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SOME PRENATAL EFFECTS ON GROWTH IN LAMBS PRODUCED BY RECIPROCAL CROSSBREEDING AND OVA TRANSFER BETWEEN LINCOLN AND SOUTHDOWN SHEEP

by

© AUTAR KRISHEN KARIHALOO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Some prenatal effects on growth in lambs produced by reciprocal crossbreeding and ova transfer between Lincoln and Southdown sheep" submitted by Autar Krishen Karihaloo in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

This study was undertaken to investigate some prenatal effects, in particular maternal environmental effects on growth of lambs. This problem was approached by:

1. reciprocal crossbreeding of large (Lincoln) and small (Southdown) breeds of sheep, and
2. transferring fertilized ova between these two breeds.

A simplified technique of ova transfer, making use of natural synchronization in the absence of superovulation gave consistent results in recovering even the single ovum shed by most ovaries.

At birth, maternal environment had a highly significant effect on the fetal weight. The difference of 17% in the birth weight due to maternal environment was virtually identical in both the reciprocal crossbreeding and ova transfer experiments. This difference increased slightly at 40 days but at 150 days and 190 days the difference was small and non-significant. The effect of maternal environment on cannon bone length, although small as compared to body weight, had a significant effect even at 150 days of age. At birth differences in the cannon bone width due to maternal environment were significant only in reciprocal cross experiment. However, after 90 days the differences were small and non-significant.

The breed of lamb at birth had a 15% effect on weight and 30% on cannon bone length but with no effect on the cannon bone width.

Litter size had a highly significant effect on fetal weight and cannon bone width at birth. The effect of litter size on cannon

bone length, although highly significant in the reciprocal cross experiment, was small and non-significant in the ova transfer experiment. However litter size had a highly significant effect on postnatal growth in all three traits. After 90 days the differences declined steadily in all cases.

Differences due to sex of lamb on body weight, and on cannon bone length and width were small at birth, but increased with age.

The effects due to breed of sire on body weight and cannon bone length were relatively small at birth, but increased as lambs grew older. Breed of sire, however, had no effect on cannon bone width at birth or postnatally.

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INTRODUCTION

Heredity and environment interact in the determination of physical characteristics of animals and especially in such characters as size and weight. Many of the early investigations led to the conclusion that body size and growth were determined in a quantitative manner by a large number of genes. These cumulative effects of divergent allelic frequencies at many loci have led to rational theories of quantitative inheritance.

In spite of advances made in quantitative genetic theory, many complications remained in the interpretation of experimental results. Several simplifying assumptions have been made in estimating genetic and environmental components of variance. These assumptions often excluded maternal effects.

Several breeding studies among animals of large and small size have shown that the maternal environment does exert a marked effect on the offspring, at least for some portion of its life. However, the manner in which maternal effects influence growth is far from clear. Mechanisms suggested to explain maternal effects include the direct non-chromosomal influence of the dam upon the development of her progeny by means of nutrients, pathogens or antibodies provided in the uterus, in the egg or in the milk.

The maternal environment may affect the growth of the offspring prenatally and/or postnatally. Before birth the fetus is entirely dependent upon its mother's nutrition, so that its growth is largely subject to her control. After birth the neonate is

largely dependent on maternal care, the most important feature of which is the available milk supply.

Methods used to study maternal effects in animals include:

i) reciprocal crossbreeding,

ii) cross fostering of young at birth,

iii) reciprocal ova transfers.

The technique of ova transfer in sheep has been reported by various investigators. All these workers have emphasized the necessity of two preparatory procedures for successful transfer. These are the synchronization of estrus in donors and recipients and superovulation of the donor.

However, a sure way of ova recovery, making use of natural synchronization and in the absence of superovulation, would be helpful for certain experimental uses of ova transfer.

The present study was designed to assess the contribution of the maternal effects on some parameters of lamb growth using reciprocal crossbreeding and ova transfer and to establish a simplified method of ova transfer to facilitate future studies.

REVIEW OF LITERATURE

During intra-uterine development and the suckling period the mother has a great influence on the development of the young as manifested in their weight at birth and weaning. Even though the realization that maternal influences were important in livestock originated with the very early breeders, it was not fully acknowledged in both humans and livestock until the 1950's. Different aggregates of factors have been used to describe maternal effects. A general description (Hunter, 1956) is "the sum of the effects of those maternal factors which influence the growth of young after fertilization of the egg."

I. Prenatal Maternal Effects

The non-genetic factors which affect prenatal growth may also include size of dam (Venge, 1950), age and parity of dam (Dickinson et al., 1962), litter size (McLaren and Michie, 1960), nutrition of dam (Wallace, 1948), placental growth (Huggett and Hammond, 1952) and cytoplasmic influences (Walton and Hammond, 1938).

A. Size of dam

1. Body weight

The size of the dam seems to be positively correlated with prenatal growth. This phenomenon was first demonstrated by Walton and Hammond (1938) who found that in a reciprocal cross between one of the world's largest and the smallest horse breeds, the Shire draft horse and the Shetland pony, the weight of the foal at birth was 3 times greater when carried by a Shire mare than when the mother was a

Shetland pony. Furthermore, this difference persisted even when the foal had attained maturity.

Similar results have been obtained in another extreme reciprocal cross between the Flemish Giant and small Polish rabbits. When the crossbreds were carried by the Flemish Giant doe, the F_1 animals weighed 533 g more at 10 months of age than when they were carried by the Polish doe. The difference in the body weight and the skeletal measurements between reciprocal F_1's was more pronounced at maturity than at birth and weaning (Johansson and Venge, 1953).

Shreffler and Touchberry (1959) recorded body weights and five different body measurements at various intervals from birth to 4 years of age for purebred Holsteins, purebred Guernsey and their reciprocal crosses. These authors reported that the breed of dam effect was significant at all ages for every measurement and was considerably larger than the breed of sire effect. They concluded that the difference between the effects of breed of sire and breed of dam could be due to genetic differences between sire and dams of the same breed, but this difference also suggests the possibility of an important prenatal environmental effect which persists from birth to maturity.

These results were later confirmed by Touchberry and Bereskin (1966) who further concluded that the difference between the magnitude of the effects of breed of dam and breed of sire did not change as age increased and the difference between reciprocal crossbreds was likely a result of prenatal maternal effect.

Apart from these crosses, there is no indication in some other experiments that the maternal influence during the gestation period has

any effect on the size of the fully grown animals. Koch and Clark (1955) analyzed data comprising 4533 birth and weaning weights of Hereford calves born during the period 1926 to 1951. The data were adjusted for sex of calf and age of dam. Differences among years were eliminated. On the basis of half-sib correlation, the maternal environment had a considerable influence on birth weight of calves and on gain from birth to weaning but little effect on gain from weaning to one year of age.

In other crossbreeding trials, it was assumed that few or no sex-linked factors influenced body size or growth, therefore the crossbred offspring resulting from mating large breed sires to small breed dams were taken for granted to be approximately the same genotype as the offspring resulting from the reciprocal cross. However, marked differences were found at birth between the reciprocal crossbred progeny groups from breeds which differed in size.

In one such experiment Joubert and Hammond (1958) reported that in reciprocal crosses between the large South Devon and small Dexter cattle which have a 2.5 fold difference in mature weight, the difference in the offspring of reciprocal crosses was about 10% of the average birth weight of the two parental breeds (mid-parent mean weight). In this study when the calves had reached 9 months of age the percentage difference in weight had gradually diminished.

Donald et al. (1962) analyzed data on 1015 liveborn calves in an experiment in which reciprocal crosses were made among the British Friesian (F), Ayrshire (A) and Jersey (J) breeds. The average birth weights of contemporary controls were F, 39.2 kg, A, 32.9 kg,

and J, 22.3 kg, calculated as the weight of the heifer calves from primiparous dams. The mean birth weight of the crossbred offspring deviated from the mid-parent in the direction of the birth weight of the maternal breed. The average deviation was 10.6% of the difference between the parental mean and the maternal breed. Dams of a small breed produced offspring smaller than expected while dams of the larger breed produced offspring heavier than expected.

In a similar study carried out by McDowell et al. (1969) where body weights for all single crosses among Ayrshire, Brown Swiss and Holsteins were compared to those of contemporary purebreds in 6 periods. From this study these authors concluded that maternal influences were not significant except on birth weight.

Birth weight of lambs has been shown to differ among breeds (Kean et al., 1949; and Kincaid, 1943), and even within the same breed, heavier ewes give birth to larger lambs (Wallace, 1948). Hunter (1956) crossed the large Border Leicester and the small Welsh Mountain breeds and found that birth weight of crossbred lambs from Border Leicester dams was 1.08 lb more than the corresponding figures from crossbred lambs from Welsh dams.

Comparable figures for the maternal influence when fertilized eggs were transferred from one breed to another were 1.96 lb. At 8 months of age, 17% of the variation in live weight was still due to maternal influence. From this study Hunter concluded that maternal effects were important only when the genotype for the size of fetus is markedly different from its mother.

Although there are other data supporting this conclusion (Dickinson et al., 1962), the accurate evaluation of maternal effects, genotype and interaction in regard to their relative importance will hardly hold true for any comparison except the particular one being studied. Dickinson et al. (1962) found that in a normal maternal environment with reasonable nutrition, the average birth weight of a single lamb is probably near its upper genetic limit. Evidence for this came when they transferred eggs between the small Welsh and the large Lincoln breed of sheep. The genotypically large fetuses developing in a large environment were much larger than genetically similar embryos which develop in a restricted environment (Welsh ewes), while genotypically small fetuses exhibited very minor size increases when they developed inside the large Lincoln dam.

2. Cannon bone length

Cannon bone (metacarpal) has been shown to reach 95% of mature length at birth in horses (Walton and Hammond, 1938). This maybe used as an index of a very early maturing characteristic that would be expected to be relatively independent of maternal influence.

Dickinson (1960), working on the same data as that of Donald et al. (1962) concluded that cannon bone length at one month of age was least affected by the maternal environment before birth and that the difference between the reciprocal crosses disappeared before one year of age. Hunter (1956) found that cannon bone of reciprocal crossbred lambs from Border Leicester ewes was 0.37 cm longer at birth than the corresponding figure from crossbred lambs from Welsh dams. The reciprocal difference in the cannon bone length disappeared before 8 months of age.

B. Age and parity of dam

The age of the dam has been shown to influence the fetal size. Thus young animals which have not reached adult size continue growth during the first pregnancy and thus provide sterner competition with the fetus for the available nutrients. In this respect Dickinson et al. (1962) have shown that first parity 'Blackface' dams, although heavier than fourth parity Welsh dams, gave birth to somewhat smaller lambs. This was also confirmed by Reynolds et al. (1965) who concluded that in beef cattle, age and weight of the dam had a degree of influence on the weight of the calf and no influence on the gestation period.

C. Litter size

Increase in litter size, results in a reduction in the birth weight of the individual fetus. Wishart and Hammond (1933) and Hammond (1934) have shown that in rabbits the size of young is inversely proportional to the number in the litter. Venge (1950) concludes that litter size in addition to breed was an important consideration in estimating maternal influence on birth weight. In sheep, twin lambs are smaller at birth than singles (Hammond, 1932; Hunter, 1956) and in twin pregnancies of mixed sex the males grow faster than females (Donald and Purser, 1956).

D. Nutrition of dam

Restricted nutrition of the dam retards prenatal growth since the fetus is dependent on its mother's plane of nutrition and energy reserve. In sheep the level of nutrition during later stages of

pregnancy has a more marked effect on the weight of twin lambs at birth than on single lambs (Wallace, 1948; Palsson and Verges, 1952). From these experiments and from his own work, Hunter (1956) concluded that competition between fetus(es) and maternal tissue for the available nutrients is a mechanism of maternal influence on the size of young at birth.

E. Placental size

During the final phase of pregnancy, fetal growth is preceded by a period when placental growth is greatest. Huggett and Hammond (1952) have suggested that placental size could be limited by various maternal processes and such placental restriction may subsequently retard fetal growth. However, McKeown and Record (1954) found evidence that maternal size has more effect than placental size on fetal growth.

F. Cytoplasmic influences

It has been suggested (Walton and Hammond, 1938) that not only the intra-uterine environment but also cytoplasmic influences may be responsible for the rate of fetal growth. Venge (1950 and 1953) transferred large numbers of eggs reciprocally between large and small breeds of rabbits. In addition egg transfers were made between medium type females genetically different in color. In the later case no clear difference could be established between the young of F_1 reciprocals in respect of growth rate or body size at maturity. From this experiment Venge concluded that cytoplasmic influence on birth weight was unlikely.

II. Ova Transfer

Heape (1890) successfully transferred two 4-cell ova from an Angora rabbit to the oviduct of a mated Belgian Hare doe rabbit and was thus first to demonstrate the possibility of development within a uterine foster mother. Two Dutch rabbit young were developed in other Belgian Hare doe rabbits and Heape (1897) concluded that the recipient female has no power to modify the breed of her foster offspring. Successful egg transfers were later carried out by other workers in laboratory animals but intensive study and the use of the technique did not start until the early 1950's. The successful utilization of ova transfer in laboratory animals not only led to refined and rigorous studies within the field of reproductive physiology, genetics, cytology, and immunology but it also provided a method for improvement of domestic livestock.

The potentials and problems associated with the application of ovum transfer to farm animals under experimental conditions have been discussed by Dowling (1949), Willet (1953) and others. Since the earlier report of ova transfer in sheep by Warwick et al. (1934), further transfers have been made with varying success (Casida et al., 1944; Hunter et al., 1955; Averill and Rowson, 1958; Moore et al., 1960; Hancock and Hovell, 1961; Rowson and Moor, 1966; Moore, 1968).

The cervix uteri of the ewe is very difficult to penetrate and in this species ova are therefore transferred surgically. Cervical stimulation is known to cause uterine contractions which might result in the expulsion of ova if transferred through it. This circumstance may account for the fact that some success has always attended reported

transfers of ova between ewes as these all have been carried out via laparotomy.

From the knowledge presently at hand, two main conditions must be met for the success of ova transfer in terms of embryo survival and growth within the uterus of the recipient. The conditions are:

1. synchronization of estrus between recipient and donor, and
2. an adequate supply of viable fertilized ova, capable of extra-utero storage for a few hours or at least for the duration of surgery.

A. Synchronization of estrus

Although there is some evidence (Averill and Rowson, 1958) that precise synchronization of the recipient and donor is unnecessary for the successful transfer of sheep ova beyond the 6-cell stage, there are other reports (Hunter et al., 1955; McLaren and Michie, 1956; Noyes and Dickmann, 1960) that the transfer of older ova into "younger" uteri is generally more favorable to success than the transfer of younger ova to the "older" uteri. On these grounds, Hancock and Hovell (1961) concluded it is a wise precaution to minimize the possibility of estrus occurring in the recipient before it occurs in the donor.

Introduction of vasectomised rams with ewes about a month before the time of normal mating, a transition period between the anestrous and estrous seasons achieves a certain degree of synchronization (Cunningham et al., 1967). Also during breeding season, in a flock of sufficient size, many donors and recipients with spontaneously

synchronous estrus occur (Hancock and Hovell, 1961).

However, classically, control of estrus in sheep has been achieved by administration of progesterone or its derivatives. Effective synchronization of estrus cycles in sheep was first reported by Dutt and Casida (1948). The optimum dose for preventing estrus and ovulation in the ewes appeared to be a daily injection of 5 to 10 mg of progesterone in oil. This method of repressing estrus and ovulation until discontinuance of the hormone brings on the breeding cycle in all treated females within a few days has proven to be quite successful in sheep (Lamond and Bindon, 1962; Moore and Shelton, 1964; Foot and Waite, 1965).

Robinson (1960) obtained satisfactory suppression of the estrous cycle with injections of progesterone in various vehicles including peanut oil, benzyl benzoate, a commercial medium 16476b, (Farbwerke Hoechst) benzyl alcohol emulsion and aqueous crystalline suspension.

After the orally administered progestational compounds became available, considerable experimentation produced good evidence of their effectiveness for synchronizing estrual cycles in sheep (Hinds et al., 1961; Southcott et al., 1962). Incorporation of 6α methyl-17α acetoxy progesterone (MAP) into the daily feed for 14 days has been shown to effectively control estrus and ovulation (Hinds et al., 1964; Dhindsa et al., 1966). Onset of estrus occurs about 60 to 90 hours after withdrawal of MAP. The intramuscular injection of 250 or 500 International Units of human chorionic gonadotrophin (HCG) 48 to 54 hours after MAP causes ovulation in 25 to 26 hours (Dziuk, 1965).

Intravaginal pessaries impregnated with a progestogen and left in the vagina for 12 to 14 days serve as perhaps the most convenient method of synchronization of estrus in sheep. Estrus occurs 3 to 4 days after the removal of the pessaries from the vagina (Robinson, 1964; Roberts, 1966; Cunningham et al., 1967).

B. Superovulation of donor

Injection of gonadotrophins to induce superovulation in the sheep has been used both during the normal breeding season (Robinson, 1951; Wallace, 1954) and during the anestrous period (Phillips et al., 1945; Robinson, 1950). Gonadotrophins have also been used to induce ovulation in ewes after progestational synchronization of the estrus cycle, but no trend in ovulation rates could be established with varying dosages of pregnant mare serum (PMS) and HCG following progesterone priming (Lamond, 1962). Hunter et al. (1955) reported that number of ovulations in 25 ewes which received 800, 1500, and 2000 I.U. of PMS ranged from 0 to 12, 1 to 10, and 0 to 9 per ewe respectively. Among these ewes, some were superovulated at either the first, second or third estrus with PMS being injected either the last day of progesterone priming, or the 12th day following the previous estrus.

Robinson (1951) and Averill (1958) reported that PMS given to ewes to cause superovulation did not cause marked cyclic abnormalities. However, Hancock and Hovell (1961) found that the onset of estrus after PMS treatment tended to be more erratic and some ewes even failed to show estrus.

Quite divergent results with very low fertility have characterized progesterone-PMS or progesterone-PMS-HCG treatments

(Moore et al., 1960). The dose, method of injection and time of injection in relation to follicular growth appear to be important factors in HCG administration.

Use of anterior pituitary extract has shown promise in effecting superovulation. A total of 75 mg for maiden ewes and 100 mg for mature ewes of horse anterior pituitary extract was administered subcutaneously in six equal injections 12 hours apart beginning on the 12th day following estrus. Ovulations ranged from 1 to 21 with an average of 11.3 for 74 treated ewes. However, only 79% of the ova shed were recovered and of these only 72% were fertilized (Moore and Shelton, 1962).

In another experiment, Shelton and Moore (1967) compared PMS and horse anterior pituitary extract (HAP) response on ewes. There was no difference in the number of ovulations, but there were greater number of persistent follicles after PMS administration. They concluded that since persistent follicles are associated with a decreased percentage of fertilized ova, HAP is more suitable than PMS for the production of a greater number of fertilized sheep ova.

C. Ovum recovery

Results of ovum transfer from laboratory animals suggest the necessity for a close synchronization between the cell stage of the ova transferred and the stage of luteal development in the ovary of the recipient (McLaren and Michie, 1956). In contrast to laboratory animals, close synchronization is difficult in sheep because ovulation occurs at a variable time in relation to both the onset and cessation of estrus.

Green and Winters (1945) have reported that first cleavage in the ovum occurs about 36 hours after ovulation; the 4-cell stage at 42 hours and the 8-cell stage as early as 42 to 44 hours after ovulation. This pattern and timing of cell division was later confirmed by Hunter et al. (1955), who recovered eggs from the oviduct 70 hours after the beginning of estrus in the donor animals and found that 8-cell eggs resulted in more pregnancies than other cell stages when transferred into the uterus of recipients which had come into estrus 16 to 20 hours after the donor animals.

Using the same synchronization pattern as that of Hunter et al. (1955), Averill and Rowson (1958) reported that a satisfactory proportion of 6 to 16-cell ova survived and developed when transferred to the uterus, but only a few 4-cell ova and none of the 2-cell ova developed in the recipient ewes which had entered estrus more than three days previously. Hunter et al. (1955) also reported the development of two 4-cell sheep ova when transferred to the uterus, but Willett et al. (1953) obtained no calves from five 4-cell ova introduced into the uterine horns of recipient cows.

In order to obtain ova at the 8-cell stage or later, Averill and Rowson (1958) recommended that laparotomy should be done about 60 hours later in ewes found at the morning inspections to be marked by the rams when ewes were examined twice daily and had free access to a fertile ram. However, Hancock and Hovell (1961) using hand mating of a donor ewe, considered it necessary to delay the operation for 72 hours after mating to ensure the recovery of ova at the 6-cell or later stage. The 72 hour delay before operation for ova recovery

is also recommended by Dziuk (1969).

1. Anesthesia

Atropine given either subcutaneously or intramuscularly 15 min before administration of anaesthetic has been used to suppress salivation (Averill, 1958; Hancock and Hovell, 1961). Nembutal has been used intravenously either to induce complete anaesthesia (Hunter et al., 1955) or to induce narcosis (Hancock and Hovell, 1961). Averill (1958) used nembutal intravenously until the conjunctival reflex was lost, followed by ether or nitrous oxide given on the closed circuit system via an endotracheal tube. In this case the endotracheal tube also served the important function of preventing inhalation of regurgitated ruminal contents. Hancock and Hovell (1961) used an oxygen-cyclopropane mixture administered by the closed circuit system.

2. Laparotomy

Reproductive organs of ewes can best be exposed through a midline incision. Ova can be recovered by flushing the oviduct towards the ovarian end or into the uterus. When flushing towards the ovary, a cannula is placed in the upper oviduct, directed into a centrifuge tube which serves to collect the fluid and eggs (Hunter et al., 1955). If the fluid is flushed through the oviduct into the uterus, a cannula can be inserted into the uterus to collect the ova and fluid (Rowson and Moor, 1966).

3. Recovery fluid and ova transfer

Homologous serum and physiological salt solutions are the basis for most media for egg recovery and storage in sheep. The serum

is heated to destroy possible ovicidal factors and filtered to remove bacteria and foreign matter (Averill and Rowson, 1958). Antibiotics such as penicillin or streptomycin can be added at the levels of 1000 units/ml and 500 to 1000 micro gm/ml respectively (Moore et al., 1960). Commercially available lamb serum can also be used effectively (Dziuk, 1969). Rowson et al. (1969) reported that in cattle homologous serum is an unsuitable medium for ova transfer, but that Tcm 199 (Glaxo Laboratories) is highly satisfactory.

While it is advisable to maintain the recovered eggs at body temperature if they are stored for only a short period, the evidence showing superiority of body temperature over room temperature for storage of sheep ova is not conclusive (Dziuk, 1969).

Eggs for transfer can be picked up with a micropipette with a vernier control or by a Pasteur pipette attached to a 1 or 2 ml syringe. Depending on its cell stage, the ovum can be deposited either into the oviduct through the fimbria or into the lumen of the uterus. While transferring into the lumen of the uterus, great care is needed to ensure that the ovum is deposited within the cavity of the uterus and not under the uterine mucosa (Hancock and Hovell, 1961).

EXPERIMENTAL

I. Objectives

The main purpose of this study was to investigate some prenatal effects, especially influence due to the breed of dam on growth of lambs. This problem was approached in two ways:

1. by reciprocal crossbreeding of large (Lincoln) and small (Southdown) breeds of sheep, and
2. by transferring fertilized ova between these two breeds of sheep.

II. Materials and Methods

A. Experimental animals

Lincolns and Southdowns had been chosen as parent stocks for a study of the recombination of quantitative traits because of their wide differences in size, fleece characteristics and their historical divergence and distinctiveness as breeds. The live weight of mature Lincoln ewes averages 80 to 100 kg, while that of Southdown ewes average only 55 to 70 kg. The animals were raised at the University of Alberta farm from a closed flock maintained since 1965.

B. Reciprocal crossbreeding

In the reciprocal cross experiment, these breeds were straightbred and crossed reciprocally so that crossbred lambs of similar genetic constitution could then be compared in both maternal environments and against straightbred contemporaries. The effects of (a) breed of sire, (b) breed of dam, (c) size of litter (singles or twins), and (d) sex of lamb were considered in this experiment. It was assumed that the genotypic contributions of the sires and dams

to the lamb were similar. The difference between the effects of the breed of sire and the breed of dam on body weight, cannon bone length and cannon bone width was taken to be the effects on these traits due to maternal environment.

1. General management

The ewes were kept on pasture during the summer, which kept them on a high nutritional plane. They were returned to breeding pens during the first week of September and allotted to various breeding groups for straightbreeding and reciprocal cross-breeding in such a way as to distribute females by various sires more or less proportionally to each breeding pen. Eight rams of each breed sired both straightbred and crossbred progeny. During the 50 day breeding period, the ewes were fed legume-grass mixed hay and oats. During the winter months, the ewes were housed in an open-fronted shed and fed alfalfa-grass mixed hay. Yearling ewes were supplementally fed oats (1/2 lb/ewe/day) until after weaning of the lambs. Adult ewes were supplemented with oats (2 lb/ewe/day) from one week before lambing until a few days before weaning.

One week before lambing, the ewes were transferred to fully covered lambing pens until post-lambing. When the newborn lambs were dry, they were weighed to the nearest 0.1 kg and the right fore cannon bone radiographed within 48 hours after birth. The lambs were weaned at about 40 days of age, kept in a dry lot and fed a complete pelleted ration ad libitum until September.

The lambs were weighed and radiographed at weaning (40 days), 90 days, 150 days and weighed but not radiographed at 190 days of age.

The cannon bone length measurement was taken from metacarpal tuberosity to the distal end of the condyle and the mid part of the metacarpal shaft was measured for the width purposes. Body weight and cannon bone length and width measurements were adjusted with respect to age.

C. Ova transfer

In the ova transfer experiment, purebred lambs of both breeds were reared in the contrasting maternal environment by transferring fertilized ova between the large Lincoln and the smaller Southdown ewes. The ewes were 2 to 4 years old and had lambed at least once by natural mating. The effect of lamb's breed, sex, litter and the maternal environment on the size of the lambs at birth was considered in this experiment.

Preliminary investigation was carried out during the breeding season of 1968 in order to establish the technique of ova transfer. During this trial all the donor and recipient ewes were estrus synchronized by vaginal pessaries impregnated with 6∝ methyl-17 ∝ acetoxy progesterone (MAP The Upjohn Company, Kalamazoo, Michigan). Pessaries were removed on the 13th day following insertion and all the treated ewes came into estrus within 3 to 5 days after the removal of the vaginal pessaries. During this investigation attempts were also made to induce multiple ovulation (superovulation) using varying doses of 'equinex' (Ayerst Laboratories, Saint-Laurent, Quebec), a purified crystalline preparation of PMS either alone or in combination with HCG, but results were inconsistent. Once the ova recovery techniques were successful, the one or two naturally shed ova were found to be sufficient.

During the main investigation carried out in 1969 no superovulation was attempted in donor ewes. One month before the time of normal mating, vasectomized rams were introduced into the experimental flock selected for ova transfer so as to achieve a degree of natural synchronization. Naturally synchronized ewes, one from each breed, were paired, such that the donor ewe was also made recipient of the ovum or ova flushed from the ewe to which she acted as a donor. This was done to make efficient use of the available ewes. However, towards the end of the breeding season it became necessary to synchronize estrus by vaginal pessaries impregnated with a synthetic progestin (Synchromate, G.D. Searle and Co. of Canada Ltd.).

The ewes were inspected twice daily and when one ewe in each breed was in estrus, each ewe of the pair was mated to a fertile ram of her own breed.

Laparotomy for ova collection and transfer was performed between 65 to 72 hours after mating. Both ewes were simultaneously operated upon so as to exchange ova without delay.

1. Experimental technique

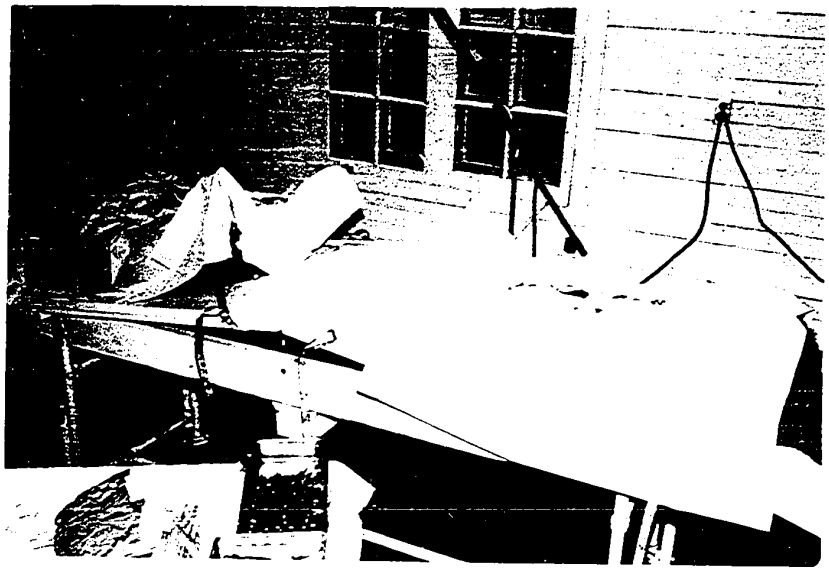
Shortly before anesthesizing the ewes, atropine sulphate (0.5 mg/kg body wt.) was injected intravenously to suppress excessive salivation or secretions of the upper respiratory tract. Also due to relaxing effect on smooth muscles of the reproductive tract, the atropine sulphate administration seemed helpful in withdrawing the short interior genitalia encountered in a few cases.

a. Anesthesia

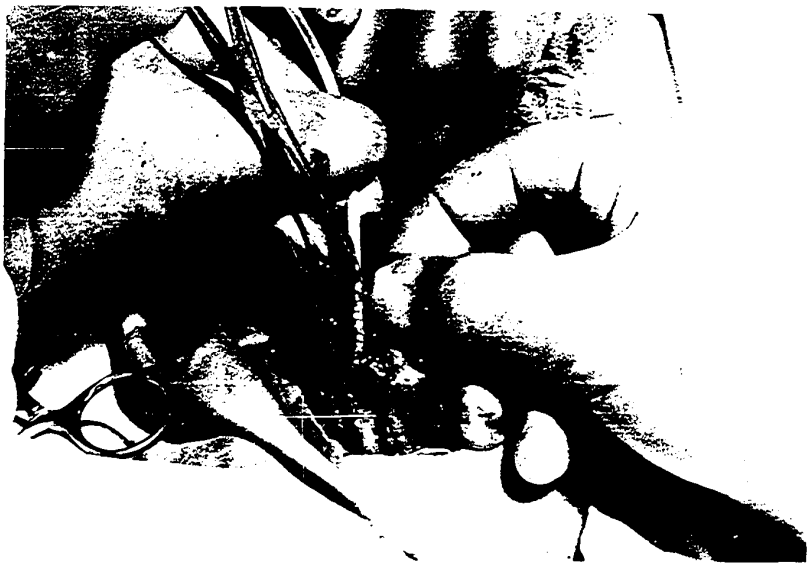
After clipping the area around the neck, a No. 13 hypodermic needle was pushed into the jugular vein through which a sterile polyethylene catheter (I.D. 1.2 mm, O.D. 1.7 mm) was inserted about 20 cm down towards the heart. The hypodermic needle was then removed leaving the catheter in the jugular vein. Sodium pentabarbital 'Nembutal' (60 mg/ml) was administered as an anesthetic through the catheter until conjunctival reflexes were lost. About 30 ml were required for Lincolns and 20 ml for Southdowns. The ewes were then placed on their backs on the surgical table which was adjusted to slightly elevate the hindquarters (Pl. 1). This forward tilt of the surgical table served the important function of preventing inhalation of regurgitated ruminal contents. After securing the animal on the surgical table the catheter in the jugular vein was hooked to a 3-way tap to which were attached two syringes one containing additional anesthetic and the other normal saline for flushing down the anesthetic.

b. Laparotomy

The abdominal area immediately anterior to the pelvis was closely shaven, cleaned and sterilized. An 8 to 10 cm incision was made just anterior to the mammary gland parallel to and about 5 mm off the Lina alba. Where necessary, the large subcutaneous vein was deflected laterally to avoid severing. The uterus and ovaries were located, withdrawn carefully and the number of ovulations and degree of follicular development recorded. Abdominal fat deposits and adhesions from previous surgery sometimes offered problems in readily exposing the internal genitalia.



Pl. 1. Donor and recipient ewes prepared for surgery.



Pl. 2. Inserting polyethylene catheter into the infundibulum.

c. Ova recovery

Three methods of ova recovery were tried.

i. A glass cannula was inserted through the uterine wall into the lumen of the uterus on the side of ovulation and held firmly up towards the utero-tubal junction. About 30 ml of sterile lamb serum (Colorado Serum Company) was flushed through the fimbria, down the oviduct and collected through the glass cannula. Even though this method of ova recovery has proven very successful with other investigators, no ova were recovered by this method in this investigation.

ii. The second method involved severing the oviduct slightly above the utero-tubal junction. This method, while the surest way of recovering ova, limits the future reproductive performance of the ewe especially when both oviducts are severed. The method was therefore discontinued.

iii. The third method consisted of flushing the oviduct towards the ovarian end. This method was developed and modified to meet the present experimental demand and once established it gave consistent results in recovering even the single ovum shed by most ovaries.

A sterile non-collapsable polyethylene catheter (I.D. 1.7 mm, O.D. 2.5 mm) was guided 2 cm into the infundibulum via the fimbria on the side of ovulation as shown (Pl. 2). This plastic catheter was firmly held in place by gently squeezing with gauze forceps the portion of the oviduct which was cannulated. The free end of the tube was directed into the flat bottom of a glass petri dish marked into 1 cm

squares (Pl. 3). Using a No. 18 hypodermic needle the uterine tip was punctured 2 cm from the utero-tubal junction and the needle point guided into the lumen to within a few mm of the junction. The horn of the uterus was elevated and finger pressure applied on around the needle to prevent back flow of flushing fluid into the uterus (Pl. 4). Two ml of serum was flushed through followed by air so as to completely empty the contents of the oviduct and the catheter into the petri dish.

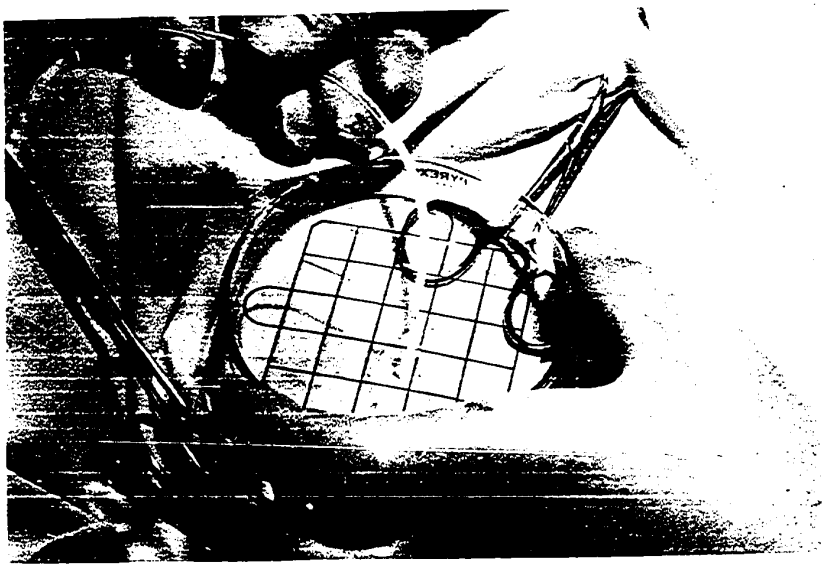
Each ovum was identified and examined for cell division under a stereoscopic, binocular microscope at 40 magnifications (Pl. 5) and left undisturbed at room temperature until the recipient was made ready for transfer. In all cases the ovum was transferred within approximately 5 min from the time it was isolated.

Use of the flat bottom petri dish facilitated the rapid location of ova particularly when the flushed material contained much debris. In most cases it took less than a minute to isolate the ovum from the flushed material.

d. Recovery media

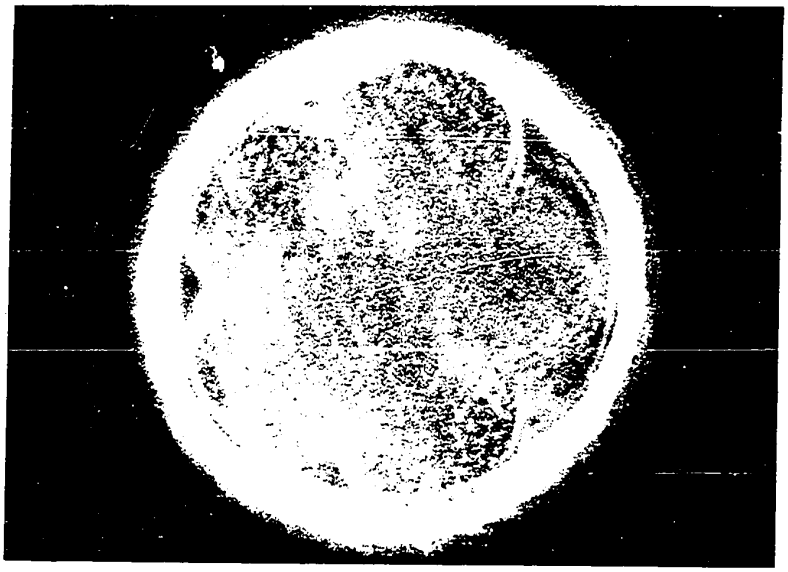
Due to the problems of importing commercial sterile lamb serum, autologus plasma was used as a flushing medium in the 1969 study.

Blood was collected from each ewe 15 minutes before the operation by sterile heparinized vacuum tubes. The tubes were centrifuged to separate the plasma and left in a water bath at 35 C. Just before use, plasma was withdrawn from the vacuum tube by piercing the rubber stopper. No antibiotics were added to the plasma.



Pl. 3. The freed end of the polyethylene catheter directed into petri dish.

Pl. 4. Flushing ova from oviduct into petri dish.



Pl. 5. Sheep ovum at 6-cell stage (x 500).

e. Ova transfer

A sharp pointed Pasteur pipette connected by rubber tubing to a short syringe was the most successful method of ova transfer. The ovum was picked up from the petri dish with a minimum of fluid and introduced either into the lumen of the tip of the uterine horn or pushed back into the oviduct by puncturing the horn with the pipette and discharging the contents with the aid of the attached syringe. Ova in the 4-cell stage were pushed back into the oviduct and later stage ova deposited into the uterus. Ova in the 4 to 32-cell stage were transferred, usually, but not always to the side of ovulation. Following collection and transfer of ova, the uterus was returned to the abdomen and the wound sutured using three layers of No. 0 chromic gut followed by mattress skin sutures with either No. 0 silk or nylon.

Ewes were left in the recovery pen for 24 hours after which they were returned to the main flock. Management practices for the ewes and lambs were identical to those described for the reciprocal cross experiment.

D. Statistical analysis

Standard errors and analysis of variance on mean values were carried out according to the method described by Steel and Torrie (1960).

Because of the unequal numbers in various groups error mean square was estimated on individual basis by the method described by Bancroft (1968). The computation was done on an IBM 360/67 computer.

III. Results

A. Reciprocal cross experiment

The data for this study were collected during 1968 and 1969. On preliminary analysis no significant differences were found in the body measurements between the years. Accordingly, the data were combined.

The average age of four groups of dams used in this experiment are presented in Table 1. Lambs in each group were born over a similar array of parities and maternal ages.

Table 1. Average Age of Dams in Years

Type of Mating	Breed of Dam	
	Lincoln	Southdown
Straight bred	2.25	2.07
Crossbred	2.39	2.40

1. Body weight

In various crossbreeding experiments with mammals, it has been shown that the size of the mother is positively correlated with prenatal growth of her progeny. The Lincoln and Southdown differ on an average by some 30 kg in their body weight. On this basis, if the embryo growth is related to the mother's size, one would expect the reciprocal cross lambs to differ in weight and size at birth.

The results of the present study followed such a pattern. The mean weights of lambs from birth to 190 days of age are given in Table 2. Means have also been plotted in Figs. 1 and 2 for males

Table 2. Mean Weight of Lambs at Various Ages

Sire	Lincoln									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	8	17	14	17	ϕ Lin Average	30	15	19	13	ϕ Lin x SD Average
Weight (kg)										
Birth (S.E.)	4.66 (±0.40)	4.55 (±0.21)	4.79 (±0.26)	4.11 (±0.14)	4.53 (±0.09)	4.29 (±0.12)	3.29 (±0.15)	3.90 (±0.14)	3.39 (±0.07)	3.72 (±0.09)
40 days (S.E.)	13.40 (±1.74)	13.18 (±0.50)	14.47 (±0.90)	12.11 (±0.58)	13.29 (±0.37)	14.01 (±0.47)	10.52 (±0.59)	12.43 (±0.51)	10.76 (±0.43)	11.91 (±0.37)
90 days (S.E.)	25.98 (±3.00)	25.67 (±1.73)	25.83 (±1.74)	22.96 (±1.31)	25.11 (±0.79)	26.60 (±1.02)	19.33 (±1.49)	23.29 (±1.17)	18.62 (±1.53)	21.96 (±0.79)
150 days (S.E.)	48.61 (±4.36)	47.80 (±2.22)	43.58 (±2.68)	40.83 (±1.62)	45.21 (±1.02)	47.35 (±1.20)	39.34 (±2.41)	40.42 (±1.28)	34.59 (±1.74)	40.43 (±1.02)
190 days (S.E.)	58.11 (±5.56)	57.01 (±2.30)	53.98 (±2.98)	49.80 (±1.66)	54.73 (±1.17)	52.84 (±1.42)	48.40 (±2.21)	46.33 (±2.43)	40.70 (±2.99)	47.07 (±1.17)

M - males; F - females; S - singles; T - twins.

ϕ includes unweighted means of males, females, singles and twins

Table 2. Mean Weight of Lambs at Various Ages (cont'd)

Sire	Southdown									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	18	24	17	28	ϕ Sd x Lin Average	5	9	6	6	ϕ Sd Average
Weight (kg)										
Birth (S.E.)	5.02 (±0.12)	4.30 (±0.10)	4.44 (±0.17)	3.89 (±0.08)	4.41 (±0.09)	3.79 (±0.16)	3.13 (±0.17)	3.08 (±0.09)	2.61 (±0.22)	3.17 (±0.09)
40 days (S.E.)	16.60 (±0.79)	12.96 (±0.54)	14.11 (±0.66)	13.28 (±0.32)	14.23 (±0.37)	12.20 (±0.78)	9.20 (±0.93)	11.03 (±0.79)	7.06 (±0.87)	9.87 (±0.37)
90 days (S.E.)	30.32 (±1.08)	22.54 (±1.19)	23.70 (±1.14)	22.08 (±0.70)	24.66 (±0.79)	22.48 (±1.52)	18.12 (±1.48)	19.23 (±0.38)	16.10 (±1.84)	18.98 (±0.79)
150 days (S.E.)	48.97 (±1.80)	44.05 (±1.73)	38.24 (±1.50)	37.44 (±1.50)	42.18 (±1.02)	38.86 (±2.57)	34.166 (±1.32)	31.85 (±0.39)	27.36 (±2.71)	33.18 (±1.02)
190 days (S.E.)	57.51 (±1.65)	51.35 (±1.54)	46.82 (±0.89)	44.90 (±0.90)	50.15 (±1.17)	43.06 (±3.08)	40.30 (±1.14)	34.71 (±1.84)	31.38 (±3.06)	37.36 (±1.17)

M - males; F - females; S - singles; T - twins.

ϕ includes unweighted means of males, females, singles and twins.

born as singles and twins and females born as singles and twins respectively.

At birth Lincoln lambs were heavier than Southdown lambs by 1.37 kg. This breed difference at birth increased two-fold over every measured period up to 150 days of age and by an additional 50% between 150 and 190 days. At 190 days Lincoln lambs were 17.37 kg heavier than Southdown lambs.

Sd x Lin lambs at birth were heavier than Lin x Sd lambs by 0.69 kg. There was an increase in weight difference at 40 days which remained relatively unchanged at 90 days. At 150 days the weight difference narrowed a little, only to increase again at 190 days at which time Sd x Lin lambs averaged 3.08 kg more than Lin x Sd lambs.

The difference in reciprocal cross lambs was much more among lambs born as twins of both sexes (Fig. 1 and 2) than between singles (Fig. 1). The difference between single females was the least with the Lin x Sd a little heavier at 150 days than Sd x Lin lambs (Fig. 2).

Crossbred lambs averaged heavier than the purebred midparent mean (MPM) at all ages studied in the present experiment. At birth the weight difference was 0.21 kg, which increased steadily at every period and at 190 days crossbreds weighed 2.58 kg more than purebred lambs.

Sd x Lin lambs on average weighed 0.56 kg, 2.65 kg, 2.61 kg, 3.0 kg and 4.1 kg more than the MPM at birth, 40 days, 90 days, 150 days and 190 days respectively. There was slight difference between the average weight of Lin x Sd and MPM from birth to 90 days. At 150 days, however, Lin x Sd lambs weighed 1.23 kg more than MPM, which remained relatively unchanged at 190 days.

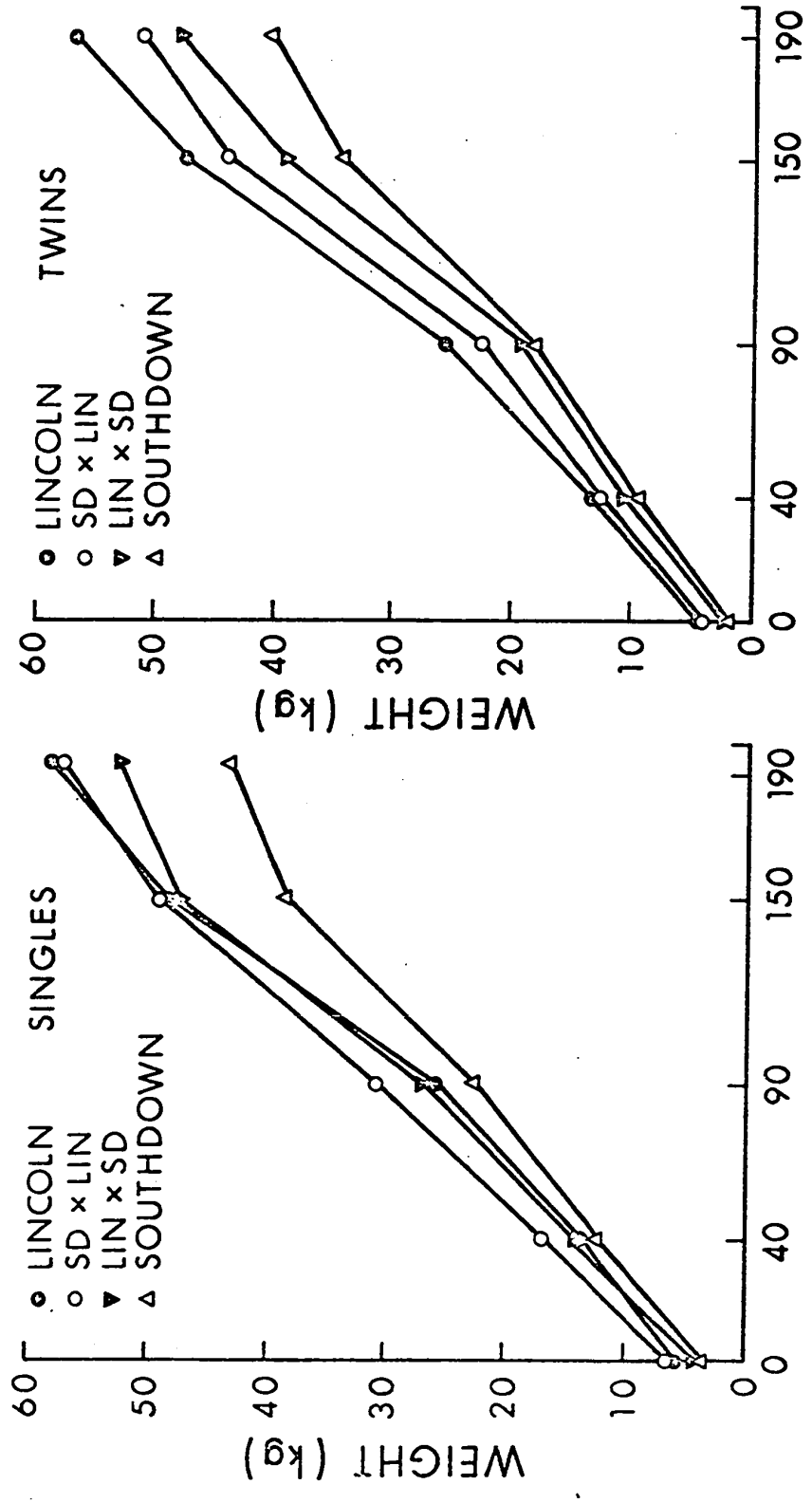


Fig. 1. Mean live weight of single and twin males plotted against age for Lincolns, Southdowns and their reciprocal crosses.

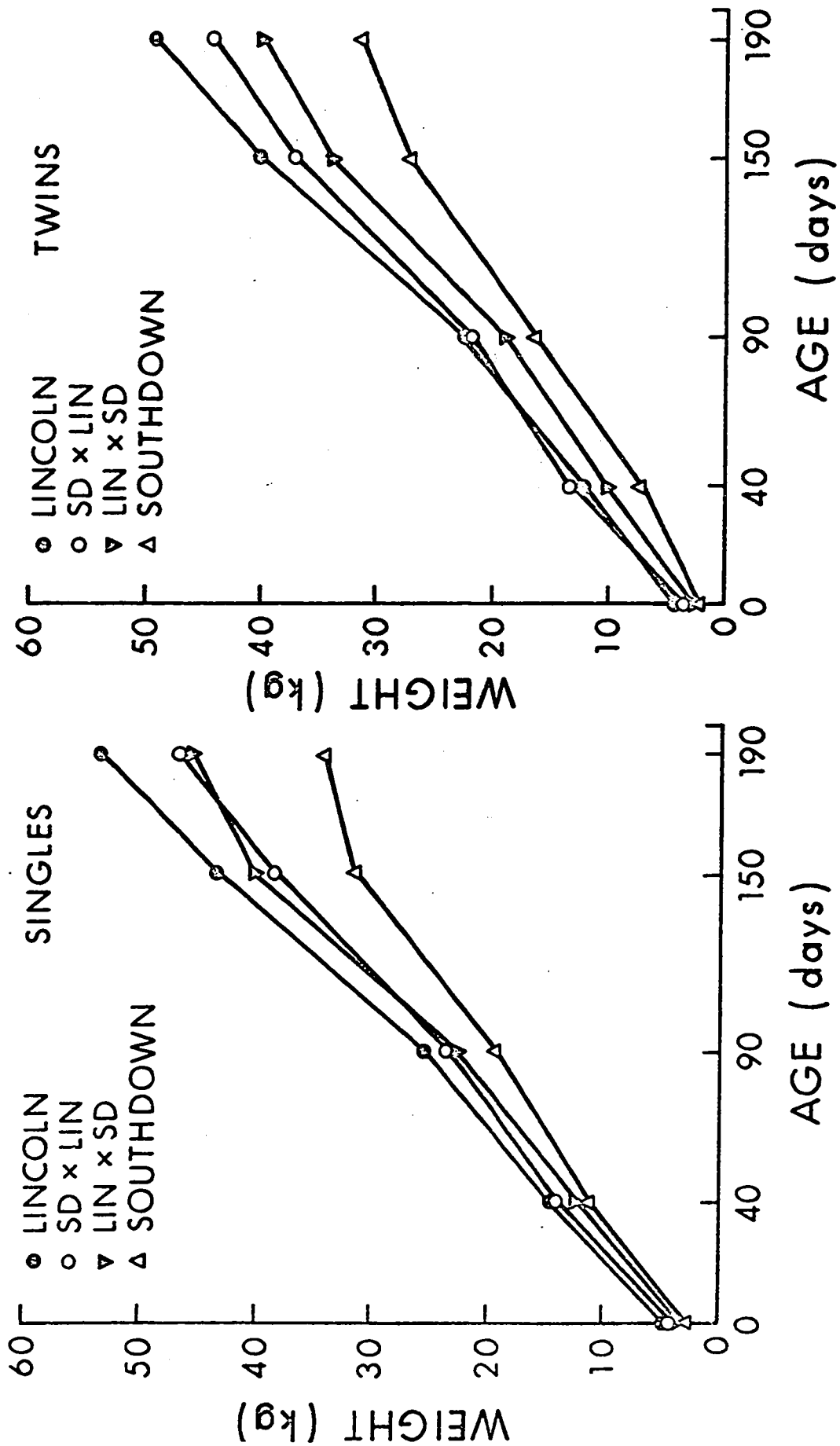


Fig. 2. Mean live weight of single and twin females plotted against age for Lincoln, Southdowns and their reciprocal crosses.

Lincoln lambs recorded the highest weights in all groups except Lincoln male single lambs weighed less than Sd x Lin male single lambs from birth to 150 days and less than Lin x Sd male single lambs from 40 to 90 days of age (Fig. 1). Also Sd x Lin female twin lambs weighed more than Lincoln female twin lambs at 40 days (Fig. 2).

Males were generally heavier than females at all ages. However, Lincoln female singles weighed slightly more at birth and 1.07 kg more at 40 days than single male Lincoln lambs. After 40 days males were heavier than females at all other periods.

At all ages singles were heavier than twins. The maximum differences recorded were at 90 and 150 days at which time singles were heavier by 4.01 and 3.95 kg. After 150 days the difference was reduced slightly.

The unweighed means of body weights at various ages of all lambs in each group have been plotted in Fig. 3. Lincoln lambs were heaviest, followed by Sd x Lin, Lin x Sd and Southdown except at 40 days when Sd x Lin lambs were slightly heavier than Lincoln lambs.

At 150 days Lincoln lambs weighed 45.21 kg, Sd x Lin, 42.18 kg, Lin x Sd, 40.43 kg, and Southdown only 33.18 kg. Considering rate of growth from 150 to 190 days, it would take an additional 12 days for Sd x Lin, 27 days for Lin x Sd, and 118 days for Southdown lambs beyond 150 days to reach the 45 kg body weight reached by Lincolns at that age.

Analysis of mean values in Table 2 was carried out in 2^4 factorial design, the factors being the:

a. breed of sire
b. breed of dam
c. sex of lamb
d. litter size

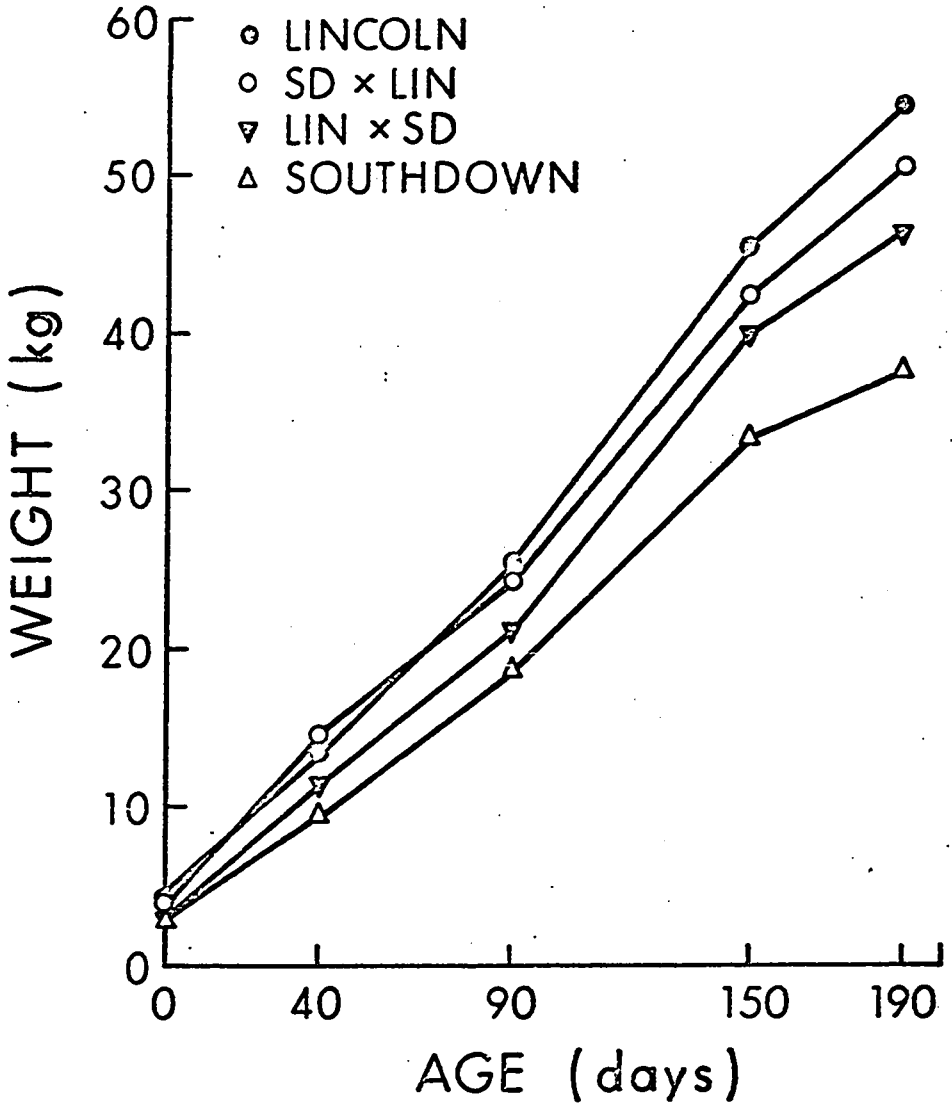


Fig. 3. Live weight averaged over unweighted means of males, females, singles and twins for Lincolns, Southdowns and their reciprocal crosses plotted against age.

Each of the four major factors considered in this experiment had a highly significant effect ($P < 0.001$) on birth weight (Table 3). The interaction between breed of sire and breed of dam was also significant ($P < 0.05$).

At 40 days of age (Table 4) effect due to breed of dam and litter size on weight were highly significant ($P < 0.001$). The effect of sex of lamb and the interaction between breed of sire and breed of dam was significant at ($P < 0.05$) and ($P < 0.01$) respectively. However, at this age there was no significant breed of sire effect.

At 90 days, breed of dam and litter size were still the major effects (Table 5) contributing to the weight of the lamb ($P < 0.001$). Differences due to sex of lamb had increased ($P < 0.001$) and breed of sire effects were significant ($P < 0.05$). No first order interactions were significant at this age.

At 150 days of age (Table 6) all main factors were highly significant ($P < 0.001$). However, differences due to breed of sire were at a higher magnitude than at 90 days of age.

The analysis of 190 day weights (Table 7) presents much the same pattern as at 150 days. Here again, the differences due to breed of sire had increased in magnitude ($P < 0.001$) and the magnitude of effects due to the litter size had considerably decreased but were still highly significant ($P < 0.001$).

The effects on the weight of the lambs of the main factors considered in the 2^4 factorial experiment are presented in Table 8.

The effect due to maternal environment was highly significant ($P < 0.001$) up to 40 days and significant ($P < 0.05$) at 90 days.

Table 3. Analysis of Variance of Mean Birth Weights (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	0.462	0.462***
Breed of dam	1	4.284	4.284***
Sex of lamb	1	0.497	0.497***
Litter size	1	1.380	1.380***
Breed of sire X breed of dam	1	0.202	0.202*
Error	230	8.438	0.036

*** $P < 0.001$
* $P < 0.05$

Table 4. Analysis of Variance of Mean 40 Day Weights (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	1.232	1.232
Breed of dam	1	32.776	32.776***
Sex of lamb	1	2.907	2.907*
Litter size	1	22.992	22.992***
Breed of sire X breed of dam	1	9.030	9.030**
Error	230	131.539	0.571

*** $P < 0.001$ **$P < 0.01$ *$P < 0.05$

Table 5. Analysis of Variance of Mean 90 Day Weights (2^4 factorial design

Source	df	SS	MS
Breed of sire	1	11.747	11.747*
Breed of dam	1	77.924	77.924***
Sex of lamb	1	23.112	23.112***
Litter size	1	64.040	64.040***
Breed of sire X breed of dam	1	6.382	6.382
Error	230	577.402	2.510

*** $P < 0.001$
* $P < 0.05$

Table 6. Analysis of Variance of Mean 150 Day Weights (2^4 factorial design

Source	df	SS	MS
Breed of sire	1	105.524	105.524***
Breed of dam	1	189.681	189.681***
Sex of lamb	1	191.338	191.338***
Litter size	1	63.242	63.242***
Breed of sire X breed of dam	1	17.741	17.741
Error	230	1085.295	4.718

*** $P < 0.001$

Table 7. Analysis of Variance of Mean 190 Day Weights (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	202.421	202.421***
Breed of dam	1	419.123	419.123***
Sex of lamb	1	226.427	226.427***
Litter size	1	53.619	53.619***
Breed of sire X breed of dam	1	16.858	16.858
Error	223	1143.484	5.497

*** $P < 0.001$

Table 8. Effects of the Main Factors on the Weight of the Lamb

	Birth	Weaning	90 days	150 days	190 days
Mean weight of all lambs (kg)	3.95	12.33	22.68	40.25	47.32
Factors					
Breed of sire (Lin x Lin + Lin x Sd)-(Sd x Lin + Sd x Sd) Lambs by Lin sires heavier by:	0.34***	0.56	1.71*	5.14***	7.14***
Breed of dam (Lin x Lin + Sd x Lin)-(Lin x Sd + Sd x Sd) Lambs born to Lin dams heavier by:	1.04***	2.86***	4.41***	6.89***	10.26***
Maternal influence due to Lin dam (Sd x Lin - Lin x Sd)	0.69***	2.31***	2.70*	1.75	3.12
Sex of lamb: males heavier than females by:	0.35***	0.85*	2.41***	6.92***	7.50***
Litter size: singles heavier than twins by:	0.59***	2.39***	4.01***	3.95***	3.69***
Interaction breed of sire x breed of dam (effects of)	0.21*	1.50**			

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$

Even though this effect was not statistically significant at 150 and 190 days it still accounted for 4.0% and 6.6% of the total variation respectively.

It has been previously shown that at birth the growth advantage of male twins (MM) over female twins (FF) is further enhanced if the litter mate is a female (MF) (Donald and Purser, 1956). The effect of sex and genotype of litter mate on birth weight is shown in Table 9.

Table 9. Effect of Sex of Co-twin on Birth Weight (kg)

Breed of the lamb	*Difference in birth wt. of sexes				Enhancement a-b
	Unlike		Like		
	N	(a)	N	(b)	
Lincoln	16	0.50	14	0.55	-0.05
Lin x Sd	14	0.03	8	-0.68	0.71
Sd x Lin	26	0.68	22	0.36	0.32
Southdown	10	0.28	6	0.87	-0.59

N - total number of animals in the group
* - difference between sexes: male - female

In this study there was in fact a diminished weight advantage in Lincoln and Southdown male lambs born co-twin with females. In the case of crossbred lambs born to Southdown dams female (FF) twins were heavier than male (MM) twins which resulted in an enhancement of 0.71 kg. In the case of crossbred lambs out of Lincoln dams there is a clear

evidence of enhancement in the weight of the male lamb when the co-twin was female.

2. Cannon bone length

Mean values for cannon bone length from birth to 150 days are presented in Table 10. These values have also been plotted in Fig. 4 and 5 for male lambs born as single and twins and for female lambs born as single and twins respectively.

At all ages Lincoln lambs had the longest cannon bones in all the groups and the Southdown lambs the shortest. The reciprocal cross lambs fell between these two groups.

At birth, Lincoln cannon bones were 22.0 mm longer than those of Southdowns. The difference increased with age and at 150 days Lincoln cannon bones were 32.0 mm longer. Southdowns at this age were still 1.5 mm shorter to the bone length recorded in Lincoln lambs at birth.

Sd x Lin lambs had longer cannon bones than Lin x Sd lambs. The initial difference of 1.6 mm at birth increased to 2.4 mm at 50 days. At 90 days the difference was the same as at 50 days and at 150 days it was 2.0 mm.

The difference between the reciprocal cross lambs was much more pronounced among male lambs (Fig. 4) than in females (Fig. 5) where there was hardly any difference in bone length.

Reciprocal cross lambs on an average had slightly longer cannon bones than MPM at birth which increased to 1.3 mm by the time lambs were 50 days old. However, at 90 days the difference narrowed considerably and at 150 days cannon bones of reciprocal cross lambs were longer by 0.3 mm than the MPM.

Table 10. Mean Cannon Bone Length of Lambs at Various Ages

Sire	Lincoln									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	8	17	14	17	ϕ Lin Average	30	15	19	13	ϕ Lin x Sd Average
Length (mm)										
Birth (S.E.)	75.92 (±1.93)	74.88 (±1.17)	74.46 (±1.57)	73.20 (±0.91)	74.62 (±0.53)	64.12 (±0.70)	61.53 (±0.81)	63.79 (±0.52)	63.31 (±1.01)	63.18 (±0.53)
50 days (S.E.)	89.12 (±3.16)	88.80 (±1.02)	88.33 (±1.85)	84.79 (±0.83)	87.67 (±0.66)	76.84 (±0.74)	72.29 (±1.05)	75.34 (±0.95)	75.03 (±1.26)	74.87 (±0.66)
90 days (S.E.)	97.19 (±3.20)	96.51 (±1.10)	95.98 (±2.19)	92.47 (±1.01)	95.53 (±0.70)	82.82 (±0.77)	79.04 (±1.22)	80.73 (±0.84)	79.50 (±1.34)	80.52 (±0.70)
150 days (S.E.)	107.20 (±2.56)	106.82 (±1.44)	103.29 (±2.56)	103.28 (±0.94)	105.14 (±0.71)	90.53 (±0.76)	87.81 (±1.26)	88.28 (±0.90)	87.15 (±1.28)	88.44 (±0.71)

M - males; F - females; S - singles; T - twins.

ϕ includes unweighted means of males, females, singles and twins.

Table 10. Mean Cannon Bone Length of Lambs at Various ages (cont'd)

Sire	Southdown									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	18	24	17	28	ϕ Sd x Lin Average	5	9	6	6	ϕ Sd Average
Length (mm)										
Birth (S.E.)	67.17 (±0.92)	64.88 (±0.53)	63.87 (±0.84)	63.34 (±0.40)	64.81 (±0.53)	54.40 (±0.75)	52.62 (±1.51)	52.92 (±1.14)	50.50 (±0.98)	52.61 (±0.53)
50 days (S.E.)	81.46 (±1.19)	76.20 (±0.67)	75.84 (±1.24)	75.69 (±0.67)	77.29 (±0.66)	64.05 (±1.29)	61.13 (±1.93)	63.60 (±1.35)	58.75 (±2.14)	61.88 (±0.66)
90 days (S.E.)	86.76 (±1.26)	81.52 (±0.85)	82.36 (±1.20)	80.62 (±0.69)	82.81 (±0.70)	68.88 (±0.93)	66.96 (±1.44)	67.67 (±1.49)	62.62 (±1.86)	66.53 (±0.70)
150 days (S.E.)	94.32 (±1.10)	90.89 (±0.83)	88.14 (±1.30)	88.63 (±0.66)	90.49 (±0.71)	74.88 (±0.88)	73.54 (±1.20)	73.43 (±1.20)	70.72 (±1.51)	73.14 (±0.71)

M - males; F - females; S - singles; T - twins.

ϕ includes unweighted means of males, females, singles and twins.

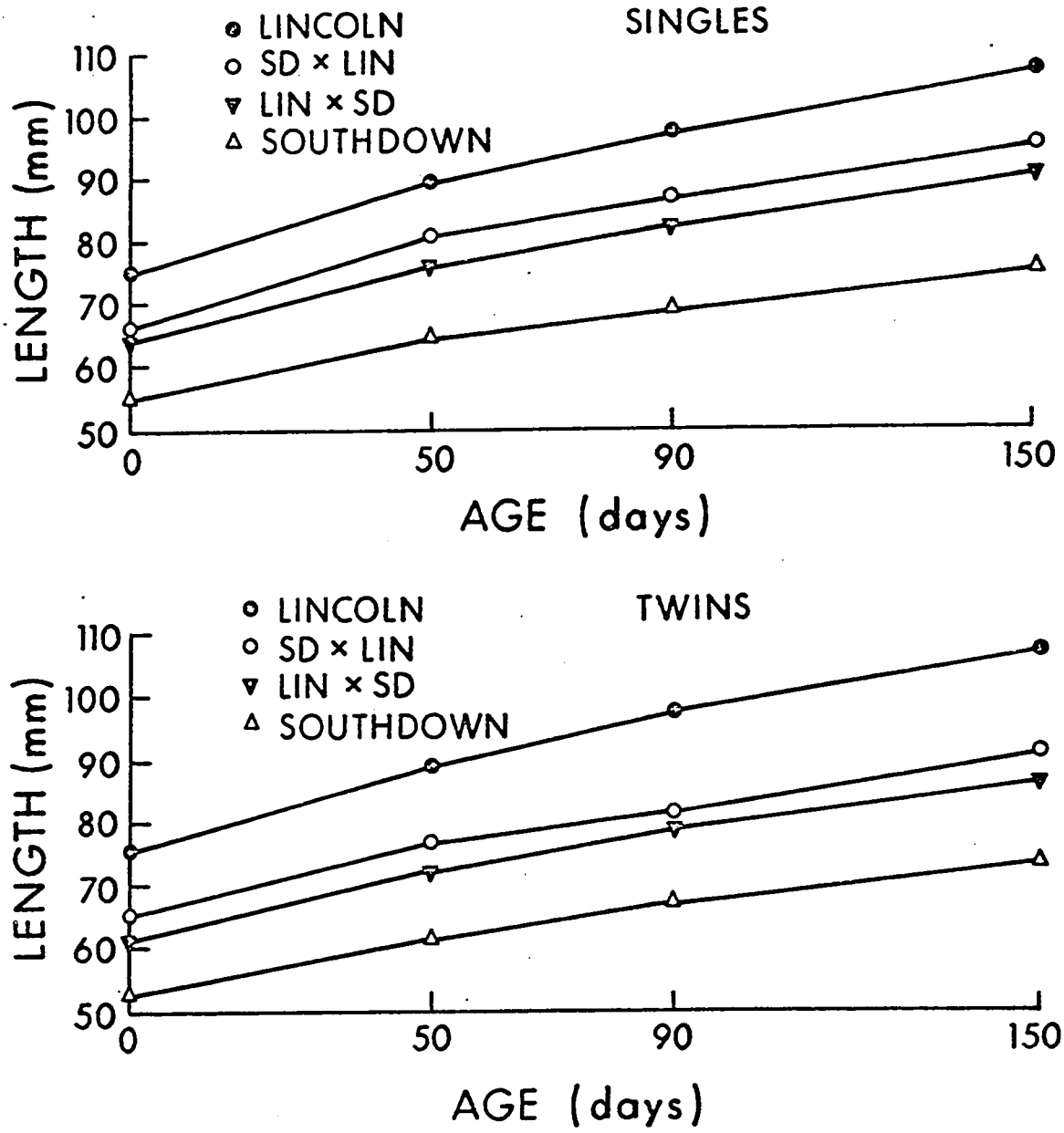


Fig. 4. Mean cannon bone length of single and twin males plotted against age for Lincolns, Southdowns and their reciprocal crosses.

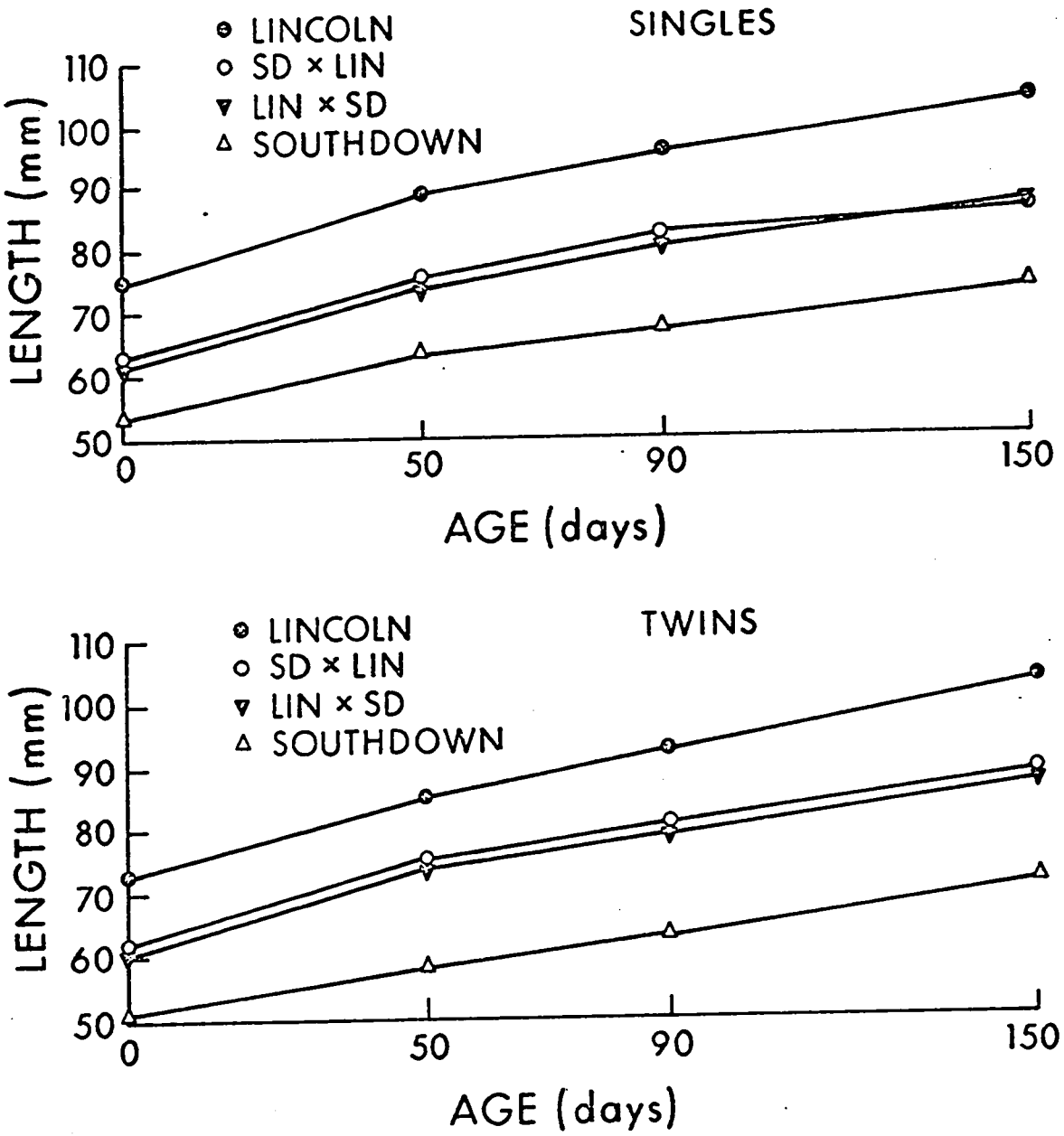


Fig. 5. Mean cannon bone length of single and twin females plotted against age for Lincolns, Southdowns and their reciprocal crosses.

Sd x Lin lambs had longer cannon bones by 1.2 mm, 2.5 mm, 1.8 mm and 1.4 mm than the MPM at birth, 50 days, 90 days, and 150 days respectively. There was no difference between Lin x Sd cannon bone length and MPM up to 90 days of age. At 150 days, however, Lin x Sd lambs had 0.7 mm shorter cannon bones than the MPM.

At all periods studied, males had longer cannon bones than females. The difference at birth was 1.3 mm which increased slightly at 50 days and from then onwards the increase was rather rapid and by 150 days the males' cannon bones were longer by 2.9 mm than females.

At birth single lambs had 1.6 mm longer cannon bones than the lambs born as twins. At 50 days the difference was 2.7 mm which remained rather unchanged at 90 days. At 150 days however, the difference narrowed down considerably, so much so that the difference at this age was less than the difference recorded at birth.

Analysis of mean values in Table 10 were carried out the same way as for body weight.

At birth breed of sire and breed of dam (Table 11) had highly significant effects ($P < 0.001$). Effects due to litter size and sex of lamb were also significant at ($P < 0.01$) and ($P < 0.05$) respectively. No first order interactions were significant at this age. Even though effects due to breed of sire and breed of dam were both highly significant, effects due to breed of dam were at a higher magnitude.

At 50 days (Table 12) effects due to breed of sire, breed of dam, and litter size were significant ($P < 0.001$). as were effects due to sex of lamb ($P < 0.05$). No first order interaction was significant.

At 90 days (Table 13) all the main factors were significant ($P < 0.001$). No first order interactions were significant. The effect

Table 11. Analysis of Variance of Mean Birth Cannon Bone Lengths (24 factorial design)

Source	df	SS	MS
Breed of sire	1	415.242	415.242***
Breed of dam	1	558.495	558.495***
Sex of lamb	1	6.413	6.413*
Litter size	1	9.594	9.594**
Error	230	261.183	1.165

*** P< 0.001 ** P< 0.01 * P< 0.05

Table 12. Analysis of Variance of Mean 50 Day Cannon Bone Lengths (24 factorial design)

Source	df	SS	MS
Breed of sire	1	550.137	550.137***
Breed of dam	1	800.890	800.890***
Sex of lamb	1	9.796	9.796*
Litter size	1	29.975	29.975***
Error	230	399.790	1.790

*** P< 0.001 * P< 0.05

due to sex of lamb had doubled as compared to 50 days of age. Again, the breed of dam was the major effect at this age.

At 150 days, breed of sire, breed of dam, and sex of lamb were still the major effects (Table 14) contributing to the bone length of lamb ($P < 0.001$). However, effects due to litter size and first order interactions were nonsignificant.

The average effects of the main factors on the length of the cannon bone are given in Table 15.

The effects due to the maternal environment were 3.67 mm at birth, 2.42 mm at 50 days, 2.29 mm at 90 days and 2.05 mm at 150 days.

Even though the effect due to maternal environment was small at birth and decreased as the lambs grew, it was nevertheless highly significant at birth ($P < 0.001$). At 50 days the significance was at ($P < 0.01$) and at 90 and 150 days it was significant at ($P < 0.05$).

The effect of sex and genotype of littermate on the cannon bone length is presented in Table 16.

In the Lin x Sd lambs the enhancement was 6.9 mm and for Sd x Lin it was only 0.2 mm. There was a negative enhancement on cannon bone length in Lincoln and Southdown lambs when the co-twin was a female.

3. Cannon bone width

The mean values for cannon bone width are presented in Table 17. There was less than 1 mm difference between the lambs in four groups. However, the differences followed on the same pattern as that of cannon bone length.

Table 13. Analysis of Variance of Mean 90 Day Cannon Bone Lengths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	713.290	713.290***
Breed of dam	1	979.220	979.220***
Sex of lamb	1	19.691	19.691***
Litter size	1	33.437	33.437***
Error	230	458.801	1.994

*** $P < 0.001$

Table 14. Analysis of Variance of Mean 150 Day Cannon Bone Lengths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	907.967	907.967***
Breed of dam	1	1157.530	1157.530***
Sex of lamb	1	31.221	31.221***
Litter size	1	6.721	6.721
Error	230	465.427	2.050

*** $P < 0.001$

Table 15. Effects of Main Factors on Cannon Bone Length

	Birth	50 days	90 days	150 days
Mean length of all lambs (mm)	64.06	75.45	81.35	89.31
Factors				
Breed of sire (Lin x Lin + Lin x Sd)-(Sd x Lin + Sd x Sd) Lambs by Lin sires longer by:	8.64***	11.73***	13.27***	14.98***
Breed of dam (Lin x Lin + Sd x Lin)(Lin x Sd)+ Sd x Sd) Lambs born to Lin dams longer by:	12.32***	14.15***	15.57***	17.03***
Maternal influence due to Lin dam (Sd x Lin - Lin x Sd)	3.68***	2.42**	2.29*	2.05*
Sex of lamb: males longer than females by:	1.27*	1.57*	2.22***	2.88***
Litter size: singles longer than twins by:	1.55***	2.74***	2.89***	1.40

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$

Table 16. Effect of Sex of Co-twin on Cannon Bone Length at Birth (mm)

Breed of the lamb	*Difference in birth length between sexes: Unlike N	Unlike (a)	Like N	Like (b)	Enhancement a-b
Lincoln	16	1.37	14	2.40	-1.03
Lin x Sd	14	-0.86	8	-7.78	6.92
Sd x Lin	26	1.73	22	1.51	0.22
Southdown	10	0.50	6	3.88	-3.38

N - total number of lambs in the group.
* - difference between sexes: males - females.

Table 17. Mean Cannon Bone Width of Lambs at Various Ages

Sire	Lincoln									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	8	17	14	17	ϕ Lin Average	30	15	19	13	ϕ Lin x Sd Average
Width (mm)										
Birth (S.E.)	11.56 (±0.28)	11.31 (±0.18)	11.11 (±0.26)	10.94 (±0.18)	11.23 ±0.11	11.17 (±0.13)	10.36 (±0.17)	11.00 (±0.21)	10.15 (±0.14)	10.67 ±0.11
50 days (S.E.)	12.80 (±0.64)	12.80 (±0.31)	13.11 (±0.37)	12.27 (±0.17)	12.74 ±0.14	13.22 (±0.16)	11.86 (±0.21)	12.72 (±0.27)	11.95 (±0.15)	12.43 ±0.14
90 days (S.E.)	14.06 (±0.65)	14.32 (±0.37)	13.91 (±0.41)	13.59 (±0.22)	13.97 ±0.17	14.50 (±0.17)	13.58 (±0.53)	13.95 (±0.25)	12.82 (±0.22)	13.71 ±0.17
150 days (S.E.)	16.82 (±0.63)	16.65 (±0.34)	15.41 (±0.46)	15.40 (±0.27)	16.07 ±0.17	16.64 (±0.14)	15.60 (±0.41)	15.60 (±0.30)	14.34 (±0.35)	15.54 ±0.17

M - males; F - females; S - singles; T - twins.

ϕ includes unweighted means of males, females, singles and twins.

Table 17. Mean Cannon Bone Width of Lambs at Various Ages (cont'd)

Sire	Southdown									
Dam	Lincoln					Southdown				
Sex	M		F			M		F		
Litter	S	T	S	T		S	T	S	T	
Number	18	24	17	28	ϕ Sd x Lin Average	5	9	6	6	ϕ Sd Average
Width (mm)										
Birth (S.E.)	11.86 (±0.20)	10.88 (±0.17)	10.75 (±0.19)	10.62 (±0.14)	11.02 ±0.11	11.40 (±0.19)	10.62 (±0.28)	10.66 (±0.17)	9.70 (±0.37)	10.59 ±0.11
50 days (S.E.)	13.94 (±0.25)	12.44 (±0.19)	12.72 (±0.22)	12.35 (±0.15)	12.86 ±0.14	13.26 (±0.28)	12.01 (±0.37)	12.21 (±0.19)	10.83 (±0.37)	12.07 ±0.14
90 days (S.E.)	14.88 (±0.21)	13.53 (±0.26)	13.94 (±0.21)	13.31 (±0.16)	13.91 ±0.17	14.24 (±0.21)	13.63 (±0.31)	13.57 (±0.37)	12.22 (±0.52)	13.41 ±0.17
150 days (S.E.)	16.77 (±0.20)	15.83 (±0.26)	15.32 (±0.26)	14.88 (±0.18)	15.70 ±0.17	16.52 (±0.44)	15.58 (±0.25)	14.45 (±0.30)	14.17 (±0.49)	15.18 ±0.17

M - males; F - females; S - singles; T - twins.

ϕ includes the unweighted means of males, females, singles and twins.

At birth, Lincoln lambs had 0.6 mm wider cannon bones than Southdown lambs. This difference increased slightly at 50 days and at 90 days the difference narrowed down only to increase again at 150 days at which time Lincoln cannon bones were wider by 0.9 mm.

Sd x Lin lambs had wider cannon bones than Lin x Sd lambs at all periods studied in the present experiment. The difference at birth was 0.4 mm, which remained unchanged at 50 days. From 50 days onwards there was a steady decline and at 150 days the difference was only 0.2 mm.

At birth, purebred lambs had 0.1 mm wider cannon bones than reciprocal cross lambs. However, at 50 days the reverse was true at which time reciprocal cross lambs had 0.2 mm wider cannon bones than purebred lambs. At 90 days the 50 day difference narrowed down to 0.1 mm and at 150 days there was no difference in the cannon bone width between purebred and reciprocal cross lambs.

Male lambs had wider cannon bones than females. The difference of 0.5 mm at birth decreased slightly at 50 days. From this period onwards the difference widened with age and at 150 days the difference was 1.3 mm.

There was a difference of 0.6 mm at birth between singles and twins lambs. The birth difference increased at 50 days at which time single lambs had cannon bones wider by 0.9 mm. However, at 90 days the difference narrowed down a little and continued to be so at 150 days, at which time male cannon bones were wider by only 0.6 mm.

Analysis of the mean values in Table 17 were carried out the same way as for body weights.

At birth (Table 18) the effects due to breed of dam, sex of lamb and litter size were highly significant ($P < 0.001$). The effects due to breed of sire and first order interactions were not significant.

The analysis of 150 day cannon bone width (Table 19) present much the same pattern as at birth. Here again, the effects due to breed of dam, sex of lamb and litter size were significant ($P < 0.001$). The effects due to breed of sire and first order interaction were not significant.

At 90 days, sex of lamb and litter size were still the major effects (Table 20) contributing to the cannon bone width of lambs ($P < 0.001$). The effect due to breed of dam was also significant ($P < 0.05$). Neither the effect due to breed of sire nor any first order interactions were significant at this age.

At 150 days (Table 21) effects due to sex of lamb and litter size were highly significant ($P < 0.001$). However, the difference due to sex of lamb were at a higher magnitude than at any other previous period. The effects due to the breed of dam were also significant ($P < 0.05$). Here again, the differences due to the breed of sire and first order interaction were not significant.

The effects on cannon bone width of the main factors considered are presented in Table 22.

The effect of maternal environment on the cannon bone width was 0.35 mm at birth, 0.42 mm at 50 days, 0.20 mm at 90 days and only 0.16 mm at 150 days.

The influence due to maternal environment was significant ($P < 0.05$) at birth and 50 days only.

Table 18. Analysis of Variance of Mean Birth Cannon Bone Widths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	0.075	0.075
Breed of dam	1	0.983	0.983***
Sex of lamb	1	1.114	1.114***
Litter size	1	1.515	1.515***
Error	230	11.880	0.052

*** $P < 0.001$

Table 19. Analysis of Variance of Mean 50 Day Cannon Bone Widths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	0.056	0.056
Breed of dam	1	1.207	1.207***
Sex of lamb	1	1.077	1.077***
Litter size	1	3.520	3.520***
Error	230	18.825	0.084

*** $P < 0.001$

Table 20. Analysis of Variance of Mean 90 Day Cannon Bone Widths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	0.125	0.125
Breed of dam	1	0.574	0.574*
Sex of lamb	1	1.849	1.849***
Litter size	1	2.283	2.283***
Error	230	27.558	0.120

*** P< 0.001 * P< 0.05

Table 21. Analysis of Variance of Mean 150 Day Cannon Bone Widths (2^4 factorial design)

Source	df	SS	MS
Breed of sire	1	0.234	0.234
Breed of dam	1	0.636	0.636*
Sex of lamb	1	8.770	8.770***
Litter size	1	2.319	2.319***
Error	230	27.915	0.121

*** P< 0.001 * P< 0.05

Table 22. Effects of Main Factors on Cannon Bone Width

	Birth	50 days	90 days	150 days
Mean width of all lambs (mm)	10.88	12.52	13.75	15.59
Factors				
Breed of sire (Lin x Lin + Lin x Sd)-(Sd x Lin + Sd x Sd) Lambs by Lin sires wider by:	0.14	0.14	0.18	0.44
Breed of dam (Lin x Lin + Sd x Lin)-(Lin x Sd + Sd x Sd) Lambs born to Lin dams wider by:	0.47***	0.57***	0.38*	0.46*
Maternal influence due to Lin dam (Sd x Lin - Lin x Sd)	0.36*	0.43*	0.20	0.16
Sex of lamb: males wider than females by:	0.53***	0.52***	0.68***	1.35***
Litter size: singles wider than twins by:	0.62***	0.88***	0.76***	0.64***

*** $P < 0.001$ * $P < 0.05$

The effect of sex of co-twin on cannon bone width at birth is presented in Table 23.

Table 23. Effect of Co-twin on Cannon Bone Width at Birth (mm)

Breed of the lamb	*Difference in bone width between sexes: Unlike N	Unlike (a)	Like N	Like (b)	Enhancement a-b
Lincoln	16	0.63	14	0.07	0.56
Lin x Sd	14	0.14	8	0.00	0.14
Sd x Lin	26	0.31	22	0.13	0.18
Southdown	10	0.83	6	0.50	0.33

N - total number of lambs in the group.
* - differences between sexes: males - females.

In all the groups there was enhancement in the cannon bone width when the sex of co-twin was a female. The enhancement was greatest in Lincoln lambs, followed by Southdown, Sd x Lin and Lin x Sd lambs.

B. Ovum transfer experiment

1. Ovulation and ova recovery

Thirty-one ewes from each breed were paired such that the donor ewe of one breed was also the recipient of ova flushed from the ewe of the opposite breed to which she acted as donor. However, only 20 Lincoln and 21 Southdown ewes were transferred with ova of the opposite breed (Appendix II).

Sixteen ewes from each breed were bred straight and their lambs kept as controls.

Among Lincolns, of a total of 49 ovulations, 29 (59.2%) were on the left ovary and among Southdowns, 28 (60.9%) of a total of 46 ovulations were on the left side (Table 24).

During the main investigation, ova recovery was higher from Southdown donors (84.8%) than from the Lincoln (73.5%). Average ova recovery from both breeds was 78.9%.

Table 24. Ovulation Pattern and Number of Ova Recovered in the Experimental Group

Breed	N	Ovulation Points on the ovaries		Total	No. of Ova Recovered	% Recovery
		L	R			
Lincoln	31	29 (59.2%)	20 (40.8%)	49	36	73.5
Southdown	31	28 (60.9%)	18 (39.1%)	46	39	84.8
Total	62	57 (60.0%)	38 (40.0%)	95	75	78.9

L - left; R - right.

The number of ova transferred into a recipient depended on the number of ova recovered from the donor. In no cases, however, were more than two ova transferred into a recipient.

The transfer of 8-cell ova into Lincoln recipients, 65 to 68 hours after mating, gave 100% results (Table 25). Six 8-cell ova were transferred to three recipients and all lambed giving birth to three sets of twins. Of three recipients of 4-cell ova, only one lambed giving birth to a set of twins. The one recipient of a 16-cell ovum did not lamb.

None of the Lincoln recipients of 4-cell ova 69 to 72 hours post mating lambed. However, all eight recipients of 6-cell, 8-cell and 16-cell ova lambed. Only one of the two recipients of 32-cell ova lambed (Table 25).

Among Southdowns at 65 to 68 hours after mating only two of four recipients of 4-cell and the two recipients of four 8-cell ova lambed giving birth to a total of six lambs. Neither of the recipients of 6 and 32-cell ova lambed.

At 69 to 72 hours post-mating, only three of seven recipients of 8-cell ova and one of two recipients of 32-cell ova lambed. The recipient of a 4-cell ovum did not lamb (Table 25).

Among Lincoln ewes carrying Southdown lambs, gestation lengthened by 1.2 days over the average gestation of the breed and for Southdown ewes carrying Lincoln lambs, gestation was shortened by 3.4 days when compared with the average gestation length of the breed (Table 26). There was no difference in the gestation length between the ewes carrying single and twin lambs. In the ova transferred group,

Table 25. Summary of Ova Transfers in Lincoln and Southdown Ewes

Lincoln						Southdown					
Stage of estrus in the recipient (from time of mating)	Stage of ova	No. of recipients	No. of recipients lambed	No. of ova transferred	No. of lambs born	Stage of estrus in the recipient (from time of mating)	Stage of ova	No. of recipients	No. of recipients lambed	No. of ova transferred	No. of lambs born
65-68 hr.	4-cell	3	1	5	2	65-68 hr.	4-cell	4	2	6	3
	6-cell	1	0	1	0		6-cell	2	0	4	0
	8-cell	3	3	6	6		8-cell	4	2	7	3
	16-cell	1	0	1	0		16-cell	-	-	-	-
	32-cell	-	-	-	-		32-cell	1	0	1	0
Total		8	4	13	8	Total		11	4	18	6
69-72 hr.*	4-cell	2	0	2	0	69-72 hr.	4-cell	1	0	1	0
	6-cell	1	1	1	1		6-cell	-	-	-	-
	8-cell	6	6	10	8		8-cell	7	3**	8	3
	16-cell	1	1	1	1		16-cell	-	-	-	-
	32-cell	2	1	2	1		32-cell	2	1	2	1
Total		12	9	16	11	Total		10	4	11	4
Overall total		20	13	29	19	Overall total		21	8	29	10

* Most of the transfers were performed at 72 hr.

** Includes one SD ewe, which died 3 weeks before parturation

gestation length was calculated from the time donor was bred until lambing of the recipient.

Table 26. Average Gestation Length in Days

Type of Pregnancy	N	Lincoln	N	Southdown
ϕ Carrying their own lambs	8	144.8 (±0.55)	8	147.2 (±1.28)
Carrying lambs of opposite breed	13	146.0 (±0.43)	8	143.6 (±1.70)

ϕ Averages of 1968 lambing due to artificial insemination.

2. Birth weight

In the present experiment gestation length did not seem to affect birth weight. However, due to small numbers in some groups this could not be very well established.

Lambs of the same breed differed in birth weight (Table 27) according to whether their uterine environment was Lincoln or Southdown and lambs reared in the same uterine environment differed in birth weight according to whether their breed was Lincoln or Southdown.

No relationship ($r = 0.04$) was found between the lamb's birth weight and donor's weight nor with the recipient's weight ($r = 0.09$) taken at the time of ova transfer.

Analysis of birth weights in Table 27 was carried out the same way as for the body weights described in the reciprocal cross experiment.

Table 27. Average Weight, Cannon Bone Length and Cannon Bone Width at Birth (with standard error)

Breed of dam	Lincoln							
Breed of lamb	Lincoln				Southdown			
Sex of lamb	M		F		M		F	
Litter size	S	T	S	T	S	T	S	T
Number	4	10	4	7	*4	4	*4	ϕ9
Birth weight (kg)	5.25 (±0.62)	4.33 (±0.15)	3.95 (±0.72)	3.96 (±0.37)	4.66 (±0.39)	3.48 (±0.22)	4.07 (±0.39)	3.43 (±0.19)
Cannon bone length (mm)	77.25 (±2.05)	74.25 (±0.91)	70.87 (±3.20)	72.57 (±1.84)	54.62 (±1.24)	53.50 (±1.25)	54.37 (±1.24)	53.11 (±0.73)
Cannon bone width (mm)	11.62 (±0.23)	11.20 (±0.21)	10.37 (±0.80)	10.57 (±0.13)	11.50 (±0.54)	10.75 (±0.32)	11.25 (±0.47)	10.72 (±0.25)

M - males; F - females; S - singles; T - twins.

* includes one lamb born as a result of ova transfers performed during 1968-1969.

ϕ includes one lamb born "twin" to a natural born Lincoln female.

Table 27. Average Weight, Cannon Bone Length and Cannon Bone Width at Birth (cont'd)

Breed of dam	Southdown							
Breed of lamb	Lincoln				Southdown			
Sex of lamb	M		F		M		F	
Litter size	S	T	S	T	S	T	S	T
Number	*2	*3	4	3	7	1	4	11
Birth weight (kg)	4.21 (±1.18)	3.17 (±0.38)	3.95 (±0.53)	4.17 (±0.09)	3.33 (±0.33)	3.00 (±0.00)	3.60 (±0.23)	2.62 (±0.18)
Cannon bone length (mm)	74.00 (±6.00)	66.17 (±2.58)	70.75 (±3.90)	71.67 (±1.20)	54.67 (±0.98)	52.00 (±0.00)	51.80 (±2.63)	51.72 (±1.34
Cannon bone width (mm)	10.75 (±1.25)	10.33 (±0.67)	10.87 (±0.65)	10.50 (±0.28)	11.16 (±0.33)	9.50 (±0.00)	10.90 (±0.48)	10.18 (±0.26)

M - males; F - females; S - singles; T - twins.

* includes one lamb born as a result of ova transfers performed during 1968-.969.

Effects due to the maternal environment, breed of the lamb and litter size (Table 28) were highly significant ($P < 0.01$). Neither the effects of sex of lamb nor any interactions were significant.

Table 28. Analysis of Variance of Mean Birth Weights (2^4 factorial design)

Source	df	SS	MS
Maternal environment	1	1.487	1.487**
Breed of lamb	1	1.563	1.563**
Sex of lamb	1	0.219	0.219
Litter size	1	1.610	1.610**
Maternal environment X Breed of lamb	1	0.105	0.105
Maternal environment X Sex of lamb	1	0.463	0.463
Maternal environment X Litter size	1	0.009	0.009
Breed of lamb X Sex of lamb	1	0.010	0.010
Breed of lamb X Litter size	1	0.088	0.088
Sex of lamb X Litter size	1	0.325	0.325
Error	65	11.916	0.183

** $P < 0.01$

Lincoln male twins seemed to be much more affected in the Southdown uterine environment than the Lincoln male singles. Lincoln male twins carried by Lincoln dams were 1.16 kg (26.8%) heavier than when reared in the Southdown uterine environment whereas male, single Lincolns were only 1.04 kg (19.8%) heavier in their natural uterine environment.

Maternal environment did not have any effect on Lincoln single females. However, Lincoln female twins weighed 0.21 kg (5.3%) more when raised in the Southdown uterine environment, than when raised in their natural dams (Table 29).

Based on unweighed sex and litter size means, Lincoln lambs raised in the Lincoln dams were 0.50 kg (10.3%) heavier than when raised in Southdown dams.

Southdown lambs, both male singles and twins and female singles and twins were heavier at birth by 1.33 kg (40.0%), 0.48 kg (16.0%), 0.47 kg (13.1%) and 0.82 kg (31.3%) respectively when reared in Lincoln dams than when carried by their natural dams. On average, Southdown lambs raised in Lincoln dams were 0.8 kg (25.1%) heavier than Southdowns carried by their natural mothers.

Looking at the overall average based on unweighted sex and litter means, lambs raised in Lincoln dams were 0.63 kg (17.6%) heavier than lambs raised in Southdown dams (Table 29).

Lincoln lambs, except single females, were heavier than their Southdown counterparts in both Lincoln and Southdown dams (Table 30).

In Lincoln dams, single Lincoln females recorded lower average birth weights than Southdown female single lambs in the same environment.

Table 29. Effect of Maternal Environment on Birth Weight of Lambs (Lincoln-Southdown)

Breed of lamb	Weight (kg)	Sex	Type of Birth	Weight (kg)
Lincoln	0.50 (±0.21)	M	S	1.04 (±0.36)
			T	1.16 (±0.28)
		F	S	0.00 (±0.30)
			T	-0.21 (±0.34)
Southdown	0.80 (±0.21)	M	S	1.33 (±0.26)
			T	0.48 (±0.36)
		F	S	0.47 (±0.30)
			T	0.82 (±0.18)
Average		M		1.01 (±0.15)
		F		0.27 (±0.12)
		S		0.71 (±0.21)
		T		0.57 (±0.21)
Overall Average				0.63 (±0.09)

M - males; F - females; S - singles; T - twins.

The difference, however, was only 0.1 kg. In Lincoln dams, Lincoln lambs were 0.45 kg (11.1%) heavier than Southdown lambs, whereas in Southdown dams Lincoln lambs were 0.73 kg (20.7%) heavier than Southdown lambs. The variance in breed of lamb accounted for 0.59 kg (15.4%) of the total variance.

Table 30. Effect of Breed of Lamb on Birth Weight (Lincoln-Southdown)

Maternal Environment	Weight (kg)	Sex	Type of Birth	Weight (kg)
Lincoln	0.45 (±0.21)	M	S	0.59 (±0.30)
			T	0.85 (±0.25)
		F	S	-0.12 (±0.30)
			T	0.51 (±0.21)
Southdown	0.73 (±0.21)	M	S	0.88 (±0.45)
			T	0.17 (±0.47)
		F	S	0.30 (±0.30)
			T	1.55 (±0.27)
Average		M		0.62 (±0.15)
		F		0.56 (±0.12)
		S		0.41 (±0.21)
		T		0.78 (±0.21)
Overall Average				0.59 (±0.09)

M - males; F - females; S - singles; T - twins.

In all cases, except Lincoln females, singles were heavier than twins of the same breed (Table 31). Lincoln female twins were heavier by 0.1 kg than the Lincoln females born as singles. Male single lambs were heavier by 0.86 kg to male twins and in females the difference was about 0.35 kg. Single lambs on the whole were heavier by 0.6 (15.0%) than twin lambs.

Table 31. Effect of Litter Size on Birth Weight (Singles) - (Twins)

Breed of lamb	Weight (kg)	Sex	Weight (kg) Difference
Lincoln	0.43 (±0.21)	M	0.98 (±0.24)
		F	-0.11 (±0.20)
Southdown	0.78 (±0.21)	M	0.75 (±0.22)
		F	0.81 (±0.18)
Average		M	0.86 (±0.14)
		F	0.35 (±0.13)
Overall Average			0.61 (±0.09)

M - males; F - females; S - singles; T - twins

3. Cannon bone length

There was small difference in the cannon bone length (Table 27) between lambs of the same breed reared in different maternal environments.

Effects due to the genotype of the lamb were highly significant ($P < 0.001$) and effects due to all other major factors and interactions were not significant (Table 32).

On average, Lincoln lambs had 3.08 mm longer cannon bones when raised in Lincoln dams than when they were raised in Southdown dams (Table 33).

Southdown lambs averaged 1.60 mm longer cannon bones when reared in Lincoln dams than when reared in their normal environment.

Table 32. Analysis of Variance of Mean Cannon Bone Lengthsat Birth (2^4 factorial design)

Source		SS	MS
Maternal environment	1	19.740	19.740
Breed of lamb	1	1438.874	1438.874***
Sex of lamb	1	5.740	5.740
Litter size	1	11.128	11.128
Maternal environment X Breed of lamb	1	3.008	3.008
Maternal environment X Sex of lamb	1	3.806	3.806
Maternal environment X Litter size	1	2.217	2.217
Breed of lamb X Sex of lamb	1	0.256	0.256
Breed of lamb X Litter size	1	0.595	0.595
Sex of lamb X Litter size	1	15.800	15.800
Error	65	325.433	5.006

*** $P < 0.001$

Table 33. Effect of Maternal Environment on Cannon Bone Length at Birth (Lincoln-Southdown)

Breed of lamb	Length (mm)	Sex	Type of Birth	Length (mm)
Lincoln	3.08 (±1.11)	M	S	3.25 (±2.50)
			T	8.08 (±1.47)
		F	S	0.12 (±1.58)
			T	0.90 (±1.79)
Southdown	1.60 (±1.11)	M	S	-0.05 (±1.39)
			T	1.50 (±2.50)
		F	S	3.57 (±1.58)
			T	1.39 (±0.97)
Average		M		3.19 (±0.80)
		F		1.49 (±0.67)
		S		1.72 (±1.11)
		T		2.96 (±1.11)
Overall Average				2.34 (±0.50)

M - males; F - females; S - singles; T - twins.

Overall maternal environment effect on cannon bone length was 2.34 mm.

In both maternal environments Lincoln lambs on average had 18.96 mm (30%) longer cannon bones than Southdown lambs (Table 34).

4. Cannon bone width

There was small and non-significant difference in the cannon bone width between lambs of the same breed reared in differenct environments (Table 27). Only effects due to litter size were significant

Table 34. Effect of Lambs Genotype on Cannon Bone Length at Birth (Lincoln-Southdown)

Maternal Environment	Length (mm)	Sex	Type of Birth	Length (mm)
Lincoln	19.81 (±1.11)	M	S	22.63 (±1.58)
			T	20.75 (±1.32)
		F	S	16.50 (±1.58)
			T	19.46 (±1.11)
Southdown	18.10 (±1.11)		S	19.33 (±2.28)
			T	14.17 (±2.50)
			S	18.95 (±1.58)
			T	19.95 (±1.45)
Average		M		19.92 (±0.75)
		F		18.71 (±0.67)
		S		19.35 (±1.11)
		T		18.58 (±1.11)
Overall Average				18.96 (±0.49)

M - males; F - females; S - singles; T - twins.

($P < 0.05$) (Table 35).

Single Lincoln lambs had 0.25 mm wider cannon bones than the twins (Table 36). However, the magnitude of difference was greater in Southdown lambs where singles had 1.0 mm wider cannon bones than twins.

Table 35. Analysis of Variance of Mean Cannon Bone Widths at Birth (2^4 factorial design)

Source	df	SS	MS
Maternal environment	1	0.896	0.896
Breed of lamb	1	0.004	0.004
Sex of lamb	1	0.131	0.131
Litter size	1	1.374	1.374*
Maternal environment X Breed of lamb	1	0.083	0.083
Maternal environment X Sex of lamb	1	0.514	0.514
Maternal environment X Litter size	1	0.174	0.174
Breed of lamb X Sex of lamb	1	0.184	0.184
Breed of lamb X Litter size	1	0.435	0.435
Sex of lamb X Litter size	1	0.210	0.210
Error	65	14.750	0.226

* $P < 0.05$

Based on overall average, singles had 0.63 mm wider cannon bones than twins.

Table 36. Effect of Litter Size on Cannon Bone Width at Birth (Singles)-(Twins)

Breed of lamb	Width (mm)	Sex	Width (mm)
Lincoln	0.25 (±0.17)	M	0.42 (±0.23)
		F	0.08 (±0.22)
Southdown	1.02 (±0.17)	M	1.43 (±0.25)
		F	0.62 (±0.20)
Average		M	0.92 (±0.16)
		F	0.35 (±0.14)
Overall Average			0.63 (±0.10)

M - males; F - females; S - singles; T - twins.

IV. Discussion

In the present experiment, breed of lamb, maternal environment and litter size were found to be the most important factors affecting birth weight of lambs. Breed of lamb was the major source of variation in cannon bone length and effects due to maternal environment were small. Cannon bone width, however, was not subject to any variation due to the breed of lamb and there was only a small effect due to the maternal environment, but litter size was a major factor.

Hammond (1932) has shown that cannon bone length is one of the earliest maturing of all body measurements and is little affected by nutrition. In the present experiment, a maternal environmental effect of about 17% on birth weight and 5% on cannon bone length at birth was identical in both the reciprocal crossbreeding and ova transfer experiments. Maternal environment had more effect on the body weight than on cannon bone length which is in full accord with the results of Hunter (1956) and Dickinson *et al.* (1962).

Lincoln lambs carried by Lincoln dams were 0.50 kg (10.3%) heavier at birth than when carried by Southdown dams, while Southdown lambs carried by Lincoln dams were 0.80 kg (25.1%) heavier than when raised in their natural environment (Table 29).

Dickinson *et al.* (1962) proposed that so long as lambs were in their normal environment and their dams under reasonable levels of nutrition, mean birth size of the lamb would be near its upper genetic limit. The evidence for this came when ova were transferred between the small Welsh and the large Lincoln breeds. Their Lincoln lambs

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carried by Lincoln ewes were much larger than Lincoln lambs carried by Welsh ewes, while Welsh lambs exhibited "only" a 9% increase when they developed in Lincoln dams. Results of the present study do not confirm this. Here it was seen that the growth responses of small breed embryos given greater environmental latitude than normal was enhanced.

Effects on birth weight due to the breed of lamb were only 0.59 kg (15.0%) while on cannon bone length 18.2 mm (30.0%). These results are in partial agreement with those of Dickinson et al. (1962), who reported that the maternal influence on birth weight is much less than the genetic influence.

Maternal age and parity, two highly correlated characters, have been shown to effect birth weight of lambs. Mature ewes give birth to heavier lambs (Hazel and Terrill, 1946; Blackwell and Henderson, 1955; Hunter, 1956; McLean et al., 1968) and first parity dams tend to restrict the growth of lambs (Dickinson et al., 1962). In the present experiments the age of dams varied from one to four years. In terms of average effect, however, these effects should have cancelled out because the lambs in each group were born over a similar array of parities and maternal ages.

Terrill and Hazel (1947) reported that towards the end of the lambing season ewes tend to have shorter gestations consequently affecting the birth weight of the lamb. On the other hand, Hammond (1932) found that weights of one week old lambs born late in the season were considerable greater in the case of singles and slightly greater in the case of twins, than those of lambs born early. This he thought

was due to the better care given to the flock when lambing commenced. In the present study no difference was found between the lamb's date of birth and birth weight. Gestation length was not correlated with the birth weight of lambs. Experiments in the past have, however, demonstrated that the length of gestation had a significant effect at birth on the body size of the lamb (Hunter, 1956).

Lincoln ewes carrying Southdown lambs had longer gestation periods than Lincoln ewes carrying Lincoln lambs ($0.1 < P > 0.05$) and conversely, Southdown ewes carrying Lincoln lambs had shorter gestations than Southdown ewes carrying Southdown lambs ($0.1 < P > 0.05$). This suggests that a fetus that is considerably smaller than the normal for the breed of the ewe is carried longer in utero. These results are in full agreement with those of Hunter (1956); Dickinson et al. (1962); and Moore (1968).

At birth, the highly significant differences ($P < 0.001$) in the body weight and cannon bone width between singles and twins was confirmed by both the reciprocal cross and ova transfer experiments. The effects of litter size on cannon bone length, although highly significant ($P < 0.001$) in the reciprocal cross experiment, were small and non-significant in the ova transfer experiment. Numerous workers have shown litter size to have a significant effect on birth weight of the lamb (Price et al., 1951; Hammond, 1952; Shelton, 1964; Harrington and Whiteman, 1967).

In the reciprocal cross experiment, effects due to sex of lamb on cannon bone length and cannon bone width were highly significant ($P < 0.001$). However, this was not the case with the ova transfer

experiment where differences due to sex of lamb were found to be very small for all three traits which agrees with the results of Hunter (1956).

From the reciprocal crossbreeding experiment it was seen that the maternal effect on body weight at 40 days had slightly increased over that at birth (Table 8). Between birth and 40 days the lamb at first is entirely dependent on maternal care the most important feature of which is the dam's milk supply. Brumby (1960) cross-fostered young between small and large strains of mice. At weaning the performance of small strain young reared by large strain females proved no better than that of small strain young reared by small strain females. From this Brumby concluded that large strain females did not markedly differ from the small strain in lactational capacity and differences in maternal performance observed between the large and small strain must originate in the prenatal environment. Hunter (1956) did not find significant regression of total milk yield of the dam on the birth weight of lambs. Previously it had been seen in the University of Alberta flock that Lincoln ewes have more total milk yield than Southdown ewes (unpublished data) and by virtue of being better milkers, it may be that lambs reared by Lincoln dams had an advantage over lambs reared by Southdowns. At 90 days, effects due to the maternal environment although significant ($P < 0.05$) were far less in magnitude than at 40 days of age. At 150 and 190 days, no significant difference due to maternal environment was found.

Dickinson (1960) reported that in cattle there was a tendency for the growth curve of crossbred animals from smaller dams to cross

over the growth curve of reciprocal crossbred animals from larger dam towards the end of the most rapid growth period. In the present experiment this did not happen at any period when mean values of all lambs were considered. However, the growth curve of Lin x Sd single females crossed that of Sd x Lin single females at 150 days of age (Fig. 2) although this increase in weight of Lin x Sd females levelled off by 190 days.

The effect of maternal environment on cannon bone length (Table 15), although small as compared to body weight, was significant even at 150 days of age. The cannon bone attained about 69% of mature size in early prenatal life but grew very slowly post-natally. Therefore the differences in cannon bone length at birth due to the maternal environment remained relatively constant for a much longer period than the differences in later maturing body weight which in this experiment were non-significant at 150 days. However, differences in cannon bone width due to maternal influence became non-significant as early as 90 days (Table 22).

Litter size had a significant effect on postnatal growth in all three traits (Table 8, 15, 22). After 90 days the differences declined steadily in all cases until at 150 days the effect of litter size on cannon bone length was non-significant although the litter size effect was highly significant for both body weight and cannon bone width. Extrapolating this decline, the difference between singles and twins should be levelling off by one year of age.

Differences in body weight due to sex of lamb was small at birth but increased very rapidly with age. These results agree with

those of Blackwell and Henderson (1955); Shelton and Campbell (1962); and Harrington and Whiteman (1967).

Joubert (1956) has shown that in sheep the difference in male and female can be attributed in part to the number of cells in each muscle fibre. This number is determined by the 100th day of pregnancy and is greater in males than in females. Nalbandov (1963) hypothesised that availability of growth hormone per unit of tissue is a basic determinant of growth.

The difference in the body weight between males and females was highest at 190 days in this experiment (Table 8). As expected at this age the role of sex hormones may be of significance for at this age lambs had attained puberty. The endocrine control of postnatal growth and development is outside the scope of this thesis.

The effects of sex of lamb on cannon bone length and width followed the same pattern as for the body weights (Tables 15, 22). The results on cannon bone length are in full accord with that of McLean (1948) and Galal et al. (1965) but do not agree with those of Hunter (1956) who found no significant differences in cannon bone length between sexes.

From Tables 8 and 15, it is seen that when weight and cannon bone length are considered against the major effects such as breed of sire, breed of dam, sex of lamb and litter size, the effects due to breed of sire on body weight are relatively small in early life due to maternal influences but increase as the lambs grow older. This confirms the results of Hunter (1956). However, with reciprocal crossbreeding of cattle Touchberry and Bereskin (1966) reported that the difference between the magnitude of the effects of breed of dam and breed of sire

did not change with increased age.

When an increase in size or rate of growth in early life of lamb is required, it is more effective to introduce it from the dam rather than from sire. However, on the economics of lamb production the maternal influence is relatively less important since at the time of slaughter the maternal environmental effects are small and non-significant. From Fig. 3 and Table 2 it is seen that at 150 days the difference in the body weight of Sd x Lin and Lin x Sd lambs was 1.75 kg. It would take 12 and 27 days more for Sd x Lin and Lin x Sd to reach slaughter weight of 45 kg reached by Lincoln lambs at this age. In effect Lin x Sd lambs would have to be maintained for 15 more days than Sd x Lin lambs to reach slaughter weight of 45 kg. Thus in a crossbreeding program for lamb production it seems that little will be gained by maintaining large ewes than small ewes for breeding purposes. Even though lambs of small dams need few more days for finishing than the lambs of large dams, the maintaince cost of the dams is smaller than for bigger ewes and should, therefore, compensate for it.

Incidental to the main objectives of this investigation, both Lincoln and Southdown ewes ovulated 60% of the time from the left ovary. This does not agree with the findings of Nalbandov (1964) who reported that in sheep ovulation takes place on the right side 52 to 59% of the time. Average ova recovery from both the breeds was 78.9% which confirms the value of the simplified ova recovery technique, in the absence of superovulation, that was used in this investigation.

Only 50% of the Lincoln recipients lambed following transfer of ova 65 to 68 hours after mating (Table 25). However at this time,

the three recipients of six 8-cell ova lambed giving birth to three sets of twins. Among transfers made at 69 to 72 hours after mating, 75% of the Lincoln ewes lambed. For Southdown recipients, the comparable figures were 36% and 50% respectively. No explanation is apparent as to why ova survival was far less in Southdown recipients than in Lincolns. However, Combs (1965) reported that during the breeding season only 73% of Southdown ewes conceived as against 97% of the Lincoln ewes by natural service.

Moore and Shelton (1964) and Shelton and Moore (1966) concluded that ova survival increases significantly with the age of ova transferred. While this was generally the case in the present investigation, the main factor affecting ova survival seemed to be the stage of estrous cycle in the recipient ewe at the time of ova transfer.

From previous experiments it has been shown that ova transferred to the uterus were more successful than the ova transferred to the Fallopian tubes (Moore et al., 1960; Moore and Shelton, 1962). However in the present investigation, site of transfer was confounded with cell stage of ova transferred and as such this observation could not be confirmed.

SUMMARY AND CONCLUSIONS

Maternal environment, breed of lamb and litter size had a highly significant effect of 0.63 kg, 0.59 kg and 0.59 kg respectively on birth weight. Magnitude of effects due to maternal environment and litter size on birth weight were virtually identical in both the reciprocal crossbreeding and ova transfer experiments. Effects on birth weight due to sex of lamb, although highly significant (0.35 kg) in the reciprocal cross experiment, were relatively small and non-significant in the ova transfer experiment.

Southdown lambs carried by Lincoln dams were 25% heavier at birth than when carried in their natural environment, but Lincoln lambs carried by Lincoln dams were only 10% heavier at birth than when carried by Southdown dams. This suggests that growth response of Southdown embryos is greatly enhanced when given greater environmental latitude than normal.

At 40 days of age there was a slight increase in maternal environmental effects in body weight. This increase might have been due to the greater milk yield of Lincoln ewes. However, at 90 days, the maternal environmental effects, although significant, were far less in magnitu-e than at 40 days. At 150 and 190 days, no significant difference due to maternal environment was found.

Effects due to breed of sire, although highly significant at birth, were small and non-significant at 40 days. From 90 days onwards the sire effects increased with age. At 190 days lambs sired by Lincoln rams were 7.14 kg heavier than lambs sired by Southdown rams.

Postnatally, litter size had a highly significant effect on body weight. After 90 days, however, the differences declined steadily and at 190 days there was only 3.69 kg difference between singles and twins. Differences in the body weight due to sex of lamb increased rather rapidly with age of lambs. At 190 days, males were 7.50 kg heavier than females.

Breed of lamb accounted for 30% of the variation in cannon bone length at birth. The differences due to maternal environment were small and significant in the reciprocal cross experiment only. However, the difference at birth diminished very slowly and at 150 days the effects due to maternal environment were still significant.

Effects due to sex of lamb and breed of sire on cannon bone length followed the same pattern as that on body weight. Effects due to litter size even though highly significant up to 90 days of age were non-significant at 150 days of age.

Effects due to litter size on cannon bone width were highly significant at birth in both the reciprocal and ova transfer experiments. Postnatally, variation due to litter size on this trait followed the same pattern as that on body weight. Effects due to maternal environment and sex of lamb were significant in the reciprocal cross experiment only. However maternal environmental effects were significant only up to 40 days of age, but the variation due to sex increased with age and at 150 days males had 1.35 mm wider cannon bones than females.

There were no effects due to the breed of lamb or breed of sire on cannon bone width.

There was an enhancement effect on both body weight and cannon bone length at birth in Lin x Sd and Sd x Lin males born as co-twins to females. However, the reverse was true with Lincoln and Southdown male lambs when born as co-twins with females. On cannon bone width, the enhancement effect was noticed in all male lambs born as co-twins to females.

Lincoln ewes carrying Southdown lambs had longer gestations than Lincoln ewes carrying Lincoln lambs and, conversely, Southdown ewes carrying Lincoln lambs had shorter gestations than Southdown ewes carrying Southdown lambs. However, gestation length was not correlated with any of the traits at birth considered in these experiments.

In both breeds, the left ovary was seen to ovulate 60% of the time. Using the simplified back flushing technique in the absence of superovaultion gave 78.9% ova recovery.

Transferring ova to recipient ewes at 69 to 72 hours after mating gave better results than transfers made at 65 to 68 hours after mating. However, this difference was not statistically significant.

Although a description has been given of some factors influencing prenatal growth of lambs, a long term study of a similar experiment involving larger number of lambs would be of value. On the economics of lamb production, little will be gained by maintaining large ewes rather than small ewes for breeding purposes, since at the time of slaughter the maternal environmental effects are small and non-significant.

Even though lambs of small dams need few more days for finishing than the lambs of larger dams, the maintaince cost of the dams is smaller than for bigger ewes and should, therefore, fully compensate for it. However, maternal environmental effects do have a marked effect on the fetal growth which last for a short period postnatally. The mechanisms of such an effect on fetal growth need to be elucidated.

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APPENDIX I

Papers published as adjuncts to the main thesis investigation:

1. Karihaloo, A. K. and W. Combs. 1970: Transfer of fertilized ova in sheep. University of Alberta, Agriculture Bulletin, summer issue.

2. Karihaloo, A. K., A. J. F. Webster and W. Combs. 1970: Effects of cold, acute starvation and pregnancy on some indicies of energy metabolism in Lincoln and Southdown sheep. Can. J. Animal Sci. 50: 191-198. (Text follows).

ABSTRACT

Blood samples were drawn at weekly intervals from 24 pregnant and 16 non-pregnant Lincoln and Southdown ewes kept over winter in a sheltered, unheated yard. Samples were analysed for plasma glucose, free fatty acid (FFA) and ketone concentrations. Records were kept of mean daily air temperature and individual food intake.

Air temperature (7-day mean) ranged from -3° to -32°C. Air temperature had no effect on food intake, FFA or ketone concentrations. However glucose concentrations did rise with increasing cold stress. Acute starvation induced large increases in plasma FFA and ketone concentrations and a fall in plasma glucose. The increases were most marked in the pregnant animals, ketones rising, on average, from 2.5 to 31 mg/100 ml.

Food intake and plasma glucose concentrations in late pregnancy fell below values recorded in non-pregnant sheep. Ketones and FFAs were slightly higher. Differences were small compared with those observed after fasting.

The results are discussed in relation to the effects of different management practices on the pathogenesis of pregnancy toxemia in sheep.

INTRODUCTION

Toxemia of late pregnancy is a well recognised cause of death in sheep (2). The condition, which usually manifests as an extreme hypoglycemia and ketoacidosis in undernourished animals, can be attributed directly to the stress imposed on the normal pathways of energy metabolism of the ewe by the high demand of the fetus for hexose sugars (8). The etiology of the disease does not, however, present an unvarying pattern. Pugh and Sellers (5) classified pregnancy toxemia according to its etiology as "nutritional pregnancy toxemia" following chronic food shortage in late pregnancy, "starvation pregnancy toxemia" following acute, total withdrawal of food, often in association with a severe metabolic stress such as cold exposure and "idiopathic pregnancy toxemia" in which overly fat ewes spontaneously developed anorexia in late pregnancy and subsequently ketoacidosis. The different clinical signs and biochemical changes in these three types of pregnancy toxemia have been thoroughly described in a recent excellent review by Reid (8).

A condition similar to pregnancy toxemia in sheep is recognised in pregnant beef cows in Western Canada. Although confirming biochemical evidence of disturbances in energy metabolism is lacking, the condition is usually attributed by clinicians to the combined chronic metabolic stresses of cold and malnutrition. Spontaneous abortions in cattle have also been attributed to the acute metabolic stress of a period of severely cold weather.

The effects of prolonged undernourishment on plasma glucose, free fatty acids and ketones, biochemical characteristics associated with the development of pregnancy toxemia in ruminants, are well known (8). The present experiment was designed to investigate the extent to which other factors such as cold, short-term food deprivation and pregnancy per se, affected plasma glucose, free fatty acids and ketones in two breeds of sheep of widely differing genetic characteristics, kept over winter in an unheated, sheltered dry lot, conditions similar to those existing in many commercial, semi-confinement sheep units in Canada.

MATERIALS AND METHODS

Animals used in this trial were adult ewes of two diverse breed types, Lincoln and Southdown. The animals were also involved in a study of maternal effects on the prenatal growth patterns of lambs born after surgical ova transfer reciprocally between these two breeds of sheep (to be reported elsewhere). As a consequence of ova transfer, four Lincoln and two Southdown ewes gave birth to lambs of the opposite breed and other lambs were born to their natural dams.

The number of non-pregnant and pregnant ewes (with type of births i.e. singles, twins and triplets) are shown in Table 1.

The experiment commenced on Nov. 25, 1968 (Week 1) and continued for 18 weeks until March 31, 1969. All ewes were maintained in an open-fronted shed, without bedding. Alfalfa hay pellets containing 16.5% crude protein on a dry matter basis were offered once daily for two hours in individual feeding stalls. At the end of this time any

Table 1. Numbers of pregnant and non-pregnant ewes and record of lambings.

Breeds	Non-Pregnant	Pregnant	Single	Twins	Triplets	Total
Lincoln	5	13	8	4	1	18
Southdown	11	11	5	6		22

uneated food was removed and weighed. In this way a record was taken of daily food intake. All the ewes were deprived of food for one day on 2nd February. Air temperature was measured continuously near the experimental area.

Once a week, blood samples were taken as quickly as possible using heparinized vacuum tubes and vacutainer needles from each ewe in the feeding stall before the meals. Ross and Kitts (10) have shown blood sampling with vacuum tubes to be the most suitable method for taking very rapid blood samples with minimum excitement to the animal.

Plasma samples were analysed for glucose, free fatty acids (FFA) and ketones (expressed as acetone). Glucose and FFA analyses were carried out on plasma samples as soon as possible after blood sampling. Plasma samples for ketone body determination were stored by deep freezing before analysis.

Glucose concentrations (mg/100 ml) were determined using the Technicon 'Auto-Analyzer' and FFA concentrations (μmoles/ml) by the colorimetric method of Mosinger (4).

Ketone bodies (as total acetone in mg/100 ml plasma) were determined in principle according to the colorimetric method of Baker and White (1). However, glass tubes with teflon coated plastic caps were used which eliminated the time consuming process of wiring glass stoppered tubes (1). Samples were heated to 120°C in an autoclave rather than in a glycerine bath. Using these minor modifications it was possible to complete 60 samples in a day as against 16 samples per day using the original method of Baker and White (1). Recovery of acetone from β-OH butyrate samples was 100 per cent.

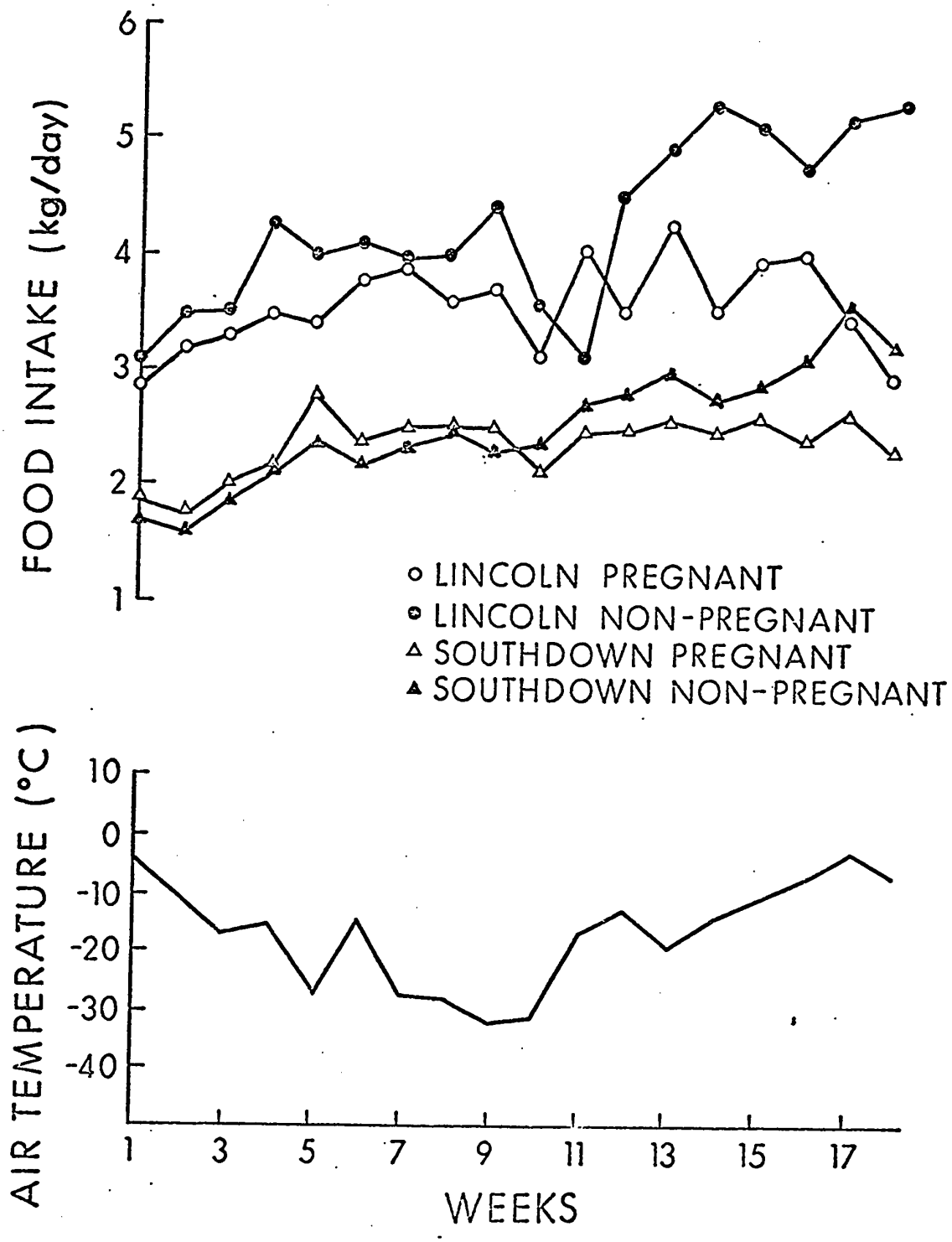

Fig. 1. Food intake of pregnant and non-pregnant Lincoln and Southdown sheep and 7-day mean values for air temperature adjacent to their enclosure. The experiment ran from 25 Nov. 1968 (Week 1) to 31 March 1969 (Week 18).

RESULTS

The winter of 1968-69 at Edmonton, Alberta was exceptionally cold (Fig. 1). Mean January air temperature was -29°C. Food intake of the ewes did not increase during the extremely cold weather but did increase in early spring (weeks 12-18) in non-pregnant animals of both breeds. This confirms an earlier report (13) of an increase in food intake in spring in whether sheep kept out of doors or in controlled environment. The spring rise in food intake did not occur in the pregnant sheep. Food intake in the Lincolns fell slightly in the last five weeks of pregnancy. The effect of pregnancy on food intake is elaborated below (Fig. 3). The decline is exaggerated in Fig. 1 since, in the final weeks, ewes were taken out of the experiment as they lambed, so that the final points represent progressively fewer individuals. Food intake in the large Lincoln ewes was, as expected, greater than in the Southdowns. Changes in the concentrations of glucose, free fatty acids and ketones were small (Fig. 2) except in the pregnant animals after the omission of a single feeding period and in late pregnancy when FFA and ketone values were elevated and glucose values reduced.

Air temperature, per se, had no significant effect on food intake, plasma FFA or ketone concentrations (Table 2). Glucose concentrations did however increase significantly as air temperature fell. The increase was significantly ($p < 0.05$) greater in non-pregnant than in pregnant animals.

Omission of a single meal, so that the ewes were kept for 46h without food, had a very marked effect on all blood measurements

Table 2. Linear regression coefficients describing the effects of air temperature of food intake and some indices of energy metabolism in pregnant and non-pregnant Lincoln and Southdown ewes.

	Lincoln		Southdown	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant
	5	13	11	11
Food intake kg/day	N.S.	N.S.	N.S.	N.S.
Glucose mg/100 ml	-0.509**	-0.282**	-0.531**	-0.375**
FFA μ moles/ml	-0.016**	N.S.	N.S.	N.S.
Ketones mg/100 ml	N.S.	N.S.	N.S.	N.S.

** $p < 0.01$

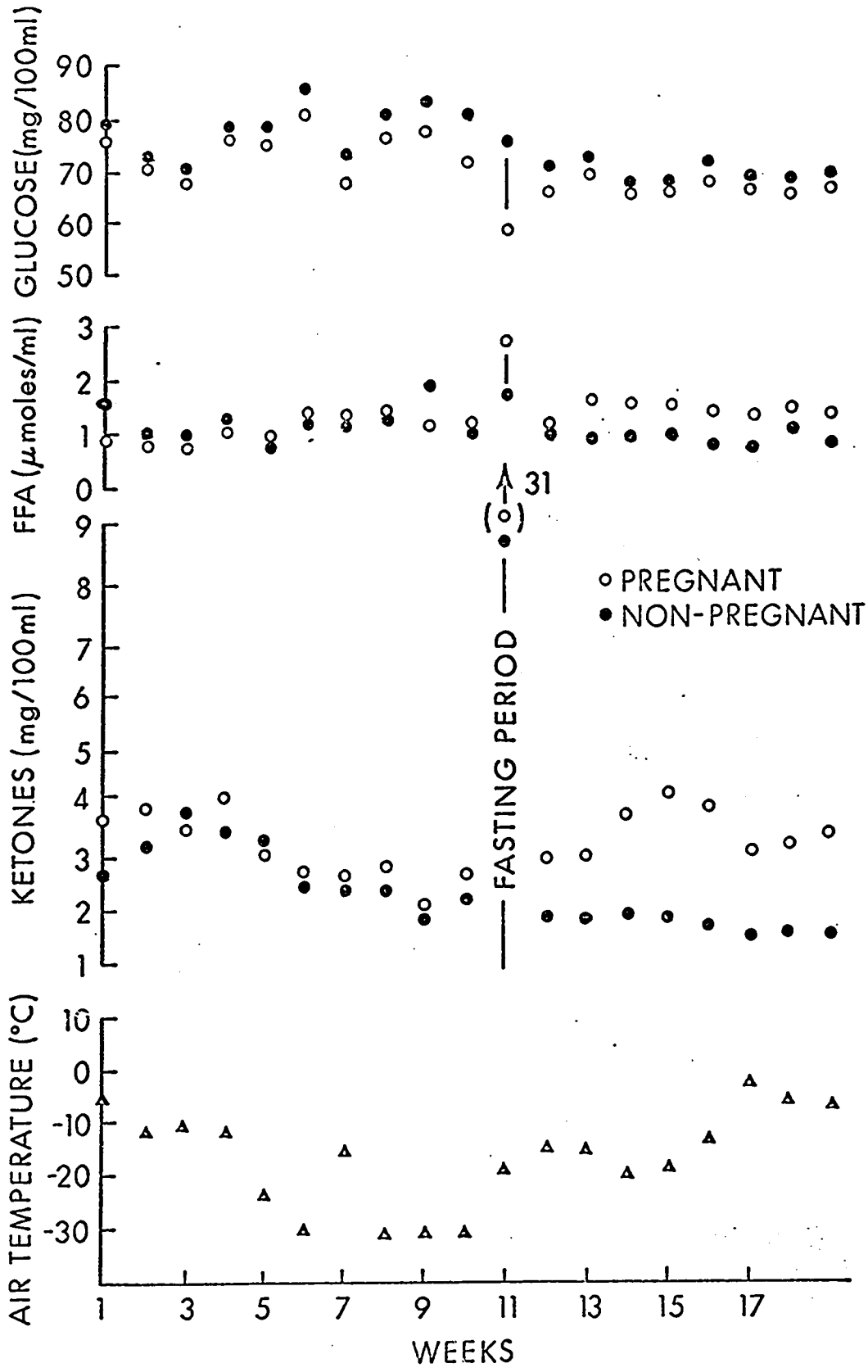

Fig. 2. Mean values for glucose, free fatty acids and ketones in blood samples taken from sheep at 7-day intervals together with air temperature in the 24h preceding. Mean plasma ketone concentration in the pregnant sheep at the end of the fasting period was 31 mg/100 ml.

(Fig. 2). Table 3 compares values for glucose, FFA and ketone concentrations after the 46h fast in pregnant and non-pregnant sheep. Plasma glucose concentrations decreased and FFA concentrations increased in relation to the number of lambs in utero. Ketone concentrations were much higher in the pregnant than in the non-pregnant animals. Ketone levels were higher in Southdown ewes carry singles than in those carrying twins. No explanation for this is apparent.

During the last six weeks of pregnancy, food intake and plasma glucose concentrations were lower and FFA and ketone concentrations higher in pregnant than in non-pregnant sheep, (Table 4). These differences were small however, compared with those recorded after the omission of one meal. Plasma ketones were elevated significantly only in ewes carrying twins. Once again, this increase was small.

Percent changes in blood characteristics during the last six weeks of pregnancy as compared with non-pregnant animals are shown in Fig. 3. Relative ketone and FFA concentrations increased progressively during the six weeks prior to parturition. These increases were statistically significant ($p < 0.01$). There were no statistically significant changes with time before parturition in food intake and glucose concentrations, although these values were nearly always less than in the controls. As was indicated in Fig. 1, the per cent differences in food intake between pregnant and non-pregnant sheep was due more to an increase in intake in the non-pregnant than to a decrease in intake in the pregnant animals.

Table 3. Glucose, FFA and ketone body concentrations after a period of 46h without food; mean values and standard errors.

	Lincoln			Southdown		
	Non-pregnant	Ewes with singles	Ewes with twins & triplets	Non-pregnant	Ewes with singles	Ewes with twins
	5	8	5	11	5	6
Glucose mg/100 ml	76.8 (±1.6)	62.3 (±1.6)	52.6 (±2.2)	73.8 (±3.1)	57.5 (±4.5)	53.7 (±3.0)
FFA μ moles/ml	1.8 (±0.2)	2.3 (±0.2)	3.0 (±0.2)	1.7 (±0.1)	2.5 (±0.1)	2.8 (±0.2)
Ketones mg/100 ml	7.5 (±0.7)	25.8 (±2.8)	35.9 (±4.3)	9.0 (±0.6)	36.7 (±8.5)	28.0 (±6.8)

Table 4. Mean values for food intake and blood characteristics during the last six weeks of pregnancy in ewes carrying one and two lambs and during the same period in non-pregnant ewes.

	Non-pregnant	Pregnant 1 lamb	Pregnant 2-3 lambs
n	16	13	11
Food intake kg/24h	3.43	3.13	2.86
Glucose mg/100 ml	73.67	68.67	66.07
Ketones mg/100 ml	2.07	2.45	4.20
FFA μ moles/ml	0.96	1.27	1.57

Means not crossed by the same line are significantly different from each other ($p < 0.5$).

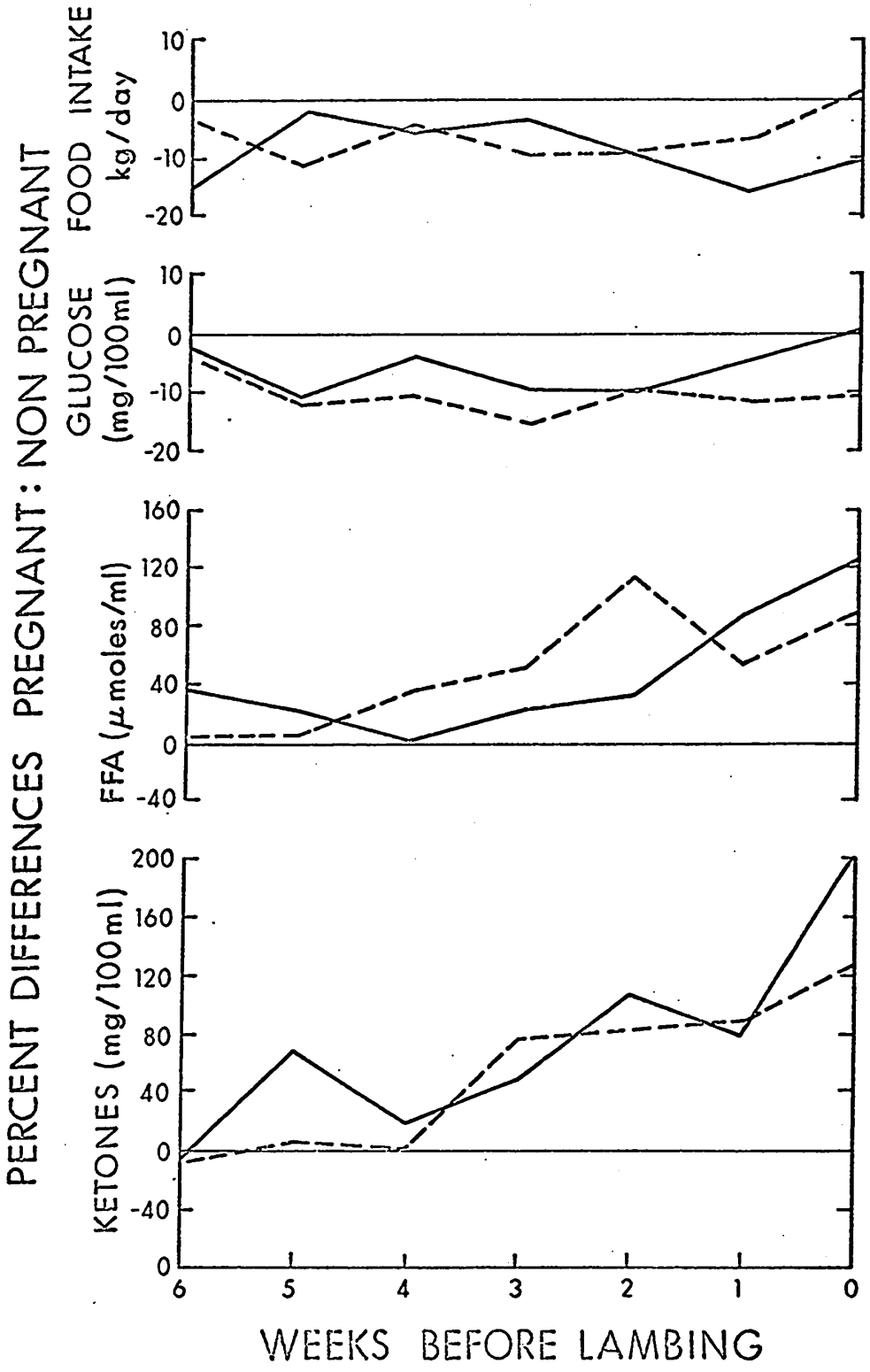

Fig. 3. Changes in food intake and blood measurements in the last six weeks of pregnancy expressed as a percentage difference from values obtained from non-pregnant sheep. _____ Lincoln ————Southdown.

DISCUSSION

The results of the experiments provide information which has direct relevance to the management of a semi-confinement flock of pregnant sheep in cold environments.

It has been shown previously (13) that adequately nourished sheep, chronically exposed to naturally occurring cold environments develop a degree of metabolic acclimatization to cold. As a consequence of this and of the increasing amount of thermal insulation provided by the growing fleece the low critical temperature of sheep has been shown to fall from about -14°C in November to -35°C in March. Below this temperature the metabolic rate must inevitably rise to meet the thermal demand of the environment. Secretion of catecholamines, and thus mobilization of the energy reserves of the body, has been shown to increase at an air temperature about 10°C above the critical (12). The elevated plasma glucose values recorded from the sheep in the present experiments during the prolonged period of intense cold in December and January confirm those of Reid (7) and support the contention that in the acclimatized, well nourished animal, cold is likely to enhance the mobilization of energy rich substrates, in particular glucose, at a greater rate than their depletion. Cold exposure per se may thus, in the acclimatized animal, be considered anti-ketogenic. It is of interest in this regard, to refer to the survey of Ekesbo (3) who observed that the incidence of lactation ketosis in dairy cows housed in unheated units in Sweden was one seventh that of cows kept in controlled environment. The effect of cold adaptation on ketogenesis in cattle is currently under investigation.

However the effect of the omission of a single feeding period on plasma glucose, free fatty acids and ketones in pregnant sheep was severe. Plasma ketone levels in individual ewes were as high as 74 mg/100 ml. Reid (6) has reported that clinical signs of pregnancy toxemia, which include anorexia, can appear at plasma ketone levels as low as 46 mg/100 ml. Although no clinical signs of pregnancy toxemia were observed in the sheep in the present experiments, the results do suggest that the occasional or regular agricultural practice of feeding pregnant cattle and sheep at intervals less frequent than 24 hours may in certain individuals constitute a metabolic stress severe enough to induce pregnancy toxemia.

The results also showed that in the late stages of pregnancy the metabolic demands of the fetus induced significant changes in the blood characteristics of both Lincoln and Southdown sheep although, at each feeding period, they were offered hay pellets in excess of appetite and they were not exposed to cold. It is of interest that food intake was, at this time, lower in the pregnant animals. Decreased food intake in late pregnancy may be due to the space limitation imposed on the rumen by the developing concepta. The effect of pregnancy on food intake was apparently similar in the two breeds studied. The metabolic changes that took place in late pregnancy were small and much less than those associated with clinical signs of pregnancy toxemia (8). This merely confirms that cases of idiopathic pregnancy toxemia (5), in which fat ewes spontaneously develop anorexia and ketoacidosis in late pregnancy, cannot be attributed to the metabolic effects of pregnancy per se. It is, however, a fairly common commercial practice to bring

ewes indoors or change their environment in some way shortly before lambing. Reid and Mills (9) have suggested that ewes, thus treated, might develop anorexia due to psychological stress. It is equally likely that cold acclimatized ewes, with a lower critical temperature of -35°C, might exhibit heat stress when brought into a building at any temperature above 0°C. Webster et al. (11) have shown that cold acclimatized cattle experience very severe heat stress at 20°C. Anorexia is an inevitable accompaniment of heat stress, and anorexia persisting for 48h may induce biochemical changes sufficient to cause clinical pregnancy toxemia. It is fair to say that any movement of sheep in late pregnancy is undesirable and that movement into any environment that induces heat stress sufficient to elevate respiration over 100/min is a potential cause of idiopathic pregnancy toxemia.

ACKNOWLEDGEMENTS

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APPENDIX II

Tables containing data for individual ewes used in ova transfer experiment.

L = Left R = Right Lin. = Lincoln Sd. = Southdown

OVA TRANSFER RECORD

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating)	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Sept. 19	Lin. 151X (3 yrs.)	90.9	72	-	2	0			217X (Sd.)	2 i-4 cell ii-8 cell	one on each side	The ovulation points on this ewe looked very advanced.	Gave birth to one Sd. male lamb 8th. Feb.,1970.
Sept. 19	Sd. 217X (3 yrs.)	54.5	64	2	-	2	i-4 cell ii-8 cell	Lin. 151X				One ovulation point looked recent which could have been mistaken for unruptured follicle.	
Sept. 20	Sd. 240X (3 yrs.)	53	72	1	-	0			123W (Lin.)	1 i-8 cell	R	Her oviduct looked black and was completely blocked - no fluid could pass through it.	Gave birth to one Lin. male lamb 2nd. Feb.,1970.
Sept. 20	Lin. 123W (4 yrs.)	90	72	2	-	1	8 cell	Sd. 240X				Only one egg was recovered and thus this ewe has one of her own egg in her.	Gave birth to her own lamb.
Sept. 22	Sd. 239X (3 yrs.)	64.8	65	1	-	1	4 cell	Lin. 182W	182W (Lin.)	1 i-32 cell	L	The ovum was left in the dish for 40 minutes at room temp. before it was transferred into her.	Did not lamb.
Sept. 22	Lin. 182W (4 yrs.)	65.6	72	1	1	1 (R)	32 cell	Sd. 239X	239X (Sd.)	1 i-4 cell	L	The ewe had badly inflamed uterus. With the flushed material some blood also came out. She has one ovum transferred and one of her own in her.	Did not lamb.
Sept. 24	Sd. 249X (3 yrs.)	61.2	66	-	1	1	unfertilized		1028Y (Lin.)	2 i-4 cell ii-4 cell	one on each side	Follicle looked very recent - so much so it was still bleeding at the time of operation.	Gave birth to Lin. female twins 19th Feb.,1970.
Sept. 24	Lin. 1028Y (2 yrs.)	83	65	1	1	2	i-4 cell ii-4 cell	Sd. 249X				Average sized ovulation points were seen on her.	
Sept. 27	Lin. 171W (4 yrs.)	83.4	65	1	1	1 (L)	4 cell	Sd. 69W	69W (Sd.)	1 i-16 cell	L	While flushing right side - the flushed material was lost and hence no ova recovered. The ovulation point looked normal.	Did not lamb.

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating)	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Sept. 27	Sd. 69W (4 yrs.)	65.0	65	1	-	1	16 cell	Lin. 171W	171W (Lin.)	1 i-4 cell	L	Nothing unusual.	Gave birth to Lin. Female 21st Feb. 1970
Sept. 29	Lin. 1040Y (2 yrs.)	70.4	64	-	1	1	8 cell	Sd. 2021Y				Nothing unusual.	
Sept. 29	Sd. 2021Y (2 yrs.)	65.2	64	-	1	0			1040Y (Lin.)	1 i-8 cell	L	She had exceptionally small reproductive tract which presented difficulty while flushing - ovulation point looked normal but ovum was not recovered.	Did not lamb.
Sept. 30	Sd. 94W (4 yrs)	65.0	68	1	-	0			134X (Lin.)	2 i-8 cell ii-4 cell	R	The ewe had ruptured oviduct on left side and therefore it could not be flushed - ovulation point looked normal in size.	Did not lamb.
Sept. 30	Lin. 134X (3 yrs.)	70.0	68	2	-	2	i-4cell ii-8cell	Lin. 94W				Both ovulation points looked of the same size.	
Oct. 2	Lin. 1056Y (2 yrs.)	69	65	2	-	2	i-8cell ii-8cell	Sd. 212X	212X (Sd.)	1 i-4 cell	L	Nothing unusual.	Did not lamb.
Oct. 2	Sd. 212X (3 yrs.)	59	66	1	-	1	4-cell	Lin. 1056Y	1056Y (Lin.)	2 i-8 cell ii-8 cell	one on each side?	Very doubtful transfers - transfer did not work all that well - the Pasteur pipette had a broken tip.	Did not lamb.
Oct. 4	Sd. 2045Y (2 yrs.)	52	66	2	-	2	i-4cell ii-4cell	Lin. 1080Y	1080Y (Lin.)	2 i-4 cell ii-4 cell	one on each side	She had small reproductive tract which presented some problem while flushing.	Did not lamb.
Oct. 4	Lin. 1080Y (2 yrs.)	93	66	2	-	2	i-8cell ii-4cell	Sd. 2045Y	2045Y (Sd.)	2 i-4 cell ii-4 cell	one on each side	Both ovulation points looked of the same size.	Gave birth to Sd. female twins 24th Feb., 1970.

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating)	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Oct. 7	Lin. 143W (4 yrs.)	83.2	66	-	2	2	i-6 cell ii-8 cell	Sd. 2003Y	2003Y (Sd.)	1 i-6 cell	R	Nothing unusual.	Did not lamb.
Oct. 7	Sd. 2003Y (3 yrs.)	49.6	66	-	1	1	6 cell	Lin. 143W	143W (Lin.)	2 i-8 cell ii-6 cell	one on each side	Ovulation point looked small	Gave birth to Lin. female twins 28th Feb., 1970.
Oct. 10	Sd. 2031Y (2 yrs.)	52.4	66	2	1	2	i-8 cell ii-8 cell	Lin. 1071Y				Nothing unusual.	
Oct. 10	Lin.1071Y (2 yrs.)	84.2	66	-	1	0			2031Y (Sd.)	2 i-8 cell ii-8 cell	one on each side	Smaller lumen oviduct than usual - it was difficult to put tube in it.	Gave birth to Sd. male & female twins 3rd Mar., 1970.
Oct. 11	Lin. 135W	55.6	65	1	1	2	i-6 cell ii-4 cell	Sd. 2012Y	2012Y (Sd.)	2 i-8 cell ii-8 cell	one on each side	Nothing unusual.	Gave birth to Sd. female twins 2nd Mar., 1970.
Oct. 11	Sd. 2012Y	54.2	65	1	1	2	i-8 cell ii-8 cell	Lin. 135W	135W (Lin.)	2 i-6 cell ii-4 cell	one on each side	Nothing unusual.	Did not lamb.
Oct. 14	Lin. 161Y	97	66	1	1	2	i-8 cell ii-6 cell	Sd. 220X	220X (Sd.)	2 i-6 cell ii-4 cell	one on each side	Both the ovulation points looked very big and advanced.	Did not lamb.
Oct. 14	Sd. 220X (3 yrs.)	60.6	66	1	1	2	i-6 cell ii-8 cell	Lin. 161X	161X (Lin.)	2 i-8 cell ii-6 cell	one on each side	One ovulation point looked very small.	Did not lamb.
Oct. 16	Lin. 92W (4 yrs.)	63.5	66	1	1	1	unfertilized					Only one unfertilized ovum could be recovered even though the tract was flushed twice. The ovulation points looked normal in size.	

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating)	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Oct. 16	Sd. 2032 Y (2 yrs.)	52.9	66	-	1	0						No ovum recovery - both the ovulation points looked normal in size.	
Oct. 17	Lin. 111X (3 yrs.)	89	65	-	1	0			251X (Sd.)	1 i-6 cell	L	Nothing unusual.	Did not lamb.
Oct. 17	Sd. 251X (3 yrs.)	51	65	-	1	1	6 cell	Lin. 111X				Nothing unusual.	Did not lamb.
Oct. 22	Sd. 71W (4 yrs.)	60	66	1	1	1 (L)	4 cell	Lin. 145X	145X (Lin.)	2 i-6 cell ii-4 cell	L	Right oviduct was completely blocked - no fluid could pass through it. Ovulation points looked normal.	Did not lamb.
Oct. 22	Lin. 145X (3 yrs.)	88.6	66	2	-	2	i-6 cell ii-4 cell	Sd. 71W	71W (Sd.)	1 i-4 cell	R?	Very doubtful transplant. While transferring the ovum the ewe moved and as a result some liquid in the Pasteur pipette was lost.	Did not lamb.
Oct. 23	Lin. 169X (3 yrs.)	99	66	-	1	1	8 cell	Sd. 2010Y	2010Y (Sd.)	2 i-8 cell ii-8 cell	one on each side	Nothing unusual.	Gave birth to Lin. male & female twins 15th Mar., 1970
Oct. 23	Sd. 2010Y (2 yrs.)	51	66	1	1	2	8 cell	Lin. 169X	169X (Lin.)	1 i-8 cell	L	Some difficulty encountered while flushing the right side - however the right oviduct was flushed from its upper half region.	Did not lamb.
Oct. 24	Lin. 174W (4 yrs.)	51.9	66	-	1	1	8 cell	Sd. 254X				Small reproductive tract. Ewe died on the table (anaesthesia failure).	
Oct. 24	Sd. 254X (3 yrs.)	52	66	1	-	1	unfertilized		174W (Lin.)	1 i-8 cell	R	Reproductive organ very small - nothing abnormal otherwise.	Gave birth to one Lin. female 15th Mar. 1970
Oct. 30	Sd. 275X (3 yrs.)	57	70	-	1	1	32 cell	Lin. 1047Y	1047Y (Lin.)	1 i-4 cell	L	Ovulation points looked small.	Did not lamb.

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to:	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Oct. 30	Lin. 1047Y (2 yrs.)	84	70	1	-	1	4 cell	Sd. 275X	275X (Sd.)	1 i-32 cell	R	Nothing unusual.	Did not lamb.
Oct. 31	Lin. 135X (3 yrs.)	84	72	1	-	1	8 cell	Sd. 232X	232X (Sd.)	2 i-8 cell ii-8 cell	One on each side	Nothing unusual.	Gave birth to one Sd. female 26th Mar., 1970.
Oct. 31	Sd. 232X (3 yrs.)	49	72	-	2	2	i-8 cell ii-8 cell	Lin. 135X	135X (Lin.)	1 i-8 cell	R	The ewe was first used about 20 days ago - as a result there were some adhesions - no difficulty encountered while flushing.	Did not lamb.
Nov. 4	Lin. 134X (3 yrs.)	75	72	1	1	0			106W (Sd.)	2 i-8 cell ii-8 cell	One on each side	The ewe was opened up 2nd time after 6 weeks. There were extensive adhesions, so much so even the reproductive tract could not be taken out Fimbrae were closed	Gave birth to Sd. male twins 25th Mar., 1970.
Nov. 4	Sd. 106W (4 yrs.)	55.0	72	2	-	2	i-8 cell ii-8 cell	Lin. 134X				The ewe was opened up 2nd time after a months interval. There were adhesions but not as bad as Lin. 134X.	
Nov. 12	Lin. 1041Y (2 yrs.)	75	72	1	-	1	32 cell	Sd. 131W				Nothing unusual.	
Nov. 12	Sd. 131W (4 yrs.)	60	72	2	-	0			1041Y (Lin.)	1 i-32 cell	R	Nothing unusual.	Did not lamb.
Nov. 17	Lin. 140X (3 yrs.)	74	72	1	-	1	16 cell	Sd. 259X				First of the synchronized group this year.	
Nov. 17	Sd. 259X (3 yrs.)	49	72	1	1	2	unfertilized		140X (Lin.)	1 i-8 cell	R	Synchronized - nothing unusual.	Did not lamb.

Date	Ewe No. and Age	Body Wt. in kg	Laparotomy (Hrs. after Mating	No. Ruptured Follicles on the Ovary L	R	No. Ova Recovered	Stage of Ova	Donor to	Recipient of	No. and Stage of Ova transferred	Side of Transfer	Remarks at the time of Laparotomy	Final Remarks
Nov. 18	Lin. 153X (3 yrs.)	82	72	-	1	1	unfertilized		244X (Sd.)	1 i-8 cell	L	Nothing unusual - she was synchronized.	Gave birth to one Sd. male 10th Apr., 1970.
Nov. 18	Sd. 244X (3 yrs.)	60	72	1	1	1 (L)	8 cell	Lin. 153X				Nothing unusual.	
Nov. 20	Lin. 160W (4 yrs.)	75	70	2	-	2	i-8cell ii-8cell	Sd. 122W	122W (Sd.)	2 i-8 cell ii-16 cell	One on each side	Nothing unusual - ovulation points looked normal.	Gave birth to Sd. female twins 14th Apr., 1970.
Nov. 20	Sd. 122W (4 yrs.)	49	70	2	-	2	i-8cell ii-8cell	Lin. 160W	160W (Lin.)	2 i-8 cell ii-8 cell	One on each side	Nothing unusual.	Did not lamb.
Nov. 21	Lin. 104X (3 yrs.)	75	70	1	1	1 (L)	16 cell	Sd. 233X	233X (Sd.)	1 i-8 cell	L	Her right side was not flushed - therefore she has one of her own ovum in her.	Gave birth to one Sd. female 16th Apr., 1970.
Nov. 21	Sd. 233X (3 yrs.)	51	70	1	-	1	8 cell	Lin. 104X	104X (Lin.)	1 i-8 cell	R	She had blockage at UTJ but oviduct was open higher up.	Ewe died - had Lin. male fetus in her.
Nov. 25	Lin. 123X (3 yrs.)	93	70	-	1	1	8 cell	Sd. 222X	222X (Sd.)	1 i-16 cell	L	There was an exceptionally big ovulation point - looked as if there were two ovulation points side by side.	Gave birth to one Sd. & one Lin. female 16th Apr., 1970.
Nov. 25	Sd. 222X (3 yrs.)	50	70	1	-	1	16 cell	Lin. 123X	102X (Lin.)	1 i-8 cell	R	Nothing unusual.	Did not lamb.
Nov. 26	Lin. 115X (3 yrs.)	89	70	2	-	2	i-16 cell ii-32 cell	Sd. 250X				Reproductive tract was small and it was hard to expose it.	
Nov. 26	Sd. 250X (3 yrs.)	53	70	1?	-	0			115X (Lin.)	2 i-16 cell ii-32 cell	One on each side?	Doubtful transfer - ewe had cystic ovary - ovulation point was very doubtful.	Did not lamb.

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Nov. 27	Lin. 133X (3 yrs.)	95	69	1	-	1	8 cell	Sd. 64W	64W (Sd.)	2 i-4 cell ii-32 cell	One on each side	Nothing unusual.	Gave birth to one Sd. female 20th Apr., 1970.
Nov. 27	Sd. 64W (4 yrs.)	58	69	-	2	2	i- 4 cell ii-32 cell	Lin. 133X	133X (Lin.)	1 i-8 cell	L	Nothing unusual.	Gave birth to one Lin. female 21st Apr. 1970.
Nov. 28	Lin. 1012Y (2 yrs.)	76	70	1	1	0			236X (Sd.)	1 i-6 cell	L	No ovum recovered from either side - due to unknown reasons.	Gave birth to one Sd. male 19th Apr. 1970
Nov. 28	Sd. 236X (3 yrs.)	52	70	1	-	1	6 cell	Lin. 1012Y				Nothing unusual.	
Dec. 8	Lin. 1007Y (2 yrs.)	107	72	1	-	1	32 cell	Sd. 229X				Nothing unusual.	
Dec. 8	Sd. 229X (3 yrs.)	60	72	-	2	2	unfertilized		1007Y (Lin.)	1 i-32 cell	R	Both the eggs recovered were unfertilized for reasons unknown.	Gave birth to one Lin. male 3rd May, 1970.