection of 500 i.u. to 1,500 i.u. P.M.S. on day 12 or 13 of the oestrous cycle regularly induced multiple ovulations at the ensuing oestrus.

5. Injection of 1,000 i.u. P.M.S. on day 12 rather than day 13 of the oestrous cycle slightly shortened the length of the current oestrous cycle ($P < 0.01$).

6. There was a significant increase in the ovarian response with increase in the dose level of P.M.S. administered. Means of the responses obtained from the injection of 1,000 i.u., 1,200 i.u. and 1,500 i.u.

were 4.2, 3.1, and 8.5 corpora lutea respectively. There was, however, considerable variation in the response obtained from ewes on the same dose level.

7. The ovarian response to P.M.S. was unaffected by the occurrence or absence of oestrus prior to the administration of P.M.S.

8. The ovarian response of ewes injected with P.M.S. on day 11 of the oestrous cycle was poorer than after injection on days 12 or 13.

9. The proportion of ova recovered from superovulated donor ewes was unaffected by the dose levels of P.M.S. administered or the number of ova shed.

10. The proportion of ova recovered _in vivo_ from donor ewes was not affected by the interval from the onset of oestrus to laparotomy up to 96 hours.

FERTILIZATION IN DONOR EWES

11. Ova shed following P.M.S. treatment were highly fertilizable. However, in some ewes which produced high numbers of corpora lutea practically all ova were unfertilized. The failure of fertilization in these ewes did not appear to be due to accelerated tubal transport of ova, but rather to the absence of spermatozoa at the site of fertilization.

12. Ewes administered with a high level of P.M.S. frequently had corpora lutea in the same ovary which were not of similar age. In some of these ewes ova at the 2-cell and 8-cell stages of development were recovered. In other ewes all ova were unfertilized.

13. Fertilization of ova in donor ewes was unaffected by increase in the number of ova shed up to a response of 10 or 11 corpora lutea.

14. A significantly greater proportion of ova developed beyond the 6-cell stage were recovered from ewes examined after, rather than before, 68 hours after the onset of oestrus.

OVULATION IN RECIPIENT EWES

15. Recipient ewes with two corpora lutea had a higher mean liveweight than ewes with one corpus luteum ($P < 0.01$). Within a flock heavier ewes had a higher mean ovulation rate than the lighter ewes but yet flocks with highest mean liveweights did not necessarily have greatest mean ovulation rates.

16. In the recipient ewes with two corpora lutea rather than with one corpus luteum there was a greater proportion of ewes graded as open-faced (2+, 3 and 3+) than as closed-faced (1, 1+ and 2). Nevertheless, no significant relationship was found between the number of corpora lutea and the face cover, side body wool or wool gradient measurements. Liveweight of the ewes was negatively correlated with their face cover ($r = -0.272$, $P < 0.01$).

PRENATAL DEVELOPMENT

17. Measurements of the length of the crown-rump and allantois of 19 - 23 day embryos were related to the age of embryo. Growth at this stage is rapid.

18. Measurements of the weight, displacement and crown-rump length of 35 day embryos showed a slightly increased size of single embryos compared to those from a multiple pregnancy.

19. A low rate of survival was obtained when 2- and 4- cell ova were transferred to the Fallopian tubes of recipient ewes. Best results followed the transfer of 8-cell and older ova to the uterus.

20. Highest rates of survival of transferred ova were obtained when there was accurate synchronization of the stage of development of the transferred ova and the stage of luteal development in the ovary of the recipient animal.

21. There were rather specific upper and lower limits of ovular and endometrial development that allowed the development of transferred ova. The results suggest that there was a greater chance of ovum survival in older rather than younger tracts.

22. Transfer of one, two or four ova did not affect the proportion of ewes which were pregnant at slaughter. In ewes which received two or four ova there was a strong tendency for mortality to fall on all ova transferred rather than on an individual ovum.

23. An ovum had a greater chance of surviving to slaughter of the ewe if transferred as a single ovum rather than one ovum in a multiple egg transfer. All ova transferred to ewes pregnant at slaughter did not have an equal chance of survival.

24. The number of corpora lutea in recipient ewes had no affect on the survival of transferred ova.

25. The highest rates of prenatal mortality occur during the first three weeks of pregnancy. In this experiment prenatal mortality did not occur between day 19 and day 35 after the onset of oestrus. Most prenatal death occurred at, or prior to, the stage of implantation and these recipient ewes thus returned to oestrus by day 17 of the cycle. No evidence was found to indicate the specific time of egg loss after transfer.

26. There was no significant relationship between liveweight and survival of ova.

27. There was a significant relationship between face cover and ovum survival in those ewes to which two ova were transferred. The results also show a higher rate of survival in open faced ewes which received one ovum.

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APPENDIX I

MEAN LIVEWEIGHTS (LBS.) OF EWE GROUPS SHOWING STANDARD ERRORS OF MEANS

Group	Date of Weighing 17 February	24 February	2 March	9 March
1964HR	125.5 ± 1.1	121.6 ± 1.2	115.4 ± 1.0	118.4 ± 1.1
1964LR	105.0 ± 0.7	102.5 ± 0.6	96.5 ± 0.7	99.1 ± 1.1
1964D_1	116.3 ± 0.7	111.9 ± 0.9	106.1 ± 0.8	109.3 ± 0.8
1964D_2	133.9 ± 2.1	132.4 ± 2.1	125.7 ± 2.0	124.1 ± 2.0

Group	Date of Weighing 17 March	23 March	30 March	6 April
1964HR	116.5 ± 1.0	117.7 ± 1.2	120.1 ± 1.3	119.5 ± 1.1
1964LR	97.5 ± 0.7	98.6 ± 0.9	101.8 ± 0.9	102.2 ± 0.9
1964D_1	106.0 ± 0.8	109.1 ± 0.8	111.1 ± 1.1	112.6 ± 1.0
1964D_2	122.9 ± 2.0	125.8 ± 2.0	129.7 ± 2.7	129.9 ± 2.6

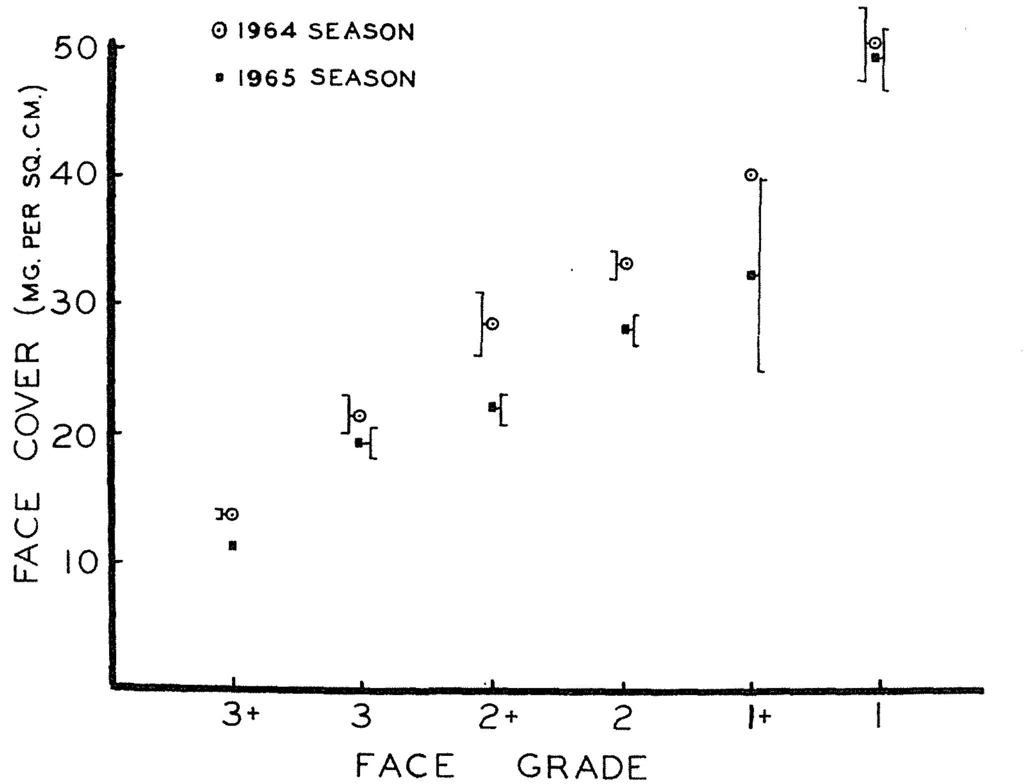
Group	Date of Weighing 23 February	3 March	11 March	31 March	11 April
1965T60	135.4 ± 2.4	132.6 ± 2.4	133.1 ± 2.4	132.7 ± 2.2	136.5 ± 2.3
1965T61	130.7 ± 2.7	126.5 ± 2.7	130.1 ± 2.5	123.9 ± 2.5	133.7 ± 3.1
1965 D	118.8 ± 1.2	114.4 ± 1.2	117.5 ± 1.2	116.5 ± 1.1	121.3 ± 1.1

APPENDIX II

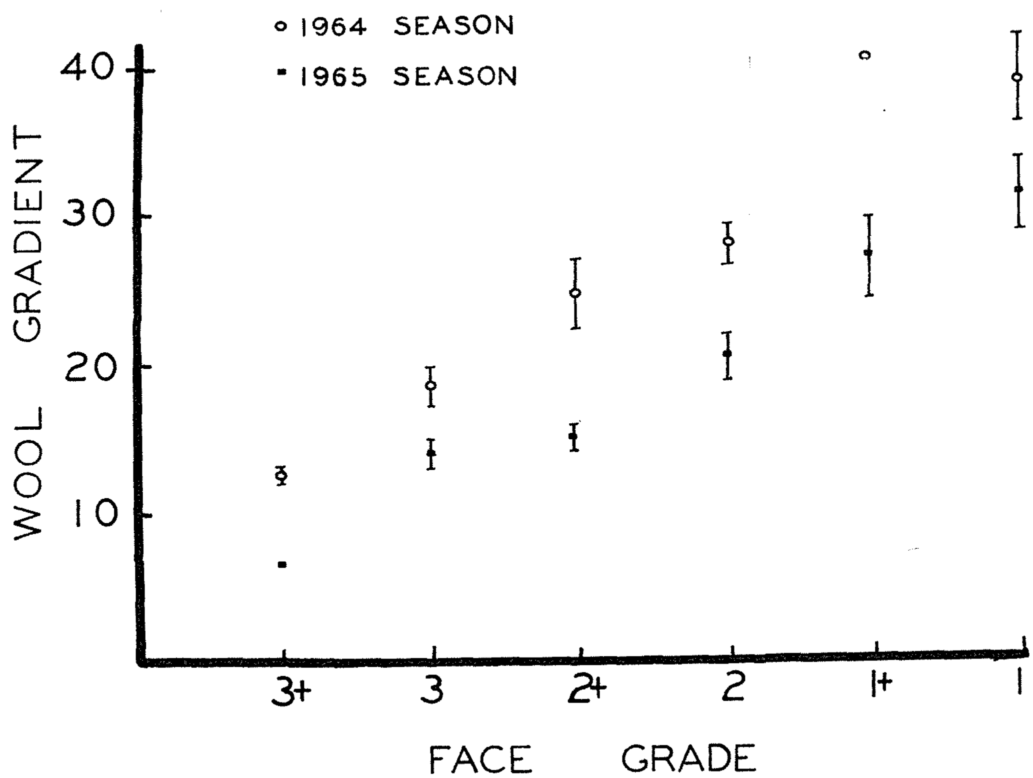
MEAN FACE COVER AND WOOL GRADIENT OF RECIPIENT EWES WITHIN FACE GRADE GROUPS

Face grade	Face covering (mg/sq. cm.) 1964 Season number of ewes	Mean	±	S.E.	1965 Season number of ewes	Mean	±	S.E.
1	11	51.0	±	3.0	5	50.0	±	2.6
1+	1	40.7		-	7	32.9	±	7.6
2	45	33.6	±	1.2	15	28.4	±	1.1
2+	13	29.0	±	2.5	17	22.3	±	1.3
3	17	21.7	±	1.4	14	19.5	±	1.2
3+	2	13.5	±	0.2	1	11.5		-

Face grade	Wool Gradient 1964 Season number of ewes	Mean	±	S.E.	1965 Season number of ewes	Mean	±	S.E.
1	11	39.6	±	3.1	5	32.1	±	2.7
1+	1	41.1		-	7	27.3	±	2.9
2	45	28.1	±	1.4	15	20.7	±	1.6
2+	13	24.9	±	2.2	17	15.0	±	0.9
3	17	18.6	±	1.4	14	14.2	±	1.1
3+	2	12.8	±	0.2	1	6.8		-



1964 SEASON
1965 SEASON
FACE COVER (MG. PER SQ. CM.)
50
40
30
20
10
3+
3
2+
2
1+
1
FACE GRADE



1964 SEASON
1965 SEASON
WOOL GRADIENT
40
30
20
10
3+
3
2+
2
1+
1
FACE GRADE