A STUDY OF SOME FACTORS AFFECTING REPRODUCTIVE PERFORMANCE IN NEW ZEALAND ROMNEY AND BORDER LEICESTER X ROMNEY TWO-TOOTH EWES

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P R E F A C E

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TABLE OF CONTENTS

Chapter		Page
VIII	DISCUSSION	50
	SYNCHRONISATION OF OESTROUS CYCLES	50
	PRODUCTION OF OVA	52
	CLEAVAGE OF FERTILISED OVA	61
	SEQUENTIAL TREATMENT OF EWES WITH P.M.S.	62
	OVULATION STUDIES	63
	SURVIVAL OF TRANSFERRED OVA	66
	UTERINE CAPACITY AFTER EGG TRANSFER	70
	PRE-NATAL LAMB GROWTH	73
	POST-NATAL LAMB GROWTH	79
	SUMMARY	83
	REFERENCES	85
	APPENDICIES	101

LIST OF TABLES

f refers to following page

LIST OF FIGURES

f refers to following page

I N T R O D U C T I O N

INTRODUCTION

The reproductive performance of the ewe is a key factor determining flock productivity and also the rate of genetic improvement possible within flocks. The lambing percentage of Romney sheep in New Zealand is variable but generally unsatisfactory. Crossbreeding with Border Leicesters may provide a method of rapidly improving lamb production.

Extensive comparisons of Romney and Border Leicester x Romney (BLX) sheep have been conducted at Whatawhata Hill Country Research Station (1954 - 1967) and Lincoln College (1952 - 1958). Results were in close agreement. On a per ewe basis, the crossbred was superior to the Romney in growth rate as a lamb and hogget, and in mature bodyweight. In addition, the lambing performance of the crossbred was at least 20% higher, and the growth rate of its lambs about 5% better, than the Romney.

Differences in reproductive performance between breeds or crosses could arise from variation in numbers of ova shed, the number fertilised, the proportion of fertilised ova which develop to full term and the extent of neo-natal lamb mortality. An understanding of the effects of the many genetic and environmental factors which can influence ewe fecundity, could lead to plans for improving reproductive performance in sheep.

An advantage in terms of the number of ova shed for BLX (F1) compared to Romney sheep has been established in both immature and mature ewes. However, studies in laboratory species and more recently in sheep have suggested that differences in uterine capacity might also contribute to observed differences between breeds in the number of lambs born. The concept of uterine capacity can be defined as the ability of a ewe to support multiple numbers of embryos through to term.

The purpose of this investigation was to examine physiological factors which might explain observed differences in reproductive performance between BLX (F1) and Romney sheep. The technique of ova transfer was used. Known numbers of fertilised ova obtained from superovulated donor ewes, were transplanted into synchronised Romney and BLX recipient ewes. Studies were made on ovulation, the survival of transferred ova and uterine capacity in recipient ewes. In addition, comparisons of the maternal environment of ewes and the pre- and post-natal growth of lambs of the two breeds were made.

CHAPTER I

REVIEW OF LITERATURE

Chapter I

REVIEW OF LITERATURE

Ova transfer is a useful technique for definitive research on maternal, genetic and physiological influence in viviparous mammals. In the study of maternal-foetal relationships in sheep, the technique offers certain advantages. It allows maximum maternal-foetal size ratios, novel maternal-foetal combinations, direct comparison of maternal environments, control of the number of offspring and the avoidance of fertilisation failure. Successful transplantation of ova within any species involves the development of techniques for synchronising oestrous cycles in donor and recipient animals and possibly for superovulation. Furthermore, effective methods must be available for the recovery, storage and transfer of ova. These different problems will be reviewed separately.

SYNCHRONISATION OF OESTROUS CYCLES

Dutt and Casida (1948) first demonstrated that progesterone suppressed ovulation and oestrus in sheep. Oestrus and ovulation occurred within five days of progesterone withdrawal. Fertility following natural or artificial insemination may be normal (Lamond and Bindon, 1962; Lamond, 1963; Roche and Crowley, 1971) but is sometimes reduced (Robinson, 1958; Davies and Dun, 1957; Davies, 1960; Lamond, 1960a). The use of progesterone and its analogues in the suppression and synchronisation of ovarian cycles in sheep and cattle, both in the breeding and anoestrous seasons has been reviewed by Lamond (1964).

Robinson (1965) described the use of progestagen impregnated sponges for synchronisation of oestrus in sheep. Fertility at the oestrus following sponge withdrawal is frequently lower than normal (Clarke et al., 1966; Robinson et al., 1967). This problem has been discussed more recently by other workers (Cumming et al., 1970; Quinlivan, 1970; Allison and Robinson, 1970; Smith and Allison, 1971).

PRODUCTION OF OVA

The administration of gonadotrophic hormones to sheep has been widely used as a method for increasing ewe fecundity and to obtain large numbers of ova for experimental studies. Pregnant mare serum gonadotrophin (P.M.S.) has been used to augment ewe fecundity during the breeding season (Robinson, 1951; Wallace, 1954; Gordon, 1958) and during the anoestrous season (Robinson, 1951; Gordon, 1958; McDonald, 1961). Workers to report successful superovulation with P.M.S. in New Zealand Romney sheep include Wallace et al. (1954) and Cumming and McDonald (1967).

P.M.S. has been used in cattle to increase the number of follicles and ovulations (Gordon, Williams and Edwards, 1962; Hafez et al., 1964; Scanlon, Sreenan and Gordon, 1968). Purified Swine Anterior Pituitary extracts are effective superovulators in both cows and pre-pubertal calves (Avery et al., 1962; Jainudeen, Hafez and Lineweaver, 1966).

Hunter (1964) has discussed the use of P.M.S. to induce superovulation in the sow.

Moore and Shelton (1964 a) reported effective superovulation in the ewe with Horse Anterior Pituitary extracts.

P.M.S. dose-ovarian response relationships have been reported in the ewe by Robinson (1951), Wallace (1954) and Averill (1958). These workers all observed marked between animal variation in response to P.M.S. which was greater with increase in dose. Wallace (1954) and Robinson (1951) both recorded an increase in the number of unruptured luteinized follicles at high dose levels of P.M.S. This effect was absent in ewes given large doses of Horse Anterior Pituitary extract (Moore and Shelton, 1964 a).

Like the ovine, steep dose-response curves for P.M.S. have been reported in the cow (Gordon <u>et al.</u>, 1962; Lamond, 1970).

The administration of gonadotrophin during the follicular phase of the oestrous cycle in sheep hastens the onset of the following oestrus (Warwick and Casida, 1943; Wallace, 1954; Robinson, 1951).

Berry and Savery (1958) found the proportion of abnormal ova from ewes treated with P.M.S. did not exceed the proportion from untreated ewes. Other workers have reported normal viability of superovulated sheep ova (Hunter, Adams, and Rowson, 1955; Averill, 1958; Moore, Rowson and Short, 1960).

A reduction in the stimulatory response of follicles to sequential P.M.S. treatment has been reported in the cow (Dziuk <u>et al.</u>, 1958) and the ewe (Hulet and Foote, 1969).

<u>OVA STORAGE</u>

Sheep ova have been successfully stored for 72 hours at 4.5 to 8.0°C (Averill, 1956) but are known to be irreversibly affected <u>in vitro</u> by exposure to rapid rates of cooling (Averill and Rowson, 1959). Harper and Rowson (1963) reported no egg transfer success after storing ova at 7°C. The storage of ova at 0 to 4°C and -79°C has also been unsuccessful (Averill and Rowson, 1959).

Averill and Rowson (1958) reported advantages of <u>in vitro</u> storage of sheep ova in dialysis chambers, but the need for this form of storage has been disputed by other workers (Hancock and Hovell, 1961). There is inconclusive evidence showing superiority of body temperature over room temperature, for temporary ova storage. Successful transfers have been reported with unheated serum (Hancock and Hovell, 1961). Sheep ova have also been successfully stored for 24 or 48 hours in stoppered tubes at room temperature in the dark (Buttle and Hancock, 1964).

Suitable media for the recovery and handling of living sheep eggs have been reviewed by Austin (1961). Hancock (1962) has reviewed criteria for ascertaining fertilisation in ova of farm species.

<u>TRANSFER OF OVA</u>

Ova transfer can be defined as the removal of gametes or zygotes from the normal maternal environment and their transfer to a recipient host which provides the pre-natal uterine environment. Of the farm species, the sheep has been extensively studied to determine factors essential to the successful implementation of the technique.

Ovum transplantation has been attempted at differing stages of the reproductive process (see McDonald, 1969) but major emphasis has been placed on gamete transfer <u>after</u> ovulation.

In vitro ova recovery methods have been described for the sheep (Austin, 1961) and cow (Scanlon *et al.*, 1968). *In vivo* laparotomy methods of recovery have been developed in the ewe (Hunter, Adams and Rowson, 1955) and sow (Hancock and Hovell, 1962). Non-surgical *in vivo* recovery of bovine ova has been reported (Dowling, 1949; Dziuk *et al.*, 1958; Sugie, 1965) but only with limited success.

Successful non-surgical transfer of ova in cattle has been reported by Mutter *et al.* (1964), Rowson and Moor (1966) and Sugie (1965). Ova can be transferred non-surgically in the cow either by puncturing the vagina wall and penetrating the uterus (Sugie, 1965) or by going directly through the cervix (Rowson and Moor, 1966). Surgical transplantation of bovine ova has been described by Willet *et al.* (1953).

Methods of ovum transplantation used in the pig have been reviewed by Polge (1966).

The cervix of the ewe is very difficult to penetrate and in this species ova must be transplanted surgically. A technique for surgical transplantation of ova in the sheep has been described by Hunter et al. (1955). Many workers have reported successful transplantation of sheep ova, from Warwick *et al.* (1934) to Cumming and McDonald (1970).

The stress of laparotomy shortly before or after ovulation in the ewe can result in a lowered conception rate (Lamond, 1963; Packham and Triffit, 1966; Cutten, 1970). Other workers have reported the effect of laparotomy need not be excessive (Alliston and Ulberg, 1961; McDonald, 1969).

Under optimum conditions 75% of transferred ova have survived (Moore, 1968).

SURVIVAL AND DEVELOPMENT OF TRANSFERRED OVA IN THE SHEEP

1. Synchronisation of oestrus in donor and recipient ewes

Transferred ova in order to survive must be placed in an environment that corresponds closely to the one in which they normally develop. In the sheep, workers have usually based synchronisation of transferred ova and genital tract on the synchronisation of oestrus in donor and recipient ewes.

Maximum ova survival following precise synchronisation (± 12 hours) has been reported (Moore and Shelton, 1964 b; Shelton and Moore, 1966) but some workers have disputed the need for close synchronisation (Averill, 1956; Averill and Rowson, 1958).

The transfer of older ova into younger uteri has generally been more favourable to success than the converse condition (Hunter et al., 1955; Hancock and Hovell, 1961; Tervit, 1967). Unsuccessful transfers have been reported where synchronisation has been optimum (Moore and Shelton, 1964 b; Shelton and Moore, 1966).

2. Site and age of transferred ova

In the ewe, most success has been reported where 8-cell ova or older, have been transferred to the uterus (Moore et al., 1960; Moore and Shelton, 1962; Shelton and Moore, 1966). However, in these studies ova of 8-cell stage or older were placed in the uterus, while ova less than 8-cell were transferred to the Fallopian tubes. Consequently, site of transfer and age of ova were confounded.

Moore and Shelton (1964 b) did not confound these two factors and reported tubal transfers to be more successful than uterine transfers.

Averill and Rowson (1958) found that no 2-cell, 16% 4-cell, and 80% 8-cell ova survived following transfer to the uterus. Other workers have also shown that ova survival increases with increasing age of ova (Moore and Shelton, 1964 b; Shelton and Moore, 1966).

3. Number of ova transferred

Increasing the number of ova transferred per ewe does not markedly influence the proportion of ewes which become pregnant, but the survival rate of transferred ova is lowered (Moore et al., 1960; Moore, 1968; Cumming and McDonald, 1970).

4. Breed of ova and/or recipient

Fertilised sheep ova can be transferred successfully within and between widely differing breeds of sheep (Hunter et al., 1955; Moore et al., 1960; Dickinson, et al., 1962; Wiener and Slee, 1965; Moore, 1968).

5. Liveweight and face cover

Cumming and McDonald (1970) did not find a significant relationship between recipient ewe liveweight and ova survival. The same workers found face cover was related to ova survival rate amongst ewes transplanted with 2 eggs each, but not in other ewes after egg transfers, or in mated ewes.

An increase in the number of corpora lutea has been reported to increase the survival rate of transferred ova (Averill and Rowson, 1958). A converse effect has been observed (Tervit, 1967), while other workers have reported no relationship (Moore et al., 1960; Cumming and McDonald, 1970).

FECUNDITY IN THE EWE

Prolificacy in sheep may be influenced by a number of environmental factors (Reeve and Robertson, 1953), by breed differences or by selection (Turner et al., 1962; Wallace, 1964).

Liveweight and nutritional status of the ewe at mating can influence reproductive performance as measured at oestrus by ovulation rate (Wallace, 1961; Killeen, 1967; Lino and Braden, 1968; Allison, 1968; Edey, 1968) and at lambing by the percentage of barren and twin-bearing ewes (Coop, 1962, 1966 a). The literature in this field has been reviewed by Coop (1966 a, 1966 b). Cockrem (1965) has commented on the interpretation of bodyweight and fertility data, in sheep.

The limitations of the slaughter technique and the advantages of using animals randomly bred and reared in the same environment, for ovulation studies, have been pointed out (McDonald and Ch'ang, 1966).

New Zealand studies have shown ovulation rate increases with advancement of the breeding season, or with each successive oestrus (McDonald, 1958; Averill, 1959, 1964; McDonald and Ch'ang, 1966; Allison, 1968). Quinlivan et al. (1966) reported ovulation rates of 1.27 to 1.65 for 2½-year Romney ewes steadily increasing with length of gestation to slaughter.

The superior reproductive performance of twin-born as opposed to single-born ewes has been demonstrated by numerous workers (Rae and Ch'ang 1955; Turner et al., 1962; Dun and Grewal, 1963; Lax and Brown, 1968; Ch'ang and Rae, 1970), and is probably due to genetic differences in the number of eggs shed (Peckham and Triffit, 1966; McDonald and Ch'ang, 1966).

In Romney ewes the extent of wool growth on the face (face cover) has been related to differences in lambing percentages within a flock (Cockrem, Barton and Rae, 1956; Inkster, 1956; Cockrem and Rae, 1966), and embryo survival (Cumming and McDonald, 1970).

Fertility in the Romney breed, has been shown to increase with age of dam and the number of lambings (Ch'ang and Rae, 1970). Differences in ovulation rate may be involved (Edgar, 1962; Wodzicka-Tomaszewska and Welch, 1969).

Differences in fertility between sheep breeds in New Zealand have been reported by many workers and are documented in the review by Dalton (1971). Several workers have reported comparisons of reproductive performance in Romney and BLX sheep (Clarke, 1962; Coop and Clark, 1965; Hight and Jury, 1970). Studies on ovulation rate comparing the Romney with Cheviot x Romney (Inkster, 1953) and BLX sheep (Lang and Hight, 1967; Allison, 1968) have been reported.

There is some evidence to suggest the ram may influence fecundity. A negative correlation between the incidence of abnormal spermatozoa in ram semen and ewe fecundity has been reported (Hulet, Foote and Blackwell, 1965). Lino and Braden (1968) observed a difference between two sires in the proportion of corpora lutea represented by embryos 1 and 2 months <u>post coitum</u>. Barker and Land (1970) found that Finn rams had no effect on litter size and lambing rate of ewes to which they were mated. An effect of the ram on mean litter size has been reported in superovulated ewes (Newton and Betts, 1968) and in ewes mated without superovulation (Parker and Bell, 1965).

UTERINE CAPACITY

A ceiling value may exist for each species and perhaps each individual female, for the number of implantations that can be successfully maintained throughout pregnancy.

In the guinea pig (Eckstein et al., 1955) mouse (McLaren and Michie, 1960) and rabbit (Adams, 1960; Hafez, 1964 a) as the number of implantations rise the vascular supply to each site is reduced; this restricts placental development and causes high embryonic and foetal deaths.

Comparative aspects of physiological and endocrinological mechanisms of implantation have been reviewed by Hafez (1963).

The limitation of uterine space or "crowding" has been implicated as a factor influencing uterine capacity in swine (Rathnasabapathy et al., 1956; Dziuk, 1968; Dhindsa and Dziuk, 1968; Fenton et al., 1968). In the same species Bazer et al. (1968) has reported differences in the proportions of "native" and "alien" embryos surviving following superinduction.

Transuterine migration of embryos is of special physiological significance in certain polytocous species for equal distribution of conceptuses between the two uterine horns. This phenomenon has been studied in the pig (Dhindsa and Dziuk, 1968) and cow (Hafez, 1964 b; Rowson et al., 1971). In the ewe, transuterine migration normally occurs quite frequently when two or more ova are shed by one ovary and none by the other (Boyd, Hamilton and Hammond, 1944).

Gordon, Williams and Edwards (1962) in the cow, Bair and Rüsse (1968) in the sheep and Lyngset (1968) in the goat, have reported that twins occur naturally at a higher proportion when a corpus luteum is present in each ovary than when two corpora lutea are present unilaterally.

Breed differences in uterine capacity were suggested by Robinson (1951) after superovulation studies with British sheep breeds. The results of other workers did not support this contention (Wallace, 1954; Wallace _et al._, 1954; Palsson, 1956; Gordon, 1958).

After transferring three ova reciprocally between two breeds Moore (1968) found Border Leicester ewes had more triplet and less single pregnancies than Merino ewes.

SIZE OF LAMBS AT BIRTH

Variation in the level of ewe nutrition during late pregnancy may influence lamb birth weight (Wallace, 1948; Thomson and Thomson, 1949; Coop, 1950).

Male lambs are generally heavier than female lambs at birth (Doney, 1957; Terrill, 1962). The birth weight of male lambs in multiple litters may be influenced by the presence of female lambs (Donald and Purser, 1956). Lambs born to mature ewes tend to be heavier than those born to young ewes (Hammond, 1932; Hunter, 1956; Dickinson _et al._, 1962). Similarly, heavier ewes may give birth to larger lambs (Hammond, 1932; Wallace, 1948; Dickinson _et al._, 1962) but not all workers have observed this effect (Hunter, 1956).

Studies have shown breed differences in lamb birth weight (Chapman and Lush, 1932; Donald and McLean, 1935; Hunter, 1956; Dickinson _et al._, 1962; Moore, 1968) but the magnitude of the effect has varied between workers.

Large differences in parental size in sheep have been utilised to illustrate maternal effects in this species (Hunter, 1956; Dickinson et al., 1962; Moore, 1968). The magnitude of the maternal environmental effect is influenced by genetic differences between dam and progeny. Theories to explain the nature of the maternal effect have been advanced by Walton and Hammond (1938) and these have been discussed with reference to the sheep by Hunter (1956).

Inverse relationships between litter size and gestation length, and litter size and lamb birth weight are known to exist in sheep (Hammond, 1932; Terrill, 1944; Forbes, 1967). Single lambs are heavier and carried longer than twins, and twins heavier and carried longer than triplets. Genetic factors are also known to influence gestation length (Terrill and Hazel, 1947; Hunter, 1956; Dickinson et al., 1962 ; Moore, 1968). The gestation length of large breeds is extended by the presence in utero of foetuses of smaller breeds (Hunter, 1956; Dickinson et al., 1962; Moore, 1968) while a converse relationship holds when these factors are reversed (Moore, 1968). It is not clear whether the size of the foetus modifies the length of gestation, or whether the length of gestation modifies the size of lambs at birth. Liggins (1968) has suggested foetal adrenal activity is of critical importance in determining the onset of parturition and thus gestation length in sheep.

Undernutrition of the ewe in late pregnancy may shorten gestation length (Thomson and Thomson, 1949; Alexander, 1956). The effect is greater amongst twin-bearing than single-bearing ewes and is influenced by the stage of gestation when the restriction is applied.

Gestation length may increase with age of ewe (Terrill and Hazel, 1947) but not all workers support this contention (Forbes, 1967).

Sex of the lamb has no effect on gestation length in sheep (Terrill, 1944; Forbes, 1967).

LIVEWEIGHT OF LAMBS AT WEANING

Many workers have found genetic differences within and between breeds of sheep in growth rate or weaning weight (Coop, 1950; Coop and Clark, 1952; Pattie, 1965; Young et al., 1965; Bassett et al., 1967). Studies of weaning weight in Romney flocks under New Zealand conditions have been reported (Ch'ang and Rae, 1961, 1970). Kirton (1970) has reviewed lamb growth rate studies with New Zealand sheep breeds. Jagusch et al. (1971) have discussed early weaning of lambs onto different pasture species.

Weaning weight comparisons of Border Leicester x Corriedale and Corriedale (Coop, 1957) and Border Leicester x Romney and Romney sheep (Coop and Clark, 1965) have been reported. In each case the liveweight of crossbred lambs at weaning was superior to straightbred lambs. Coop (1957), Donald et al. (1968) and Carter (1968) found that lambs sired by Border Leicester rams had higher liveweights near weaning than those sired by smaller breeds, although considerable variation of the progeny between sires within breeds was found.

Sex differences in the liveweight of lambs at weaning are small and early castration of ram lambs reduces these differences (Hazel and Terrill, 1945, 1946; Ch'ang and Rae, 1961).

Effects of age, type of birth, and rearing of the lamb, and age of dam on weaning weight have been reported by many workers (Hazel and Terrill, 1945, 1946; Blackwell and Henderson, 1955; Lax and Brown, 1967; Ch'ang and Rae, 1961, 1970). The estimates of age of dam and type of rearing effects are considered to be reflections of the milk production of the dam and milk consumed by the litter (Hunter, 1956; Barnicoat, 1947; Wallace, 1948; Barnicoat <u>et al.</u>, 1949).

The actual amount of milk produced by a ewe is influenced both by her own potential to produce milk and by the potential of the lamb to obtain it (Moore, 1966 a).

Several authors have studied differences between breeds and strains of ewes in milk production (Coombe <u>et al.</u>, 1960; Munro, 1962; Moore, 1966 a; Coop and Drew, 1963) and milk quality (Barnicoat <u>et al.</u>, 1956; Moore, 1966 b).

C H A P T E R I I

M A T E R I A L S A N D M E T H O D S

Chapter I I

MATERIALS AND METHODS

ANIMALS

The experimental units in this study, were an unselected group of eighty eight Romney and seventy two BLX (F1) non-parous eighteen-month-old sheep (recipient ewes). These animals had been run together since birth and were of similar genetic origin having been derived from a randomly bred flock maintained at Massey University since 1944. A Romney and BLX ewe are shown in Figures 1 and 2 respectively.

An additional flock of one hundred and fifty six, 5-year-old Romney ewes, served as donors of fertilised ova. These animals were mated to either a Romney or Border Leicester sire of proven fertility. Two vasectomised Romney rams were each run with donor and recipient ewe groups during the breeding season.

MANAGEMENT OF ANIMALS

At the commencement of the experiment in March the district was experiencing severe drought conditions. In the absence of pasture feed animals were fed concentrate and hay, during March and April. The level of feeding was sufficient to maintain live-weight of recipient ewes, as shown in Table 1. Following an improvement in pasture growth during early May supplementary feeding was terminated. All sheep grazed predominantly ryegrass-white clover pasture for the duration of the experiment.

TABLE 1

LIVEWEIGHTS (kg.) OF ROMNEY AND BLX TWO-TOOTH EWES IN THE PRE-EXPERIMENTAL PERIOD

Date of weighing	Romney	BLX
5. 2. 70	40.5 ± 0.4 *	46.8 ± 0.6
4. 3. 70	40.6 ± 0.5	46.5 ± 0.4

* Mean ± Standard Error

Each ewe was individually identified by a serially numbered ear tag and a corresponding number branded on the flank wool. Entire and vasectomised rams were fitted with a Sire Sine mating harness. Observations to record oestrous ewes were made twice daily (7 a.m. and 7 p.m.). Donor ewes in oestrus were withdrawn from the 'donor pool' and individually mated to one of four sires of known identity. Each ewe was mated twice daily until the end of oestrus.

Prior to lambing, animals were stocked at about 8 ewe equivalents per acre. This rate increased to approximately 11 ewe equivalents between lambing and weaning.

EXPERIMENTAL PLAN

The experimental plan is set out in Figures 3 and 4 and is discussed in the following sections.

HORMONES AND TREATMENTS

1. Pilot Trial

On the 20th February, 1970, a pilot trial was set up. This trial, which involved 20 5-year-old Romney ewes, 2 Romney and 2 Border Leicester entire rams, had two objectives. Information was required on gonadotrophin - ovarian response relationships which might assist in determining the dose of pregnant mare serum gonadotrophin (P.M.S.)* to be used in the main experiment. In addition, the trial provided a means of objectively testing the fertility of 4 sires intended for later use.

Cyclic Romney ewes were injected with either 1000 or 1500 i.u. P.M.S. on day 12 or 13 of the oestrous cycle which followed progestagen

* Paines and Byrne Ltd.

Fig. 1. Ewe 163. 18-month-old Romney ewe

Fig. 2. Ewe 133. 18-month-old Border Leicester x Romney ewe

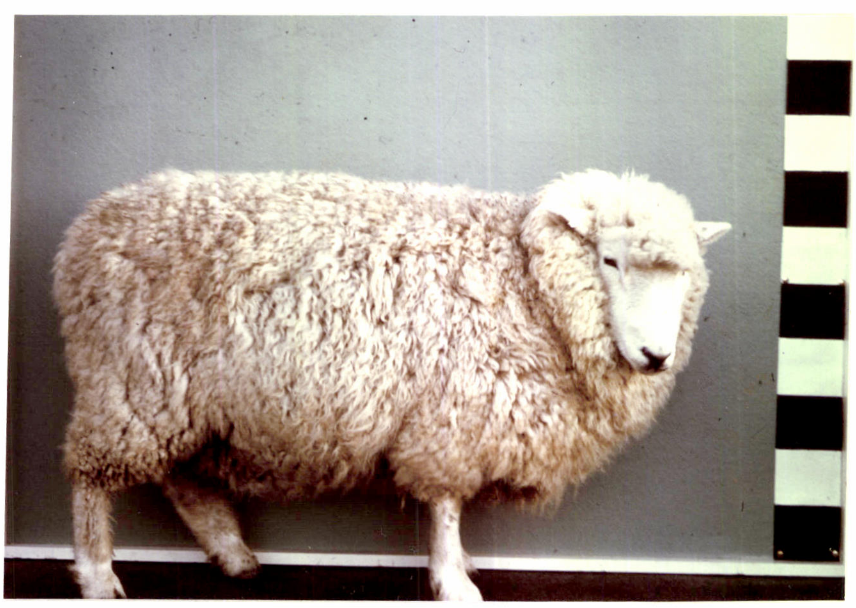


Fig. 5. Ewe 96. Romney ewe face grade 1

Fig. 6. Ewe 144. Romney ewe face grade 4+

treatment. These animals were laparotomised three days after mating to a single sire. The ovarian response was recorded and recovery of ova attempted.

2. <u>Synchronisation of Oestrous Cycles</u>

To facilitate the conduct of ova transfer experiments it is necessary to control the number of ewes in oestrus at any one time. To this end, oestrous cycles in donor and recipient ewes were synchronised following 15 day treatments with intra-vaginal sponges. Each sponge contained 40 mg. 6α-methyl-17α-acetoxyprogesterone (M.A.P.)*. From 4th to 23rd March, vaginal pessaries were inserted into 123 donor and 160 recipient ewes. Where possible a constant ratio of donor to recipient ewes (9:12) were treated each day.

3. <u>Superovulation of Donor Ewes</u>

Superovulation was induced in donor ewes by a single subcutaneous injection of P.M.S., given on Days 11, 12 or 13 of the oestrous cycle which followed progestagen treatment. A dose of 1500 i.u. P.M.S. was initially employed, (based on pilot trial results) but as results became available treatments of 1100 and 1300 i.u. P.M.S. were also used. For donor ewes which did not show oestrus after sponge withdrawal the time of ovulation was estimated and P.M.S. administered 12 days after this time. The day of oestrus detection was taken as Day 0.

Since ova transfers were performed from the 5th April to 20th June, it was necessary to give 63 ewes a second injection of P.M.S. during a subsequent oestrous cycle. Seven ewes received three treatments; sequentially treated ewes received either 1500 or 1800 i.u. P.M.S.

* Upjohn Company

RAM FERTILITY

An assessment of the fertility of the rams to be used was made prior to the start of the experiment. Semen samples, obtained using an electro-ejaculation technique, were examined for evidence of vigorous wave motility. In addition, evidence of fertile sperm was provided by the finding of cleaved ova in the flushings from ewes served in the pilot trial.

OVA RECOVERY

Donor ewes were laparotomised approximately 72 hours after first being observed in oestrus and the number of recent corpora lutea and Graafian follicles (> 5 mm. in diameter) recorded. Ova were recovered from the reproductive tract by flushing the Fallopian tube and about 5 cm. of the most distal portion of the uterine horn. The technique used was as described by Hunter, Adams and Rowson (1955).

Sterile sheep serum, the medium used for ova recovery, was obtained from whole blood collected at slaughter or from the jugular vein of live rams. The blood was collected in sterilized beakers, allowed to clot, then stored at 3°C for 24 hours. The serum fraction was decanted off, centrifuged, antibiotic added (1000 units Streptomycin per ml.), Seitz-filtered, and dispensed into sterilised bottles before storage at -10°C. When required for use the serum was thawed and held at 35°C in a water bath.

Immediately following recovery flushings were examined under a binocular microscope. Observations were recorded on the number and stage of cleavage, of ova present. Apparently normal segmentation for the age of ova was taken as the criterion for fertilisation. Ova were temporarily stored in an incubator at 35°C until required for transfer. The storage period seldom exceeded one hour.

OVA TRANSFER

Recipient ewes were laparotomised approximately 72 hours after first being observed in oestrus, and the number of recent corpora lutea recorded. Reciprocal transplants of Romney and BLX ova, at rates of one or three ova per ewe, were made. In most cases 8-cell ova were transferred, and always to the uterus of the recipient ewe.

The recipient's tract was exteriorised and ova transferred using essentially the same technique as described by Hunter et al. (1955). Ova were gently evacuated into the lumen of the uterine horn through a puncture on the antimesometrial border, 2 to 3 cm. from the utero-tubal junction. Care was taken to ensure the pipette point was free in the lumen of the uterine horn before evacuation, and not above the uterine mucosa. After deposition of ova, the pipette was checked to ensure all ova had been transferred.

A maximum of two ova were placed in any uterine horn. In ewes receiving one ovum with a single corpus luteum, the transfer was made to the uterine horn adjacent to the ovary containing the corpus luteum.

Ewes which returned to oestrus following transfer were either subjected to a second transfer if ova were available, or naturally mated to one of the sires already used. All pregnant recipient ewes were allowed to go to term.

FACE WOOL GRADING

The system of Cockrem (1966) was used for the grading of Romney recipient ewes, on face wool cover (1 = maximum cover, 5 = open faced). In Figures 5 and 6 Romney ewes corresponding to face grades 1 and 4+ respectively are shown.

OBSERVATIONS ON PROGENY

One week prior to lambing, and until after parturition, ewes which had received three ova at transfer were closely observed in separate pens. This procedure was followed to avoid possible confusion of parentage where multiple births occurred.

Lambs were identified by a serially numbered metal ear tag. This was inserted within 12 hours of birth when observations on lamb weight (to the nearest 0.45 kg.) and sex, were also recorded. The length of gestation was measured to the nearest day, commencing from the time of mating of donor ewes.

Where multiple births occurred an attempt was made to allow the ewe to rear all the offspring. However, in some cases it was necessary to rear all or part of a litter on a foster dam. Lambs reared in this manner were excluded from subsequent experimental observations.

Male lambs were not castrated. All lambs were weaned approximately 100 days after birth and their weights recorded.

METHODS OF ANALYSIS

1. Discrete Classifications

Two different methods of carrying out independence tests were used. Either Chi-square tests or the G-statistic were employed. The former is the traditional way of analysing such data but G (log likelihood ratio test) has theoretical advantages (Sokal and Rohlf, 1969) and is computationally simpler. When only 2 classifications were involved, Yates' correction for continuity was applied.

2. Multiple and Nested Classifications

Bartlett's Test for homogeneity of variances was used for testing the equality of variances (Snedecor and Cochran, 1967). Differences between pairs of means were inspected using Duncan's Multiple Range Test, extended to group means with unequal sample sizes (Kramer, 1956). Estimates of variance components were computed following most analyses of variance (see Appendix I).

The general linear mathematical model for the two-way classification with interaction was used for the analysis of maternal effects data (Chapter VI).

$$Y_{ijk} = u + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where

u	=	the overall mean, when equal frequencies exist in each subclass
a_i	=	effect of maternal genotype (i = 1 or 2)
b_j	=	effect of lamb genotype (j = 1 or 2)
$(ab)_{ij}$	=	individual interaction effects expressed as deviations from the mean u
e_{ijk}	=	error peculiar to each Y_{ijk}

As the subclass numbers were unequal in the data, the least squares method of fitting constants (Harvey, 1960) was used to estimate the main effects and interaction. Tests for statistical significance were by analysis of variance. For this purpose the e_{ijk} were assumed to have a zero mean, constant variance, and a normal distribution.

For the data on lamb weaning weight (Chapter VII) a slightly more complex model was used.

$$Y_{ijkl} = u + a_i + b_j + c_k + (ab)_{ij} + e_{ijkl}$$

where

c_k = effect of sex of lamb (k = 1 or 2)

Estimates of variance components were not computed for either maternal effects or weaning weight data. The number of degrees of freedom for both maternal and lamb genotype were inadequate to obtain accurate estimates of variance components for the two main effects or the interaction.

3. Relating Discrete and Continuous Variables

In the analysis of data relating numbers of corpora lutea and ewe liveweight (Chapter IV), the technique of biserial correlation as described by Peters and Van Voorhis (1940) was used.

4. Adjusted Weaning Weights of Lambs

The results of analysis of data on lamb weaning weights are contained in Chapter VII.

Lambs at weaning were from 95 to 105 days of age. Adjustments were made to the weaning weight of each lamb to remove known environmental effects. The corrections implemented were those used in the New Zealand National Flock Recording Scheme.

Adjustments made were as follows : -

Single lamb reared as a single	-	no correction
Single reared as a twin	-	+ 4.53 kg.
Twin reared as a twin	-	+ 4.53 kg.
Twin reared as a single	-	+ 3.17 kg.
Triplet reared as a triplet	-	+ 6.80 kg.
Triplet reared as a twin	-	+ 5.44 kg.
Triplet reared as a single	-	+ 4.53 kg.
Every day under or over 100 days of age at weaning	-	± 0.136 kg.

The parameter of Weight Gain Per Day for each lamb was computed as follows : -

$$\text{Wt. Gain Per Day (kg.)} = \frac{\text{Wt. at Weaning} - \text{Wt. at Birth}}{\text{Age at Weaning (Days)}}$$

FIGURE 3

EXPERIMENTAL PLAN

Part (a) The Breeding Season

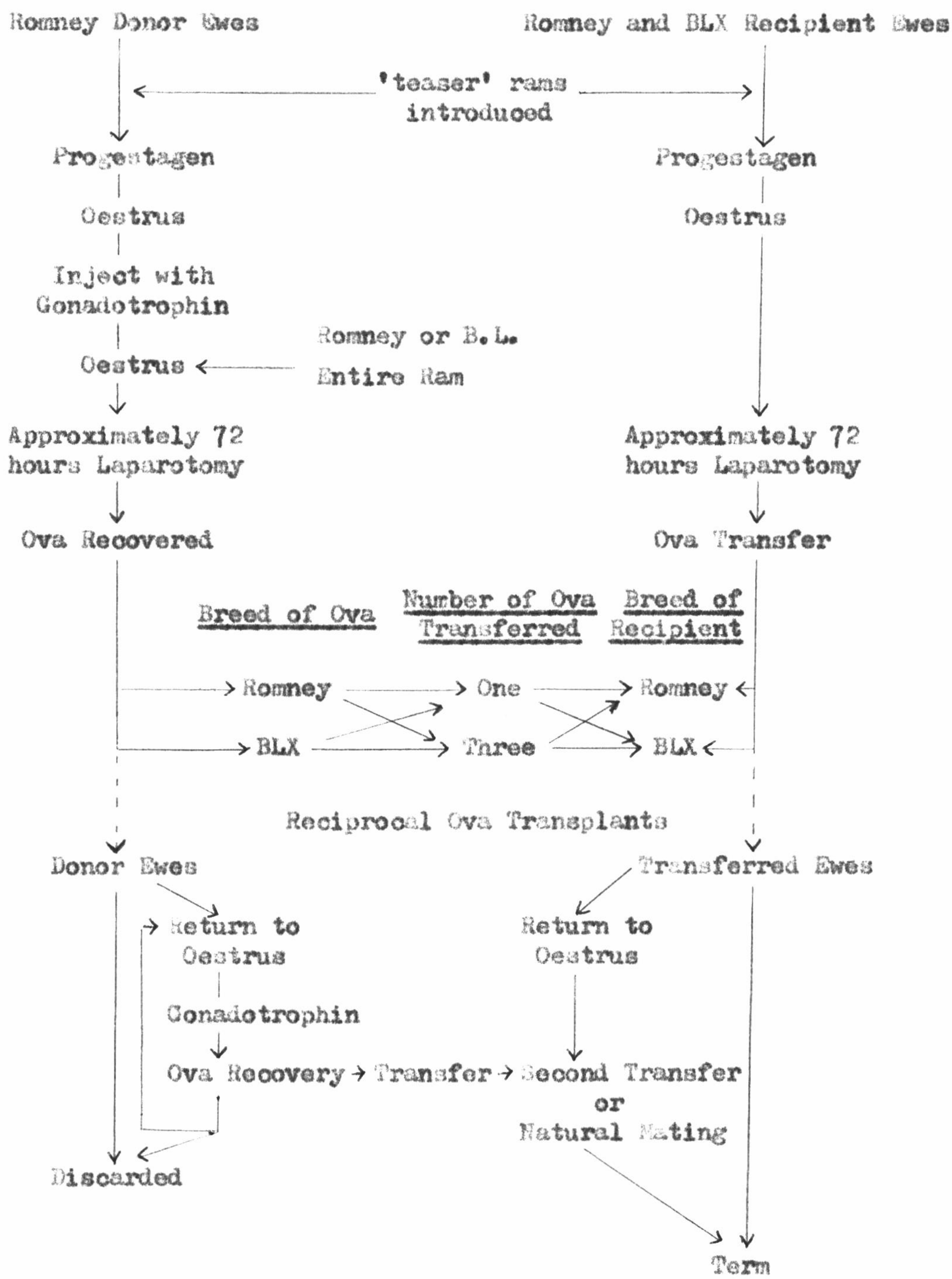


FIGURE 4

EXPERIMENTAL PLAN

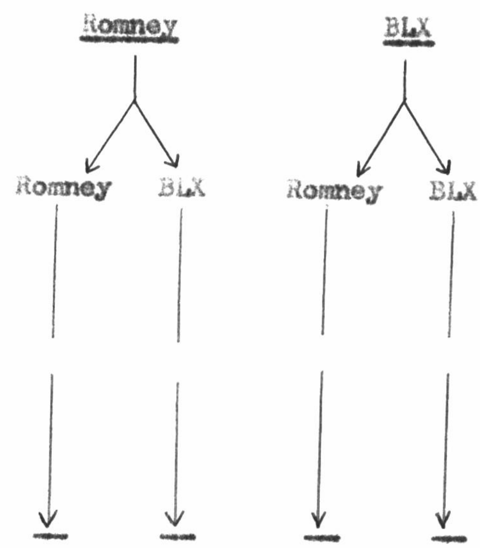
Part (b) Observations on Progeny

Maternal Genotype :

Lamb Genotype :

Parturition : Birth Weight and Gestation Length Recorded

100 Days : Weaning Weight Measured



CHAPTER III

SYNCHRONISATION AND HORMONAL STIMULATION OF EWES FOR OVA TRANSFER

Chapter III

SYNCHRONISATION AND HORMONAL STIMULATION OF EWES FOR OVA TRANSFER

The results presented in this chapter were obtained during the breeding season, following hormonal treatment of donor and recipient ewes, for ova transfer.

INDUCTION OF OESTRUS FOLLOWING PROGESTAGEN TREATMENT

1. Effect of breed and age of ewe on the manifestation of oestrus

The incidence of oestrus in 158 Romney and BLX Two-Tooth ewes following sponge withdrawal is shown in Table 2. Analysis of these data did not show a significant difference between breed, in the number of ewes showing overt or silent oestrus.

Data pooled for the Two-Tooth animals and results from 120 5-year-old Romney ewes are presented in Table 3. A significantly greater proportion ($Chi^2 = 7.80$; $p < 0.01$) of older (92.5%) compared to younger (80%) ewes, showed overt oestrus after progestagen treatment.

2. Effect of breed and age of ewe on the time-interval between sponge withdrawal and oestrus

Table 4 and Figure 7 show the number and percentage respectively, of Two-Tooth ewes in oestrus on each of 6 days after sponge withdrawal. An analysis of these frequency distributions

TABLE 2

EFFECT OF BREED OF EWE ON THE MANIFESTATION OF OESTRUS FOLLOWING PROGESTAGEN TREATMENT

Breed	Number of Ewes Showing Overt	or Silent Oestrus	Total Number of Ewes	% of Ewes Showing Overt Oestrus
Romney	71	17	88	80.6
BLX	55	15	70	78.5
Total	126	32	158	

$Chi^2 = 0.016$ - Not Significant

TABLE 3

EFFECT OF AGE OF EWE ON THE MANIFESTATION OF OESTRUS

Age of Ewes	Number of Ewes Showing Overt or	Silent Oestrus	Total Number of Ewes	% of Ewes Showing Overt Oestrus
5 - yrs	111	9	120	92.5
18- months	126	32	158	79.7
Total	237	41	278	

$Chi^2 = 7.80 - p < 0.01$

TABLE 4

EFFECT OF BREED OF EWE ON THE TIME INTERVAL BETWEEN SPONGE WITHDRAWAL AND OESTRUS

Breed	Number of Ewes in Oestrus Days from Sponge Withdrawal						Total Number of Ewes
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
Romney	4	44	17	3	3	0	71
BLX	4	38	7	1	2	3	55
Total	8	82	24	4	5	3	126

G = 11.92 - $p < 0.05$

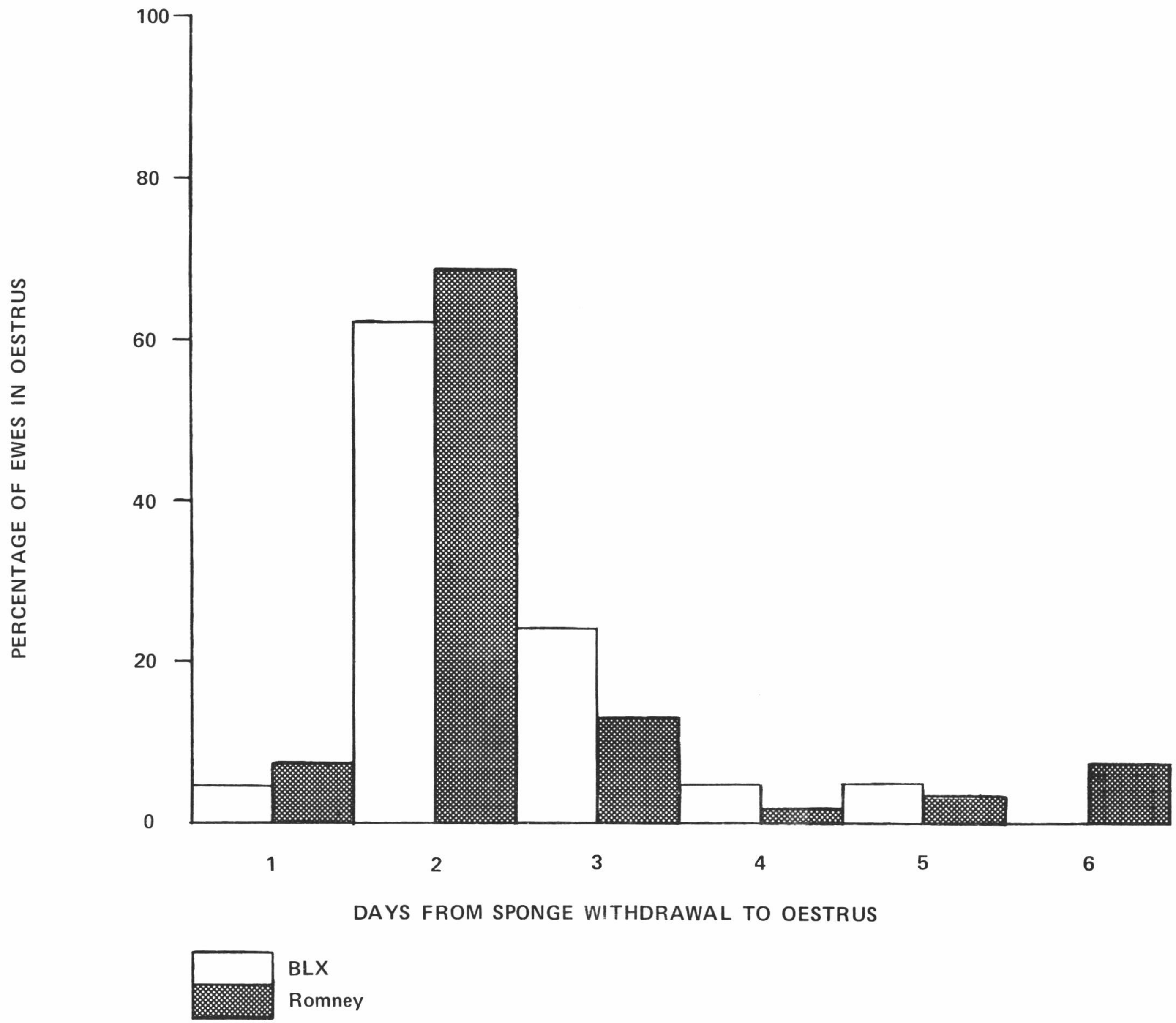
TABLE 5

EFFECT OF AGE OF EWE ON THE TIME INTERVAL BETWEEN SPONGE WITHDRAWAL AND OESTRUS

Age of Ewe	Number of Ewes in Oestrus Days from Sponge Withdrawal						Total Number of Ewes
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
5 - yrs	15	65	30	1	0	0	111
18 - months	8	82	24	4	5	3	126
Total	23	147	54	5	5	3	237

G = 15.74 - $p < 0.01$

Figure 7: Effect of breed of ewe on the time interval between sponge withdrawal and oestrus



revealed a significant breed effect ($G = 11.92$; $p < 0.05$).

Similar frequency distributions for the 5-year-old and 18-month-old ewes are contained in Table 5 (numbers) and Figure 8 (percentages). Age of ewe, and the distribution of ewes showing oestrus, were not independent ($G = 15.08$; $p < 0.01$). Within three days of sponge removal, 99% and 80% of 5-year and 18-month sheep were in oestrus, respectively. Older ewes appeared to be released from pituitary inhibition more rapidly than younger ewes following the withdrawal of the inhibitory factor.

PRODUCTION OF OVA FROM DONOR EWES

The results in the section were from 5-year-old Romney ewes given a single injection of P.M.S.

1. Oestrous cycle length following P.M.S. injection

The length of the oestrous cycle following the injection of 1100, 1300 or 1500 i.u. P.M.S. on day 11, 12 or 13 of the cycle, are presented in Table 6. An analysis of variance showed that neither the dose, nor the day of injection of P.M.S. had a significant effect on the length of the oestrous cycle. Estimates of variance components (Appendix I) revealed that dose, and day of injection of P.M.S., accounted for 2% and 3% respectively, of the total variation.

2. Ovarian response

Table 7 shows the ovarian response of the 120 ewes treated with P.M.S. in terms of the mean number of corpora lutea, and corpora lutea plus large follicles (> 5 mm. diameter) per ewe. A further 3 ewes failed to respond to treatment. Frequency distribution curves were plotted and these data did not follow normal distribution

Figure 8: Effect of age of ewe on the time interval between sponge withdrawal and oestrus

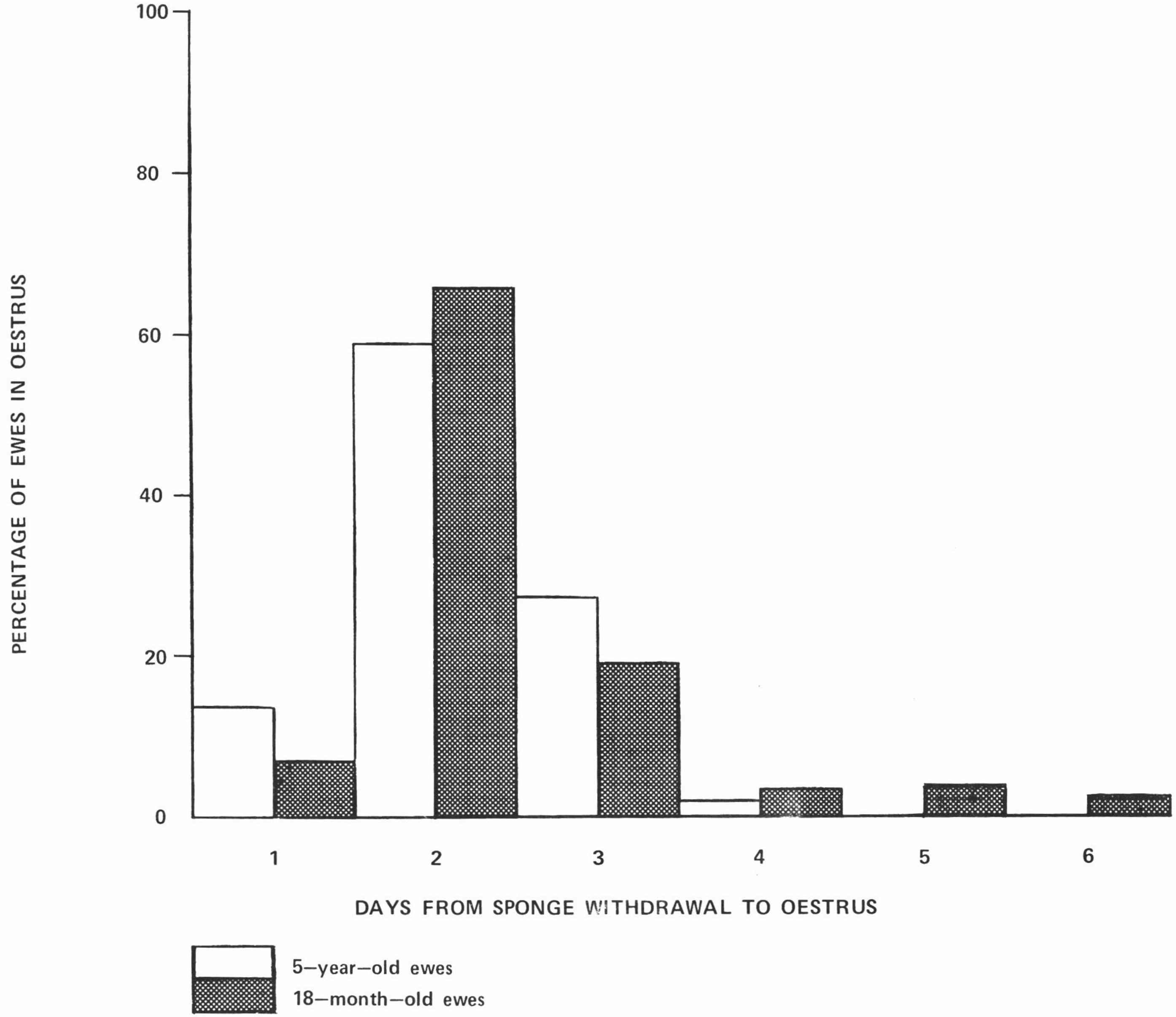


TABLE 6

EFFECT OF DOSE OF P.M.S. AND DAY OF INJECTION ON OESTROUS CYCLE LENGTH

Dose of P.M.S.	Day of Oestrous Cycle of Treatment	Number of Ewes	Cycle Length - days (Mean ± S.E.)
1100	11	7	16.42 ± 0.21
	12	3	16.33 ± 0.40
	Combined days	10	16.40 ± 0.18
1300	11	15	15.80 ± 0.22
	12	15	15.86 ± 0.16
	Combined days	30	15.83 ± 0.14
1500	11	40	15.80 ± 0.28
	12	33	16.06 ± 0.18
	13	7	16.71 ± 0.41
	Combined days	80	15.98 ± 0.11

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Mean Squares	Partition of Variation
Between doses	2	1.20 N.S.	2%
Between days within doses	4	1.31 N.S.	3%
Error	113	0.87	95%

N.S. = Not Significant

TABLE 7

OVARIAN RESPONSE OF EWES TO TREATMENT WITH P.M.S.

Dose of P.M.S. (i.u.)	Number of Ewes	No. Corpora Lutea (Mean ± S.E.)	No. Corpora Lutea plus Large Follicles (Mean ± S.E.)
1100	10	2.10 ± 0.37 (1 - 4)	2.80 ± 0.55 (1 - 6)
1300	30	3.[illegible]6 ± 0.31 (1 - 7)	3.63 ± 0.33 (1 - 7)
1500	80	5.81 ± 0.44 (1 - 19)	7.52 ± 0.54 (1 - 22)

(   ) = Range of Values

curves. In addition, Bartlett's Test for homogeneity of variance disclosed that the variance differed significantly between P.M.S. treatments (Appendix II). However, a square root transformation of the data showed similar variances in the different treatments (Appendix II). The results for ovarian response after transformation are given in Table 8.

The ovarian response to 1500 i.u. P.M.S. was higher than that to both 1300 i.u. and 1100 i.u. P.M.S. An analysis of variance (Table 8) revealed a significant effect of dose of P.M.S. on ovarian response, both for the number of corpora lutea ($p < 0.025$; 26% of total variation) and the number of corpora lutea plus large follicles ($p < 0.001$; 75% of total variation). A Duncan's Multiple Range Test showed that for both these measures of ovarian response there were significant differences ($p < 0.01$) between 1300 i.u. and 1500 i.u., and 1100 i.u. and 1500 i.u. P.M.S. (Appendix II). The effect of day of oestrous cycle of treatment, on both measures of ovarian response, was almost negligible (Table 8).

3. Recovery of ova

An attempt was made to recover ova from 117 ewes which had been treated with P.M.S. An additional 2 ewes had Fallopian tube obstructions. One further ewe had a congenitally deficient uterine horn. From 7 (6%) ewes no ova were recovered. The data were expressed as percentages and transformed using an arcsin transformation. Unless otherwise indicated the results were presented on a per ewe basis.

TABLE 8

OVARIAN RESPONSE AFTER P.M.S. TREATMENT - TRANSFORMED DATA

Dose of P.M.S. (i.u.)	Day of Oestrous Cycle of Treatment	Number of Ewes	No. Corpora Lutea (Mean ± S.E.)	No. Corpora Lutea plus Large Follicles (Mean ± S.E.)
1100	11	7	1.50 ± 0.17	1.77 ± 0.14
	12	3	1.13 ± 0.13	1.27 ± 0.13
	Combined days	10	1.39 ± 0.12	1.62 ± 0.13
1300	11	15	1.73 ± 0.13	1.83 ± 0.12
	12	15	1.75 ± 0.11	1.85 ± 0.12
	Combined days	30	1.74 ± 0.08	1.84 ± 0.02
1500	11	40	2.16 ± 0.11	2.50 ± 0.13
	12	33	2.45 ± 0.14	2.74 ± 0.16
	13	7	2.02 ± 0.23	2.41 ± 0.13
	Combined days	80	2.31 ± 0.07	2.59 ± 0.09

ANALYSIS OF VARIANCE

Source of Variation	d.f.	No. Corpora Lutea Mean Squares		Partition of Variation	No. Corpora Lutea plus Large Follicles Mean Squares		Partition of Variation
Between doses	2	5.55	*	26%	50.57	***	75%
Between days within doses	4	0.57	N.S.	0.75%	0.44	N.S.	0.00
Error	113	0.48		73.25%	0.59		25%

N.S. = Not Significant * = $p < 0.025$ *** = $p < 0.001$

(a) Effect of dose of P.M.S. on the percentage of ova recovered per ewe

The percentage recovery of ova per ewe for the three dose levels of P.M.S. are shown in Table 9. A Bartlett's Test for homogeneity of variance of these transformed data revealed that the variance was homogeneous ($Chi^2 = 2.74$; not significant). The dose of P.M.S. had a significant effect ($p < 0.005$; 17% of total variation) on the percentage recovery of ova. Significant differences between 1100 i.u. and 1500 i.u. P.M.S. ($p < 0.05$), and 1300 i.u. and 1500 i.u. P.M.S. ($p < 0.01$) were disclosed by a Duncan's Multiple Range Test (Appendix II).

If the percentage of ova recovered was calculated from the proportion of ova recovered of the total number of ova shed (based on a count of corpora lutea), from all ewes receiving each dose of P.M.S. the recovery percentages would be 86% (1100 i.u.), 84% (1300 i.u.) and 63% (1500 i.u. P.M.S.). Analysis showed a significant association ($Chi^2 = 19.30$; $p < 0.001$) between recovery percentage and P.M.S. dose. The difference in percentage recovery between data pooled for 1100 i.u. and 1300 i.u. ($Chi^2 = 0.37$; not significant) and 1500 i.u. P.M.S., was significant ($Chi^2 = 18.37$; $p < 0.001$).

(b) Effect of Ovarian Response on the percentage of ova recovered per ewe

Figures 9 and 10 show that neither the number of corpora lutea, nor the number of corpora lutea plus large follicles per ewe, had any marked effect on the percentage of ova recovered from that ewe. However, a decline in the percentage recovery was apparent when

TABLE 9

EFFECT OF DOSE OF P.M.S. ON THE PERCENTAGE OF OVA RECOVERED AND ON THE PERCENTAGE OF RECOVERED OVA FERTILISED PER EWE (DATA TRANSFORMED)

Dose of P.M.S. (i.u.)	Number of Ewes	Percentage Ova Recovered (Mean ± S.E.)	Number of Ewes	Percentage Recovered Ova Fertilised (Mean ± S.E.)
1100	10	79.5 ± 5.5	10	90.0 ± 0.0
1300	28	72.7 ± 4.4	27	67.7 ± 7.1
1500	79	56.4 ± 3.0	73	54.5 ± 4.7

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Percentage Ova Recovered Mean Squares	Partition of Variation	d.f.	Percentage Recovered Ova Fertilised Mean Squares	Partition of Variation
Between doses	2	4392.49 **	17%	2	6359.04 *	11%
Error	114	647.75	83%	107		89%

* = $p < 0.025$
** = $p < 0.005$

Figure 9: Effect of the number of corpora lutea per ewe on the percentage of ova recovered and the percentage of recovered ova fertilised from that ewe

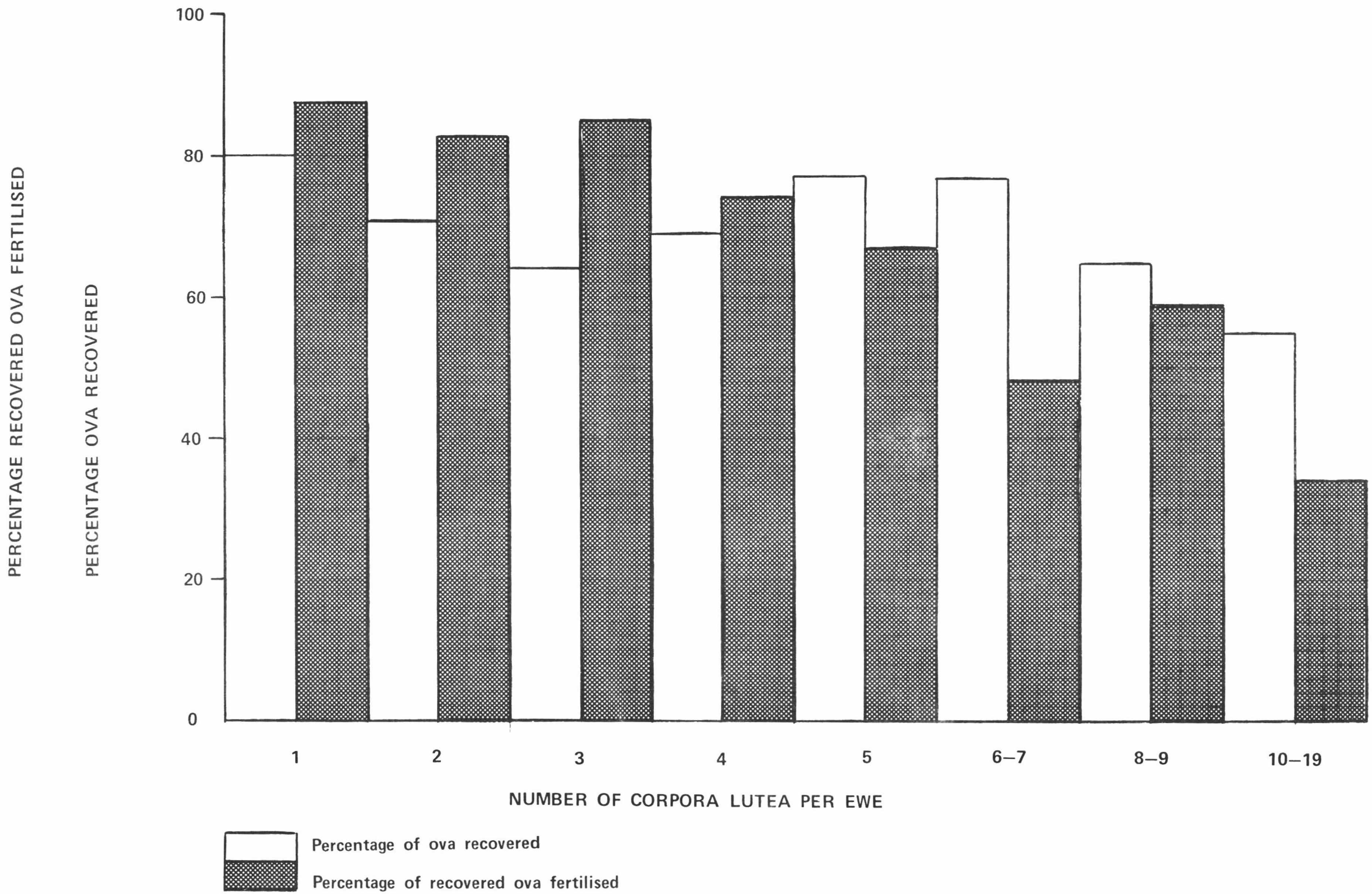
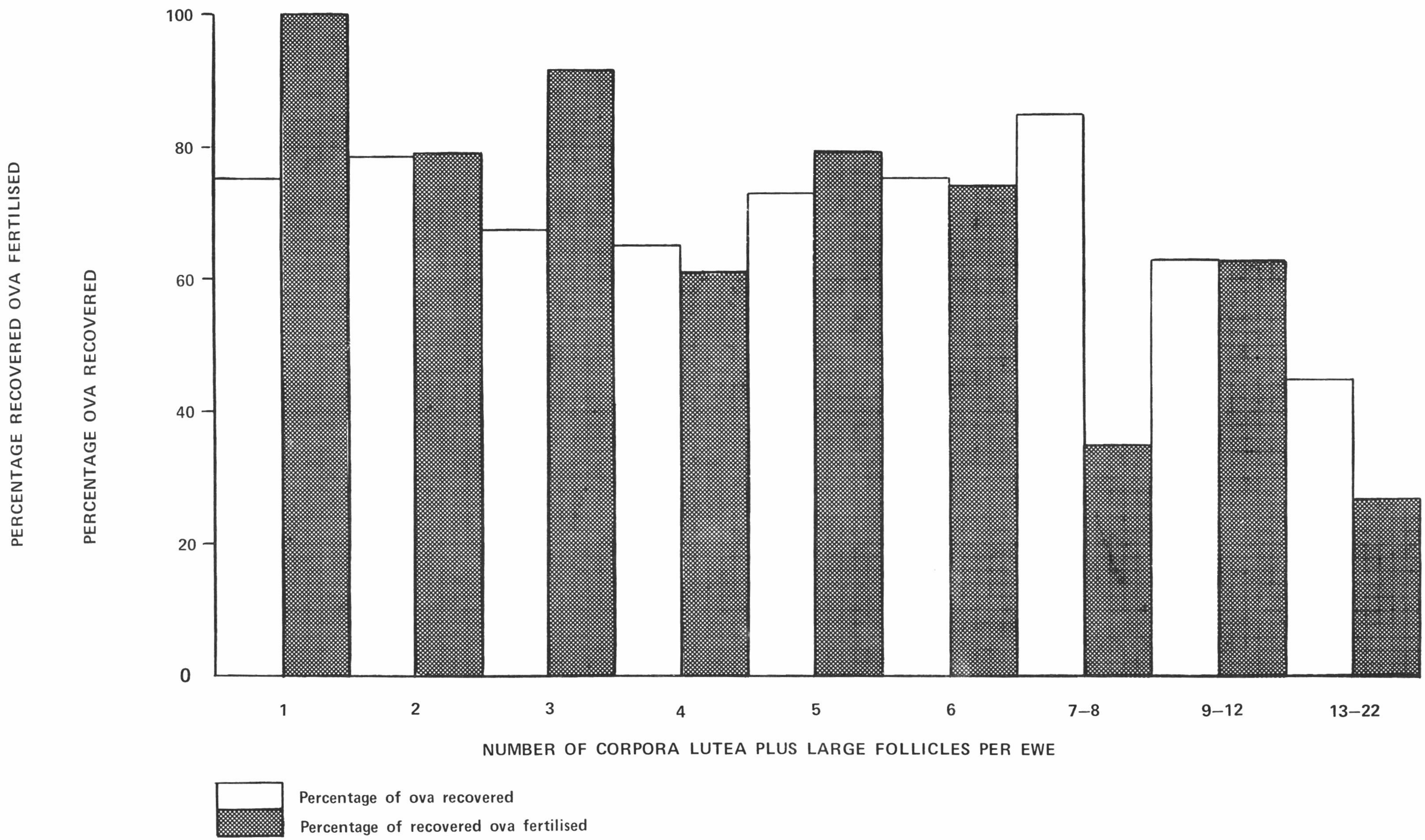


Figure 10: Effect of the number of corpora lutea plus large follicles per ewe on the percentage of ova recovered and the percentage of recovered ova fertilised from that ewe



the ovarian response exceeded 6 to 7 corpora lutea, and 7 to 8 corpora lutea plus large follicles per ewe, respectively.

(c) Effect of the interval from onset of oestrus to laparotomy on the percentage of ova recovered

Ova were recovered 67 to 77 hours after ewes were first observed in oestrus. The time interval between onset of oestrus and laparotomy had no marked effect on the percentage of ova recovered per ewe (Figure 11).

4. Fertilisation of recovered ova

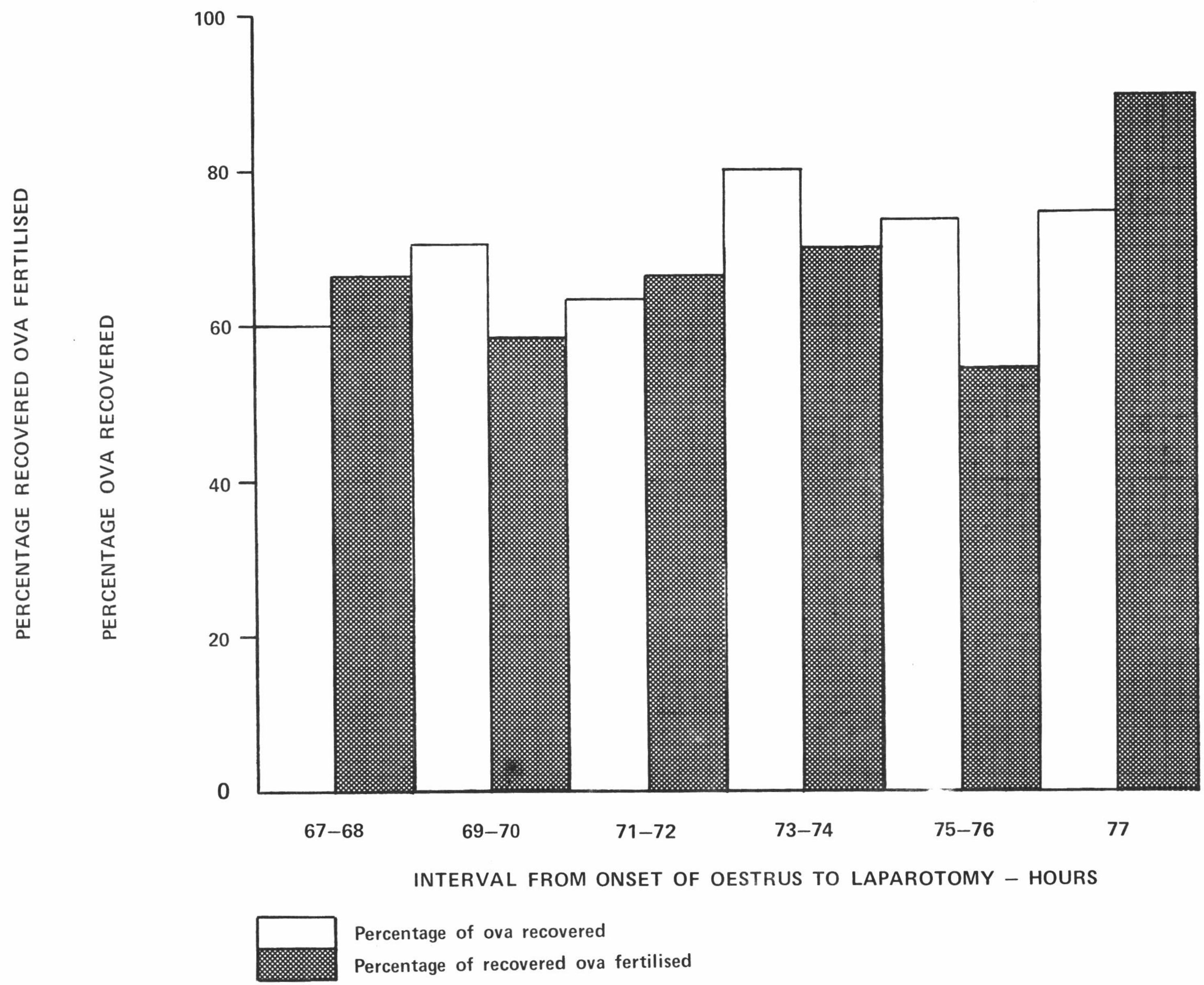
Normally cleaved ova at recovery were classified as being fertilised.

Of 110 ewes yielding ova, 67 (61%) gave only cleaved ova: 27 (24.5%) uncleaved ova only, while both cleaved and uncleaved ova were recovered from 16 (14.5%) ewes. The data were transformed using an arcsin transformation and unless otherwise stated were presented on a per ewe basis.

(a) Effect of dose of P.M.S. on the percentage of recovered ova fertilised per ewe

Bartlett's Test for homogeneity of variance of these transformed data showed that the variance was homogeneous (Chi^2 = 1.83; not significant). The percentage of recovered ova fertilised per ewe, for each dose of P.M.S. is presented in Table 9. An analysis of variance revealed a significant effect ($p < 0.025$; 11% of total variation) of P.M.S. dose on the percentage of recovered ova fertilised. The difference between 1100 i.u. and 1500 i.u. P.M.S. was shown to be significant ($p < 0.01$) by a Duncan's Multiple Range Test (Appendix II). If however, the percentage of recovered ova

Figure 11: Effect of the interval from onset of oestrus to laparotomy on the percentage of ova recovered and the percentage of recovered ova fertilised per ewe



fertilised was computed by an alternative method (i.e. was calculated from the proportion of ova recovered fertilised, of the total number of ova recovered, from all ewes receiving each dose of P.M.S.) the fertilisation rates would be 100% (1100 i.u.), 61% (1300 i.u.) and 51.5% (1500 i.u. P.M.S.). Analysis showed fertilisation rate and P.M.S. dose were strongly associated ($Chi^2 = 17.17$; $p < 0.001$). The difference in fertilisation rate between data pooled from ewes given 1100 i.u. and 1500 i.u. P.M.S. ($Chi^2 = 1.76$; not significant) and those receiving 1300 i.u. P.M.S. was significant ($Chi^2 = 13.20$; $p < 0.001$).

(b) Effect of Ovarian Response on the percentage of recovered ova fertilised per ewe

Figures 9 and 10 show that when the number of corpora lutea, or corpora lutea plus large follicles exceeded 3 per ewe, a decline occurred in the percentage of recovered ova fertilised. The result for 8 to 9 corpora lutea and 9 to 12 corpora lutea plus large follicles was high, but only 8 ewes contributed to this result.

(c) Effect of the time interval from the onset of oestrus to laparotomy on the percentage of recovered ova fertilised per ewe

After and including 67 hours from the onset of oestrus, increasing intervals to laparotomy up to 77 hours, had no marked effect on the percentage of recovered ova which were fertilised (Figure 11). However, the result for 77 hours, was high.

CLEAVAGE OF FERTILISED OVA

Of the 388 ova recovered, 216 (55.6%) were cleaved at laparotomy. Ova of differing cleavage stages were found in the same flushing from 8 ewes. In 4 of these animals the corpora lutea were observed to be of differing ages. Figures 13, 14 and 15 show a 1-, 2- and 6-cell ovum, respectively.

1. Shortest and longest interval after the onset of oestrus to the recovery of each cleavage stage

The shortest and longest interval to the recovery of each cleavage stage is shown in Table 10. Within each cleavage stage ova were obtained almost throughout the total time interval (10 hours). This could indicate a wide possible time-range during which each cleavage stage might be recovered. Only 3 ewes contributed to the result for the 2-cell ova.

2. Mean interval after the onset of oestrus to the recovery of each cleavage stage

Table 11 and Figure 12 show the mean interval after the onset of oestrus to the recovery of each ova cleavage stage. These results indicate that the fertilised ova cleaved at a rapid rate, at least to the 8- to 12-cell stage.

(a) Effect of dose of P.M.S.

The mean interval after the onset of oestrus to the recovery of most cleavage stages for each dose of P.M.S. are given in Table 12. Dose of P.M.S. had no marked effect on the interval to recovery of either 2- or 8-cell ova. With 4- and 6-cell ova, the lower the dose of P.M.S. the shorter was the interval to recovery. However, the converse applied to 8- to 12-cell ova.

TABLE 10

SHORTEST AND LONGEST INTERVAL (HOURS) AFTER THE ONSET OF OESTRUS TO THE RECOVERY OF EACH CLEAVAGE STAGE

Cleavage Stage (Number of cells per ovum)	Shortest Interval	Longest Interval
2	68	68
4	68	77
6	68	77
8	68	77
8 - 12	69	77

TABLE 11

MEAN INTERVAL AFTER THE ONSET OF OESTRUS TO RECOVERY OF EACH CLEAVAGE STAGE

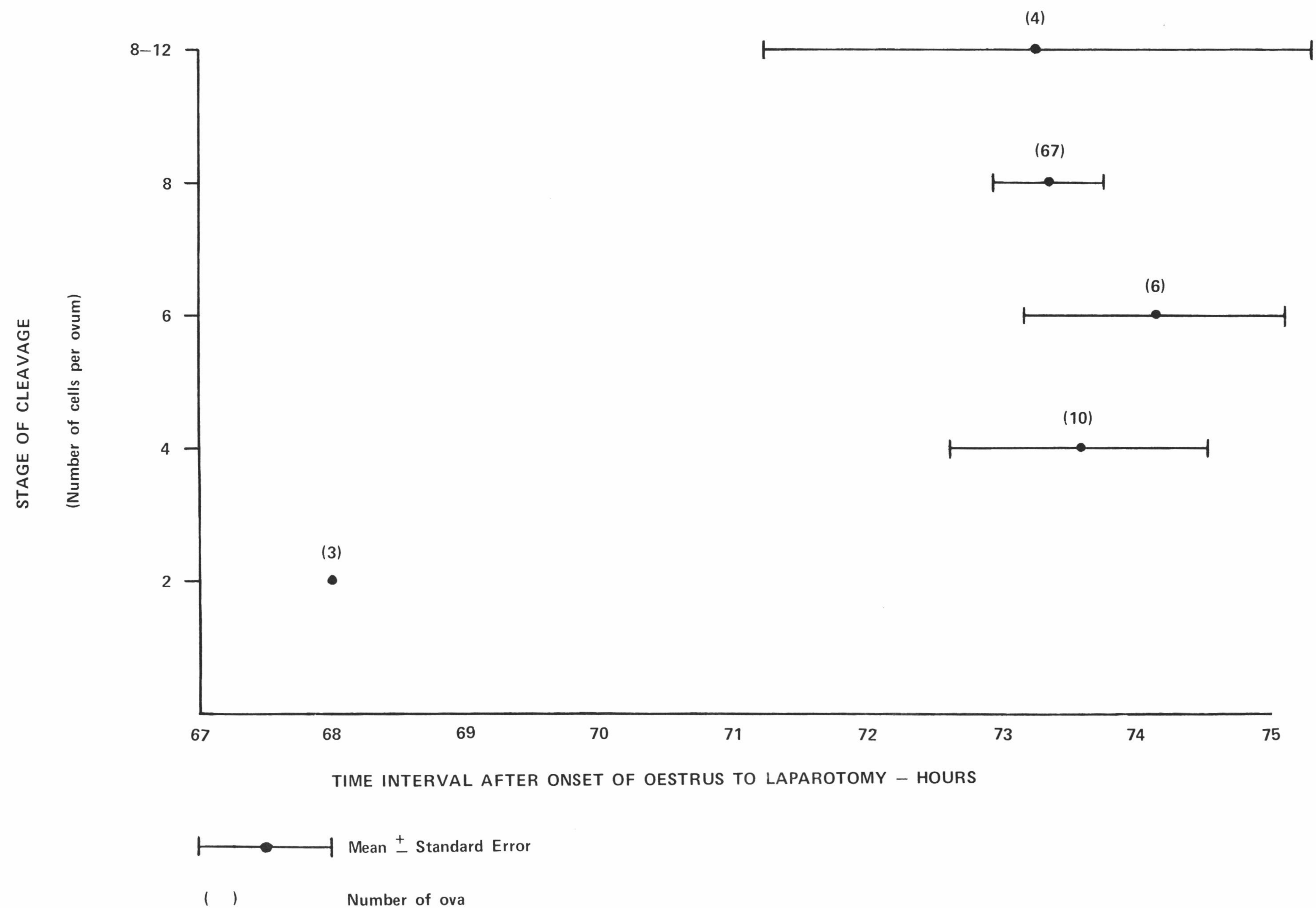
Cleavage Stage (Number of cells per ovum)	Number of Ova	Interval to Recovery (hours)	
		Mean ± S.E.	Variance
2	3	68.00 ± 0.0	-
4	14	73.60 ± 0.98	9.66
6	10	74.16 ± 1.03	10.20
8	179	73.34 ± 0.38	10.18
8 - 12	10	73.25 ± 2.01	19.00

TABLE 12

EFFECT OF DOSE OF P.M.S. ON THE MEAN INTERVAL (HOURS) FROM ONSET OF OESTRUS TO RECOVERY OF EACH CLEAVAGE STAGE

Cleavage Stage (Number of cells per ovum)	Dose P.M.S.		
	1,100 i.u. Mean ± S.E.	1,300 i.u. Mean ± S.E.	1,500 i.u. Mean ± S.E.
2	-	68.00 ± 0.0	68.00 ± 0.0
4	71.50 ± 1.43	72.00 ± 2.0	75.40 ± 0.54
6	-	72.33 ± 2.19	76.00 ± 0.57
8	72.00 ± 1.02	73.13 ± 0.85	73.69 ± 0.46
8 - 12	-	77.00 ± 0.00	72.00 ± 2.5

Figure 12: Mean interval after onset of oestrus to the recovery of ova cleavage stages



SEQUENTIAL TREATMENT OF EWES WITH P.M.S.

Data became available during this study, on the ovarian response of Romney ewes sequentially treated with P.M.S. in the breeding season. The results in Table 13, were from 63 ewes given two, and 7 ewes three injections of P.M.S. These data are partly confounded since the dose of P.M.S. was not constant between treatments, for each animal.

In animals given two injections of 1500 i.u. P.M.S., the number of corpora lutea and corpora lutea plus large follicles observed, declined after the second treatment. Also, an increase in dose of 300 i.u. P.M.S. (to 1800 i.u.) at the second injection failed to produce any marked increase in ovarian response. These results show that a general decline in ovarian response occurred with successive treatments of P.M.S.

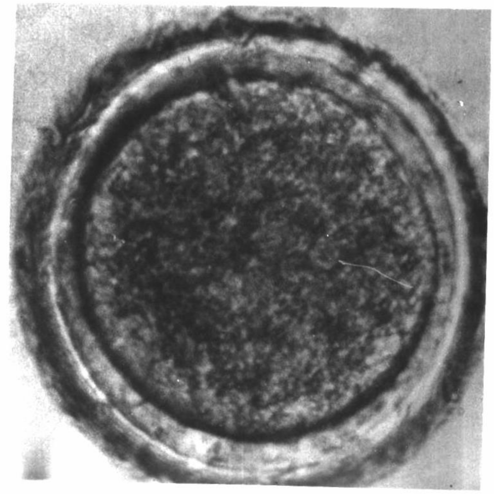
An increase in ovarian size and in the number of luteinized follicles were observed after the second and third P.M.S. treatments. In some cases this condition was associated with a failure to ovulate. Of the 7 ewes given three injections of P.M.S. 3 (43%) failed to ovulate.

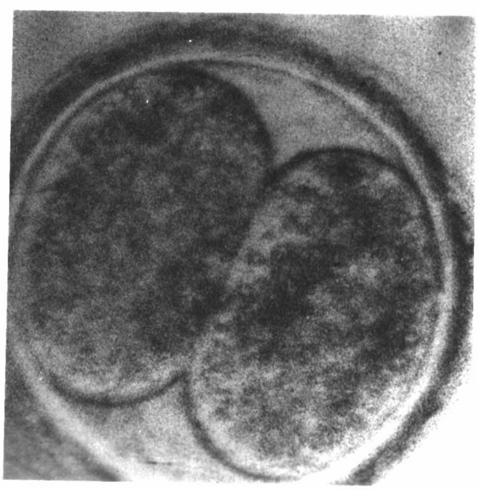
The genital tract of ewe 99, removed three days post-oestrus, after treatment with 1800 i.u. P.M.S. (third injection) is shown in Figure 16. This tract provides an illustration of the type of ovarian response (marked increase in ovarian size and in the number of unruptured follicles) commonly observed in ewes after the second or third P.M.S. treatment. For comparative purposes, a tract from a non-stimulated ewe, removed three days post-oestrus, is also shown (Figure 17).

Fig. 13. Unfertilised ovum recovered 72 hours after onset of oestrus in donor ewe

Fig. 14. 2-cell ovum recovered 68 hours after onset of oestrus in donor ewe

Fig. 15. 6-cell ovum recovered 77 hours after onset of oestrus in donor ewe





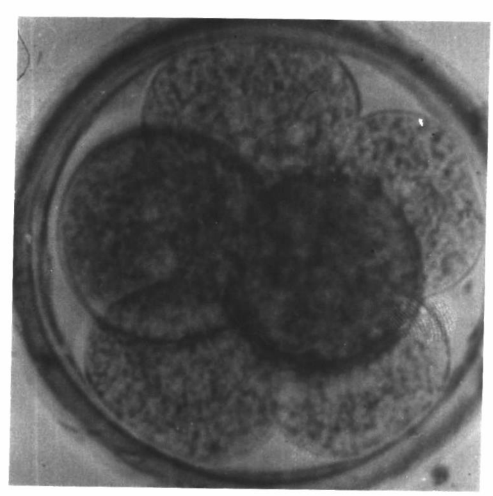
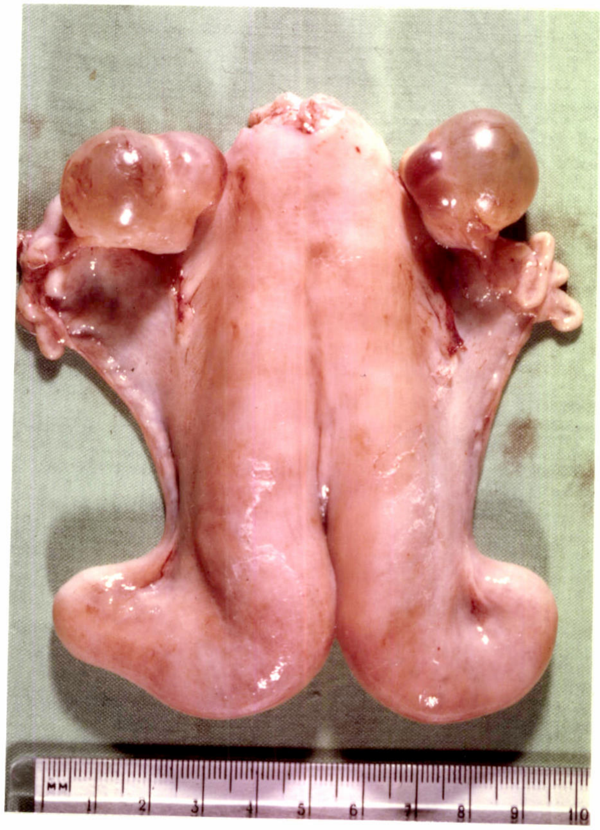


Fig. 16.   Ewe 99. Excised genital tract removed three days post-oestrus after treatment with 1800 i.u. P.M.S.

Fig. 17.   Ewe 146. Excised genital tract removed three days post-oestrus. Non-stimulated ewe.


MM
1 2 3 4 5 6 7 8 9 10

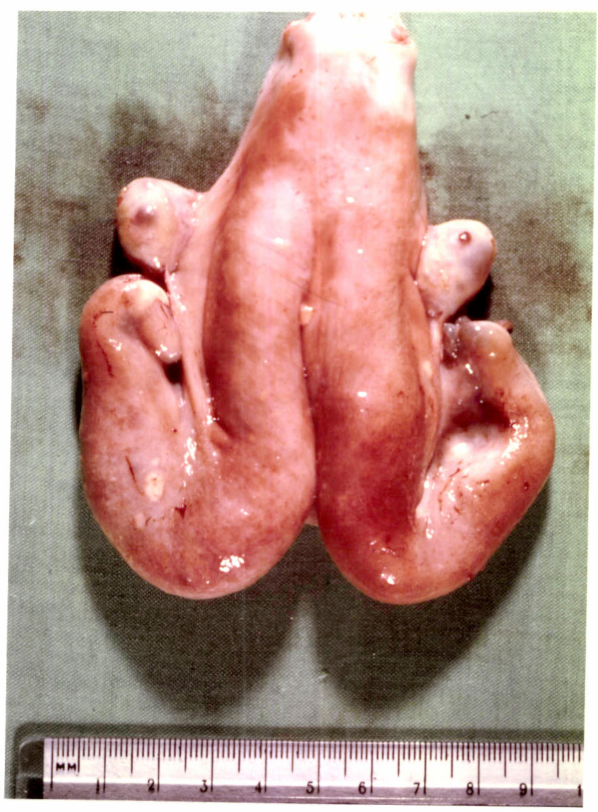

MM
1 2 3 4 5 6 7 8 9

TABLE 13

EFFECT OF SEQUENTIAL TREATMENT WITH P.M.S. DURING THE BREEDING SEASON ON OVARIAN RESPONSE

Treatment	Dose of P.M.S. (i.u.)	Number of Ewes	Number of Corpora Lutea (Mean ± S.E.)	Number of Corpora Lutea plus Large Follicles (Mean ± S.E.)
One	1100	6	2.00 ± 0.44	2.16 ± 0.40
	1300	17	2.88 ± 0.37	3.47 ± 0.41
	1500	40	4.37 ± 0.50	5.82 ± 0.74
	Combined doses	63	3.74 ± 0.35	4.84 ± 0.51
Two	1500	28	3.32 ± 0.41	4.21 ± 0.51
	1800	35	3.37 ± 0.36	4.08 ± 0.46
	Combined doses	63	3.34 ± 0.26	4.14 ± 0.34
Three	1800	7	2.42 ± 0.57	6.42 ± 0.89

CHAPTER IV

OVULATION STUDIES IN ROMNEY AND BLX TWO-TOOTH EWES

Chapter IV

OVULATION STUDIES IN ROMNEY AND BLX TWO-TOOTH EWES

One hundred and fifty-eight Romney and BLX Two-Tooth ewes were laparotomised, in most cases for ova transfer. Both the number of recent corpora lutea and the corpora lutea of the previous cycle were recorded. These results were obtained over a three month period during the breeding season. In the analysis of these data an attempt was made to relate most of the information to one month (April) of the breeding season.

1. Effect of breed of ewe on ovulation rate

Table 14 shows the number of corpora lutea observed at laparotomy. The difference of 0.24 ovulations per ewe, between breeds, was highly significant ($Chi^2 = 12.21$; $p < 0.001$). Two BLX ewes failed to show regular cyclic activity during the breeding season. Ewe 330 showed oestrus during progestagen treatment. At laparotomy, a corpus luteum corresponding to this ovulation was found. Fluid filled distentions of both uterine horns were observed in ewe 57 at laparotomy. This animal came in oestrus 48 hours after surgery and a subsequent investigation revealed that the fluid had disappeared.

2. Effect of ewe birth rank on ovulation rate

Ewes were classified according to birth rank, breed and the number of corpora lutea found. These results are presented in Table 15. Within the BLX breed, there was a suggestion of an effect of birth rank (41% born as twins v. 32% born as singles had two corpora lutea) on ovulation rate ($Chi^2 = 1.12$; $p < 0.5$). Birth rank and

TABLE 14

NUMBERS OF CORPORA LUTEA AT LAPAROTOMY

Breed	Number of Corpora Lutea		Total Ewes	Ovulation Rate
	One	Two		
Romney	79	9	88	1.10
BLX	46	24	70	1.34
Total	125	33	158	

$Chi^2 = 12.21 - p < 0.001$

TABLE 15

EFFECT OF BIRTH RANK ON OVULATION RATE WITHIN BREED OF EWE

	Breed of Ewe			
	Romney		BLX	
Birth Rank	Number of Corpora Lutea			
	One	Two	One	Two
Single	65	14	31	15
Twin	8	1	14	10
Total Ewes	73	15	45	25

Romney : Chi^2 = 0.93 - Not Significant

BLX : Chi^2 = 1.12 - Not Significant

TABLE 16

INFLUENCE OF FACE GRADE ON OVULATION RATE - ROMNEY EWES

Face Grade	Number of Corpora Lutea One	Number of Corpora Lutea Two	Total Ewes	Ovulation Rate
1, 1+, 2-, 2	28	3	31	1.09
2+, 3-, 3	18	5	23	1.21
3+, 4-, 4, 4+, 5	31	1	32	1.03
Total	77	9	86	

G = 4.95 - Not Significant

ovulation rate were independent for Romney ewes ($Chi^2 = 0.93$; not significant). The difference in ovulation rate between __all__ ewes (data from both breeds pooled) born as singles or twins was not significant ($Chi^2 = 0.93$; not significant). There were no significant interactions.

3. __Relationship between face grade and ovulation rate - Romney ewes__

Ovulation and face grade (see Chapter II) data were available on 86 Romney ewes. These animals were initially grouped into three classes on the basis of face grade (Table 16). Analysis of these data showed a suggestion of an effect of face grade on ovulation rate ($G = 4.95$; $p < 0.10$). However, when only two classes of face grade were considered (i.e. grades 1 to 3- v. 3 to 5) the two factors were independent ($Chi^2 = 0.11$; not significant).

4. __Influence of liveweight on ovulation rate__

The mean liveweights of Romney and BLX ewes were 40.6 ± 0.6 and 46.5 ± 0.5 kg., respectively (Table I). Possible associations between liveweight and ovulation rate were examined on a within breed basis. The technique of biserial correlation (see Chapter II) was used in this analysis. Coefficients of correlation between liveweight and ovulation rate for the Romney and BLX breeds were -0.015 ± 0.18 and -0.124 ± 0.15, respectively. In view of the large standard errors, these correlation values can be regarded as negligible.

CHAPTER V

SURVIVAL OF TRANSFERRED OVA AND UTERINE CAPACITY

Chapter V

SURVIVAL OF TRANSFERRED OVA AND UTERINE CAPACITY

The results of transplants of fertilised ova made to Romney and BLX Two-Tooth ewes are given in this chapter. Unless otherwise indicated the results refer only to the initial transfer attempt for any ewe. All factors were measured in terms of lambs born.

SURVIVAL OF TRANSFERRED OVA

A total of 140 transplants at rates of one or three ova per ewe were attempted (Table 17). However, an additional 16 transplants (10 at one,; 4 at two, and 2 at three ova per ewe) were made to ewes which failed to conceive to the initial transfer attempt. Of these ewes, 12 became pregnant and 17 lambs were born.

Eight ewes which did not show oestrus after transfer, failed to lamb. A further 5 ewes were given three opportunities to become pregnant (two transfer attempts and a natural mating) but never lambed. In addition, 2 ewes returned to oestrus about day 50 after transfer.

1. Conception rate following transfer

Table 18 shows the effect of breed of recipient, breed and number of ova transferred, on conception rate following transfer. Only the number of ova transferred had a significant effect. Of the 80 ewes receiving one egg, 53 (66.2%) became pregnant, while 54 (90%) of 60 ewes given three eggs, subsequently lambed (Chi^2 = 9.44;

TABLE 17

NUMBER OF OVA TRANSPLANTS

Breed	Number of Ova Transplanted		Total
	One	Three	
Romney	45	32	77
BLX	35	28	63
Total	80	60	140

TABLE 18

EFFECT OF BREED OF RECIPIENT, BREED AND NUMBER OF EGGS TRANSFERRED, AND SIRE, ON CONCEPTION RATE FOLLOWING TRANSFER

Main Effect	Number of Ewes Pregnant	Number of Ewes Non-pregnant	Total Ewes	% of Ewes Pregnant
Breed of Recipient				
Romney	62	15	77	80.5
BLX	45	18	63	71.4
$Chi^2 = 1.124$ - N.S.				
Breed of Egg				
Romney	49	16	65	75.3
BLX	58	17	75	77.3
$Chi^2 = 0.004$ - N.S.				
Number of Ova Transferred				
One	53	27	80	66.2
Three	54	6	60	90.0
$Chi^2 = 9.448$ - $p < 0.005$				
Sire				
I	31	8	39	79.5
II	29	11	40	72.5
III	27	9	36	75.0
IV	20	5	25	80.0
$Chi^2 = 0.758$ - N.S.				
Total	107	33	140	74.6

N.S. = Not Significant

$p < 0.005$). The analysis revealed a significant interaction between breed of recipient and the number of ova transferred ($Chi^2 = 4.77$; $p < 0.05$).

2. Ova survival

(a) Ewes receiving one ovum

The number and percentages of ova surviving in ewes which received one egg are presented in Table 19. There was a suggestion of an effect of breed of recipient (73% v. 57% survived) on the proportion of ova surviving.

(b) Ewes receiving three ova

In ewes given three eggs the proportion of ova surviving was not significantly effected by breed of recipient, breed of egg, or sire (Table 20).

(c) All ewes

Table 21 shows the survival of ova in all ewes was not significantly affected by any factor examined. Of 260 ova transferred, 160 (61.5%) survived: 53 of 80 (66.2%) in ewes which received one egg, and 107 of 180 (59.4%) in ewes given three eggs. With single transplants 73.3% and 57.1% of ova survived in Romney and BLX ewes, respectively. Conversely, when three ova were transferred a greater proportion survived in BLX (63%) than Romney (56.2%) ewes.

UTERINE CAPACITY AFTER EGG TRANSFER

In this section the results apply only to ewes which received three ova at transfer.

TABLE 19

EFFECT OF BREED OF RECIPIENT, BREED OF EGG, AND SIRE, ON OVA SURVIVAL IN EWES RECEIVING ONE EGG

Main Effect	Number of Ova Surviving	Number of Ova Lost	Total Ova	% of Ova Surviving
Breed of Recipient				
Romney	33	12	45	73.3
BLX	20	15	35	57.1
Chi^2 = 1.70 - N.S.				
Breed of Egg				
Romney	21	13	34	61.7
BLX	32	14	46	69.5
Chi^2 = 1.64 - N.S.				
Sire				
I	18	6	24	75.0
II	13	9	22	59.0
III	14	8	22	63.6
IV	8	4	12	66.6
Chi^2 = 1.60 - N.S.				
Total	53	27	80	66.2

N.S. = Not Significant

TABLE 20

EFFECT OF BREED OF RECIPIENT, BREED OF EGG, AND SIRE, ON OVA SURVIVAL IN EWES RECEIVING THREE EGGS

Main Effect	Number of Ova Surviving	Number of Ova Lost	Total Ova	% of Ova Surviving
Breed of Recipient				
Romney	54	42	96	56.2
BLX	53	31	84	63.0
$Chi^2 = 1.17$ - N.S.				
Breed of Egg				
Romney	56	37	93	60.2
BLX	51	36	87	58.6
$Chi^2 = 1.06$ - N.S.				
Sire				
I	25	20	45	55.5
II	31	23	54	57.4
III	26	16	42	61.9
IV	25	14	39	64.1
$Chi^2 = 0.83$ - N.S.				
Total	107	73	180	59.4

N.S. = Not Significant

TABLE 21

EFFECT OF BREED OF RECIPIENT, BREED AND NUMBER OF OVA, AND SIRE, ON OVA SURVIVAL - ALL EWES

Main Effect	Number of Ova Surviving	Number of Ova Lost	Total Ova	% of Ova Surviving
Breed of Recipient				
Romney	87	54	141	61.7
BLX	73	46	119	61.3
Chi^2 = 0.004 - N.S.				
Breed of Egg				
Romney	77	50	127	60.6
BLX	83	50	133	62.4
Chi^2 = 0.177 - N.S.				
Number of Eggs Transferred				
One	53	27	80	66.2
Three	107	73	180	59.4
Chi^2 = 1.39 - N.S.				
Sire				
I	43	26	69	62.3
II	44	32	76	57.8
III	40	24	64	62.5
IV	33	18	51	64.7
Chi^2 = 0.703 - N.S.				
Total	160	100	260	61.5

N.S. = Not Significant

Both litter size and lamb weight at birth can be considered to be functions of uterine capacity. To facilitate a between breed comparison in uterine capacity, these data were separately classified according to distribution of litter size, and the weight of lamb born per ewe.

1. Distribution of litter size

Table 22 shows the effects of maternal and embryo genotype on the distribution of litter size in ewes receiving three eggs. A Chi-square analysis failed to show an effect of embryo genotype. However, there was a suggestion of an effect of maternal genotype since BLX ewes had less single and more twin pregnancies than Romney ewes.

The results of a further 6 multiple transplants are given in Table 23. These transfers were made to ewes which failed to become pregnant to the initial transfer attempt. Analysis of these data pooled with the results in Table 22 also showed that distribution of litter size, maternal and embryo genotype, were independent.

Ewe 118 (Romney) produced at parturition one live lamb plus two mummified foetuses. In addition, ewe 101 (BLX) gave birth to a live lamb between 2 stillborn foetuses. The ewe and lamb, plus the foetuses are shown in Figures 18 and 19, respectively.

2. Weight of lamb born per ewe

In Table 24 the effects of maternal and embryo genotype on the weight of lamb born per ewe were examined. An analysis of variance revealed a suggestion of an effect of maternal genotype ($p < 0.10$; 9.2% of total variation. The mean weight of lamb born per ewe was greater for BLX than Romney ewes. The negative variance

TABLE 22

EFFECT OF MATERNAL AND EMBRYO GENOTYPE ON DISTRIBUTION OF LITTER SIZE IN EWES RECEIVING THREE EGGS

Maternal Genotype	Embryo Genotype	Number of Ewes with Litter Size of One	Two	Three	Total Ewes
Romney	Romney	6	8	3	17
	BLX	4	5	3	12
	Total	10	13	6	29
BLX	Romney	0	8	3	11
	BLX	3	8	3	14
	Total	3	16	6	25
Grand Total		13	29	12	54

Partition of Chi^2 : Distribution of Litter Size

Source of Variation	d.f.	Chi^2	P	
Between Maternal Genotype	2	3.7935	N.S.	$p < 0.5$
Between Embryo Genotype	2	0.3161	N.S.	
Interaction	2	1.4744	N.S.	$p < 0.5$
Total	6	5.5840		

N.S. = Not Significant

TABLE 23

MULTIPLE TRANSPLANTS - SECOND TRANSFER ATTEMPT

Maternal Genotype	Embryo Genotype	Number of Ova Transferred	Number of Lambs Born
Romney	BLX	2	2
Romney	BLX	2	2
BLX	Romney	3	3
BLX	BLX	3	1
BLX	BLX	2	2
BLX	BLX	2	1

TABLE 24

EFFECT OF MATERNAL AND EMBRYO GENOTYPE ON THE WEIGHT OF LAMB BORN PER EWE IN EWES RECEIVING THREE EGGS

Maternal Genotype	Embryo Genotype	Number of Ewes	Mean Weight of Lamb Born Per Ewe (kg.) (Mean ± S.E.)
Romney	Romney	17	5.35 ± 0.46
	BLX	12	5.48 ± 0.55
	Combined	29	5.40 ± 0.35
BLX	Romney	11	6.36 ± 0.42
	BLX	14	6.12 ± 0.54
	Combined	25	6.26 ± 0.34

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Mean Squares	Partition of Variation
Between Maternal Genotype	1	44.44 N.S.	9.2%
Between Embryo Genotype within Maternal Genotype	2	1.16 N.S.	-6.8%
Error	50	17.13	97.6%

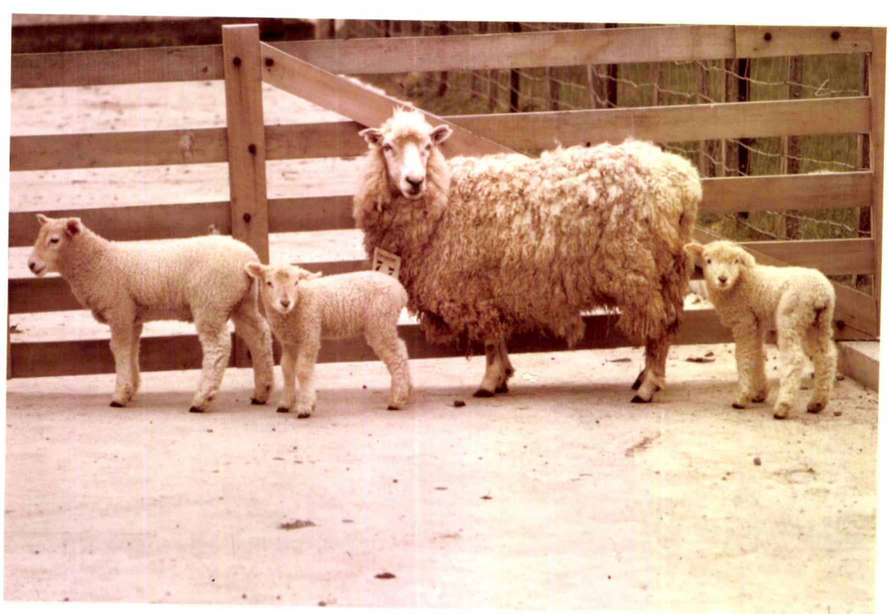
N.S. = Not Significant

Fig. 18. Ewe 101. Ewe and surviving lamb following transfer of three ova

Fig. 19. Ewe 101. Stillborn foetuses and placental membranes of live lamb following transfer of three ova

Fig. 20. Ewe 181. Romney ewe and three Romney lambs after multiple transplant

Fig. 21. Ewe 240. Border Leicester x Romney ewe with three Border Leicester x Romney lambs after multiple transplant



component estimated for embryo genotype within maternal genotype could be ascribed to sampling error.

3. Relationship between number of lambs born after transfer of three ova and natural ovulation rate

Litter size after transfer of three eggs and the natural ovulation rate of these ewes at laparotomy (pregnant ewes only), are presented in Table 25. In both breeds, litter size after transfer of three eggs was significantly greater than the potential litter size determined by natural ovulation rate. However, the difference was of greater magnitude in Romney ($Chi^2 = 21.87$; $p < 0.001$) compared to BLX ($Chi^2 = 12.38$; $p < 0.01$) ewes.

Figures 20 and 21 show a Romney and BLX ewe respectively, each with triplet lambs at foot, born following ova transplants.

TABLE 25

OVULATION RATE AT LAPAROTOMY AND LITTER SIZE IN EWES

RECEIVING THREE EGGS - DATA PREGNANT EWES ONLY

	Maternal Genotype									
	Romney					BLX				
	No. of Corpora Lutea or Lambs Born			Total Ewes	Mean	No. of Corpora Lutea or Lambs Born			Total Ewes	Mean
Factor	1	2	3			1	2	3		
Natural Ovulation Rate	27	2	0	29	1.06	15	10	0	25	1.40
Litter Size After Transfer	10	13	6	29	1.86	3	16	6	25	2.12

Romney : $Chi^2 = 21.87$; $p < 0.001$

BLX : $Chi^2 = 12.38$; $p < 0.01$

C H A P T E R   V I

P R E - N A T A L   L A M B   G R O W T H

Chapter VI

PRE-NATAL LAMB GROWTH

A total of 173 lambs were available for studies on pre-natal lamb growth. Of these lambs, 78 were born as singles, 56 as twins and 39 as triplets. All but 8 lambs were born as a result of ova transfer.

EFFECT OF LITTER SIZE ON GESTATION LENGTH AND BIRTH WEIGHT

The mean length of gestation and birth weight of single, twin and triplet-born lambs are presented in Table 26.

1. Gestation Length

Only small differences in the mean gestation length between single, twin and triplet-born lambs were observed. An analysis of variance showed that these differences were not significant (Table 26).

2. Birth Weight

An inverse relationship between mean birth weight and litter size is shown in Table 26. Single lambs were heavier than twins, and twins heavier than triplets. A highly significant effect ($p < 0.001$) of litter size on birth weight was disclosed by an analysis of variance (Table 26). In addition, a Duncan's Multiple Range Test (Appendix III) showed that the mean birth weights for the 78 single, 56 twin and 39 triplet-born lambs all differed significantly ($p < 0.01$).

TABLE 26

EFFECT OF LITTER SIZE ON GESTATION LENGTH (DAYS) AND LAMB BIRTH WEIGHT (kg.)

Litter Size	Number of Ewes	Gestation Length (Mean ± S.E.)	Number of Lambs	Birth Weight (Mean ± S.E.)
Singles	78	146.75 ± 0.21	78	4.35 ± 0.09
Twins	28	146.35 ± 0.31	56	3.13 ± 0.09
Triplets	13	146.53 ± 0.48	39	2.60 ± 0.09

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Gestation Length Mean Squares	d.f.	Birth Weight Mean Squares
Between Litter Size	2	1.66 N.S.	2	231.59 ***
Error	116	3.48	170	2.70

*** $p < 0.001$

N.S. = Not Significant

EFFECT OF SEX OF LAMB ON GESTATION LENGTH AND BIRTH WEIGHT

1. Single-Born Lambs

Mean values for the length of gestation and birth weight of single-born lambs within each subgroup, are given in Table 27.

The differences in gestation length between sex, within each subgroup, were small and non-significant (Table 28). An examination of data pooled for all single-born lambs also failed to reveal a significant sex effect (Table 28). The between sex variance component estimated from this data was negligible.

Table 27 shows that except for BLX lambs born to Romney ewes, male lambs were heavier than their female counterparts. However, none of these subgroup differences were significant as disclosed by the analysis of variance summarised in Table 29. A similar result was obtained when pooled data (all single-born lambs) was examined (Table 29).

2. All Lambs

The mean birth weights for twin and triplet-born lambs within each subgroup are presented in Table 30. Again males tended to be heavier than females both within subclasses and birth rank. However, these differences were not significant (Table 29). Similarly, an analysis of variance of birth weight data for all lambs, showed the effect of sex to be non-significant (Table 29).

TABLE 27

LENGTH OF GESTATION (DAYS) AND BIRTH WEIGHT (kg.) - SINGLE-BORN LAMBS

Maternal Genotype		Romney				BLX			
Lamb Genotype		Romney		BLX		Romney		BLX	
Sex		Males	Females	Males	Females	Males	Females	Males	Females
Number of Lambs		5	13	13	17	5	6	11	8
Gestation Length	- Mean	147.40	147.46	147.23	146.41	145.60	145.83	147.09	146.62
	S.E.	0.81	0.38	0.45	0.55	0.87	1.05	0.66	0.49
Birth Weight	- Mean	4.51	4.05	4.14	4.29	4.66	4.49	4.79	4.35
	S.E.	0.32	0.11	0.28	0.17	0.24	0.12	0.40	0.17

S.E. = Standard Error

TABLE 28

EFFECT OF SEX OF SINGLE-BORN LAMBS ON GESTATION LENGTH - (DAYS)

Summary of Analyses of Variance

Genotype		Total Number of Lambs	Mean Squares
Maternal	Lamb		
Romney	Romney	18	0.29 N.S.
Romney	BLX	30	3.34 N.S.
BLX	Romney	11	0.03 N.S.
BLX	BLX	19	1.00 N.S.

ANALYSIS OF VARIANCE - ALL SINGLE-BORN LAMBS

Source of Variation	d.f.	Mean Squares	Partition of Variation
Between sex	1	1.25 N.S.	-
Error	76	3.81	100%

N.S. = Not Significant

TABLE 29

SEX DIFFERENCES IN LAMB BIRTH WEIGHT

Summary of Analyses of Variance

Genotype		Type of Birth		
Maternal	Lamb	Single	Twin	Triplet
			Mean Squares	
Romney	Romney	2.02 N.S.	0.00	0.00
Romney	BLX	0.69 N.S.	1.19 N.S.	0.05 N.S.
BLX	Romney	0.36 N.S.	0.10 N.S.	0.02 N.S.
BLX	BLX	4.27 N.S.	0.02 N.S.	6.24 N.S.
Data Pooled for Type of Birth		3.69 N.S.	1.00 N.S.	2.03 N.S.

ANALYSIS OF VARIANCE - ALL LAMBS

Source of Variation	d.f.	Mean Squares	Partition of Variation
Between Sex	1	6.90 N.S.	0.3%
Error	173	5.32	97.7%

N.S. = Not Significant

TABLE 30

BIRTH WEIGHT (kg.) - TWIN AND TRIPLET-BORN LAMBS

Maternal Genotype	Romney				BLX			
Lamb Genotype	Romney		BLX		Romney		BLX	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Twins								
Number of Lambs	6	8	6	6	7	7	9	7
Birth Weight - Mean	2.99	2.98	3.29	3.00	3.04	2.96	3.40	3.36
S.E.	0.46	0.19	0.32	0.22	0.16	0.12	0.28	0.33
Triplets								
Number of Lambs	2	7	3	6	7	5	4	5
Birth Weight - Mean	2.63	2.64	2.44	2.37	2.68	2.73	3.07	2.31
S.E.	0.22	0.19	0.45	0.10	0.27	0.34	0.38	0.37

S.E. = Standard Error

INFLUENCE OF MATERNAL AND LAMB GENOTYPE ON GESTATION LENGTH AND BIRTH WEIGHT

Maternal influence can be defined as the total effect of all maternal factors which influence the growth of the embryo and subsequently the lamb. In this treatise, the dam's role as it affects foetal growth, has been referred to as the effect of maternal genotype. Similarly, the embryo's growth has been considered in terms of the lamb's genotype. The methods of analysis of these data are described in Chapter II.

1. Single-Born Lambs

Table 31 shows the effects of maternal and lamb genotype on the gestation length and birth weight of single-born lambs.

Irrespective of their genotype, lambs were carried significantly longer ($p < 0.025$) in the Romney as opposed to the BLX maternal environment. The effect of lamb genotype on gestation length was small and non-significant, especially for BLX lambs. However, Romney lambs born to Romney ewes, were carried almost 2 days longer than lambs of the same genotype born to BLX ewes. There was a strong interaction ($p < 0.10$) between the two main effects.

Lambs developing in a BLX maternal environment, were 8% heavier at birth, than lambs developing in a Romney maternal environment, irrespective of their genotype (Table 31). This effect of maternal genotype on lamb birth weight just failed to reach significance ($p < 0.10$). However, the effect of lamb genotype was very small and non-significant. The interaction term was negligible.

TABLE 31

EFFECT OF MATERNAL AND LAMB GENOTYPE ON GESTATION LENGTH (DAYS) AND LAMB BIRTH WEIGHT (kg.) - SINGLE-BORN LAMBS

Genotype: Maternal	Genotype: Lamb	Number of Lambs	Gestation Length (Mean ± S.E.)	Birth Weight (Mean ± S.E.)
Romney	Romney	18	147.44 ± 0.34	4.20 ± 0.13
Romney	BLX	30	146.76 ± 0.37	4.23 ± 0.15
	Combined	48	147.02 ± 0.26	4.22 ± 0.10
BLX	Romney	11	145.54 ± 0.66	4.57 ± 0.12
BLX	BLX	19	146.47 ± 0.43	4.60 ± 0.24
	Combined	30	146.13 ± 0.37	4.59 ± 0.15

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Gestation Length Mean Squares	Birth Weight Mean Squares
Maternal Genotype	1	20.70 **	11.37 N.S.
Lamb Genotype	1	0.20 N.S.	0.10 N.S.
Interaction	1	11.00 N.S.	0.001 N.S.
Error	74	3.58	3.25

** $p < 0.025$
N.S. = Not Significant

2. All Lambs

The influence of maternal and lamb genotype on the length of gestation, and birth weight of all lambs, is shown in Table 32.

As with single pregnancies, maternal genotype had a significant effect ($p < 0.001$) upon length of gestation. Irrespective of their genotype, lambs were carried longer in Romney compared to BLX ewes. Romney lambs were born significantly later (146.73 v. 146.38 days; $p < 0.05$) than BLX lambs, independent of their maternal environment. There was a strong, although non-significant interaction ($p < 0.10$) between the two main effects.

When all lambs were considered, lamb genotype had a marked effect on lamb birth weight (Table 32). This is in contrast to the result obtained for single-born lambs only. BLX lambs were heavier at birth (3.71 v. 3.41 kg.; $p < 0.001$) than Romney lambs, irrespective of breed of dam. However, the birth weights of lambs born to Romney and BLX ewes were similar (3.56 v. 3.59 kg.). The interaction was not significant.

COMPETITION IN UTERO BETWEEN TWIN LAMBS

The performance of a male foetus in multiple litters may be dependent at least in part on whether or not female foetuses are present. During this investigation data became available on the birth weights of 28 pairs of twin lambs. Within the obvious limitations of a small sample size and possible extraneous variation, the results were examined in an attempt to show *in utero* competition effects between the twin pairs.

TABLE 32

EFFECT OF MATERNAL AND LAMB GENOTYPE ON GESTATION LENGTH (DAYS) AND LAMB BIRTH WEIGHT (kg.) - ALL LAMBS

Genotype		Number of Lambs	Gestation Length (Mean ± S.E.)	Birth Weight (Mean ± S.E.)
Maternal	Lamb			
Romney	Romney	41	147.36 ± 0.29	3.44 ± 0.13
Romney	BLX	51	146.78 ± 0.26	3.65 ± 0.10
	Combined	92	147.04 ± 0.18	3.56 ± 0.08
BLX	Romney	37	146.02 ± 0.28	3.37 ± 0.15
BLX	BLX	44	145.93 ± 0.27	3.78 ± 0.16
	Combined	81	145.97 ± 0.20	3.59 ± 0.09

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Gestation Length Mean Squares	Birth Weight Mean Squares
Maternal Genotype	1	51.50 ***	0.145 N.S.
Lamb Genotype	1	5.00 *	19.99 ***
Interaction	1	2.70 N.S.	2.07 N.S.
Error	169	2.94	5.24

* $p < 0.05$
*** $p < 0.001$
N.S. = Not Significant

There are four classes of twin lambs: males twin to males, and to females; females twin to females, and to males. The mean birth weights of these four classes are shown in Table 33. Males twin to females were heavier than both males twin to males, and females twin to males. However, an analysis of variance revealed that the effect of sex combination on birth weight was not significant (Table 33).

From the mean birth weights, four sets of differences were computed and these appear in Table 34. The largest difference was that for males twin to females minus females twin to males, but it did not reach significance. However, the birth weight superiority of males over females was generally greater in <u>mixed</u> sex than <u>like</u> sexed litters.

TABLE 33

BIRTH WEIGHTS (kg.) FOR CLASSES OF TWIN-BORN LAMBS

	Classes of Twin-Born Lambs			
	Males Twin to Males	Males Twin to Females	Females Twin to Males	Females Twin to Females
Number of Lambs	14	14	14	14
Birth Weight - Mean	3.10	3.29	3.08	3.07
S.E.	0.23	0.19	0.13	0.18

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Mean Squares
Sex Combination	3	0.77 N.S.
Error	52	2.50

S.E. = Standard Error

N.S. = Not Significant

TABLE 34

DIFFERENCES IN MEAN BIRTH WEIGHT (kg.) BETWEEN CLASSES OF TWIN-BORN LAMBS

Classes of Twin-Born Lambs			Differences in Mean Birth Weight	Analysis of Variance Mean Squares for Birth Weight Differences
Males Twin to Females	-	Males Twin to Males	0.19	1.33 N.S.
Females Twin to Females	-	Females Twin to Males	- 0.009	0.0
Males Twin to Males	-	Females Twin to Females	0.027	0.04 N.S.
Males Twin to Females	-	Females Twin to Males	0.21	1.62 N.S.

N.S. = Not Significant

CHAPTER VII

POST-NATAL LAMB GROWTH

Chapter V I I

P O S T - N A T A L   L A M B   G R O W T H

The liveweight of a lamb at weaning is a convenient measure of the growth of the lamb up to the stage at which weaning occurs. Weaning weight records of 154 lambs (74 singles; 50 twins and 30 triplets) were available for analysis. Each record was adjusted for known environmental effects. These adjustments and the method of computing weight gain per day are described in Chapter II. A Standard Error for all lambs, within each birth rank, was calculated from the error variance for both weaning weight, and weight gain per day.

EFFECT OF SEX ON LAMB WEANING WEIGHT AND WEIGHT GAIN PER DAY

1. Single-Born Lambs

The liveweight at weaning and weight gain per day of single-born lambs are presented in Table 35. Within each subgroup, male lambs (non-castrated) were consistently heavier and gained weight faster than female lambs. Analyses of variance summarised in Table 36 (weaning weight only) showed that none of these differences were significant. However, a significant difference in weaning weight between males and females (25.95 v. 23.77 kg.; $p < 0.025$) was observed when all single-born lambs were examined (Table 36).

As a consequence of the above result a sex classification was included in the analysis of maternal and lamb genotype effects on weaning weight and weight gain per day (Table 37). In this model (see Chapter II) the effect of sex on weaning weight just failed to

TABLE 35

WEIGHT AT WEANING AND WEIGHT GAIN PER DAY (kg.) - SINGLE, TWIN AND TRIPLET-BORN LAMBS

Maternal Genotype		Romney				BLX			
Lamb Genotype		Romney		BLX		Romney		BLX	
Sex		Males	Females	Males	Females	Males	Females	Males	Females
Number of Lambs	S.E.								
Singles		4	13	12	16	5	6	11	7
Twins		5	8	5	5	5	6	9	7
Triplets		2	7	3	2	7	4	3	2
Weaning Weight									
Singles	0.90	22.84	22.23	25.06	24.64	26.90	23.43	27.62	24.75
Twins	1.25	16.05	14.16	19.37	14.55	18.82	17.76	22.09	20.40
Triplets	2.44	16.55	15.21	21.70	13.29	12.51	13.09	22.02	14.60
Weight Gain Per Day									
Singles	0.004	0.184	0.183	0.209	0.204	0.221	0.188	0.227	0.203
Twins	0.009	0.131	0.111	0.161	0.114	0.157	0.147	0.186	0.170
Triplets	0.012	0.157	0.125	0.200	0.115	0.116	0.109	0.202	0.131

S.E. = Standard Error

TABLE 36

SEX DIFFERENCES IN LAMB WEANING WEIGHT

Summary of Analyses of Variance

Genotype: Maternal	Genotype: Lamb	Type of Birth: Single		Type of Birth: Twin		Type of Birth: Triplet		All Lambs	
		Mean Squares							
Romney	Romney	3.72	N.S.	53.63	N.S.	13.47	N.S.	18.41	N.S.
Romney	BLX	5.73	N.S.	283.02	N.S.	412.18	N.S.	157.80	N.S.
BLX	Romney	158.91	N.S.	14.84	N.S.	6.20	N.S.	12.48	N.S.
BLX	BLX	170.83	N.S.	105.43	N.S.	361.92	N.S.	96.84	N.S.
Data Pooled for Type of Birth		416.89	**	106.63	N.S.	154.27	N.S.		

ANALYSIS OF VARIANCE - ALL LAMBS

Source of Variation	d.f.	Mean Squares
Between Sex	1	530.93 **
Error	152	81.09

** $p < 0.025$

N.S. = Not Significant

TABLE 37

EFFECT OF MATERNAL AND LAMB GENOTYPE AND SEX ON WEANING WEIGHT AND WEIGHT GAIN PER DAY (kg.) - SINGLE-BORN LAMBS

Genotype: Maternal	Genotype: Lamb	Number of Lambs	Weaning Weight (Mean ± S.E.)	Weight Gain Per Day (Mean ± S.E.)
Romney	Romney	17	22.45 ± 0.49	0.183 ± 0.010
Romney	BLX	28	24.82 ± 0.61	0.206 ± 0.013
	Combined	45	23.93 ± 0.43	0.197 ± 0.009
BLX	Romney	11	25.00 ± 0.65	0.202 ± 0.010
BLX	BLX	18	26.50 ± 0.73	0.218 ± 0.012
	Combined	29	25.93 ± 0.52	0.212 ± 0.004

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Weaning Weight Mean Squares	Weight Gain Mean Squares
Between Sex	1	195.39 N.S.	0.0152 N.S.
Maternal Genotype (K)	1	253.08 *	0.0128 N.S.
Lamb Genotype (L)	1	216.91 N.S.	0.0225 *
Interaction (KL)	1	13.57 N.S.	0.0009 N.S.
Error	69	62.73	0.0054

* $p < 0.05$

N.S. = Not Significant

reach significance ($p < 0.10$). Although males gained weight more rapidly than females, the difference was not significant.

2. <u>All Lambs</u>

For both twin and triplet-born lambs, males were superior to females in both weaning weight and growth rate (Table 35). However, with triplet lambs the subclass numbers were in many instances very low. Table 36 shows that the differences in weaning weight both within subgroup, and type of birth, for twin and triplet-born lambs were not significant. An analysis of variance of weaning weight for <u>all lambs</u> (corrected for type of birth and rearing effects) is contained in Table 36. Males were significantly heavier than females (24.21 v. 22.53 kg. $p < 0.025$).

In the three way model (Table 38) sex differences in weaning weight approached significance ($p < 0.10$). However, the rate of growth of males was markedly superior to females ($p < 0.05$).

EFFECT OF MATERNAL AND LAMB GENOTYPE ON WEANING WEIGHT AND WEIGHT GAIN PER DAY

1. <u>Single-Born Lambs</u>

The effects of maternal and lamb genotype on weaning weight and weight gain per day of single-born lambs are shown in Table 37.

Irrespective of their genotype, lambs reared by BLX dams were significantly heavier at weaning than lambs reared by Romney ewes (25.93 v. 23.93 kg.; $p < 0.05$). In addition, BLX lambs were heavier than Romney lambs at weaning (25.48 v. 23.45 kg.; $p < 0.10$) irrespective of breed of dam. The interaction between the two main effects was small and non-significant.

TABLE 38

EFFECT OF MATERNAL AND LAMB GENOTYPE AND SEX ON WEANING WEIGHT

AND WEIGHT GAIN PER DAY (kg.) - ALL LAMBS

Genotype: Maternal	Genotype: Lamb	Number of Lambs	Weaning Weight (Mean ± S.E.)	Weight Gain Per Day (Mean ± S.E.)
Romney	Romney	39	21.41 ± 0.58	0.180 ± 0.010
Romney	BLX	43	23.97 ± 0.49	0.202 ± 0.012
	Combined	82	22.75 ± 0.42	0.191 ± 0.008
BLX	Romney	33	22.41 ± 0.54	0.188 ± 0.012
BLX	BLX	39	25.27 ± 0.54	0.222 ± 0.012
	Combined	72	23.96 ± 0.43	0.207 ± 0.009

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Weaning Weight Mean Squares	Weight Gain Mean Squares
Between Sex	1	241.25 N.S.	0.0441 **
Maternal Genotype (K)	1	164.03 N.S.	0.0254 *
Lamb Genotype (L)	1	1176.81 ***	0.1213 ***
Interaction (KL)	1	9.57 N.S.	0.0090
Error	149	73.71	0.0055

* $p < 0.05$
** $p < 0.005$
*** $p < 0.001$
N.S. = Not Significant

The growth rate of BLX and Romney lambs were 0.210 and 0.190 kg. per day, respectively ($p < 0.05$). Lambs with BLX mothers, had a faster although non-significant growth rate compared to lambs reared by Romney ewes (0.212 v. 0.197 kg. per day). The interaction term was negligible.

2. <u>All Lambs</u>

Table 38 shows the influence of maternal and lamb genotype on weaning weight and weight gain per day for all lambs.

Lambs (irrespective of genotype) reared by BLX dams were heavier at weaning than lambs reared by Romney ewes (23.96 v. 22.75 kg.). This difference was not significant. However, as for single-born lambs, there was a marked difference in weaning weight due to lamb genotype, independent of maternal genotype. The liveweights at weaning of BLX and Romney lambs were 24.58 and 21.87 kg., respectively ($p < 0.001$). There was no significant interaction.

Maternal genotype had a marked influence on lamb growth rate (Table 38). Lambs from BLX ewes were clearly superior to their counterparts reared by Romney dams (0.207 v. 0.191 kg. per day; $p < 0.05$). Similarly, BLX lambs grew at a faster rate than Romney lambs, irrespective of maternal genotype (0.211 v. 0.184 kg. per day; $p < 0.001$). The interaction term was non-significant.

CHAPTER VIII

DISCUSSION

Chapter VIII

DISCUSSION

SYNCHRONISATION OF OESTROUS CYCLES

Oestrous cycles in donor and recipient ewes were synchronised using progestagen impregnated sponges. Fertility at the oestrus following sponge withdrawal is frequently lower than normal (Clarke et al., 1966; Robinson et al., 1967). Consequently, donor ewes in this experiment were allowed one oestrous cycle following progestagen treatment prior to mating and ova recovery. Hancock and Hovell (1961) adopted this procedure for similar reasons. However, this may be unnecessary since Shelton and Moore (1966) have shown the survival of ova transferred to recipient ewes to be unaffected by previous progesterone treatment.

A significantly higher incidence of silent heats following sponge withdrawal were observed in 18-month compared to 5-year-old ewes (Table 3). Laparotomy at the subsequent oestrous cycle confirmed that ovulation had occurred in those ewes showing silent heat. Furthermore, older ewes were released from pituitary suppression more rapidly than younger ewes, following the withdrawal of the inhibitory factor (Table 5).

Cumming (1965) reported that 20% of mature Romney ewes showed silent heat after progesterone injections, when treatment was commenced in late February. With immature ewes, Ch'ang et al. (1968) observed 38% incidence of silent heat following treatment with progestagen sponges.

The effects of age on the response by the animal to hormonal synchronisation are generally confounded with the reproductive status of the animal. Thus immature or non-parous animals are more likely to be in anoestrous than adults, when both are treated at an identical time early in the breeding season. A recent report (Gibson and Robinson, 1971) has suggested the nutritional status of the ewe can influence the incidence of ovulation without oestrus. The injection of 500 i.u. P.M.S. 24 hours after the final progesterone injection will improve the oestrus - ovulation response in non-parous ewes (Robinson, 1961).

A constant daily ratio of donor to recipient ewes were treated with progestagen sponges. However, because of the differential response described above there were occasions when insufficient donor ewes were available. This problem might be overcome by the continued treatment of donor animals for at least 2 days after the final recipient has been treated.

The sponges used were air dried. Each contained 40 mg. of M.A.P. impregnated in 3 ml. of ethanol. Successful use of progestagen-impregnated intra-vaginal sponges depends upon the absorption of an effective dose. This in turn is influenced by the method of drying the sponge and the initial dose of steroid(Robinson, Quinlivan and Baxter, 1968); density of the sponge (Morgan et al., 1967) and the volume of ethanol used in impregnation (Robinson, 1971). Oestrus synchronisation might have been improved by increasing the volume of ethanol used in impregnation (to 5 ml. per sponge) and by oven drying sponges. It is now clear that these factors can influence crystal size and the consequent uptake by the ewe of the progestagen.

PRODUCTION OF OVA

The onset of the following oestrus is advanced when gonadotrophin is administered during the follicular phase of the oestrous cycle (Warwick and Casida, 1943; Robinson, 1951; Wallace, 1954; Gordon, 1958). A significant reduction in oestrous cycle length following gonadotrophin treatment on day 12 rather than day 13, was reported by Cumming (1965). However, these results (Table 6) and those of Wallace (1954) and Tervit (1967) have revealed that oestrous cycle length was not significantly affected by the day of P.M.S. injection. A relationship between P.M.S. dose and the extent of shortening of the cycle was suggested by Wallace, Lambourne and Sinclair (1954). The findings of Robinson (1951), Tervit (1967) and the present experiment showed no evidence of such a relationship.

Robinson (1951) and Wallace (1954) have reported P.M.S. dose-ovarian response relationships. Both workers observed that dose-response curves were extremely steep and the response in any ewe largely unpredictable.

Ovarian response (as measured by the number of corpora lutea and the number of corpora lutea plus large follicles) was greater with increase in dose of P.M.S. (Table 7). This is contrary to the findings of Cumming (1965) and Tervit (1967) who reported a lower response to 1200 i.u. compared to 1000 i.u. P.M.S. The P.M.S. was assumed to be of labelled potency, and that the methods of administration did not affect this potency.

The mean ovulation rates (ovulations per oestrus) and range of ovulations for the 120 ewes treated with either 1100, 1300 and 1500 i.u. P.M.S. were 2.10 (1-4), 3.26 (1-7) and 5.81 (1-19), respectively. This represents a marked increase in the number of multiple ovulations relative to the normal ovulation rate found in Romney ewes (Wallace, 1954; Averill, 1959, 1965; McDonald and Ch'ang, 1966; Allison, 1968). Variation in response between animals was considerable, especially in ewes receiving 1500 i.u. P.M.S. Both Wallace (1954) and Robinson (1951) observed an increase in the number of large unruptured luteinized follicles when high doses of P.M.S. were given. Similar observations were recorded in this study (Table 7). In an attempt to reduce the incidence of overstimulation treatments at lower levels of P.M.S. were included.

At the time of the present experiment, P.M.S. (material, dose and time of administration similar for donor ewes) given to 2½-year-old Romney and BLX ewes caused a greater superovulation response than that already recorded in donor ewes (unpublished data). The physical condition of the 2½-year-old animals was markedly superior to the donor ewes involved in this experiment. Yet Wallace (1954) reported that the plane of nutrition did not affect the ovarian response of sheep treated with P.M.S. Nevertheless, a recent report by Gibson and Robinson (1971) has suggested that nutrition is a factor involved in the seasonal variation in the sensitivity of the ewe to oestrogen. Consequently, the nutritional status of the ewe, in the period preceding and at the time of gonadotrophin treatment, might influence ovarian response to a greater degree than is currently recognised.

Wallace (1954) reported no difference in ovarian response in ewes injected with P.M.S. on days 11, 12, 13 or 14 of the oestrous cycle. The results of this study support those findings (Table 8).

The steep dose-response curve and unpredictability of response is a major limitation to the successful use of P.M.S. for controlled multiple ovulation induction. One of the difficulties with this hormone is its long half-life, at least in the species so far studied (Catchpole, Cole and Pearson, 1935; Lamond, 1960 b). A hormone with this characteristic might be expected to interfere with the neuro-endocrine mechanisms responsible for ovulation.

A further difficulty in the use of P.M.S. is the possibility that the ratio of F.S.H. - to L.H.-like action in this preparation is inappropriate for stimulation of normal growth and maturation of follicles in the ewe. It has been determined in both the mouse (Lamond, 1960 c) and the rabbit (Fox and Krinsky, 1968) that the ratio of F.S.H.- to L.H.-like activity in P.M.S. is about 5 : 1. Attempts to modify P.M.S. preparations to suit the ewe, rather than achieve an optimum ewe-environment to suit the unique characteristics of the hormone, would seem to be worthwhile.

Of a total of 578 ova shed (based on the number of corpora lutea) 388 were recovered. This represents a recovery rate of 67% which compares favourably with the 58% reported by Hunter <u>et al.</u> (1955); the 61% of Hancock and Hovell (1961) and 57% for Averill (1958). However, Cumming (1965) and Tervit (1967) reported ova recovery rates of 72% and 78%, respectively. All workers have used essentially the same technique.

Dose of P.M.S. had a significant effect on the percentage recovery of ova (Table 9). Little difference occurred between the recovery rates for 1100 i.u. (79%) or 1300 i.u. P.M.S. (72%) but the result for 1500 i.u. P.M.S. (56%) was depressed. This is contrary to the findings of Cumming (1965) and Tervit (1967) but in agreement with the results of Robinson (1951).

There can be no doubt that the poor ova recovery rates recorded in ewes given 1500 i.u. P.M.S. was due to the low numbers of ova obtained from animals showing evidence of overstimulation. In 4 ewes randomly chosen from those affected, the ova recovery rates were 25% (12 C.L.), 14% (7 C.L.), 28% (13 C.L.) and 40% (17 C.L.), respectively. It is possible that in such animals the mechanisms of ovum reception were faulty. The enlargement of the superovulated ovary, in many instances would have exceeded that of the infundibulae even though the latter become enlarged at ovulation. Furthermore, in some ewes the incidence of unruptured luteinized follicles was high. Where this condition occured the number of corpora lutea recorded may not have accurately reflected the number of ova shed. Consequently, the <u>individual</u> ovulation rate of the ewe, (i.e. the response to any particular dose of P.M.S.), is a factor of greater importance influencing ova recovery <u>than any direct effect</u> of P.M.S.

When the recovery of ova was calculated from the proportion of ova recovered of the total number of ova shed, by all ewes receiving each dose of P.M.S., significantly less ova were recovered after 1500 i.u. (63%) than either 1300 i.u. (84%) or 1100 i.u. P.M.S. (86%). However, only 10 ewes contributed to the result for 1100 i.u. P.M.S. and the maximum number of ovulations in any ewe was 4.

The reports of Hancock and Hovell (1961), Cumming (1965) and Tervit (1967) agree that the percentage of ova recovered is not significantly affected by any increase in the number of ova shed. In the present experiment the recovery of ova was not markedly influenced by the number of ova shed until the latter exceeded 6 to 7 corpora lutea or 7 to 8 corpora lutea plus large follicles per ewe, respectively (Figures 9 and 10). However, when the ovarian response exceeded this level a decline in recovery percentage occurred.

Laparotomies for recovery of ova were conducted 67 to 77 hours after ewes were first observed in oestrus. The objective was to recover as many ova as possible at the 8-cell stage. Cumming (1965) reported no decrease in recovery rate when laparotomy was conducted up to 96 hours after onset of oestrus. Averill (1958) found recovery to be most efficient when performed 50 to 70 hours after mating. However, like Tervit (1967) he observed a decline in the proportion of ova recovered when laparotomy was conducted 80 or more hours after the onset of oestrus. Sheep ova normally enter the uterus between 77 and 96 hours after the onset of oestrus (Clark, 1934). The recovery of ova from the uterus is likely to be less satisfactory than from the oviduct, since a larger area must be flushed. In the present experiment the time-interval between onset of oestrus and laparotomy had no marked effect on the percentage of ova recovered per ewe (Figure 11). This result could be expected since almost all ova should have been present in the oviduct when recovery was attempted.

Of the 388 ova recovered 55.6% were fertilised. In addition, 61% of ewes gave only cleaved ova: 24.5% yielded uncleaved ova only, while both classes of ova were found in 14.5% of ewes.

The percentage of recovered ova which were fertilised was significantly influenced by the dose of P.M.S. (Table 9). Lowest fertilisation rates (either on a per ewe or a proportion basis) were recorded in ewes which received 1500 i.u. P.M.S., or alternatively, in animals showing greatest ovarian stimulation.

Hancock and Hovell (1961) reported no significant correlation between the numbers of ova shed and the proportion of recovered ova fertilised. Nevertheless, the results of Robinson (1951), Averill (1958), Cumming (1965), Tervit (1967) and the present experiment indicate a reduction in fertilisation rate with increase in either dose of P.M.S. or the number of ovulations per ewe.

Ova recovered from ewes treated with P.M.S. are fully viable (Hunter et al., 1955; Averill and Rowson, 1958; Hancock and Hovell, 1961). Berry and Savery (1958) reported that the proportion of abnormal ova from ewes treated with P.M.S. did not exceed the proportion from untreated ewes.

The criterion used to ascertain fertilisation of ova in this experiment was apparent normal segmentation for the age of the ovum. Hancock (1962) pointed out that segmentation is not an absolute gauge of fertilisation since degenerative or spontaneous fragmentation superficially resembling cleavage of fertilised ova, may occur in unfertilised sheep ova. Care was taken in this experiment to avoid mistaken classification of fragmenting ova as fertilised ova.

There has been much speculation but little direct information reported in the literature on the causes of fertilisation failure in superovulated animals. The mating and ova recovery observations in this study were sufficiently critical to cast some light on the problem.

No single explanation can satisfactorily account for the fertilisation failure observed. In some ewes in which the response to gonadotrophin was excellent (i.e. absence of persistent follicles and no marked increase in ovarian size) fertilisation failure occurred. Alternatively, in ewes which did show over-stimulation, fewer ova were fertilised at recovery. Still further, in 16 ewes, some ova were fertilised while others remained uncleaved.

Cumming (1965) observed that in ewes from which ova of differing cleavage stages were recovered, the corpora lutea appeared to differ in age. A similar observation was made in the present experiment.

An additional objective of this investigation necessitated the hand-service of donor ewes to individual sires. On occasions as many as 10 ewes were required to be mated to one ram at a single mating. Attempts were made to have each ewe served twice daily until the end of oestrus. One of the 4 sires used, perhaps not unexpectedly showed a marked deterioration in fertility during the mating programme. This was evidenced by the recovery of a high proportion of unfertilised ova in flushings from ewes served by this ram. Gordon (1970) has reported that some rams, when faced with a large number of ewes on heat at one time exhausted their sperm reserves long before they exhausted their mating capabilities. Consequently, fertilisation failure in some ewes, in particular those showing a normal ovarian response, might be directly attributable to inferior ram fertility.

Hand-mating of sheep is a procedure demanding considerable handling of the animals involved. There is some evidence (Lang, 1964) which suggests conditions of "stress" may interfere with normal reproductive function in the ewe. It follows that animals unused to prolonged handling might be subjected to some form of "stress" with possible resultant reproductive malfunction. This could also be a contributing factor to the impaired fertilisation observed in donor ewes in this experiment.

Robinson (1951) found in sheep given high doses of P.M.S. (1000 to 2000 i.u.) a large number of ova were released, accelerated tubal transport occurred and low fertilisation rates were obtained. Averill (1958) observed similar results but only when more than 15 ova were shed per ewe. However, in the present experiment there was no evidence of rapid tubal transport since ova recovery rates did not decline until ova were expected in the uterus. Similar findings were reported by Cumming (1965) and Tervit (1967).

Numerous workers (Robinson, 1951; Tervit, 1967; Cumming and McDonald, 1967; Holst, 1969) have reported fertilisation defects in P.M.S. treated ewes, and some have implicated inferior spermatozoa transport as a likely cause. A common observation, (also noted in this study) has been the absence of spermatozoa attached to the zona pellucida of uncleaved ova. This suggests insufficient spermatozoa reach the site of fertilisation.

In the ewe, the establishment of a large population of spermatozoa in the cervix is necessary to ensure continuity of migration of spermatozoa to the Fallopian tubes and good fertility (Mattner, 1963). The production, and possibly the viscosity of cervical mucus can influence the development of the cervical population of spermatozoa and the subsequent migration of spermatozoa to the Fallopian tubes (Gibbons and Mattner, 1966; Mattner and Braden, 1969). Smith and Allison (1971) have observed differences in the characteristics of cervical mucus secretion in progestagen treated as compared with normal cyclic ewes. It follows that the endocrine or physiological environment at the time of oestrus and ovulation in the P.M.S.-treated ewe might be unsatisfactory for the normal transport, capacitation and survival of spermatozoa.

Cumming et al. (1970) have shown that in progestagen treated ewes, the release of Luteinizing Hormone occurred early (relative to non-treated ewes) in relation to the onset of oestrus. In the ewe, ovulation occurs at or soon after the end of oestrus (Robinson, 1959), and the fertile life of the ovum lasts for 12 to 15 hours (Mauzier and Wintenberger, 1952). It seems reasonable to suggest that faulty timing of ovulation might also contribute to the low fertilisation rate in P.M.S.-treated ewes. This may be reflected either in a decrease in fertilisability of the ageing ovum or insufficient spermatozoa being available at the site of fertilisation.

In practice, an ejaculate of sperm adequate for ensuring fertilisation in the normal ewe might be quite inadequate for the P.M.S.-treated sheep. To overcome this problem, each ewe could be hand-mated to several vigorous, fertile rams twice daily until the end of oestrus. This procedure would ensure a supply of spermatozoa to the genital tract of the ewe, probably sufficient to counteract any aberrations in gamete transport.

CLEAVAGE OF FERTILISED OVA

Ova were recovered between 67 and 77 hours after ewes were first observed in oestrus. Because of the limited period during which sheep were examined, these results do not provide an accurate estimate of the times when 2-, 4-, 6- and 8- to 12-cell ova are normally found in the ewe.

In the sheep the time of ovulation shows considerable variation (Hancock, 1962) but occurs at or soon after the end of oestrus (Robinson, 1959). No information was available on the time of ovulation in the present experiment. However, the results for the shortest and longest interval from the onset of oestrus to the recovery of each cleavage stage, (Table 10) indicate that both the time of ovulation and fertilisation in the Romney ewe may show considerable variation.

The mean interval after the onset of oestrus to the recovery of 2-cell ova was 68 hours. This is later than the 47.8 and 58.3 hours reported by Chang and Rowson (1965), and Tervit (1967) respectively, but earlier than the 79 hours reported by Cumming (1965). However, detection of oestrus may vary between observers.

Numerous workers have reported rapid segmentation of sheep ova from the first to the third or fourth division (Green and Winters, 1945; Chang and Rowson, 1965; Cumming, 1965; Tervit, 1967). These findings were confirmed, and the results also indicated that each cleavage stage can be recovered over a wide time range.

The dose of P.M.S. appeared to influence the mean interval from onset of oestrus to recovery for 4-, 6- and 8- to 12-cell ova but not for 2- or 8-cell ova (Table 12). However, except for 8-cell ova the number of ewes contributing to these observations was very low, and the variance high. In the case of 4-, 6- and 8- to 12-cell ova the effect of P.M.S. could be due to the small number of ewes contributing to each result.

Variation in cell stages of fertilised ova within ewes with multiple numbers of ovulations, was found in 10 ewes. However, in each case the cleavage stages of the ova were very similar. This observation is in agreement with the finding of Averill (1958) that the cell stages of cleaving eggs in individual ewes seldom exceed one-and-a-half complete cleavages.

SEQUENTIAL TREATMENT OF EWES WITH P.M.S.

The need to obtain additional numbers of ova necessitated the administration of P.M.S. to some ewes on more than one occasion.

There is little information on the response of domestic animals to repeated injections of gonadotrophin. Dziuk et al. (1958) observed in cattle that P.M.S. induced an antibody reaction thereby reducing the stimulatory response of follicles to subsequent P.M.S. administration. A reduction in the ovulation response of ewes given repeated injections of P.M.S. was reported by Hulet and Foote (1969). The latter workers observed that ewes exposed to repeated injections of P.M.S. developed a refractory condition believed to be associated with an immune-like reaction to a protein hormone (Greenwald, 1963).

The results of the present experiment are not unequivocal in that the dose of P.M.S. was not constant between treatments for each animal. In addition, the interval between injections for each ewe varied from 16 to 48 days. Nevertheless, the results did show clearly that ovarian response per ewe declined with successive treatments of P.M.S. (Table 13). This confirms the findings of Hulet and

Foote (1969). The latter workers also observed an increase in the failure of ewes to ovulate following sequential injections of P.M.S. Similarly, in the present study, of the 7 ewes given three injections 3 failed to ovulate. An increase in the incidence of ovarian abnormalities (adherent fimbriae, grossly enlarged ovaries, persistent follicles) was also observed as the number of treatments was increased.

Welch (1969) stated that with efficient superovulation techniques, one donor ewe if subjected to 3 laporatomies for egg recovery in 6 cycles in one season, should be able to leave 25 lambs per season. In the light of the results of this experiment and other work, it seems unlikely that a P.M.S. treated flock of Romney ewes could <u>maintain</u> an ova production rate of this order.

OVULATION STUDIES

Ovulation rate was measured by counting the number of corpora lutea in each ewe at laparotomy. The number of corpora lutea was assumed to represent the number of ova shed.

BLX ewes shed a significantly greater number of ova than Romney ewes (Table 14). This result is in agreement with previous comparisons of ovulation rate involving the same two breeds. The superiority of 0.24 ovulations per ewe for BLX sheep in this study compares favourably with similar differences of 0.06 and 0.31 ovulations per ewe reported by Lang and Hight (1967) in hoggets, and Allison (1968) in mature ewes, respectively. The ovulation rate of 1.10 for Romney ewes is similar to the values of 1.00, 1.22 (Inkster, 1953, 1959) and 1.10 (Edgar, 1962) reported in ewes of the same age and breed.

Liveweight of the ewe at mating is known to influence the number of ova shed (Wallace, 1961; Allison, 1968). BLX ewes in this experiment were heavier than their Romney counterparts. However, the difference in ovulation rate observed, can be explained as a genetic difference due to breed rather than an effect of liveweight *per se*.

No relationship was observed between ovulation rate and liveweight within either breed. This result is perhaps not unexpected in view of the small number of animals involved, the nature of the variables being measured, and the type of analysis used. Cockrem (1965) pointed out that the ideal analysis which can relate discrete variables to continuous variables has yet to be arrived at, if it is indeed possible.

Ovulation rate is known to increase with advancement of the breeding season or with each successive oestrus (McDonald and Ch'ang 1966; Allison, 1968). In the present study observations on the number of ova shed were obtained over three months of the breeding season. In analysis of these data the variation due to stage of the breeding season was reduced by relating the information to a period of one month. To achieve this, in some animals corpora lutea relating to the oestrous cycle previous to that observed at laparotomy were considered. Furthermore, the proportion of animals of the two breeds examined throughout the experimental period was approximately constant.

Variation in the plane of nutrition was assumed to have a negligible effect on the present results as the liveweight of the animals remained similar during the breeding season. Indeed, the district was experiencing severe drought conditions during most of the experimental period and this probably influenced the incidence of multiple ovulation in ewes of both breeds. It cannot be discounted that the severe environmental conditions prevailing, were perhaps tolerated less readily by one breed than the other. However, this could not be determined.

The animals used in the present experiment were randomly bred, themselves unselected for any productive traits, and had been run together since birth. Some workers investigating ovulation rate in sheep (Averill, 1959, 1965; Edey, 1968) have obtained animals from many widely differing environments and observed them at a common location. McDonald and Ch'ang (1966) found that ewes of similar genetic origin although bred at two farms for 5 years, when brought into a common environment showed marked differences in ovulatory activity several months later. This result highlights the need to allow for extraneous sources of variation in studies of reproduction in sheep.

Mean ovulation rates for single and twin-born BLX ewes were 1.32 and 1.41, respectively (Table 15). Although non-significant these results are in agreement with the findings of earlier workers (Packham and Triffit, 1966; McDonald and Ch'ang, 1966) that twin-born ewes shed more ova than single-born ewes. This effect was not apparent within Romney sheep but only 9 animals of this group were born as twins, as opposed to 79 born as singles. Conversely, of the 70 BLX ewes, 24 were born as twins. The failure to observe an effect of birth rank on ovulation rate in Romney ewes was probably due to the low number of twin-born ewes in the sample.

Studies in New Zealand Romney (Cockrem, Barton and Rae, 1956; Inkster, 1956) and Corriedale (Coop, 1956 a, 1956 b) sheep have indicated that ewes showing extensive wool growth on the face had a lower fertility as measured by the number of lambs born, than open-faced ewes. The lower number of lambs was the result of a greater number of barren ewes and a smaller number of twins born. The lower incidence of twinning might be due to fewer ova being shed by woolly-faced ewes.

Cumming (1965) found no relationship between the number of corpora lutea and face cover in Romney ewes and the results of the present experiment support this finding. The number of animals involved however, was probably inadequate to satisfactorily test such a relationship.

SURVIVAL OF TRANSFERRED OVA

1. Conception Rate

Of the 80 ewes receiving one egg, 53 (66%) became pregnant while 54 (90%) of 60 ewes transferred with three ova subsequently lambed. This difference in conception rate was significant, and contrary to the findings of other workers (Moore *et al.*, 1960; Moore, 1968; Cumming and McDonald 1970) who have reported that an increase in the number of ova transferred did not markedly influence the proportion of ewes which became pregnant. Indeed, Moore *et al.* (1960) suggested that the success or failure of any ovum transfer was dependant on the inherent ability of the ewe to support a pregnancy, rather than the number of ova transferred. It might perhaps be argued that the chances of a successful pregnancy occurring are greater when the number of ova transferred is increased, since the presence of one or more defective gametes need not necessarily mean conception failure. Alternatively, the possibility of exact synchronisation between transferred ova and the recipient genital tract might be greater in multiple as opposed to single ova transplants. When 3 ova were transferred, the eggs were frequently obtained from 2, and sometimes 3, individual donor ewes. The chance of at least one ovum being in phase with the recipient tract and hence a successful pregnancy occurring, might increase as the number of ova transferred per ewe is raised.

Differences in ova viability due to variation in the length of ova storage might also be implicated. However, the storage time for ova in this experiment did not exceed 90 minutes and the results of Averill (1956) indicate that ova viability is unlikely to be impaired during a storage period of this order.

Generally accepted optimum conditions for egg transfer in sheep include precise synchronisation (± 12 hours) of oestrus in donor and recipient (Moore and Shelton, 1964 b; Shelton and Moore, 1966), and the transfer of 8-cell ova slightly advanced in "age" of the recipient genital tract (Hancock and Hovell, 1961) to the uterus of the recipient ewe. These conditions were closely adhered to in the present study. However, marked between ewe variation can occur in the time of ovulation and fertilisation in relation to the onset of oestrus in the ewe (Hancock, 1962). Consequently, even though the onset of oestrus in donor and recipient animals may be closely synchronised, the luteal stage of the recipient genital tract and transferred ova may differ widely. This might explain why some ewes did not become pregnant to transfer even though conditions were considered to be optimum.

Neither the breed of recipient nor the breed of egg transferred affected the proportion of ewes becoming pregnant (Table 18). This confirms earlier reports (Hunter et al., 1955; Moore et al., 1960; Dickinson et al., 1962; Wiener and Slee, 1965; Moore, 1968) that fertilised ova can be successfully transferred between different sheep breeds.

Where one egg was transferred, a greater proportion of Romney as opposed to BLX ewes became pregnant. However, the converse was true when three eggs were transferred. This gave rise to a significant interaction between breed of recipient and the number of ova transferred.

One objective of this experiment was to investigate any differences in ova viability attributable to the ram. Where multiple transplants were performed all ova had to have a common sire. In some instances this limited the number of multiple transplants performed, which might otherwise have been possible. There was no difference between sires in the proportion of ewes becoming pregnant, but the numbers of ova in each subclass were probably inadequate to satisfactorily test this effect.

Altogether, 7[illegible].6% of transferred ewes became pregnant which is close to the 79.5% reported by Moore (1968) in a similar experiment. These figures compare favourably with the number of ewes reported to conceive to a natural mating at a single oestrous period (Quinlivan et al., 1966). It appears that 20 to 30% of ewes are incapable of conceiving at a single oestrous period. In this experiment 5 ewes given three opportunities to become pregnant (two transfer attempts and a natural mating) failed to lamb. No obvious genital tract abnormalities were observed at laparotomy yet it is possible these animals were permanently infertile. However, a further 10 ewes, which failed to become pregnant to an initial transfer, subsequently conceived to a second transfer or natural mating. This result indicates that the inability of some ewes to conceive at a single oestrus is only transient. Eight animals did not show oestrus after transfer or mating, but failed to lamb. The onset of the anoestrous season may have occurred however, before these animals had an opportunity to return to oestrus.

2. Ovum Survival

Neither breed of recipient nor breed of egg had a significant effect on ovum survival, either in ewes receiving one or three eggs, or in all ewes. However, a greater proportion of ova survived in Romney compared to BLX ewes given a single egg (Table 19). A converse relationship was observed when three eggs were transferred. An increase in the number of ova transferred resulted in a lowering of the ovum survival rate (Table 21). A similar observation has been reported by other workers (Moore *et al.*, 1960; Cumming and McDonald, 1970; Moore, 1968). The survival rates of ova transferred at one or three per ewe in the present experiment were [illegible]6.2 and 59.4%, respectively; similar to the results reported by Moore (1968) of 75% and 55% following the transfer of the same numbers of ova per ewe.

Brambell (1948) suggested that pre-natal loss of ova would be distributed at random between ewes with one, two or three ovulations. The results of Moore *et al.* (1960), Cumming and McDonald (1970), Moore (1968) and the present experiment showed that the proportion of ova suffering pre-natal mortality increased as the number of ova transferred increased.

Lino and Braden (1968) reported a difference between two sires in the proportion of corpora lutea represented by embryos 1 to 2 months <u>post coitum</u>. In the present study there was no evidence of a relationship between sire and the proportion of transferred ova represented by lambs born. An effect of the ram on mean litter size has been reported in superovulated ewes (Newton and Betts, 1968) and in normally mated ewes (Parker and Bell, 1966). In animals each receiving three eggs, the mean litter size (lambs born per ewe) of ewes in the present study mated to the four rams were 1.66, 1.72, 1.85 and 1.92, respectively. These differences were not significant. In the experiments reported by Parker and Bell (1966) and Newton and Betts (1968) no measurement of ovulation rate was made. Consequently, the relationship between ewe fecundity and the ram reported by these workers might be simply due to a difference in ovulation rate in the ewes to which the respective sires were mated.

UTERINE CAPACITY AFTER EGG TRANSFER

Since Two-Tooth ewes do not normally produce a large number of twins, the transfer of 3 ova per ewe was considered an adequate "load" of the reproductive tract to test uterine capacity in these animals. The parameter was measured in terms of litter size and weight of lamb born per ewe.

Although BLX ewes had less single and more twin pregnancies than Romney ewes neither maternal nor embryo genotype significantly affected the distribution of litter size (Table 22). This result is in contrast to that of Moore (1968) who found in ewes receiving three eggs, Border Leicester ewes had more triplet and less single pregnancies than Romney ewes. However, the latter study involved the comparison of two breeds which differed widely in body weight, body size, wool characteristics and fecundity.

When weight of lamb born per ewe was considered, BLX ewes were again superior to Romney ewes in terms of uterine capacity but the difference failed to reach significance (Table 24).

Robinson (1951) following studies in P.M.S. treated sheep suggested that breeds of low fertility may have a uterine environment that after P.M.S. treatment would reduce the number of embryos to a lower level than in more highly fertile breeds. The results of Wallace (1954), Palsson (1956) and Gordon (1958) do not support this contention. In these experiments Romney, Ryeland, Southdown and Cheviot sheep (representative of low fertility breeds) produced multiple-births as readily as more highly fertile breeds such as the Clun and Suffolk. There was no evidence from these studies that uterine capacity, provided sufficient eggs were shed, was affected by breed. However, the results of Moore (1968) and the present experiment suggest that the effect of breed is by no means clear cut. It must also be remembered that the animals compared in the present study were of similar genetic origin, a factor not in common with earlier studies. Experiments involving larger numbers of animals are needed to clarify the situation.

In the present experiment a maximum of two ova were placed in any uterine horn. Ewes with a single corpus luteum received two ova to the uterine horn adjacent to the ovary containing the corpus luteum. Rowson, Lawson and Moor (1971) recently reported that a much higher percentage of viable twins were obtained in heifers that conceived following the transfer of a single ovum to each uterine horn than if two ova were placed in a single horn. The authors explained the difference in twinning rate as a failure of one of the two eggs transferred to a single uterine horn, to migrate to the contralateral horn, with consequent overcrowding and sometimes embryonic death. The number of cotyledons to which conceptuses were attached was usually much greater when the pregnancies were bilateral. Ewe 110 in the present investigation produced at parturition one live lamb plus 2 mummified foetuses, while a further ewe (No. 101) gave birth to a live lamb between 2 near full-term lambs (Figures 18 and 19). Obviously, it was not possible to ascertain in what uterine horn these lambs and foetuses developed yet it seems likely that pregnancy failure occurred in one horn only, in both ewes. The cause of foetal death remains unknown. However, overcrowding can be implicated as a likely cause in view of the findings of Rowson et al. (1971) and that the surviving lamb from both ewes was a normal healthy individual.

In recent unpublished work at Cambridge University, Rowson and Lawson found after transferring 5 eggs each into mature Romney Marsh ewes, (as well as Suffolk and Finn ewes) that at litter sizes of three or less, performance of the Romney Marsh ewe did not seem to be hampered by any limitation imposed by the uterus.

In relation to the present work which applies to Two-Tooth ewes, at litter sizes which might be acceptable in practice (2 to 3) capacity of the uterus did not seem to be a factor limiting reproductive performance in either breed studied (Table 25). In both breed groups, litter size after transfer of three eggs was significantly greater than the potential litter size determined by natural ovulation rate. However, the difference between natural ovulation rate and mean litter size was of greater magnitude in the Romney as opposed to the BLX breed.

While there were indications from this study that breed differences in uterine capacity might exist, the results clearly show potential reproductive performance to be limited by the number of ova shed in ewes of both breeds. In the case of a breed with a low ovulation rate such as the Romney this result has important practical implications. Factors of sheep management which increase the numbers of ova shed at mating, or methods of selection which identify those animals with a propensity for high ovulation rate are worthy of close consideration in Romney sheep.

PRE-NATAL LAMB GROWTH

Studies in sheep (Terrill, 1944; Forbes, 1967), cattle (Salisbury and Van Demark, 1961) and other mammalian species (McLaren and Michie, 1963) have demonstrated an inverse relationship between litter size and gestation length. Single progeny are carried longer *in utero* than twins, and twins longer than triplets. In the present experiment the gestation length of single, twin and triplet-born lambs were very similar (Table 26).

Undernutrition of the ewe during late pregnancy is known to influence gestation length (Thomson and Thomson, 1949; Alexander, 1956). However, this factor can be excluded as a possible explanation for the failure to observe a relationship between litter size and length of gestation in this study. All ewes were run together prior to lambing and liveweight measurements indicated that the animals were adequately fed.

Gestation length is a parameter which shows little variation. Consequently, to satisfactorily measure this variation large numbers of animals must be sampled. In the present study the numbers of twin and triplet-bearing ewes contributing to the measurements of gestation length were 28 and 13, respectively. Such numbers were probably inadequate to obtain a reliable estimate of the variation in gestation length.

An inverse relationship between litter size and lamb birth weight was observed. Single lambs were heavier than twins, and twins heavier than triplets. This relationship has been widely documented in multiparous species and several theories have been advanced to explain the effect. There is considerable evidence that competition between foetus(es) and the maternal tissues is a probable mechanism of the maternal influence on the size of young at birth (Hammond, 1944; Wallace, 1948; Hunter, 1956). However, McLaren and Michie (1960) have argued that the "limited nutrient pool" theory cannot fully account for the effects of litter size on foetal growth. Eckstein et al. (1955) found that in a species such as the guinea pig which has a bicornuate uterus, the number of embryos in the same uterine horn was more important than the number in the opposite horn in determining foetal and placental weight. On this basis McLaren and Michie (1960) postulated foetal growth was largely controlled by the size attained by the placenta in early pregnancy, which in turn was influenced by the supply of blood and dissolved nutrients, available for placental growth.

Differences in gestation length due to sex of the lamb were small and non-significant (Tables 27 and 28). This result is in agreement with the findings of other workers (Terrill, 1944; Forbes, 1967). However, Dickinson et al. (1962) reported a difference in gestation length due to lamb sex. Males were carried one day longer than females. This is the case in many mammalian species but has generally not been found in sheep (Terrill and Hazel, 1947). In view of the small number of animals involved in the study of Dickinson et al. (1962) and the nature of the variables measured, the result should be accepted with caution. In the present experiment sex effects on gestation length were analysed in single-born lambs and in all lambs. The results were similar in both cases.

Generally, male lambs were heavier at birth than female lambs and again probably in view of the small number of animals involved none of the differences reached significance (Tables 27, 29 and 30). Other workers have reported similar findings (Hunter, 1956; Doney, 1957; Dickinson et al., 1962; Terrill, 1962).

In the analysis of data on the influence of maternal and lamb genotype on gestation length and lamb birth weight a sex classification was not included in the model. Earlier analyses (Table 28 and 29) had shown that sex was not an important source of variation in either gestation length or birth weight. Consequently, any variation in these parameters due to sex is included in the error term of analyses of variance. Again analyses were performed for both single-born lambs only, and for all lambs. The discussion will be confined initially to single-born lambs since these data remain free from variation due to litter size.

Lambs were carried almost one day longer in the Romney than the BLX maternal environment (Table 31). Conversely, the effect of lamb genotype was very small. A strong interaction was apparent between the two main effects. This occurred since Romney lambs were carried almost 2 days longer in Romney ewes than in BLX ewes, while BLX lambs had very similar lengths of gestation irrespective of the maternal environment in which they developed.

Previous studies (Hunter, 1956; Dickinson et al., 1962; Moore, 1968) have indicated that the gestation length of larger breeds was extended by the presence in utero of foetuses of smaller breeds. In addition, Moore (1968) found gestation length was decreased when the small breed carried the larger breed. In the present experiment an effect of shortening of gestation length was observed when BLX ewes carried Romney lambs. If BLX animals are considered to be genetically larger than Romney sheep then this result does not support the findings of earlier studies. However, when Romney ewes carried BLX lambs gestation length was again decreased, which is in agreement with the results of Moore (1968). It must be pointed out that the experiments of Hunter (1956), Dickinson et al. (1962) and Moore (1968) all involved comparisons of sheep breeds which differed widely in parental size, and other characteristics. However, the animals involved in the present study were of similar genetic origin. It is true that BLX ewes were on average 6 kg. heavier than Romney ewes. Nevertheless, it is also true that the size difference between these two breeds was considerably less than the differences in breed size employed in earlier experiments referred to above. The magnitude of the maternal and genetic effects and their interaction on gestation length will largely depend on the specific breed combinations chosen.

The results for all lambs (Table 32) were similar to those obtained for single-born lambs, with one exception. A significant effect of lamb genotype on gestation length became apparent. This is probably due to the inclusion of a greater number of observations since the difference in mean gestation length between Romney and BLX lambs remained similar in both analyses. Litter size differences cannot be implicated since earlier analyses (Table 26) had shown the gestation length of single, twin and triplet-born lambs to be similar.

Lambs developing in a BLX maternal environment were 8% heavier at birth than lambs developing in a Romney maternal environment (Table 31). However, the effect of lamb genotype was very small and non-significant.

In this study the maternal environment provided by the ewe was the most important single factor influencing lamb birth weight. This result is in contrast to the findings of other workers (Hunter, 1956; Dickinson <u>et al.</u>, 1962) who found lamb genotype to be the most important factor influencing the size of lambs at birth. Again however, the relative importance of the maternal and genetic contributions and their interactions will depend to a considerable extent on the specific breed combinations chosen.

A factor of important practical significance is the demonstration that the maternal environment provided by the Romney ewe <u>restricted</u> potential foetal growth of Romney lambs (Table 31). To explain further, the birth weight of Romney lambs given greater environmental latitude than normal (i.e. developing in a BLX ewe) <u>increased</u> by 0.37 kg. relative to the birth weights of Romney lambs born to Romney ewes. Indeed, the birth weight of Romney lambs born to BLX ewes was only slightly less (0.03 kg.) than BLX lambs born to the same breed of dam. This difference of 0.03 kg. in birth weight can be considered a genetic difference due to lamb genotype. Conversely, BLX lambs when placed in a Romney maternal environment were prevented from exhibiting their full growth potential. The birth weight of BLX lambs born to Romney ewes was 0.37 kg. <u>less</u> than BLX lambs born to ewes of the same breed. However, the growth performance of BLX lambs even in the restrictive Romney maternal environment was still slightly better (0.03 kg.) than Romney lambs developing in the same environment. Again this difference of 0.03 kg. can be considered to reflect genetic differences between the two lamb genotypes in pre-natal growth.

Thus the maternal environment seems capable of limiting the size of a genetically larger lamb but may increase the size at birth of a genetically smaller lamb. The results of this experiment have demonstrated the restrictive nature of the pre-natal maternal environment offered by the Two-Tooth Romney ewe, relative to that offered by the F1 crossbred ewe of the same age.

The results for all lambs (Table 32) in terms of birth weight showed a marked disparity to those obtained for single-born lambs. However, the effects of lamb genotype and maternal environment cannot be accurately assessed from the data for all lambs since a previous analysis (Table 26) showed a significant relationship between litter size and birth weight.

Beatty (1956) suggested the difference in birth weight between genetically large and small offspring was greater when both types developed in the same litter than it was when they developed in different litters. Consequently, the performance of a male foetus in litters of more than one may be dependent in some degree on whether or not a female foetus is present.

The differences between the classes of twin-born lambs in the present experiment were small relative to the variability of birth weight and so failed to reach significance (Table 34). Sex differences in birth weight are probably revealed most accurately by the mixed-sex twins (males twin to females - females twin to males) since this comparison does not involve two completely distinct sets of dams. The results showed that males were about 0.20 kg. heavier than females in mixed sex litters. Donald and Purser (1956) reported a similar result. The largest differences occurred between males and females of mixed-sex twin pairs and these differences were considerably greater than those between males and females from like-sexed pairs.

Overall the results indicate that the largest and smallest classes of twin occurred in the same litter. The birth weight superiority of males over females was greater in mixed-sex than like-sexed litters. Thus the pre-natal growth of the lamb is influenced not only by its sex but perhaps also by the sex of its co-twin. It must be remembered however, that the sample size of these data was small and other factors such as lamb genotype may be involved. Nevertheless, the possibility exists that males in mixed-sex litters are able to turn to their own advantage the smaller demands of their female co-twins without depressing the growth of the latter at all.

POST-NATAL LAMB GROWTH

Male lambs (non-castrated) were heavier at weaning and gained weight faster than female lambs (Table 35). The difference between ram and ewe lambs in weaning weight was similar to that reported in New Zealand Romney sheep (Ch'ang and Rae, 1961), but smaller than that found in other studies (Hazel and Terrill, 1945, 1946). Since sex was shown to be an important source of variation in weaning weight a sex classification was included in the model used to estimate maternal and lamb genotype effects (see Chapter II). This model was not suitable for an accurate significance test of sex effects and these are best shown by the analyses in Table 35.

Results of the effects of maternal and lamb genotype on weaning weight and weight gain were similar for both single-born and reared lambs, and for all lambs. Consequently, the discussion will be confined to single-born and reared lambs since these data remain relatively free of complicating environmental factors.

Irrespective of their genotype, lambs reared by BLX ewes were heavier at weaning and had a greater daily weight gain than lambs reared by Romney ewes (Table 37). Furthermore, BLX lambs were superior to Romney lambs both in weaning weight and rate of growth to weaning, irrespective of the breed of dam. These results clearly show that the maternal environment provided by BLX ewes was markedly superior to that offered by Romney ewes. In addition the results demonstrated a breed difference in post-natal lamb growth. Given a common maternal environment the performance of BLX lambs always exceeded that of Romney lambs.

The weaning weight of BLX lambs reared by Romney ewes was slightly inferior to Romney lambs reared in a BLX maternal environment. However, the results for weight gain per day showed a converse relationship (Table 37). This apparent paradox occurred because of the higher mean birth weight of Romney lambs born to BLX ewes, relative to BLX lambs born to Romney ewes (Table 31).

The most important single factor shown to influence <u>pre-natal</u> lamb growth was the maternal genotype of the ewe. The effect of lamb genotype was small. At weaning however, while the important influence of maternal environment on lamb growth was maintained the effect of <u>lamb</u> genotype was much stronger than at birth.

Considerable variation in performance was observed between animals within each breed. A Romney ewe (No. 181) with triplet Romney lambs, weaned a total weight of 57.1 kg. of lamb, 2.2 kg. more than the ewes own liveweight at weaning. This represented a combined lamb growth rate of 0.52 kg. per day. While the performance of this animal was outstanding, there were other examples of poor mothering ability, especially amongst Romney ewes.

The growth rate of lambs in this experiment were comparable to those reported in other New Zealand studies (Kirton, 1970). BLR lambs were superior to Romney lambs in weaning weight to the extent of 2.0 kg. per lamb; lower than the advantage of 2.2 to 4.4 kg. reported by Coop and Clark (1965) for lambs of the same breed. However, in the latter study the separate effects of lamb and maternal genotype were not partitioned.

Barnicoat et al. (1949) suggested that the greater weight of single lambs at weaning could be explained partly by their higher birth weight and partly by their higher milk intake. Genetic differences in milk quality are relatively unimportant as factors influencing lamb growth rate (Barnicoat et al., 1956; Moore, 1966 b). Several studies have shown differences between breeds and strains of ewes in milk production (Coombe et al., 1960; Munro, 1962). These workers however, did not distinguish between the potential of the ewe to produce milk and the potential of the lamb to obtain it. Moore (1966 a) described an experiment in which these two factors were not confounded. The results showed that for single lambs in the first 6 to 7 weeks of lactation, the lambs ability to obtain milk was more important in relation to milk yield than the ewes ability to produce it.

In relation to the present experiment, the maternal environmental effect on post-natal lamb growth can reasonably be explained as being due to differential levels of milk production by the two breeds of dam. Coop and Drew (1963) reported that $2\frac{1}{2}$-year-old BLX (F1) ewes produced more milk than Romney ewes of the same age, when suckling either twin or single lambs.

The observed effect of lamb genotype implied genetic differences between the two breeds in growth ability; this being reflected in the higher birth and weaning weights of BLX compared to Romney lambs. Coop (1957), Donald <u>et al</u>. (1968) and Carter (1968) found that lambs sired by Border Leicester rams had higher liveweights near weaning than those sired by smaller breeds. However, the possibility exists of the presence of a further genetic component, this being reflected in the superior ability of BLX lambs to <u>obtain</u> milk produced by the dam. The influence of this component, could be expected to decline as the lamb became less dependant on the milk supply of the ewe.

S U M M A R Y

S U M M A R Y

The objective of this investigation was to examine physiological factors which might explain observed differences in reproductive performance between Romney and Border Leicester x Romney sheep.

A marked difference was found in the numbers of ova shed between breeds. This difference in ovulation rate is probably the most important single factor contributing to the greater number of lambs born to BLX ewes. The effect of ovulation rate is likely to be manifested by a higher proportion of ewes with multiple births. A tendency was also observed amongst BLX sheep for ewes born as twins to shed more ova than ewes born as singles. This result would indicate that identification of ewes with a propensity for high ovulation rate could lead to gains in reproductive performance in future generations as well as in the current flock.

There was a suggestion of a breed difference in the ability of ewes to support multiple numbers of embryos to term, but this effect was not clear. Results did show that potential reproductive performance was limited by the number of ova shed, in both breeds. In the Romney breed, which generally has a low ovulation rate the result has serious practical implications. Management procedures or selection methods which lead to increased numbers of ova shed, are worthy of close consideration in Romney sheep.

BLX lambs showed superior pre-natal growth to Romney lambs. The maternal environment of the ewe was the most important factor examined influencing lamb birth weight. Lamb genotype was relatively unimportant. The maternal environment of Romney ewes

appeared to restrict potential pre-natal lamb growth.

Lamb growth from birth to weaning was greater in BLX compared to Romney lambs. Both the maternal environment of the ewe and the genotype of the lamb influenced differences in lamb weaning weight. It is likely the milk production of BLX ewes was superior to Romney ewes. BLX lambs appeared better able to obtain and utilise milk produced by the dam, than Romney lambs.

Maternal and lamb genotype effects on pre- and post-natal growth are likely to influence the number of lambs weaned per ewe, and the number of lambs surviving to weaning, both important components of reproductive rate.

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A P P E N D I C I E S

Appendix I

ESTIMATION OF VARIANCE COMPONENTS IN A TWO-LEVEL NESTED ANALYSIS OF VARIANCE WITH UNEQUAL SAMPLE SIZES

Effect of dose of P.M.S. and day of injection on oestrous cycle length

ANALYSIS OF VARIANCE

Source of Variation	d.f.	Mean Squares	Expected Mean Squares
Between doses	2	1.20	$s^2 + n_o' s^2_{BCA} + (nb)_o s^2_A$
Between days within doses	4	1.31	$s^2 + n_o s^2_{BCA}$
Error	113	0.87	s^2

1. Computation of the coefficients of the variance components

$$n_o' = \frac{55.02 - \frac{3246}{120}}{\text{d.f. btwn doses}} = \frac{27.97}{2} = 13.98$$

$$n_o = \frac{120 - 55.02}{\text{d.f. btwn days within doses}} = \frac{64.98}{4} = 16.24$$

$$(nb)_o = \frac{120 - 61.66}{\text{d.f. btwn doses}} = \frac{58.34}{2} = 29.17$$

2. Solving for estimates of variance components

M.S. (btwn doses) $= 1.20 = s^2 + 13.98\ s^2_{BCA} + 29.17\ s^2_A$

M.S. (btwn days within doses) $= 1.31 = s^2 + 16.24\ s^2_{BCA}$

M.S. (error) $= 0.87 = s^2$

Therefore

$s^2 = 0.87$

$s^2_{BCA} = 0.027$

$s^2_A = 0.020$

Appendix I cont'd

3. <u>Relative magnitude of the variance components</u>

Total variation $= s^2 + s^2_{BCA} + s^2_A$

$= 0.87 + 0.027 + 0.020$

$= 0.917$

(a) <u>Percent of variation between doses</u>

$$= \frac{0.020}{0.917} \times \frac{100}{1} = 2.1\%$$

(b) <u>Percent of variation between days within dose</u>

$$= \frac{0.027}{0.917} \times \frac{100}{1} = 2.9\%$$

(c) <u>Percent of variation - error variance</u>

$$= \frac{0.87}{0.917} \times \frac{100}{1} = 95.0\%$$

Appendix II

TABLE 1

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE OF OVARIAN RESPONSE DATA

a) Number of corpora lutea

Dose of P.M.S. (i.u.)		d.f. (n-1)	Variance s^2	Coded s^2	(n-1) log s^2
1,100	(1)	6	1.62	16.20	7.25
	(2)	2	0.33	3.35	1.05
1,300	(1)	14	3.21	32.10	21.09
	(2)	14	3.07	30.70	20.82
1,500	(1)	39	13.18	131.80	82.67
	(2)	32	20.38	203.80	73.89
	(3)	6	7.96	79.60	11.40

d.f. = 6 $\quad$ Chi^2 = 31.79 ***

The variance is significantly heterogeneous.

b) Number of corpora lutea plus large follicles

Corresponding values for Bartlett's Test on these data were -

d.f. = 6 $\quad$ Chi^2 = 35.61 ***

The variance is significantly heterogeneous.

(1) = Day 11 of the oestrous cycle
(2) = Day 12 of the oestrous cycle
(3) = Day 13 of the oestrous cycle
*** = $p < 0.001$

Appendix II cont'd

TABLE 2

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE OF OVARIAN RESPONSE DATA AFTER SQUARE ROOT TRANSFORMATION

a) Number of corpora lutea

Dose of P.M.S. (i.u.)		d.f. (n-1)	Variance s^2	Coded s^2	(n-1) log s^2
1,100	(1)	6	0.178	17.8	7.50
	(2)	2	0.050	5.0	1.39
1,300	(1)	14	0.257	25.7	19.73
	(2)	14	0.200	20.0	18.21
1,500	(1)	39	0.567	56.7	68.39
	(2)	32	0.718	71.8	59.39
	(3)	6	0.383	38.3	9.49

d.f. = 6 Chi^2 = 7.7 N.S.

The variance is homogeneous.

b) Number of corpora lutea plus large follicles

Corresponding values for Bartlett's test on these data were -

d.f. = 6 Chi^2 = 3.70 N.S.

The variance is homogeneous.

(1) = Day 11 of the oestrous cycle

(2) = Day 12 of the oestrous cycle

(3) = Day 13 of the oestrous cycle

N.S. = Not Significant

TABLE 3

DUNCAN'S MULTIPLE RANGE TEST ON DOSES OF P.M.S. FOR NUMBER OF CORPORA LUTEA AND NUMBER OF CORPORA LUTEA PLUS LARGE FOLLICLES

Mean Number of Corpora Lutea and Corpora Lutea plus Large Follicles for Doses of P.M.S.

	1,100 i.u. P.M.S.		1,300 i.u. P.M.S.		1,500 i.u. P.M.S.	
	(A)	1.39	(A)	1.74	(A)	2.31
	(B)	1.62	(B)	1.84	(B)	2.59
				- - - -	- - - -	- - - -
$p < 0.01$		- - - -	- - - -	- - - -	- - - -	- - - -

(A) = Mean Number of Corpora Lutea per Ewe

(B) = Mean Number of Corpora Lutea plus Large Follicles per Ewe

\- - - Significant

Appendix II cont'd

TABLE 4

DUNCAN'S MULTIPLE RANGE TEST ON DOSES OF P.M.S. FOR PERCENTAGE OF OVA RECOVERED PER EWE

Mean Percentage of Ova Recovered per Ewe for Doses of P.M.S.

1,100 i.u. P.M.S.	1,300 i.u. P.M.S.	1,500 i.u. P.M.S.
79.5	72.7	56.4

$p < 0.05$ - (spanning all three doses)

$p < 0.01$ - - - - - - - - - - - - - (spanning 1,300 and 1,500 i.u.)

- - - - = Significant

TABLE 5

DUNCAN'S MULTIPLE RANGE TEST ON DOSES OF P.M.S. FOR PERCENTAGE OF RECOVERED OVA FERTILISED PER EWE

Mean Percentage of Recovered Ova Fertilised per Ewe for Doses of P.M.S.

1,100 i.u. P.M.S.	1,300 i.u. P.M.S.	1,500 i.u. P.M.S.
90.0	67.7	54.5

$p < 0.01$ - (spanning all three doses)

- - - - = Significant