INVESTIGATIONS INTO PROCEDURES FOR THE IMPLEMENTATION OF A MULTIPLE OVULATION AND EMBRYO TRANSFER SCHEME USING EWE LAMBS

A thesis presented in partial fulfilment of

the requirements for the degree of

Doctor of Philosophy

in Animal Science

at Massey University

RAYMUNDO RANGEL-SANTOS

1991

ABSTRACT

Three trials using 6-7 month-old Romney ewe lambs and adult ewes were conducted to evaluate the feasibility of implementing a juvenile MOET scheme. Older lambs were used to evaluate ways of improving the ovulation rates in 3 additional trials, one of them involved Booroola-cross lambs. Studies were also carried out to develop and evaluate a technique for splitting sheep embryos, and these were transferred to recipient ewes.

In the three trials involving superovulation and transfer a range of gonadotrophin treatments were used. The more highly purified preparations (FSH-P, Ovagen) gave lower responses compared with PMSG (1.78, 1.06 vs 4.18 respectively in Trial 3, $P<0.01$). Ovulation rate of ewe lambs relative to adult ewes in the 3 trials were respectively 1.20 vs 2.00 ($P<0.01$), 4.18 vs 6.35 and 1.61 vs 3.15. Overall, administration of GnRH did not significantly increase the ovulation rates, although lambs treated with PMSG + GnRH tended to give higher responses.

Egg recovery was higher in most cases in adults than in lambs (91% vs 55%, $P<0.01$; 72% vs 47%, $P<0.01$; 34% vs 43%, for trials 1, 2 and 3 respectively). Fertilization rate in trial 1 was lower in lambs than in adults (50% vs 82%, $P<0.05$) but not different in trials 3 and 5, probably due to the use of intrauterine insemination.

Ovulation rate was not improved by treating lambs at 8-9 months of age or by administering PMSG 2 or 4 days before sponge removal. Ovulation rates following gonadotrophin stimulation were higher in androstenedione-immunized ewe lambs than in non-immunized ewe lambs (2.22 vs 1.59, $P<0.05$). Booroola-cross lambs treated at 6-7 months of age gave considerably higher ovulation rates compared to those recorded in Romney lambs from another experiment conducted at the same time (3.79 vs 1.61).

Lower embryo survival and incidence of twins were found for demi-embryos generated from 9-10 month-old lambs compared to those obtained from 24 months or older animals. Higher pregnancy rate and demi-embryo survival were found when bisecting blastocysts rather than morulae harvested in the same flush.

A deterministic model incorporating parameters provided by the study showed that the limited number of lambs generated from lamb donors restricted the improvement in genetic progress.

ACKNOWLEDGEMENTS

I gratefully acknowledge my supervisors, Associate Professors Dr. M.F. McDonald and Dr. G.A. Wickham, for the guidance they have provided during the investigation and the preparation of this manuscript. It has been a great privilege to work with you, thank you very much for all.

My special thanks are due to Dr. W. Vivanco for stimulating and rewarding discussions on different aspects of embryo transfer throughout the study.

I want to thank Miss M. Dattena, Mr M. Anwar, Mr M.A. Witcherley and D.L. Burnham for practical assistance during the experimental work.

To Mr L. Williams, Miss L. Watt, Mr M. Hogan and Mr K. Kilmister for their help with the management of the animals.

I want to thank Dr. H. Varela-Alvares and Dr. D. J. Garrick for advice on statistical analysis.

I want to express my appreciation to the members of the staff in the Animal Science Department for sharing their knowledge and for their support during my studies. I also want to thank the postgraduate students in the Animal Science Department for their friendship and support while at Massey.

I acknowledge the financial support received from the Ministry of External Relations and Trade (M.E.R.T) New Zealand, Universidad Autonoma Chapingo and Consejo Nacional de Ciencia y Tecnologia (CONACYT) Mexico.

Finally and most importantly my special thanks go to my wife Luz Maria, my son Raymundo Jr and my daughter Luz Maria Jr for their love, support and understanding and for the difficulties that they had while I was completing this thesis overseas. I also want to thank my parents, brothers and sisters for their continuous support and encouragement throughout my life, and particularly while I was in New Zealand.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

Chapter IV.

LIST OF FIGURES

LIST OF APPENDICES

Appendix		**Page**

CHAPTER I:

INTRODUCTION

CHAPTER I:

INTRODUCTION

The need to improve the rate of genetic gain in the livestock industries has lead scientists to develop methodologies such as artificial insemination. More recently the technique of multiple ovulation and embryo transfer (MOET) has been used in farm-animal improvement schemes. Land and Hill (1975) have shown that, through the use of MOET, selection could be more intense and the generation interval considerably reduced compared to normal reproduction, particularly in ruminant farm animals which have low reproductive rates and long generation intervals. The theoretical benefits of such methodologies have been examined for sheep (Smith, 1986), and a juvenile MOET scheme shown to be more promising. Toro et al. (1988) calculated that the rate of genetic change for wool production could be more than double compared to schemes using normal reproduction provided the generation interval could be reduced to one year.

The implementation of such a scheme under practical conditions requires good embryo transfer rates from 6 to 8 month-old ewe lambs. This means treating animals around the time when puberty is normally achieved. Onset of puberty is a complex process in which the growing lamb monitors and integrates internal and external factors to time the sequence of endocrine events that underlie the transition into adulthood. The success of such a programme depends on the ability to induce multiple ovulations consistently and for the eggs to be fertilized and develop to term.

Attempts to induce ovulations in prepubertal sheep have been successful (Mansour, 1959), their eggs have been fertilized (Land and McGovern, 1968), transplanted and have resulted in offspring (Quirke and Hanrahan, 1977). Nevertheless, there is some evidence indicating that embryos from ewe lambs have a lower developmental capacity compared to that of embryos from adult ewes. This has been demonstrated by in vitro (Wright et al., 1976) and in vivo (McMillan and McDonald, 1985) studies. Although results from these studies have shown the feasibility of superovulating ewe lambs and for those embryos to develop to term, no attempts have been made to evaluate the

practical feasibility of implementing a juvenile MOET scheme on a large scale. Although there is a great amount of information published on the technique of multiple ovulation and embryo transfer, most of it has been generated on adult ewes. Major physiologic differences between adults and lambs make it difficult to extrapolate experimental results from one to the other.

Attempts have been made to improve the efficiency of embryo transfer programmes in sheep. These have included the use of different superovulatory treatments to increase the number of ovulations, improvement of the mating systems to increase fertilization rate and development of appropriate flushing solutions to improve embryo survival. Due to the lack of information in ewe lambs, procedures developed in adults have usually been applied to ewe lambs. Comparable ovulation rates (Bradford et al., 1971) and numbers of embryos recovered (Tervit et al., 1989) have been reported when postpubertal ewe lambs and mature ewes were superovulated in a similar way, suggesting that similar responses may be expected between mature ewes and young sheep that have already reached puberty. Treating prepubertal ewe lambs could give similar responses to those obtained in adults but there are no data available. Therefore, in order to superovulate prepubertal ewe lambs, the first approach was to examine the efficacy of standard superovulatory treatments developed in adults. Immunization against androstenedione in an attempt to increase the ovulation rates in gonadotrophin-treated ewe lambs was also examined. Because the implementation of the scheme depends primarily on the capacity to induce multiple ovulations, the availability of genotypes with high natural ovulation rate (such as animals carrying the Booroola gene) or showing high sensitivity to exogenous gonadotrophins could be a valuable resource to use for examining the feasibility of the scheme.

There are a number of possible techniques such as embryo splitting, embryo sexing and cloning that could be used to increase the efficiency of a MOET scheme. The potential advantages of using those techniques to increase the rate of genetic improvement have been demonstrated in cattle (Nicholas and Smith, 1983). It was also indicated that a MOET scheme producing young sires could double the rate of genetic improvement, compared with normal schemes, and on a national basis would have a high benefit-cost ratio even with the present high cost of embryo transfer. Refined techniques will likely reduce the current costs and make use of the method commercially viable. This could

have considerable impact in countries such as New Zealand where the main source of income is from the export of animal products.

Five to six transferable embryos on average have been obtained from superovulated sheep although there is a great variability in the response (Torres and Cognie, 1984; Torres et al., 1987). Achievement of similar results in ewe lambs would make a juvenile MOET scheme attractive and likely to succeed.

The present work was an investigation of the practical feasibility of a juvenile MOET scheme to increase the rate of genetic improvement in sheep. The first part of this is a review of literature regarding puberty and embryo transfer procedures. The review on puberty describes the neuroendocrine sequence of events during the transition into adulthood, as well as some of the main factors affecting age at puberty. The review on embryo transplantation procedures describes information generated mainly on adult ewes and includes the topics of synchronization of oestrus, superovulation and factors affecting the response to the superovulatory treatments, service of donors, embryo recovery, embryo evaluation and embryo transfer. Some of the main factors affecting the success of embryo transfer are also described. The final part of the review describes the development of embryo splitting techniques as well as some of the main factors affecting the survival of demi-embryos.

A series of research trials are reported which examine aspects related to the efficiency of a MOET scheme using ewe lambs as donors. Superovulation, embryo collection and transplantation of embryos from the ewe lambs of a Romney flock used in a long term fleece weight selection experiment (Massey University PT flock) were performed during a period of three consecutive years. In the study there was a limited number of lambs available to work on. Nevertheless, because of the importance of the flock, the first priority was to evaluate the feasibility of the scheme on this flock. It was critical to treat the lambs at 6-7 months of age so that their offspring would be born at the same time as offspring from adult ewes. In this way ewe lambs generated using normal reproduction as well as those produced by MOET could be submitted to the MOET programme during the subsequent breeding season causing no interference with the normal reproduction of the flock and keeping a generation interval of approximately 1 year on the female side.

The specific objectives of the study were:

a) To evaluate the ovulatory response of Romney ewe lambs to various gonadotrophins.

b) To evaluate possible ways of improving the superovulation response such as the prior immunization against androstenedione. It was also of interest to establish whether, by using animals with the potential for high fecundity, such as those with the Booroola gene, superovulation might by improved relative to the response obtained from Romneys. Data obtained from such studies could be used to estimate the genetic benefits for wool production in a Romney flock.

c) To study the ovum fertilization rate in donors and conception rate in recipients after transfer of fertilized eggs collected from superovulated ewe lambs.

d) To develop a technique to split sheep embryos and to evaluate the feasibility of applying the technique in conjunction with a MOET programme.

To aid in the presentation of the experimental work throughout the thesis Table 1.1 lists the various trials and studies conducted at Massey University and at a commercial embryo transfer site (LambXL) in the Manawatu.

The final part of the thesis reports a preliminary analysis of the likely genetic gains of applying a MOET programme in a flock selected for fleece weight and utilizing estimates of the parameters from the experimental trials.

Table 1.1 Chronological sequence of the trials: Number of trial or study (Trial or Study), Time when conducted (Time), Flocks used (Flock) and experimental site (Site).

Trial	Time		Flock	Site
1	March-April	1988	PT Romney	Massey
2	May-June	1988	Commercial Romney	Massey
3	March-April	1989	PT Romney	Massey
4	April-June	1989	Commercial Romney	Massey
5	March-April	1990	PT Romney	Massey
6	March-July	1990	B-R x P	Massey
Study				
1	Aug-Sept.	1988	D.T., F.T., O.D.	LambXL
2	April-June	1989	Commercial Romney	Massey
3	November	1989	D.T., F.T.	LambXL
4	December	1989	Commercial Romney	Massey
5	April	1990	C.R., Drysdale	Massey
6	March-May	1990	D.T., F.T.	LambXL

P.T. = Fleece weight selection and control.
B-R x P = Booroola-Romney x Perendale.
D.T. = Danish Texel.
F.T. = Finnish Texel.
O.D. = Oxford Down.
C.R. = Commercial Romney.

CHAPTER II:

REVIEW OF FACTORS AFFECTING PUBERTY AND EMBRYO TRANSFER SUCCESS IN SHEEP

CHAPTER II:

REVIEW OF FACTORS AFFECTING PUBERTY AND EMBRYO TRANSFER SUCCESS IN SHEEP

2.1 Puberty.

Neuroendocrine sequence during the transition into adulthood.

Prepubertal sheep show luteinizing hormone (LH) pulses with an amplitude as great or greater than those of the adult, but the frequency is low, e.g. 2-3 pulses / h (Bindon and Turner, 1974; Foster et al., 1975a, b; Echternkamp and Laster, 1976; Huffman and Goodman, 1985) and does not provide the necessary stimulus to induce follicular development. The LH pulse frequency is increased three- to four-fold during the last week before first ovulation (Huffman and Goodman, 1985). Three preovulatory LH surges occur in most of the lambs during the transition into adulthood (Foster et al., 1986). The first behavioural oestrus occurs once the brain is exposed to high concentrations of progesterone for several days during the first normal luteal phase. Generally it has been accepted that the presence of the brief progesterone rise ensures that subsequent luteal function will be normal, although it is not a prerequisite for regular ovarian activity because it is not present in all the lambs (Keisler et al., 1985; Foster and Ryan, 1987).

According to the "Gonadostat" hypothesis developed in the rat (Ramirez and McCann, 1963) the concentration of circulating gonadotrophins is low in the prepubertal female because the system controlling their secretion is very sensitive to the inhibitory feedback action of ovarian steroids such as oestradiol. The sensitivity decreases as the time of puberty approaches and consequently the ovary is stimulated to function in an adult-like manner. Evaluation of the hypothesis in the lamb showed a decrease in the response to oestradiol feedback inhibition during the pubertal period (Foster and Ryan, 1979).

In adults, each pulse of LH has been associated with a pulse of gonadotrophin releasing hormone (GnRH) (Levine et al., 1982; Clarke and Cummins, 1982). Cells integrating the GnRH pulse generator have been found in the medial basal hypothalamus (Jackson et al., 1978; Domanski et al., 1980; Pau et al., 1982) and the preoptic area (Lehman et al.,

1986). Thus the hypothalamus plays a central role in regulating the time of sexual maturation. The activity of the GnRH pulse generator will be determined by the influence of internal and external cues (Foster et al., 1986). The key event leading to sexual maturity will occur only when the integration of internal and external cues indicate that both size and season are favourable for initiating a pregnancy (Foster, 1988).

Age at puberty is influenced by many factors. Some of them are considered here.

Season of birth: Regardless of the season of birth, lambs reach puberty only during the breeding season (Hammond, 1944). Thus winter-born lambs reach puberty at an older age compared to spring-born lambs (Hammond, 1944; Hafez, 1952; Watson and Gamble, 1961; Mallampati et al., 1971; Dyrmundsson and Lees, 1982). Although birth later in the year may reduce age at puberty further (Mallampati et al., 1971; Fitzgerald and Butler, 1982; Foster and Ryan, 1987), extremely late births result in lambs delaying onset of reproductive cycles until the following year (Hammond, 1944; Watson and Gamble, 1961; Mallampati et al., 1971; Foster, 1981; Dyrmundsson and Lees, 1982).

Photoperiod: The pathway for transmission of photoperiodic cues that time reproduction in the adult sheep has been reviewed (Karsh et al., 1984; Martin, 1984). It was proposed that the pineal gland mediates photoperiod cues through its pattern of melatonin secretion (proportional to the hours of darkness). The pattern of circulating melatonin codes for day length and the information is used to increase or decrease the sensitivity of the GnRH pulse generator to oestradiol negative feedback and to alter the frequency of LH secretion. The pineal gland is active early in life (Wiggins et al., 1970) and prepubertal lambs can produce a melatonin rhythm that is responsive to the light/dark cycles (Foster et al., 1985).

The relation of the secretion pattern of melatonin to the prevailing photoperiod endows it with the potential to serve as a time keeping hormone in the sexually immature lamb, as well as in adults. Several studies demonstrated the involvement of melatonin in the timing of puberty by photoperiod in the female sheep (Kennaway and Gilmore, 1984; Nowak and Rodway, 1985; Yellon and Foster, 1986). Although melatonin is naturally produced in a circadian pattern, chronic treatment may be interpreted as a series of short days in adults (Lincoln and Ebling, 1985). In lambs, melatonin implants have been

found to have no effect, to advance puberty or to delay puberty in different experiments on pineal-intact lambs raised outdoors (Kennaway and Gilmore, 1984; Nowak and Rodway, 1985). The response was related to the age when the treatment was started; delayed puberty was associated with an early onset of melatonin administration, and precocious puberty was associated with a later insertion of implants.

Body size: Ad-libitum fed ewe lambs attain puberty at the usual age (~30 weeks), but exposing lambs to a restricted diet for variable periods of time after weaning, before they were fed ad libitum, prevented puberty occurring despite the lambs having experienced an appropriate photoperiod (Foster et al., 1985). This was because the females were below some physiological size required for puberty. Ewe lambs also achieved puberty when they were fed ad libitum during the autumn or winter, but this was not the case when the animals were fed ad libitum during the anoestrous season. Those animals did not start cycling until the next autumn. These facts indicate that both season and feeding level (body size) during the breeding season are involved in the timing of the initiation of reproductive cycles in the lamb through photoperiod and growth-related cues. Alteration of the timing of puberty in the lamb during the breeding season by the level of nutrition is through modulation of gonadotrophin secretion (Fitzgerald et al., 1982; Fitzgerald, 1984; Foster and Olster, 1985). The undernourished female fails to begin reproductive cycles during the breeding season at normal age apparently because it is hypersensitive to oestradiol inhibition of LH secretion (Foster and Olster, 1985). However, undernourished lambs can accumulate photoperiod information and start cycling once the appropriate size has been achieved (Foster and Yellon, 1985). In many cases first oestrus in ewe lambs is attained at weights ranging from 50-70 % of the adult body weight (Hafez, 1952, 1953; Dyrmundsson, 1972) and within an age range of 6-18 months (Dyrmundsson, 1973).

An integrated model for puberty has been proposed (Foster, 1988). In summary, the activity of the gonadotrophin releasing hormone (GnRH) pulse generator, and hence the frequency of LH secretion, is modulated by cues that relate information about physiologic size and well-being and the time of the year. Metabolic signals that are translated as inadequate development or poor nutrition reduce the activity of the neural oscillator by increasing the sensitivity to negative oestradiol feedback and, more directly, through steroid-independent inhibitory mechanisms. Such low-frequency stimulation of

the pituitary gland by GnRH produces insufficient LH to develop a preovulatory follicle. Additional development or improved nutrition is translated metabolically to the GnRH pulse generator to increase its activity. Thus the inherent ability to produce high frequency discharges increases, the sensitivity to the inhibitory steroid feedback lessens and the neural oscillator is susceptible to acceleration by oestradiol. The resultant rapid LH pulse frequency, characteristic of the follicular phase, drives the pubertal process by developing one or more follicles to the preovulatory stage. The associated increase in oestradiol production activates the preovulatory gonadotrophin surge mechanism, and ovulation occurs. This sequence happens only during the part of the year when adults are capable of breeding because, in addition to developmental determinants, there is a seasonal requirement for the timing of puberty.

The young lamb uses the nocturnal rise in pineal melatonin secretion to code the length of the day and construct a photoperiod history. Only when a long-day/short-day sequence of melatonin is experienced can sensitivity to oestradiol feedback inhibition be reduced to allow the establishment of the frequency of LH pulses characteristic of the follicular phase.

Opioids (Mathews and Murdoch, 1984; Finnie et al., 1985) and dopamine (Finnie et al., 1985) have also been implicated in the control of LH in the prepubertal lamb, although whether their action changes during sexual maturation remains unknown.

Follicle stimulating hormone (FSH) levels are well within the adult range by 10-12 weeks of age (Foster et al., 1975b; Fitzgerald and Butler, 1982). A subtle rise in peripheral FSH concentration, however, has been detected during the peripubertal period (Ryan and Foster, unpublished study cited by D.L. Foster, 1988). Thus, a role for FSH in the transition into adulthood in the sheep cannot entirely be excluded although, even in the mature ewe, FSH may be only permissive with respect to the timing of follicular maturation (Karsh et al., 1984).

Ovarian development: In sheep antral follicles are evident before birth, but they do not respond to exogenous gonadotrophins until after 2 to 4 weeks of age (Mansour, 1959; Mauleon, 1969) when cells of the granulosa and thecal layers become well developed (Kennedy et al., 1974). At a slightly greater age (5-6 weeks) exogenous gonadotrophins

can induce ovulation and formation of corpora lutea (Worthington and Kennedy, 1979).

2.2 Embryo transplantation procedures.

2.2.1 Oestrous synchronization.

Two main methods have been developed to control oestrous activity in sheep. The first involves the use of progesterone or progestagens (e.g. Medroxy-progesterone acetate, MAP; Fluorogestone acetate, FGA) and the second the use of prostaglandin $F_{2\alpha}$ (PG) and its analogues (e.g. Cloprostenol).

Progesterone and progestagens.

Progesterone or some of its related compounds have been administered as multiple injections, orally, by intravaginal dispositives or subcutaneous implants (Lamond, 1964). Some factors identified as affecting the efficiency of control of ovarian cycles in sheep include the hormone used (progesterone or an analogue), its dose, method of administration, stage of the breeding season, nutrition, introduction of the ram and time of the day of final treatment.

The use of polyurethane ester sponges impregnated with one of the progesterone analogues has been adopted as a standard way to achieve oestrous synchronization in sheep. The dispositives are normally inserted for 12 to 14 days.

Clarke et al. (1966) and Robinson et al. (1967) reported reduced fertility when oestrus was synchronized using polyurethane ester sponges containing progestagens (MAP or SC-9880), probably due to impaired sperm transport and survival (Quinlivan and Robinson, 1967, 1969; Allison and Robinson, 1970) and perhaps enhanced by an abnormal pattern of mucous secretion during the time of oestrus (Smith and Allison, 1971). Fertility has been shown to be normal at the next oestrus after synchronization (Robinson, 1967; Allison and Robinson, 1970).

Observations on the use of an internal drug release device (CIDR: containing 9% or 12% progesterone or 0.3 g or 0.5 g respectively) indicated normal fertility (Harvey et al., 1984), earlier onset of oestrus and closer synchrony of oestrus (Welch et al., 1984; Rhodes and Nathanielsz, 1988) as well as earlier LH surge and ovulation (Shackell, 1991) when compared to sponges containing an analogue of progesterone.

Three factors should be considered in an oestrous synchronization programme: suppression, synchronization and fertility. Before the method can be considered satisfactory, 80 to 90% of the treated animals must show heat within 36 to 48h of device removal (Lamond, 1964).

Prostaglandin and its analogues.

In order to achieve control of oestrus using prostaglandins or any of its analogues the presence of a responsive corpus luteum is required and this is found in sheep between days 4 and 14 of the oestrous cycle (Douglas and Ginther, 1973). The efficiency of this method has been demonstrated using natural prostaglandins (Douglas and Ginther, 1973) and the synthetic analogue, cloprostenol (Trounson et al., 1976; Acritopoulou and Haresign, 1980), given as a single injection. Because only 66% of the treated animals responded to a single prostaglandin injection, a double injection given 9-10 days apart was suggested to ensure response from all the treated animals.

2.2.2 Superovulation.

Superovulation in farm animals has been induced using three types of gonadotrophins: gonadotrophins extracted from the pituitary gland of several animal species (pig, sheep, horse), pregnant mare serum gonadotrophin (PMSG) or equine chorionic gonadotrophin (eCG) and human menopausal gonadotrophin (hMG).

Attempts to superovulate sheep have been made by administering the follicle stimulating preparation either towards the end of the normal oestrous cycle (days 11-13) or around the end of the progestagen treatment used to control the time of oestrus and ovulation.

Pregnant mare serum gonadotrophin.

PMSG is a complex glycoprotein with FSH-like and LH-like activities (Gonzalez-Mencio et al., 1978) produced in the endometrial cups (Allen and Moor, 1972) of pregnant mares between days 40 and 130 of gestation (Cole and Hart, 1930). The highest rate of production is between 55 and 65 days of gestation (Walker, 1977). The concentration of PMSG is higher in pony mares compared to larger breeds of horses (Rowlands, 1963) and has been reported to be affected by the genotype and number of foetuses, sire of foetus, degree of endometrial folding and age and parity of the mare (Day and Rowlands, 1945; Clegg et al., 1962; Allen, 1969; Allen and Moor, 1972; Allen, 1982;

Murphy et al., 1985).

Superovulation in sheep has been commonly attempted using PMSG either as raw material (Hunter et al., 1955; Rowson and Adams, 1957; Gherardi and Martin, 1978; Gherardi and Lindsay, 1980) or as more purified freeze dried preparations reconstituted before use (Moore et al., 1960; Holst, 1969; Clarke, 1973; Eastwood and McDonald, 1975; Tervit et al., 1976; Hanrahan and Quirke, 1982).

Due to its long half-life (McIntosh et al., 1975), PMSG has been generally administered as a single injection. Although a wide range in dose levels of PMSG has been investigated, about 2000 i.u. has been indicated as the highest permissible dose (Gordon, 1983). Alternatively in some studies the amount of gonadotrophin administered has been determined on a weight basis at a rate of 20-50 i.u. / kg of body weight (Willadsen, 1979a).

Pituitary gland preparations.

There are several partly purified homogenates of pituitary origin (FSH-P; Folltropin; Ovagen), containing FSH and LH available on the market to induce superovulation in sheep. The rate of clearance of pituitary gonadotrophins from the blood stream is very fast (Akbar et al., 1974). Hence, for successful superovulation, they must be administered in multiple injections either once or twice daily during 2 to 4 days (Moore and Shelton, 1962, 1964a,b; Crosby et al., 1980; Torres and Cognie, 1984; Torres et al., 1987).

Human menopausal gonadotrophin.

Human menopausal gonadotrophin (hMG) has been shown to induce superovulation successfully in cattle (Murphy et al., 1984; McGowan et al., 1985). However, since it is no more efficient than pituitary gland preparations and is costly, it has not been used widely.

2.2.3 Factors affecting the response to the superovulatory treatment.

2.2.3.1 Gonadotrophin used.

PMSG has been the gonadotrophin preparation most widely used for superovulation in farm animals. Since the first report regarding use of PMSG to increase fertility in sheep

more than 50 years ago (Lopyrin, 1938) much research has been conducted using this gonadotrophin to superovulate sheep (e.g. Robinson, 1951; Gordon, 1958; Evans and Robinson, 1980; Rangel-Santos, 1987). Its widespread use has reflected its availability, effectiveness and ease of administration.

The use of pituitary preparations for the same purpose was reported around the same time (Hammond et al., 1942), but they were not used widely due to costs. However, improvements in isolation and purification methods for pituitary gonadotrophins have resulted in the availability of highly purified gonadotrophin preparations. Some of these have been used intensively in recent years. Thus, sheep have been successfully superovulated using the following gonadotrophin preparations: FSH-P (Wright, 1981; Armstrong and Evans, 1984a; Torres and Cognie, 1984; Cognie et al., 1986; Torres et al., 1987), Folltropin (Thompson and Smith, 1988; Dattena, 1989; Buckrell et al., 1990; Mapletoft et al., 1990) and Ovagen (W.H. Vivanco, personal communication). A crude extract from horse anterior pituitary (HAP) has also been used successfully (Moore and Shelton, 1962, 1964a,b; Shelton and Moore, 1967; Crosby et al., 1980).

There is general agreement that pituitary gland preparations induce higher superovulatory responses than PMSG. The lower efficacy of PMSG has been associated with its long half-life causing continued ovarian stimulation, unovulated follicles, abnormal endocrine profiles and reduced embryo quality (Saumande et al., 1978; Betteridge et al., 1981/82; Mikel-Jenson et al., 1982; Moor et al., 1984, 1985). In cattle some of these problems have been considerably reduced by the intravenous injection of antibodies to PMSG (Dieleman et al., 1987) at the time of the first insemination, 12-18h after the onset of heat (Wang et al., 1987, 1988). In sheep anti-PMSG given 24 to 72h after PMSG gave similar results (Bindon and Piper, 1982a). The same effect was observed in goats given anti-PMSG at the time of onset of oestrus (Armstrong et al., 1982). However, anti-PMSG does not appear to reduce the variability of the response in any case.

Although folliculogenesis in mammals requires FSH and LH, the optimum proportions to administer to achieve superovulation consistently are not established. A great variation in the ratio of FSH:LH activity has been reported not only in PMSG obtained from different mares, but also between bleedings of the same mare at different stages

of pregnancy (Gonzalez-Mencio et al., 1978). Similar results have been reported when several batches of FSH-P (Murphy et al., 1984; Lindsell et al., 1986; Donaldson, 1990) and hMG (Murphy et al., 1984) were evaluated.

A common finding with all superovulatory hormones has been unpredictability and great variation in response. These problems are encountered not only in superovulated sheep, but also in cattle and goats. The objective of obtaining a large and consistent superovulatory response depends on the presence of responsive follicles and the administration of appropriate hormones. Although the importance of follicular dynamics and the chemical and biological properties of the gonadotrophin preparations have been identified as key factors to superovulate animals (Monniaux et al., 1983; Moor et al., 1984) neither of these key determinants has yet been optimized.

Studies conducted in cattle (Monniaux et al., 1983; Chupin et al., 1984; Murphy et al., 1984; Donaldson and Ward, 1985, 1986) and sheep (Armstrong and Evans, 1984a) support the general finding that increased amounts of LH in the superovulatory treatments reduce ovulation rate.

Low fertilization rate has also been reported in superovulated cattle, probably due to premature stimulation of the maturing oocyte (Moor et al., 1984). The problem was not overcome using multiple inseminations (Donaldson, 1985a).

The results confirm previous reports (Donaldson, 1985b; Callesen et al., 1986) that the traditional superovulatory treatment with gonadotrophins containing LH disturbs the normal oocyte and follicular development and leads to oocytes of inferior quality. Furthermore, a decrease in the percentage of transferable embryos due to changes in the number of fertilized eggs and an increase in the percentage of degenerated embryos was observed as LH levels were increased (Donaldson and Ward,1986).

Studies of the the endocrinology of the pre and periovulatory periods of superovulated animals reported disturbed plasma levels of progesterone and/or LH around those periods, which invariably led to poor egg or embryo quality (Mikel-Jenson et al., 1982; Greve et al., 1983, 1984a, b; Callesen et al., 1986).

2.2.3.2 Dose-level of gonadotrophin.

A dose response relationship has been reported when superovulation was attempted in sheep using PMSG (Robinson, 1951; Wallace, 1954; Holst, 1969; Bindon et al., 1971: Smith, 1976; Evans and Robinson, 1980; Rainio, 1991) or pituitary gland preparations (Moore and Shelton, 1964a; Boland, 1973). Similar relationships have also been observed in other farm animal species (Gordon et al., 1962; McGaugh et al., 1974; Moore, 1975).

Increasing the dose-level of gonadotrophin increases the variability of the response. This is more marked in the case of PMSG-treated animals. This large variability has been reported as the major limiting factor in all embryo transfer programmes (Cahill, 1982; Mapletoft and Murphy, 1989). If the level of gonadotrophin is too high, it will induce an overstimulation of the ovaries, particularly when PMSG is used. Ovarian overstimulation has been associated with a high incidence of persistent large follicles (Moore and Shelton, 1962; Mutiga and Baker, 1982; Ryan et al., 1984; Armstrong and Evans, 1983).

2.2.3.3 Breed.

Breed differences in sensitivity to PMSG were suggested by Wallace (1954) and have been confirmed in subsequent studies (Averill, 1958; Bradford et al., 1971; Tervit et al., 1976; Armstrong and Evans, 1983). Differences in sensitivity to PMSG have also been reported between strains within breeds (Braden et al., 1960; Bindon et al., 1971; Trounson and Moore, 1972). Strains with high natural ovulation rate seem more sensitive to PMSG stimulation than strains with low natural ovulation rate.

A French study (Torres et al., 1987) reported significantly higher ovulation rates in Romanov x Prealpes (prolific animals) compared to Prealpes or Lacaune ewes (non-prolific animals) superovulated with FSH-P.

Studies conducted using Booroola Merino sheep and/or their crosses have clearly shown higher ovulatory response in ewes carrying the high fecundity gene (Bindon and Piper, 1982b; Piper et al., 1982; Kelly et al., 1983/84; Rangel-Santos, 1987).

The Booroola ewe's high sensitivity to PMSG is apparent even in the prepubertal ovary. Ovulation rates of 5 month-old ewe lambs carrying the Booroola gene and injected with

500 i.u. of PMSG were higher when compared to the ovulation rates of ewe lambs generated from ewes classified as non-carriers (Bindon and Piper, 1986; unpublished study cited by Bindon et al., 1986). Similar results had been reported by Oldham et al. (1984) after injecting the same amount of hormone into 5 to 6 month-old ewe lambs which were carriers of the high fecundity gene. However, the higher sensitivity of the Booroola ewes carrying the fecundity gene was not confirmed when the animals were stimulated with FSH-P (Bindon and Piper, 1986; unpublished study cited by Bindon et al., 1986).

Apart from the genetic effect, it has also been indicated that differences in the degree of seasonality between the breeds involved could influence the ovulatory response (Bradford et al., 1971). Genetic as well as environmental factors were suggested to be involved when several flocks of the same breed but from different locations were treated (Armstrong and Evans, 1983). McDonald and Ch'ang (1966) reported significant differences in the natural ovulation rate of ewes of the same breed, but different origin, even after several months of being kept in a common environment.

Superior ovulatory responses to PMSG treatments have also been reported in high fecundity cattle (Thimonier et al., 1979) and mice (Bindon and Pennycuick, 1974). In summary these results show high sensitivity to PMSG stimulation of animals that were inherently highly fecund.

2.2.3.4 Age.

Although much research has been conducted on superovulation of animals, there are only a few studies where attempts to separate the possible effect of age have been made.

Averill (1958) reported no significant difference in ovulation rate between 2-tooth and mature Border Leicester and Welsh Mountain ewes. However, a consistently steeper slope of the regression line of ovulation rate on PMSG dose for the older than for the younger ewes was found. A similar tendency was reported by Torres et al. (1987) after FSH-P stimulation, but the difference was not statistically significant. Finnish Landrace ewe lambs weighing less than 60% of the weight of mature ewes produced an ovulation rate similar to that of the adult ewes (Bradford et al., 1971).

2.2.3.5 Immunization against steroids.

Immunization against steroid hormones has been successfully applied to modify normal physiological states during the last 40 years. In sheep, low ovulation rate is the main factor limiting prolificacy and productivity (Hanrahan, 1980). Immunity against steroids has been induced to overcome this problem. This can be accomplished by either active or passive immunization.

Increases in ovulation rate have been reported in ewes immunized against androstenedione (Scaramuzzi et al., 1977; Gibb et al., 1981; Land et al., 1982; Scaramuzzi and Hoskinson, 1984; Quirke et al., 1986; Philipon and Driancourt, 1987), testosterone (Scaramuzzi, 1979), oestrone (Scaramuzzi et al., 1980, 1982) and oestradiol-17β (Scaramuzzi, 1976; Scaramuzzi et al., 1980). These reports indicated increases in ovulation rate from 0.2 to 0.8 of a corpus luteum. The lambing rate can be increased by at least 20 lambs per 100 ewes joined using this simple procedure (Smith et al., 1981; Cox et al., 1982; Land et al., 1982; Scaramuzzi et al., 1982). The increased lambing percentages have been mainly due to an increased number of twins. Only moderate levels of antibody titres are required to achieve gains in lambing performance, as overstimulation of the immune system leads to reproductive failure (anoestrus, anovulation and dry ewes) and poor lambing performance (Scaramuzzi and Hoskinson, 1984).

Ovarian examination of androstenedione-immunized ewes indicated an increase in the number of large non-atretic follicles and it was concluded that the increased availability of these follicles was the immediate cause of the elevated ovulation rate (Scaramuzzi, 1984; Scaramuzzi and Hoskinson, 1984). It was also indicated that the enhanced survival of large follicles may be mediated via a reduction in the rate of androgen-induced atresia by the presence of androgen-binding antibodies in the follicular fluid of immunized ewes. Accordingly Driancourt et al. (1985) reported an alteration in the pattern of differentiation of ovulatory follicles in the 3 days preceding ovulation, and concluded that immunization against androstenedione might increase ovulation rate by modulating the process of selection. This process is thought to be controlled by the balance between gonadotrophin concentrations, ovarian sensitivity to gonadotrophins and intra-ovarian regulation (Driancourt and Fry, 1988).

Numerous reports have demonstrated increased LH levels during the luteal phase of the cycle in androstenedione-immunized ewes (Martensz and Scaramuzzi, 1979; Scaramuzzi and Hoskinson, 1984; Pathiraja et al., 1984; Campbell et al., 1987). The levels are thought to rise because of a lack of negative feedback by oestradiol on LH secretion (Martensz et al., 1976).

The levels of FSH have generally been either lowered or remained unchanged (Scaramuzzi and Hoskinson, 1984). However, McNatty et al. (1988) reported significantly higher levels of FSH in androstenedione-immunized ewes emphasizing that FSH plays an important role in determining the ovulation rate in sheep.

Androstenedione-immunized ewes have also shown a reduction in the interval from luteal regression to oestrus, but no effect on the interval from oestrus to LH surge or from LH surge to ovulation (Scaramuzzi and Hoskinson, 1984). Fertilization rate may be slightly depressed. Furthermore steroid-immune ewes have increased rates of embryo wastage which appear to be associated with elevated ovulation rates (Scaramuzzi and Hoskinson, 1984).

Other studies have reported a reduction in egg recovery rates, an increased proportion of abnormal embryos, and retarded embryo growth in androstenedione-immunized ewes (Boland et al., 1984; Murray et al., 1985; Nancarrow et al., 1985; Scaramuzzi et al., 1987). It was concluded that immunological alteration of the normal steroidogenic pathways involving androstenedione reduces ovum capture by the fimbria and interferes with embryogenesis.

There are indications that immunization against oestrone (Hoskinson et al., 1982; Smith et al., 1983), oestradiol (Boland et al., 1985; Eppleston et al., 1988) and androstenedione (Robinson and Scaramuzzi, 1986; Keeling et al., 1986) increases the ovarian response to exogenous gonadotrophins.

Although there is a general agreement that androstenedione immunization increases ovulation rate and lambing percentages, the mechanisms of action remain unclear.

2.2.4 Service of donors.

There is a general consensus that high fertility semen should be used to inseminate superovulated animals. Attempts to achieve fertilization in superovulated ewes have been made by using natural mating, keeping rams and donor ewes together over the period during which oestrus is expected (Hunter et al., 1955; Moore et al., 1960; Moore and Shelton, 1962, 1964b; Allison, 1975; Eastwood and McDonald, 1975) or using single sire hand mating (Keane, 1973; Tervit et al., 1976; Torres et al., 1987). In this system the ewes are placed with the entire ram in a mating pen and observed for mating.

Despite the use of proven high fertility rams, fertilization failure is frequent in superovulated ewes. This has been attributed to a decreased sperm transport through the cervix associated with the superovulatory treatments (Trounson and Moore, 1974a; Evans and Armstrong, 1984a). The problem has been partly overcome by placing the semen directly into the uterus (Killeen and Moore, 1971; Trounson and Moore, 1974a; Boland and Gordon, 1978). Fertilization rates above 90% were recorded by these authors. Intrauterine deposition of semen by laparotomy, however, resulted in low embryo recovery rates (Trounson and Moore, 1974a; Boland and Gordon, 1978). This has been corrected when insemination was conducted laparoscopically (Armstrong and Evans, 1984b). An effect of the time when the intrauterine insemination is performed on egg recovery and fertilization rates has been demonstrated in several studies (Robinson et al., 1989; Walker et al., 1989; Scudamore et al., 1991).

Fertilization failure after superovulation has been identified as the main factor limiting embryo transfer in sheep (Betteridge and Moore, 1977; Armstrong and Evans, 1983). Low fertilization rates have been reported frequently in ewes with more than 16 corpora lutea. The problem seems more readily related to the magnitude of the superovulatory response rather than to the type of exogenous gonadotrophin used.

2.2.5 Embryo recovery from donors.

The procedures commonly used to collect embryos in sheep were first described by Hunter et al. (1955). Further modifications of the basic flushing procedures were reported by Tervit and Havik (1976).

Embryos can be collected from the oviducts or the uterine horns, depending on the

stage of embryonic development required. In sheep, embryos normally enter the uterus 3 to 4 days after oestrus (Moore, 1977). For this reason, when attempts have been made to collect embryos on these days, the last portion of the uterine horns has also normally been included.

Nowadays in commercial embryo transfer operations most embryos are collected by flushing the uterine horns between days 5 and 7 after oestrus. Embryos can be flushed out until day 14 after oestrus (Willadsen, 1979a).

Attempts to flush embryos from the uterus in situ with the help of a laparoscope have been described by McKelvey and Robinson (1984). An average of 50% of embryos were recovered from ewes flushed 7 days after oestrus (McKelvey et al., 1986).

Studies where attempts have been made to collect embryos from the oviducts (Averill, 1958; Killeen and Moore, 1971; Tervit and Havik, 1976; Boland and Gordon, 1978) have indicated a decrease in the percentage of embryos recovered as the time after oestrus increased. This was associated with an accelerated rate of transport through the oviduct (Robinson, 1951; Averill, 1958). Bondurant (1986) indicated that the general decrease in recovery rates with the increase in oestrus-to-collection interval, may result from the death of genetically nonviable embryos, the increased difficulty in removing the embryo from a relatively voluminous uterus or premature luteal regression and expulsion of the embryo.

2.2.6 Embryo searching and evaluation.

Normally a stereoscopic microscope is used to search for and evaluate the embryos. The developmental stages of sheep embryos obtained when flushing was conducted either during the first 5 days after oestrus (Hancock and Hovell, 1961; Tervit, 1967; Trounson and Moore, 1974a; Willadsen, 1979a) or from day 5 to day 14 (Rowson and Moor, 1966a) are well characterized. Attempts to collect embryos between days 5 and 7 after onset of oestrus will generally give rise to morulae (early or compact) and blastocysts (early, expanded or hatched). Although the difference between fertilized and unfertilized eggs is quite obvious at early stages of development, this is not the case with later stages of development (morulae and blastocysts).

Several grading systems based on embryo morphology have been used to delineate embryo quality under practical conditions (Shea et al., 1976; Elsden et al., 1978; Linder and Wright, 1983). Results based on pregnancy rate confirm their effectiveness without apparent difference between them (Seidel, 1981). The efficacy of the grading systems has also been supported by more specific studies using either phase contrast microscopy (Linares and King, 1980) or electron microscopy (Linares and Ploen, 1981). The parameters commonly used to evaluate embryo quality include shape, colour, number and compactness of the cells, size of the perivitelline space, number of extruded and degenerated cells and the number and size of vesicles (Linder and Wright, 1983).

Abnormalities found in a normal flush can be classified as morphological or chromosomal.

The incidence of morphological abnormalities in sheep varies between 4 and 23% (Dutt, 1954; Hart, 1956; Averill, 1958; Braden, 1964; Allison, 1967; Tervit and McDonald, 1969). The most commonly found descriptions were fragmentation, degeneration, involution of vitellus and broken zonae pellucidae. The presence of anucleated particles has also been reported (Killeen and Moore, 1970, 1971) but, since these embryos were able to develop normally, it was suggested they be considered atypical rather than abnormal. The incidence of chromosomal abnormalities determined by karyotype analysis was only 6% (Long and Williams, 1980) and this was similar to percentages reported in cattle (McFeely, 1967) and pigs (McFeely and Rajakosky, 1968).

Because embryos at different stages of development are commonly found in the washings from a single flush, the criterion most extensively used to evaluate embryos is whether an embryo has attained an appropriate stage of development (Elsden et al., 1978; Kunkel and Stricklin, 1978; Shea, 1981). It has been suggested that sheep embryos be considered as retarded if they are one (Moore, 1982; Bondurant, 1986) or more cleavage divisions behind their normal cleaved litter mates (Willadsen, 1979a). In vitro embryo culture results (Moore, 1982) indicated that such embryos rarely show continued development suggesting that they are not viable.

2.2.7 Embryo transfer.

Embryo transplantation in sheep has been normally carried out by midventral laparotomy

under general or local anaesthesia (Moore, 1982). Frequently the ewes are restrained in a laparotomy cradle like the one described by Lamond and Urquhart (1961). More recently, techniques of transferring embryos with the help of a laparoscope have been reported (Mutiga and Baker, 1984; McKelvey and Robinson, 1984). Pregnancy rates close to normal have been achieved after the transfer of day 5 to 7 embryos using laparoscope-aided techniques (Schiewe et al., 1984; Walker et al., 1985). The technique is quick (5-8 minutes per ewe, McKelvey et al., 1985) and avoids much of the trauma that often leads to postoperative adhesions when embryos are transplanted by laparotomy.

2.2.8 Factors affecting the success of embryo transfer.

2.2.8.1 Degree of synchronization of oestrus in donor and recipient ewes.

The interval from onset of oestrus to transfer has been the criterion used to select donors and recipients to be used in an embryo transfer programme. Although the highest pregnancy rate has been reported with an exact degree of synchronization between the donors and recipients (Hancock and Hovell, 1961; Moore and Shelton, 1964b; Cumming, 1965), acceptable pregnancy rates have also been achieved when there is a synchronization of ± 1 day (Moore and Shelton, 1964b; Rowson and Moor, 1966b; Hunter et al., 1955) or ± 2 days (Averill, 1956; Moore and Shelton, 1964b; Rowson and Moor, 1966b). Higher degrees of asynchrony appear to be incompatible with pregnancy (Moor, 1965; cited by R. Newcomb, 1977) or result in extremely low conception rates (Rowson and Moor, 1966b). The latter authors indicated that embryonic loss could occur when the donor and recipient ewes are not closely synchronized because the uterine environment may not be suitable for the embryos, or because the "out-of-phase" embryo is incapable of exerting a sufficient luteotropic action on the recipient's corpus luteum, which is then not maintained. Accordingly, Wilmut et al. (1985) indicated that embryos which are too asynchronous to compensate for an inappropriate uterine environment become abnormal, fail to inhibit luteolysis and are lost from the uterus.

Wilmut and Sales (1981) and Lawson et al. (1983) reported an increase in embryo development after the transfer of embryos into more advanced uterine environments and a decrease in the development of embryos transferred into less advanced uterine environments. The same effect was also reported in pigs (Pope et al., 1986).

Marked variation between ewes can occur in the time of ovulation and fertilization in relation to oestrus (Hancock, 1962). This increases the likelihood that the stage of development of the transferred embryo and the genital tract of the recipient ewe will not be closely synchronized even if the onset of oestrus in the donor and recipient ewes are exactly synchronized (Tervit, 1967). The above could probably explain why some ewes do not become pregnant (Larsen, 1971) or produce unsuccessful transfer results (Moore and Shelton, 1964b; Shelton and Moore, 1966) despite close synchrony based on onset of oestrus between donor and recipient ewes.

Cumming (1965) suggested that there are specific lower and upper limits for the embryonic and endometrial development that will allow the development of the transplanted embryos; and Wilmut et al. (1985) indicated that pregnancy rates should be greater than those achieved at present if there were a perfect degree of synchronization between donors and recipients.

2.2.8.2 Quality of the embryos transplanted.

Different grading systems based mainly on morphological appearance have been extensively used to delineate the quality of the embryos. The usefulness of gross morphological evaluation of the embryos in predicting pregnancy rates for groups of embryos has been widely recognized, despite its limited value in determining the likelihood of survival of individual embryos. Regardless of the grading system used, pregnancy rates have been shown to be highest (around 60%) for embryos classified in the highest grading (e.g. excellent, good). The lowest pregnancy rates (approximately 20%) were reported for embryos in the lowest rating (e.g. poor), with intermediate pregnancy rates for embryos graded in between (e.g. fair) (Shea et al., 1976; Elsden et al., 1978; Tervit et al., 1980; Wright, 1981; Linder and Wright, 1983).

2.2.8.3 Number of embryos transplanted.

Increasing the number of embryos transferred per recipient has generally failed to improve pregnancy rates (Moore et al., 1960; Moore and Shelton, 1962; Cumming, 1965; Tervit, 1967; Cumming and McDonald, 1970; Armstrong and Evans, 1983), although Larsen (1971) reported a significant elevation in pregnancy rate when the number of embryos transplanted was increased from one to three (66% and 90%, respectively).

It has been suggested that the success or failure of any transfer depends mainly on the inherent ability of the recipient ewe to support a pregnancy rather than on the number of eggs transplanted (Moore et al., 1960). Nevertheless Larsen (1971) indicated that increasing the number of embryos transplanted could increase the chance of at least one of them being in phase with the recipient, thus increasing the probability of a successful pregnancy. On the other hand, it has been stated that in a suitable maternal environment, embryo losses are at random and not excessive (Moore and Shelton, 1964b).

Embryo survival has been shown to decrease as the number of embryos transplanted is increased (Moore et al., 1960; Cumming, 1965; Tervit, 1967; Moore, 1968; Cumming and McDonald, 1970; Larsen, 1971; Land and Wilmut, 1977).

Ashworth et al. (1984) reported that reduced embryo survival in sheep was associated with low concentration of progesterone on day 0 and 1, as well as during the luteal phase. Likewise, significantly higher levels of progesterone on day 0 to 3 were found in pregnant cows (Lee and Ax, 1984). Conversely Evans and Robinson (1980) found no significant difference in embryo survival between ewes with high or low plasma progesterone concentrations. They concluded that over a wide range progesterone has no quantitative effect on fertility. Moore et al. (1960) did not find significant differences in pregnancy rate, embryo survival or number of lambs born between recipients induced to superovulate (treated with 1500 i.u. of PMSG) and non-superovulated recipients.

2.2.8.4 Site of transfer.

The site of transfer should depend on the stage of development. Thus, 8-cell embryos must be transferred into the oviducts, and embryos at more advanced stages of development into the uterine horns (Moore and Shelton, 1962; Moore, 1982). A higher pregnancy rate has been reported after tubal compared to uterine transfers (Moore and Shelton, 1964b). Increased embryo survival as the age of the transferred embryo increases has also been found (Moore and Shelton, 1964b; Shelton and Moore, 1966).

Consistently higher pregnancy rates have resulted after transferring bovine embryos ipsilateral to the corpus luteum (Newcomb and Rowson, 1976; Sreenan, 1976; Newcomb et al., 1978; Del Campo et al., 1979). No significant difference was observed

in another study (Tervit et al., 1977). Almost certainly the lowered pregnancy rate is due to failure to maintain the corpus luteum (Christie et al., 1979). Several reports have suggested that position within the uterine horn was important (Newcomb, 1979; Christie et al., 1980; Newcomb et al., 1980) and additional evidence has been shown suggesting that the tip of the ipsilateral horn was a favoured site for embryo development (Newcomb and Rowson, 1980).

The results of two studies conducted in sheep, in which the effect of the distribution of ovulation rate of the recipient on embryo survival has been examined, were reported by Torres and Sevellec (1987). Results of the first study indicated no significant difference in the percentages of lambs born between recipients with a single CL on each ovary compared to recipients with 1 or 2 corpora lutea on the same ovary (68% vs 62%, respectively) following the transfer of an embryo into each uterine horn. Similarly in the second study when 2 embryos were transferred into the same horn, the percentages of lambs born were not significantly different whether the corpora lutea were on each ovary or on the same ovary (71% vs 93%, respectively).

2.2.9 Embryo splitting.

Embryo splitting is a technique that has been developed to increase the number of offspring per embryo. The technique can result in the production of identical twins or clones of offspring. Successful embryo bisection and production of offspring, using day-6 sheep embryos which were divided and cultured in vitro before transfer, was first reported more than 16 years ago (Trounson and Moore, 1974b). There have been attempts to split either pre-compaction or post-compaction embryos. The blastomeres in the pre-compaction embryo are easily separated with minimal damage, but their survival rate is low once the zona pellucida has been opened or removed (Modlinski, 1970; Moor and Cragle, 1971; Trounson and Moore, 1974b; Massey et al., 1982). Factors such as disaggregation due to the low adherence between the cells (Bronson and McLaren, 1970; Willadsen and Fehilly, 1983) and detrimental effects of the direct contact between the cells and endometrium (Modlinski, 1970) have been associated with the low survival rate. Poor survival of demi-embryos generated from morula stage embryos could be due to the cracked zonae pellucidae (Williams et al., 1984). These studies supported the idea that an intact zona pellucida is required for development of pre-compaction stage embryos. Willadsen (1979b, 1980) reported a technique that

considerably improved the survival rate of bisected pre-compaction stage sheep embryos. In his technique bisected embryos were placed into evacuated zonae pellucidae embedded in agar cylinders in an attempt to maintain blastomeres together. Then they were temporarily transferred to ligated oviducts of ewes to allow compaction to occur before their definitive transfer into recipient ewes. In this study the first sets of monozygotic twins were also reported.

Willadsen and Polge (1981) divided 8-cell cow embryos using the same technique and reported the production of monozygotic twins and triplets. This showed the feasibility of the technique and the ability of blastomeres from 2-, 4- and 8-cell embryos to progress to term. The fact that, in commercial systems, most of the embryos were collected at later stages of development, generally after compaction (from compact morula to expanded blastocyst), made necessary the evaluation of the splitting technique using such later stage embryos. Willadsen et al. (1981) conducted a study on day 5 and 6 cow embryos. In-vitro embryo survival was 90% on average and there was no difference between embryos bisected into halves or quarters. Survival rate of demi-embryos after transfer (75%) was similar to that of normal embryos but survival rate of quarter embryos (41%) was considerably below that obtainable with normal cow embryos. The authors indicated the possibility of omitting the agar embedding, as well as the need to place the bisected embryos into the oviduct of ewes for culture before their transfer to definitive recipients, when fully compacted late morulae were used to produce demi-embryos. This can be accomplished provided that the micromanipulated embryos are incubated at 38° C for a few hours in order that re-compaction may take place, before transplantation to recipient cows.

Studies in which both steps were avoided have been reported using sheep (Gatica et al., 1984) and cattle (Ozil et al., 1982; Lambeth et al., 1983; Ozil, 1983; Picard et al., 1986) embryos. The average pregnancy rates ranged from 53% to 73%, and 17% to 48% respectively, after the demi-embryos were transferred as twins or individually into each recipient. All these techniques, although less complicated than the first, still required the use of complex equipment, great personal skills and were tedious and time consuming.

A further simplification to splitting techniques has been reported (Chesne et al., 1987;

Warfield et al., 1987; Seike et al., 1989) where no surrogate zonae pellucidae were used; but the use of a holding pipette to maintain the embryo in position for splitting was still required.

An even quicker and more simple technique was reported by Williams and Moore (1988). In their technique, the bisection was performed using a razor blade joined to glass tubing placed onto a hand-held micromanipulator. For splitting, the blade was manoeuvered above the embryo and, with a downward movement, forced through the zona pellucida, the final movement being a forward cutting of the embryonic tissue along the bottom of the petri dish. The technique requires only a hand-made microblade, a manipulator and a stereomicroscope. The procedure takes only a few minutes and results in normal pregnancy rates.

Some of the factors affecting the pregnancy and survival rate of demi-embryos include embryo quality before bisection, developmental stage or embryo age, presence of zona pellucida, transfer site and number of demi-embryos transferred.

Embryo quality: Embryos to be split need to be selected carefully since it has been shown that good quality whole embryos will produce good quality demi-embryos and higher pregnancy and survival rates compared to demi-embryos obtained from poor quality whole embryos (Seidel, 1982; Lambeth et al., 1983; Ozil, 1983; Picard et al., 1986; Arave et al., 1987; McEvoy and Sreenan, 1990). Fair and poor quality embryos are not good candidates for bisection. Pregnancy rate after the transfer of demi-embryos generated from excellent or good quality whole embryos was not significantly different (Williams et al., 1984).

Stage of development: The pregnancy rate after the transfer of bisected embryos increased with age (5.5 to 7.5 days) and stage of development (early morula to expanded blastocyst). An increase in the percentage of twin pregnancies was also reported with an increase in the stage of embryonic development (Williams et al., 1984).

Pregnancy rate, survival rate and the number of foetuses per original embryo were reported to be lower (McEvoy and Sreenan, 1990) for morula-derived than for blastocyst-derived demi-embryos (20 vs 73%, 15 vs 45%, 30 vs 91%, respectively). A

considerable increase in the efficiency of the splitting technique with day of flushing (8 to 10 days after onset of oestrus) has also been indicated (Chesne et el., 1987). Williams et al. (1984) suggested that the blastocyst was the best stage for embryo splitting and De Armas et al. (1986) reported over 80% of demi-embryos generated from blastocysts developing after 2h of in-vitro culture. From these studies it can be concluded that stage of development is more important than embryo age, when embryos are to be selected for splitting. This is supported by results from Williams et al. (1984) where it was shown that, regardless of the day of flushing, blastocysts always gave better results than embryos at any other stage of development.

Presence of the zona pellucida: The zona pellucida does not play a role in embryonic development from the compact-morula stage onwards (Chesne et al., 1987). This has been shown after its enzymic or mechanical removal in studies conducted in vitro and in vivo (Trounson and Moore, 1974b; Hope and Bavister, 1983; Mertes and Bondioli, 1985; De Armas et al., 1986). Pregnancies have been reported from zona-free demi-embryos that were tight morulae, early blastocysts, blastocysts and expanding blastocysts at the time of transfer (Warfield et al., 1987). On the other hand, survival rate of demi-embryos was similar after they were transferred into their own zonae pellucidae or after they were placed into evacuated zonae pellucidae (Williams et al., 1984). Furthermore, embedding zona-free demi-embryos in gelatin did not show any advantage over transferring embryos without zonae pellucidae (Warfield et al., 1987).

Empty zonae pellucidae, when required, have been obtained from ovaries of slaughtered animals through aspiration, from degenerated embryos or from unfertilized eggs (Willadsèn, 1980; Lambeth et al., 1983; Picard et al., 1986) obtained at the same time that the embryos to be bisected were collected. They can also be kept stored at -20°C in PBS plus 20% foetal calf serum (Willadsen, 1980) or steer serum (Williams et al., 1984).

Site of transfer: Cattle studies have shown higher pregnancy rates (Sreenan, 1976) and embryo survival rates (Heyman and Renard, 1978) after the transfer of whole embryos to the uterine horn ipsilateral to the ovary with the corpus luteum than to the contralateral site. Results from 4 studies, where demi-embryos were transferred singly to the ipsilateral uterine horn or as twins placing one demi-embryo at the tip of each

uterine horn, failed to show any significant difference in their survival rate (36.9% and 36.5% respectively; Warfield et al., 1987). Overall the results were low, probably due to the transfer of demi-embryos into the uterine horn contralateral to the corpus luteum, when two demi-embryos were implanted. It has previously been indicated that if the demi-embryo ipsilateral to the corpus luteum fails to survive, it is unlikely that the contralateral embryo will survive (Seidel, 1981). In addition, in the trials conducted by Warfield et al. (1987), the fact that embryos were occasionally transferred up to 8h after flushing could also be involved. Similar results were reported after the transfer of whole (Renard et al., 1980) and demi-embryos (Baker and Shea, 1985). In other studies an overall higher pregnancy rate was reported, but no significant difference between embryos transferred singly or as twins was found (Williams and Moore, 1988; Seike et al., 1989). Ozil (1983) reported a considerably higher pregnancy rate after twinning but the difference was not statistically significant.

A higher survival rate has been generally found when 2 demi-embryos were transferred into the ipsilateral horn (Willadsen et al., 1981; Ozil, 1983; Chesne et al., 1987) compared to when one embryo was transplanted into each side (Gatica et al., 1984; Warfield et al., 1987), but this has not always been the case (Seike et al., 1989). When survival rate is compared after transferring 1 or 2 demi-embryos to the ipsilateral horn, the results have been quite variable. While Ozil (1983) and Warfield et al. (1987) failed to find any difference, Lambeth et al. (1983) reported a higher survival rate after transferring 2 demi-embryos and Seike et al. (1989) indicated the opposite. Although identifying the reasons for the disagreement among the different authors is quite difficult, there is no doubt that factors such as day of flushing or stage of development, media used for flushing, handling and splitting, splitting technique, and time from collection to microsurgery and transfer could all be involved.

The viability of a demi-embryo is influenced by the number of blastomeres left after splitting (Picard et al., 1986) especially if the bisected cell masses are cultured in vitro for more than 4h. Heyman (1985) indicated that the intensity of the embryonic signals given to the recipient may be related to the number of embryonic cells in the uterus. A minimal number of embryonic cells may be necessary to ensure that the recipient uterus receives signals strong enough to initiate pregnancy. The author indicated that the addition of 1 or 2 trophoblastic vesicles when transferring half-embryos can increase the

intensity of the embryonic signals and enhance the establishment of pregnancy after transfer. On the other hand, it has been determined that splitting post-compaction embryos will produce the loss of between 9 and 13% of the blastomeres (Skrzyszowska and Smorag, 1987; Nibart et al., 1988). The latter authors also indicated that a higher percentage of cells from the inner cell mass than from the trophoblast are damaged during bisection. The results also showed that the percentage of damaged cells in the demi-embryos depends to some extent on the stage of development of the bisected embryo. This problem was decreased with advanced stages of development.

The occurrence of twins from a single demi-embryo has been reported in several studies (Williams et al., 1984) but the incidence has been low (Williams et al., 1984; Warfield et al., 1987). Although the reasons are unknown, it may result from segregation of the embryo into two functional domains as it grows through the cracked zona pellucida (Hsu and Gonda, 1980; Massip et al., 1983). Other possibilities involve disorganization and reorganization of the inner cell mass (Williams et al., 1984). In this regard extruded cells or even apparent cellular debris might contain enough viable cells to proliferate and reorganize to form another functional embryo.

CHAPTER III:

MOET STUDIES USING EWE LAMBS

CHAPTER III:

MOET STUDIES USING EWE LAMBS

3.1 Materials and methods.

3.1.1 Experimental animals and their management.

The trials were conducted at the Sheep and Beef Cattle Research Unit (S.B.C.R.U.), Massey University.

The animals used in these trials were maintained on ryegrass-white clover pastures. Drinking water was supplied ad libitum. The adult ewes and ewe lambs were identified individually with brass ear tags, and with plastic ear tags and serial numbers sprayed on the flank or plastic collars too.

Donor ewe lambs used in this MOET programme came from a Romney fleece weight selection flock (F.W.) or its control flock (C.F.). A small group of mature Romney ewes from the same flocks was always included for comparison. The recipient ewes came from several commercial Romney flocks, as well as from a Drysdale flock all kept at Massey University.

Other studies conducted during the programme involved the use of the following groups of animals: a) Two groups of commercial Romney ewe lambs, b) One group of Booroola-Romney x Perendale ewe lambs, and c) One group of Drysdale ewe lambs.

Additional work, conducted in conjunction with a commercial enterprise included the use of three groups of mixed-age Romney ewes as recipients, while ewes from several breeds (Romney, Danish Texel, Finnish Texel, Gotland Pelt and Oxford Down) were used as donors.

The entire rams used in the MOET programme were two tooths selected from either the F.W. or the control flock. Some of these rams were also used in other embryo transfer studies.

Adult rams of several breeds (Danish Texel, Finnish Texel, Gotland Pelt and Oxford Down) were used in the studies conducted in conjunction with a commercial enterprise.

Vasectomized Romney and/or Suffolk teaser rams were used for detection of oestrus in recipient ewes while only Romney teaser rams were used for heat detection in the donors.

3.1.2 Synchronization of oestrus.

To enable animals to be programmed for embryo transfer (E.T.) oestrous cycles were synchronized using intravaginal sponges impregnated with 40 mg of M.A.P. (17∝-acetoxy- 6∝-methyl pregn-4 -ene-3, 20-dione) inserted for 12-14 days. The sponges were made by applying the progestagen dissolved in ethanol and allowing them to air dry. An applicator was used to place the sponges coated with antiseptic cream in the adult ewe but in the ewe lamb a finger was initially passed through the vaginal "ring" prior to the sponge being inserted and gentle pressure applied so as to avoid vaginal damage. At sponge removal care was taken not to leave the sponge or part of it inside the vagina especially in the ewe lamb. Sponges were usually withdrawn from recipient ewes 12-15h earlier than from donor ewes, unless stated otherwise. This was done to achieve a better degree of synchronization between them.

Detection of oestrus was carried out using entire or vasectomized rams (teaser rams). The entire or the teaser rams fitted with Sire Sine harnesses holding crayons of different colours were introduced to the sheep at sponge removal or within the following 12h. The male to female ratio in the case of recipient ewes was between 1:12 and 1:20 and it was reduced to 1:4 or 1:6 in case of donor animals. The ewe mobs were inspected for oestrus 24h after sponge removal, normally twice a day at 08.00 and 17.00 h and sometimes at midday when a high incidence of oestrus was expected. A ewe was considered in heat when clearly marked on her rump.

The ewes programmed as 'recipients' remained with the teaser ram until required at the time of embryo transfer, while the potential donor ewes were separated from the entire or teaser rams shortly after detection of oestrus. Initially donors running with entire rams and not recorded in heat by 72h after sponge removal were laparoscoped 6 to 7 days later to record the number of ovulations. Later, when intrauterine artificial insemination

(A.I.) was used, donors not detected in heat by 60h after sponge removal were artificially inseminated 12h later (i.e. 72h after sponge removal).

3.1.3 Superovulation.

Gonadotrophins to induce superovulation were administered intramuscularly (i.m.) starting 2 days before sponge withdrawal, unless stated otherwise. Several preparations of pregnant mare serum gonadotrophin (PMSG) were used:

Massey-PMSG: Blood was collected by jugular venipuncture from pregnant mares using Acid citrate dextrose (ACD) as anticoagulant. Following centrifugation, plasma was removed and stored at -20°C until required. Its potency was determined by radioimmunoassay using Folligon as standard for comparison[†].

Folligon: PMSG preparation (Intervet, Holland).

Consept45: PMSG preparation (Heriot Agvet Pty Ltd, Australia).

Pregnecol: PMSG preparation (Heriot Agvet Pty Ltd, Australia).

Two sources of FSH were used:

FSH-P: Porcine origin purified follicle stimulating hormone (Schering Corporation, U.S.A).

Ovagen: Ovine origin purified follicle stimulating hormone (Immuno-Chemical Products, New Zealand).

In addition to the gonadotrophins two other drugs were used in an attempt to enhance the ovulatory response:

Fertagyl: Synthetic gonadotrophin releasing hormone (GnRH, Intervet, Holland).

Fecundin: Immunogen consisting of androstenedione-7a-carboxy-ethylthioether: human serum albumin (androstenedione-7a-HSA) and DEAE-dextran as an adjuvant (Coopers Animal Health Ltd, Australia).

[†] Kindly carried out by Dr. K.P. McNatty, Wallaceville Animal Research Station.

3.1.3.1 Gonadotrophins and ovulatory responses.

Trial 1.

The trial was conducted in March-April of 1988. Fifty six 6 to 7 month-old ewe lambs were used as donors; 31 were from a Romney fleece weight selection flock and 25 were from the control flock. An additional group of 23 mixed-age Romney ewes was included for comparison. The animals were divided into 3 time of treatment groups (Periods). Groups 1 and 2 were further subdivided into 2 subgroups each, according to the time of sponge removal. Sponges in subgroups 2 and 4 were withdrawn 2 days after they had been removed from sheep of subgroups 1 and 3. This difference in timing of synchronization between groups and among subgroups within each group was intended to facilitate the conduct of the study and also to allow recipients from subgroups 1 and 3, which came into oestrus later than expected, to be used as recipients in subgroups 2 and 4. The allocation and number in each of the treatments, groups and subgroups are shown in Tables 3.1, 3.2 and 3.3. Because lambs were born within a 6 weeks period, generally those born in the first 2 weeks were included in group 1, the ones born in the second 2 weeks were assigned to group 2 and those born in the last 2 weeks to group 3. However, in some cases when lambs born in the first 2 or the second 2 weeks were considerably lighter than the average weight of their respective group they were assigned to the following group to allow them to grow further. These were replaced with average live-weight lambs from the following group. The same criteria of animal distribution was used in trials 3 and 5 in which lambs from the progeny tested Romney flock were also used. The distribution of the animals to the different treatments in the present and subsequent trials was always at random. Superovulation in subgroups 1 and 2 was attempted by injecting 900 or 1200 i.u. of Consept45 at sponge removal (T1 and T2, respectively). However, the time of injection was changed because of the poor response observed so subgroups 3 and 4 (Group 2) were treated with 900 and 1200 i.u. of Consept45 given 24h before sponge removal (T3 and T4, respectively). Animals in group 3 were treated to induce a non-superovulatory oestrus first. This was achieved by administering 300 i.u. of Consept45 at sponge removal to ensure occurrence of oestrus. Superovulation was attempted by administering 900 or 1200 i.u. of Consept45 on day 13 of the following oestrous cycle (T5 and T6, respectively).

Eight 18-month-old Romney rams, of which half were from the F.W. and the other half from the controls, were used to serve the donors.

A group of 172 mixed-age Romney ewes was used as recipients. Oestrus was detected in these animals using 3 Suffolk teaser rams. Entire and/or teaser rams were introduced to their donor and recipient groups respectively at sponge removal.

Table 3.1 Distribution of animals of group 1 to their respective treatments and subgroups (Trial 1).

			SUBGROUP		
AGE	TRT	DOSE(i.u.)	1	2	
Lambs	1	900	5	5	10
	2	1200	6	6	12
Adults	1	900	2	2	4
	2	1200	2	2	4
TOTAL			15	15	30

Table 3.2 Distribution of animals of group 2 to their respective treatments and subgroups (Trial 1).

			SUBGROUP		
AGE	TRT	DOSE(i.u.)	3	4	
Lambs	3	900	6	6	12
	4	1200	6	5	11
Adults	3	900	2	3	5
	4	1200	3	2	5
TOTAL			17	16	33

Table 3.3 Distribution of animals of group 3 to their respective treatments (Trial 1).

AGE	TRT	DOSE(i.u.)	
Lambs	5	900	5
	6	1200	6
Adults	5	900	2
	6	1200	3
TOTAL			16

Trial 2.

The trial was carried out in May-June of 1988 and included a group of 62 animals as donors. Fifty of these animals were 8 to 9 month-old commercial Romney ewe lambs and the remaining 12 were mixed-age Romney ewes. Superovulation in ewe lambs was attempted by injecting 1200 i.u. of Consept45, 2 (T1) or 4 (T2) days before sponge removal or 1200 i.u. of Folligon, 2 (T3) or 4 (T4) days before sponge removal. A group of ewe lambs remained untreated and was kept as a control (T5). T1 was also administered to the group of ewes and was recorded as T6. Donors were mated naturally using 4 Romney rams from the fleece weight selection flock. The sires were introduced to the group of donors at sponge removal. The distribution of the animals to the experimental groups is shown in Table 3.4.

A group of 58 mixed-age Romney ewes was used as recipients and run with 2 Romney teaser rams.

Table 3.4 Distribution of animals to the treatments (Trial 2).

		Time of injection (days before sponge removal)		
AGE	HORMONE	2	4	
Lambs	Consept 45	T1=10	T2=10	20
	Folligon	T3=10	T4=10	20
	Control	T5=10		10
Adults	Consept 45	T6=12		12

Trial 3.

The trial was conducted in March-April of 1989. The study involved as donors a total of 73 six to seven month-old ewe lambs of which 39 were from the Romney fleece weight selection flock and the remaining 34 were controls. An additional group of 20 mixed-age Romney ewes was included for comparison. Superovulation in ewe lambs was attempted by submitting the animals to one of the following treatments.

(a) 1200 i.u. of Folligon given 2 days before sponge removal.
(b) Treatment (a) + 100 μg GnRH.
(c) 24 mg of FSH-P given as 6 injections in a descending dose (5,5,4,4,3,3 mg) twice daily every 12h, and starting 2 days before sponge removal.
(d) Treatment (c) + 100 μg GnRH.
(e) 8 ml of Ovagen given in 8 injections as a constant dose (1 ml each) twice daily every 12h, and starting 2 days before sponge removal.
(f) Treatment (e) + 100 μg GnRH.
(g) 1200 i.u. of Folligon given 2 days before sponge removal + 2.5 ml of Ovagen given in the morning of day 4 after sponge insertion (as priming).
(h) Treatment (g) + 100 μg GnRH.

Mature ewes were treated with Folligon either with or without GnRH as for Treatments (a) and (b), respectively. The trial was conducted over 3 periods (1,2 and 3) at intervals of 2 weeks. Table 3.5 shows the distribution of the animals to the experimental groups. GnRH was given within 3h of oestrous detection. Six Romney rams, of which 4 were from the F.W. flock and the other 2 from the control flock, were used to serve the donors. The first group of donors (23 lambs and 6 ewes) was mated naturally and laparoscopic A.I. was used for the remaining animals (Groups 2 and 3) in an attempt to increase the fertilization rate and embryo quality.

One hundred and thirty nine mixed-age ewes were used as recipients of which 108 were Romney and 31 were Drysdale.

Oestrous detection was carried out using 2 Romney teaser rams in the donor animals and 3 Suffolk teaser rams in the group of recipient ewes.

Table 3.5 Distribution of animals to their respective treatments and groups (Trial 3).

	LAMBS				ADULTS			
	GROUP				GROUP			
TREATMENT	1	2	3	TOTAL	1	2	3	TOTAL
1200 i.u. Folligon	3	3	3	9	2	3	4	9
1200 i.u. Folligon + 100 µg GnRH	3	3	3	9	4	4	3	11
24 mg FSH-P	3	3	4	10				
24 mg FSH-P + 100 µg GnRH	3	3	3	9				
8 ml Ovagen	3	2	3	8				
8 ml Ovagen + 100 µg GnRH	3	2	3	8				
1200 i.u. Folligon + 2.5 ml Ovagen	3	3	3	9				
1200 i.u. Folligon + 2.5 ml Ovagen + 100 µg GnRH	2	3	6	11				
TOTAL	23	22	28	73	6	7	7	20

Trial 5.

The trial was conducted in March-April of 1990. The study involved as donors a total of 64 six to seven month-old ewe lambs of which 34 were from the Romney fleece weight selection flock and the remaining 30 were controls. An additional group of 13 mixed-age Romney ewes was included for comparison. Ewe lambs were submitted to the following treatments in an attempt to induce superovulation.

(1) 1200 i.u. of Folligon given 2 days before sponge removal plus 100 μg GnRH.
(2) 300 i.u. of Folligon given 2 days before sponge removal plus 8 ml of Ovagen in 8 injections as a constant dose (1 ml each) twice daily every 12h, starting immediately after the Folligon injection.
(3) Treatment (2) + 100 μg GnRH.
(4) 500 i.u. of Folligon given 2 days before sponge removal plus 8 ml of Ovagen in 8 injections as a constant dose (1 ml each) twice daily every 12h, starting immediately after the Folligon injection.
(5) Treatment (4) + 100 μg GnRH.

Mature ewes were treated with Folligon as for Treatment (1).

In order to conduct the study, the mob was divided into 3 treatment groups and each group was treated with an interval of 2 weeks from each other. The distribution of the animals to the experimental groups is shown in Table 3.6. GnRH was given within 3h of oestrous detection. The donors received intrauterine A.I. using semen from 4 rams of the fleece weight selection flock.

The recipients were 128 mixed-age Drysdale ewes. Two Romney teaser rams were used for heat detection in the group of donors and 3 Suffolk teaser rams in the recipient ewes. Teasers and ewes were joined 12h after sponge removal.

Table 3.6 Distribution of animals to their respective treatments and groups (Trial 5).

	LAMBS				ADULTS			
	GROUP				GROUP			
TREATMENT	1	2	3	TOTAL	1	2	3	TOTAL
1200 i.u. Folligon	5	5	3	13	3	5	5	13
300 i.u. Folligon + 8 ml Ovagen	5	5	3	13				
300 i.u. Folligon + 8 ml Ovagen + 100 µg GnRH	5	5	3	13				
500 i.u. Folligon + 8 ml Ovagen	5	5	3	13				
500 i.u. Folligon + 8 ml Ovagen + 100 µg GhRH	4	5	3	12				
TOTAL	24	25	15	64	3	5	5	13

3.1.3.2 Trial 4 (Immunization against androstenedione and superovulation).

The trial was conducted in April-June of 1989. The study involved 90 eight to nine month-old Romney ewe lambs used as donors and 31 mixed-age Romney ewes as recipients. Immunization against androstenedione was carried out in half of the animals by administering 4 ml of Fecundin as 2 equal subcutaneous injections given 4 weeks apart. For practical convenience the mob was divided into 2 time of treatment groups. Oestrous synchronization in group 1 was started on the day of the second Fecundin injection and group 2 was programmed 1 week later. Superovulation was attempted by injecting 1200 i.u. of one of the following sources of PMSG: Massey-PMSG, Consept45 or Folligon. Animals allocated to each source of PMSG were further subdivided and half of them received 100 μg of GnRH (Fertagyl) at onset of oestrus, while the other half remained untreated. The distribution of the animals to the experimental groups is shown in Table 3.7.

Semen from 2 Romney rams of the F.W. flock was used for intrauterine A.I. of the donor animals. The same number of Romney teaser rams was used to detect the incidence of heat in the donors and 1 Suffolk teaser ram was placed win the group of recipients.

Table 3.7 Distribution of animals to their respective treatments and groups (Trial 4).

	GROUP		
TREATMENT	1	2	TOTAL
1200 i.u. Massey-PMSG	3	3	6
1200 i.u. Massey-PMSG + 4 ml Fecundin	4	4	8
1200 i.u. Massey-PMSG + 100 μg GnRH	4	3	7
1200 i.u. Massey-PMSG + 4 ml Fecundin + 100 μg GnRH	4	4	8
1200 i.u. Pregnecol	4	3	7
1200 i.u. Pregnecol + 4 ml Fecundin	3	4	7
1200 i.u. Pregnecol + 100 μg GnRH	4	3	7
1200 i.u. Pregnecol + 4 ml Fecundin + 100 μg GnRH	4	4	8
1200 i.u. Folligon	4	4	8
1200 i.u. Folligon + 4 ml Fecundin	4	4	8
1200 i.u. Folligon + 100 μg GnRH	4	4	8
1200 i.u. Folligon + 4 ml Fecundin + 100 μg GnRH	4	4	8
TOTAL	46	44	90

3.1.3.3 Trial 6 (Booroola gene effects and superovulation).

The Booroola-Merino is a prolific strain with a high natural ovulation rate and litter size (Bindon, 1984). This study evaluated the ovulatory response to gonadotrophins in ewe lambs from a flock based on interbred (Booroola x Romney) x Perendale sheep which had also been selected for fecundity.

The trial was conducted from March to July of 1990 and involved 120 ewe lambs. Superovulation was attempted by injecting 1200 i.u. Folligon (Treatment 1) or 1200 i.u. Folligon + 100 µg GnRH (Treatment 2). Ninety of the animals were treated in March-April when they were 6 to 7 month-old. The remaining 30 lambs were treated in June-July, when they were 9 to 10 month-old. Natural ovarian activity was recorded during May and June in lambs being tupped. The distribution of the animals to the experimental groups is shown in Table 3.8.

The number of recent corpora lutea and large follicles were recorded at laparoscopy 5 to 7 days after detection of oestrus in treated animals and 5 to 10 days after oestrous detection in case of natural ovulation. Laparotomy was performed when difficulty was encountered in recording the ovulatory response by laparoscopy (3 animals).

Three Romney teaser rams were used for oestrous detection in animals treated at 6 to 7 months of age as well as when the natural incidence of heat was investigated. However, only 2 teaser rams were used when the ewe lambs were treated at 9 to 10 months of age.

Table 3.8 Distribution of animals to their respective treatments and groups (Trial 6).

AGE	TRT	HORMONE	No.	TOTAL
6-7 months	1	1200 i.u. Folligon	45	
	2	1200 i.u. Folligon + 100 µg GnRH	45	90
9-10 months	1	1200 i.u. Folligon	16	
	2	1200 i.u. Folligon + 100 µg GnRH	14	30
TOTAL				120

3.1.4 Service of donors.

3.1.4.1 Natural mating.

Donor ewes were naturally mated using 1 ram per mating paddock during the 1988 breeding season and the first group of trial 3 conducted in 1989. Ram/ewe pairing was always at random. Whenever possible at least 1 adult ewe was placed with the group of lambs at mating. This was done in an attempt to increase the intensity of oestrous behaviour in ewe lambs.

3.1.4.2 Intrauterine A.I.

Due to the low incidence of oestrus and a low fertilization rate in ewe lambs mated during 1988 and group 1 of trial 3 conducted in 1989, intrauterine A.I. was used in groups 2 and 3 of trial 3 and in subsequent embryo transfer trials.

Semen procedures.

Semen was collected by artificial vagina and held at 35°C in a water bath. After evaluation for volume, colour, microscopic wave pattern and individual sperm motility, it was diluted in a ratio 1:2 using as diluent standard ultra-heat-treated (UHT) milk containing 1000 i.u. of Penicillin per ml. The diluent was prepared every day before semen collection. Only samples of semen showing good motility before and after dilution were used.

Insemination procedures.

Ewes to be inseminated were injected intramuscularly with 0.3 - 0.5 ml of acetyl promazine as tranquillizer and 2 ml of 2% xylocaine as local anaesthesia. The animals were restrained in a laparotomy cradle during the operation. Laparoscope-aided insemination was carried out 6-14h after onset of oestrus using the technique described by Killeen and Caffery (1982), modified as reported by Dattena (1989).

3.1.5 Embryo recovery from donors.

Embryo recovery was attempted 5-6 days after onset of oestrus (oestrus = day 0) in all the animals with one or more ovulations. The flushing technique was surgical as described by Tervit and Havik (1976). Depending on the ovarian response, one or both uterine horns were flushed with 20 ml of Dulbecco's Phosphate Buffered Saline (PBS) (Immuno-Chemical Products, New Zealand) containing 5% sheep serum, and warmed

to 37°C. When the ovulatory response was high or when problems at flushing were encountered, a second flush was performed. The sheep serum (S.S.) was prepared at the beginning of each breeding season as described by Rangel-Santos (1987) and was heat-inactivated for 30 minutes at 56°C before use to remove the embryo toxic factor (Chang, 1949). After embryo recovery was attempted, all donor ewes were returned to the teasers and observations made to check returns to heat for at least a further cycle.

3.1.6 Embryo searching and evaluation.

A stereoscopic microscope was used for embryo searching (at 20X) and embryo evaluation (at 80X). An additional plastic petri dish cover marked with a grid of lines was used under the glass petri dish to facilitate the searching. After an embryo was found, it was transferred into a small petri dish containing fresh PBS plus 10% sheep serum. The embryos were evaluated on the basis of their stage of development and morphology, as has been suggested by Lindner and Wright (1983). They were kept in the small petri dish inside an incubator at 37°C until transfer.

3.1.7 Transfer of embryos.

Embryos were transferred by mid-ventral laparotomy using standard surgical procedures. The number of embryos transferred per recipient was dependent on the availability, genotype and quality of the embryos. The site of transfer was always the horn ipsilateral to the ovary with the corpus luteum (CL) when there was a single ovulation, and it was chosen at random when ovulation was recorded on both ovaries.

After embryo transfer and completion of surgery, the recipient ewes were grazed with teaser rams and the possible occurrence of oestrus observed for at least a further cycle. Every recipient ewe was used only once, though it could be programmed again in the same year if found nonpregnant.

3.1.8 Analysis of data.

Ovulatory response data.

Bartlett's test conducted on the ovulatory response data indicated heterogeneity of variance for almost all of the comparisons in most of the trials conducted. Therefore transformation of the data was needed before conducting the analysis of variance to test the significance of the factors involved. In the present case, because the data contained zeros as observed values, the ovulatory response data were transformed using the Log10 (Y+1) transformation to avoid the problem of negative infinity when taking the logarithm of zero values (Sokal and Rohlf, 1981). Multiple comparisons of means were carried out using Duncan's multiple range test. To facilitate interpretation and discussion of the results, the data are presented as means with their standard errors.

The analyses of factors affecting the ovulatory responses (recorded as ovulation rate, number of large follicles or total ovarian response following the administration of the respective gonadotrophin treatment) were carried out using general linear models. The same general linear model was fitted to evaluate the effects of the appropriate factors on the three variables of response within each trial, unless stated otherwise. Therefore only one model is given for each trial.

For every case in which two or more factors were examined, a full model was fitted in the preliminary analyses. Second and higher order interactions found not significant were dropped from the model. Similarly, in most cases when first order interactions were found non-significant they were dropped from the model, however, in some cases these were kept in the model to show likely tendencies.

The results presented for ovulatory responses are from the application of the following linear models. In each case μ is the general mean and E_{ijklm} is the error peculiar to each observation.

Trial 1.

$Y_{ijkl} = \mu + A_i + B_j + C_k + E_{ijkl}$

where

A_i = effect of dose of PMSG, i=1 (900 i.u.) or 2 (1200 i.u.).

B_j = effect of time of PMSG injection, j=1 (0h) or 2 (-24h).

C_k = effect of genotype, k=1 (Control) or 3 (Fleece weight).

Trial 2.

$Y_{ij} = \mu + A_i + E_{ij}$

where

A_i = effect of gonadotrophin treatment (i=1, 2, 3, 4 or 5).

Trial 3.

$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + (AB)_{ij} + (AC)_{ik} + (AD)_{il} + E_{ijklm}$

where

A_i = effect of gonadotrophin treatment (i=1, 2, 3 or 4).

B_j = effect of GnRH treatment, j= 1 (treated) or 2 (not treated).

C_k = effect of genotype, k=1 (Control) or 3 (Fleece weight).

D_l = effect of group (l=1 or 2).

$(AB)_{ij}$ = effect of the interaction of the i^{th} gonadotrophin treatment and the j^{th} GnRH treatment.

$(AC)_{ik}$ = effect of the interaction of the i^{th} gonadotrophin treatment and the k^{th} genotype.

$(AD)_{il}$ = effect of the interaction of the i^{th} gonadotrophin treatment and the l^{th} group.

Trial 4.

$Y_{ijkl} = \mu + A_i + B_j + C_k + (AC)_{ik} + E_{ijkl}$

where

A_i = effect of source of PMSG, i=1 (Massey-PMSG), 2 (Pregnecol) or 3 (Folligon).

B_j = effect of androstenedione immunization j= 1 (immunized) or 2 (non-immunized).

C_k = effect of GnRH treatment k= 1 (treated) or 2 (not treated).

$(AC)_{ik}$ = effect of the interaction of the i^{th} source of PMSG and the k^{th} GnRH treatment.

Trial 5.

$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + E_{ijkl}$

where

A_i = effect of gonadotrophin treatment (i=1, 2, 3, 4 or 5).

B_j = effect of genotype, j=1 (Control) or 3 (Fleece weight).

C_k = effect of group (k=1 or 2).

$(AB)_{ij}$ = effect of the interaction of the i^{th} gonadotrophin treatment and the j^{th} genotype.

Trial 6.

$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$

where

A_i = effect of gonadotrophin treatment (i=1 or 2).

B_j = effect of age/season (j=1 or 2).

$(AB)_{ij}$ = effect of the interaction of the i^{th} gonadotrophin treatment and the j^{th} age/season.

The general linear model used to analyze the effect of age on the ovulatory responses in trials 1, 2, 3 and 5 was:

$Y_{ij} = \mu + A_i + E_{ij}$

where

A_i = effect of age i= 1 (lambs) or 2 (adults).

The following model was utilized to evaluate the factors affecting the ovulatory responses of adult ewes (Splitting; study 1) treated with PMSG (Massey-PMSG).

$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + E_{ijkl}$

where

A_i = effect of dose of PMSG, i=1 (700i.u.), 2 (1000i.u.) or 3 (1300 i.u.).

B_j = effect of time of PMSG injection (j=1, 2, 3, 4 or 5).

C_k = effect of mare (k=1, 2 or 3).

$(AB)_{ij}$ = effect of the interaction of the i^{th} dose and the j^{th} time of injection.

$(AC)_{ik}$ = effect of the interaction of the i^{th} dose and the k^{th} mare.

$(BC)_{jk}$ = effect of the interaction of the j^{th} time of injection and the k^{th} mare.

Data on the percentage of eggs recovered, fertilized or embryos transferable.

The data were expressed on a per ewe basis for statistical analysis. Because the original data included values between 0 and 100% making its distribution non-normal, it was necessary to transform the data using the arcsin transformation before an analysis of variance was carried out as suggested by Sokal and Rohlf (1981) and Gomez and Gomez (1984). The significance of the factors affecting the respective response variable was tested using the F-test on the transformed data. However, to facilitate the interpretation and discussion of the results arithmetic means of the data are reported. Duncan's multiple range test was used to perform multiple comparisons of means.

Egg recovery and transfer were attempted in trials 1, 2, 3, 4 and 5. The general linear models applied to evaluate the factors affecting egg recovery, fertilization rate or the percentage of embryos transferable for each trial are presented below.

Trial 1.

$Y_{ijk} = \mu + A_i + B_j + E_{ijk}$

where

A_i = effect of dose of PMSG, i= 1 (900i.u.) or 2 (1200 i.u.).

B_j = effect of time of PMSG injection, j= 1 (0h) or 2 (- 24h).

Trial 2.

Egg recovery and fertilization rate.

$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$

where

A_i = effect of source of PMSG, i=1 (Consept45) or 2 (Folligon).

B_j = effect of time of PMSG injection j= 1(- 2 days) or 2 (- 4 days).

$(AB)_{ij}$ = effect of the interaction of the i^{th} source of PMSG and the j^{th} time of PMSG injection.

Due to the low number of observations the model used to evaluate the percentage of embryos transferable included only the main effects.

Trial 3.

$Y_{ijkl} = \mu + A_i + B_j + C_k + E_{ijkl}$

where

A_i = effect of gonadotrophin treatment (i=1, 2, 3 or 4).

B_j = effect of GnRH treatment j= 1 (treated) or 2 (not treated).

C_k = effect of group (k=1 or 2).

Trial 4.

Fertilization rate.

$Y_{ijkl} = \mu + A_i + B_j + C_k + (AC)_{ik} + E_{ijkl}$

where

A_i = effect of source of PMSG, i=1 (Massey-PMSG), 2 (Pregnecol) or 3 (Folligon).

B_j = effect of androstenedione immunization j= 1 (immunized) or 2 (non-immunized).

C_k = effect of GnRH treatment k= 1 (treated) or 2 (not treated).

$(AC)_{ik}$ = effect of the interaction of the i^{th} source of PMSG and the k^{th} GnRH treatment.

The interaction term was not included for the analyses of egg recovery and the percentage of embryos transferable.

Trial 5.

Egg recovery.

$Y_{ij} = \mu + A_i + E_{ij}$

where

A_i = effect of gonadotrophin treatment (i=1, 2 or 3).

Fertilization rate and the percentage of embryos transferable.

$Y_{ijkl} = \mu + A_i + B_j + C_k + E_{ijkl}$

where

A_i = effect of gonadotrophin treatment (i=1, 2 or 3).

B_j = effect of inseminator (j=1 or 2).

C_k = effect of ram (k=1 or 2).

Trials 1, 3 and 5.

The effect of age on egg recovery, fertilization rate or the percentage of embryos transferable.

$Y_{ij} = \mu + A_i + E_{ij}$

where

A_i = effect of age i= 1 (lambs) or 2 (adults).

Discrete data.

The significance of the factors affecting the incidence and distribution of onset of oestrus, pregnancy rate and embryo survival were examined using Chi-square test by means of a contingency table analysis. Whenever the number of factors involved was three or more, log-linear model analysis was used as suggested by Zar (1984). Statistical analyses were performed using one of the following statistical computing packages: SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA, 1988), REG (Generalized Regression Package, A. Gilmour, NSW. Dept. of Agriculture, Aust, 1985) or SPSS^x (Statistical Package for Social Sciences, SPSS^x Inc., Chicago, Il, USA, 1984).

The following symbols were used to indicate the levels of significance throughout the trials.

Symbol	Level of significance
NS	P >0.05
*	P <0.05
**	P <0.01
***	P <0.001

Where multiple comparisons of means have been shown different superscripts were used to indicate significant differences at the appropriate levels of significance shown in the table.

3.2 Results.

3.2.1 Incidence and distribution of onset of oestrus following progestagen sponge treatment.

Trial 1.

The incidence of oestrus following removal of sponges from the 172 Romney ewes used as recipients is shown in Table 3.9. Four sponges were not recovered at the end of the synchronization treatment, giving a 98% efficiency of sponge retention. Reasons for the losses were not determined. In total 72% of the animals were recorded in heat. The analysis of the data showed no significant effect of group of treatment on the percentage of animals detected in heat.

Figure 3.1 shows the distribution of onset of oestrus by group of treatment. One animal from group 1 showing heat at 96h has been counted with animals detected in heat at 84h. Statistical analysis of these results did not show any significant effect of group of treatment on the proportion of animals recorded in heat at the times indicated. Overall, 52% of the animals were detected in heat within 2 days of sponge removal, 44% on day 3 and 4% more than 3 days after sponge withdrawal.

Table 3.9 Effect of group of treatment on the incidence of oestrus following sponge removal (Trial 1).

Group (Period) of treatment	Total number of ewes	Number of ewes detected in heat	% of ewes detected in heat
1	60	40	67
2	70	56	80
3	42	27	64
Total	172	123	72

$Chi^2 = 4.24$, 2 df NS

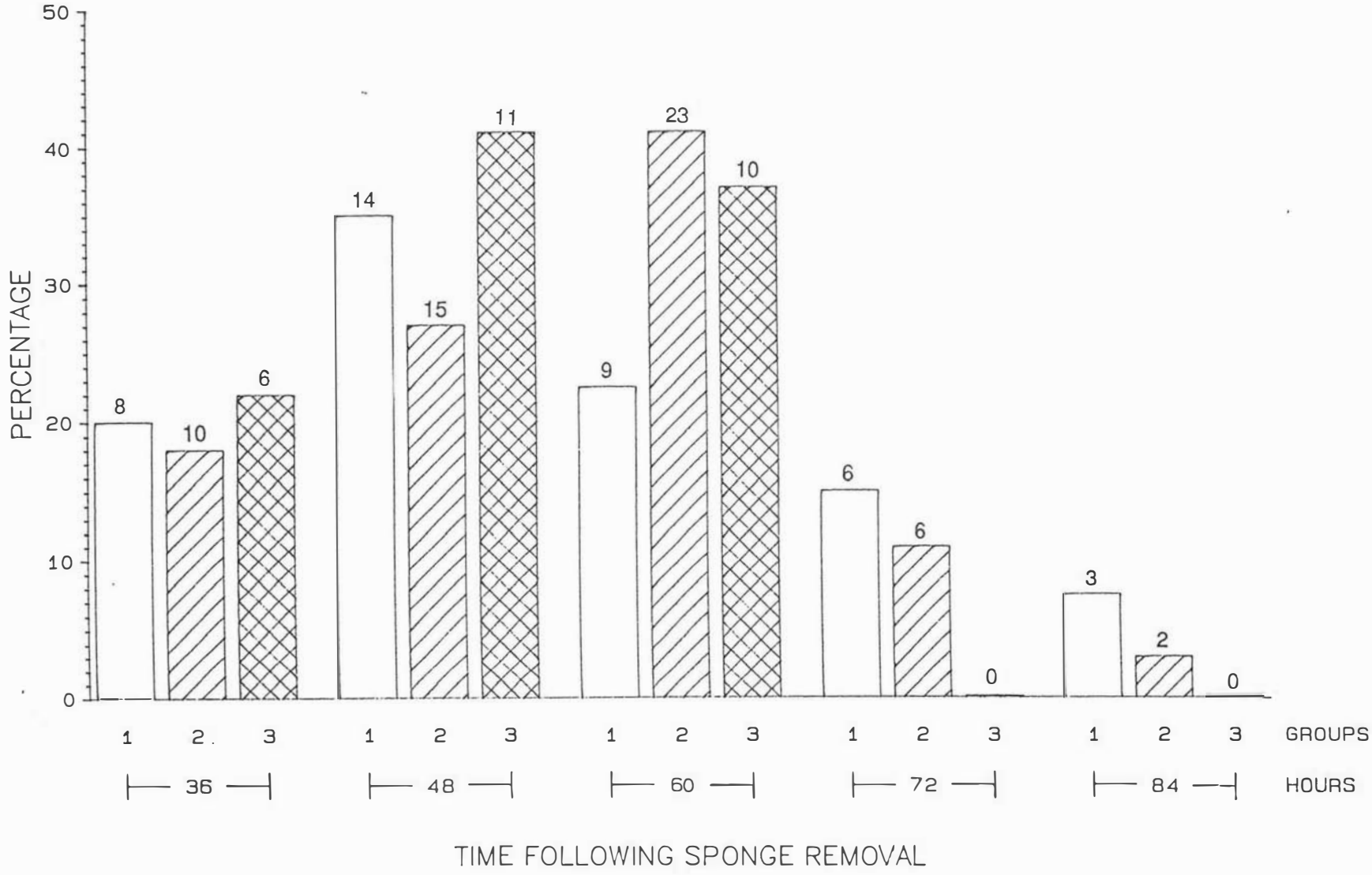

Figure 3.1 Percentage of animals showing oestrus at different times following sponge removal for groups 1 (open bars), 2 (hatched bars) and 3 (crosshatched bars). Numbers of animals are indicated above the bars (Trial 1).

Of the 63 sheep (45 lambs and 18 adults) used as donors in the trial, 6 lost the sponge (4 lambs and 2 adults) giving 90% sponge retention. Heat was detected in 68% (43/63) of the animals including 1 lamb that had lost the sponge.

The distribution of onset of oestrus for donors and recipients is shown in Figure 3.2. This was dependent on the type of animal (donor or recipient), donors showing heat on average 10h earlier than recipients. The average time of onset of oestrus for donors and recipients was 43h and 53h respectively.

Trial 2.

All the recipients included in this trial were detected in heat within 72h of sponge removal This included 3 animals that lost the sponge and were detected in heat 36h following the expected time of sponge withdrawal. The efficiency of sponge retention was 95% (55/58).

The incidence of oestrus of the 50 donor lambs is shown in Table 3.10. One sponge was not recovered giving a 98% rate of sponge retention. The animal that lost the sponge was recorded in heat 36h following the expected time of sponge removal.

Two analyses were conducted to evaluate the percentage of animals showing heat. In the first analysis, data from animals of treatments 1 and 2 (Consept45 given 2 or 4 days before sponge removal, respectively) and treatments 3 and 4 (Folligon given 2 or 4 days before sponge removal) were combined. This was done as the same percentage of animals showed heat and they had been treated with the same hormone, though given at different times. Analysis of these data did not show any significant differences in the percentage of animals detected in heat between those treated with Consept45 (Treatments 1 + 2) or Folligon (Treatments 3 + 4) (90% vs 100%, respectively).

In the second analysis all the lambs treated with PMSG (Consept45 or Folligon) were combined and compared with animals of treatment 5 which did not receive PMSG. There were no significant differences in the percentage of animals showing heat between lambs treated with or without PMSG (95% vs 90%, respectively). However, the distribution of onset of oestrus (Figure 3.3) was affected by PMSG treatment. The average time of onset of oestrus for lambs receiving or not receiving PMSG was 36h

and 48h respectively.

Table 3.10 Percentage of animals showing heat by treatment (Trial 2).

Gonadotrophin treatment		Total number of lambs	Number of lambs detected in heat	% of lambs detected in heat
Source of PMSG	Time when given			
Consept45	- 2 days	10	9	90
Consept45	- 4 days	10	9	90
Folligon	- 2 days	10	10	100
Folligon	- 4 days	10	10	100
No PMSG	—	10	9	90
Total		50	47	94

The distribution of onset of oestrus for donors treated with PMSG and for recipients is shown in Figure 3.4. This was dependent on the type of animal. Donors showed heat on average 8h earlier than recipients. The average time of onset of oestrus for donors and recipients was 36h and 44h respectively.

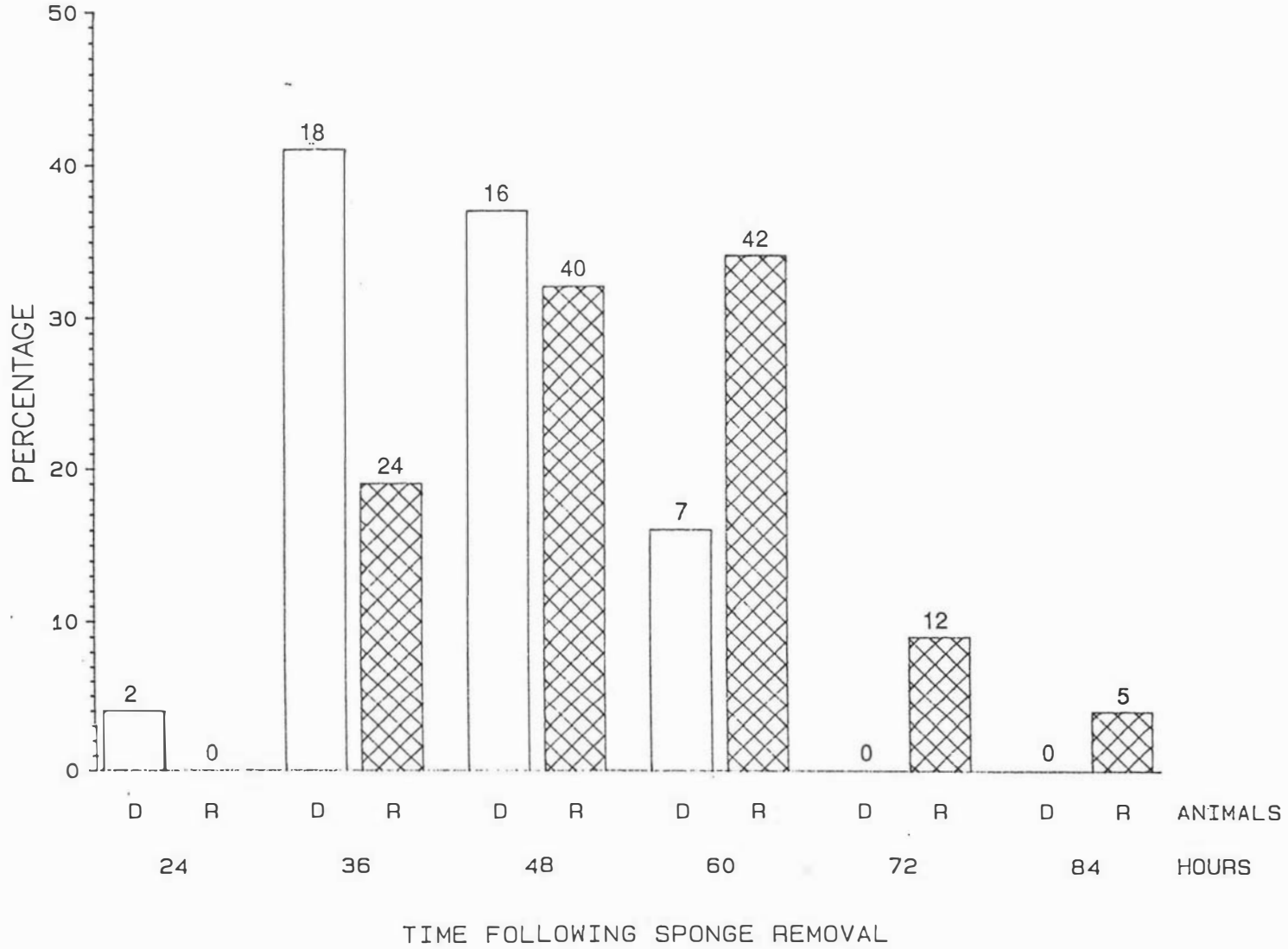

Figure 3.2 Percentage of animals showing oestrus at different times following sponge removal for animals used as donors (D: open bars) or recipients (R: crosshatched bars). Numbers of animals are indicated above the bars (Trial 1).

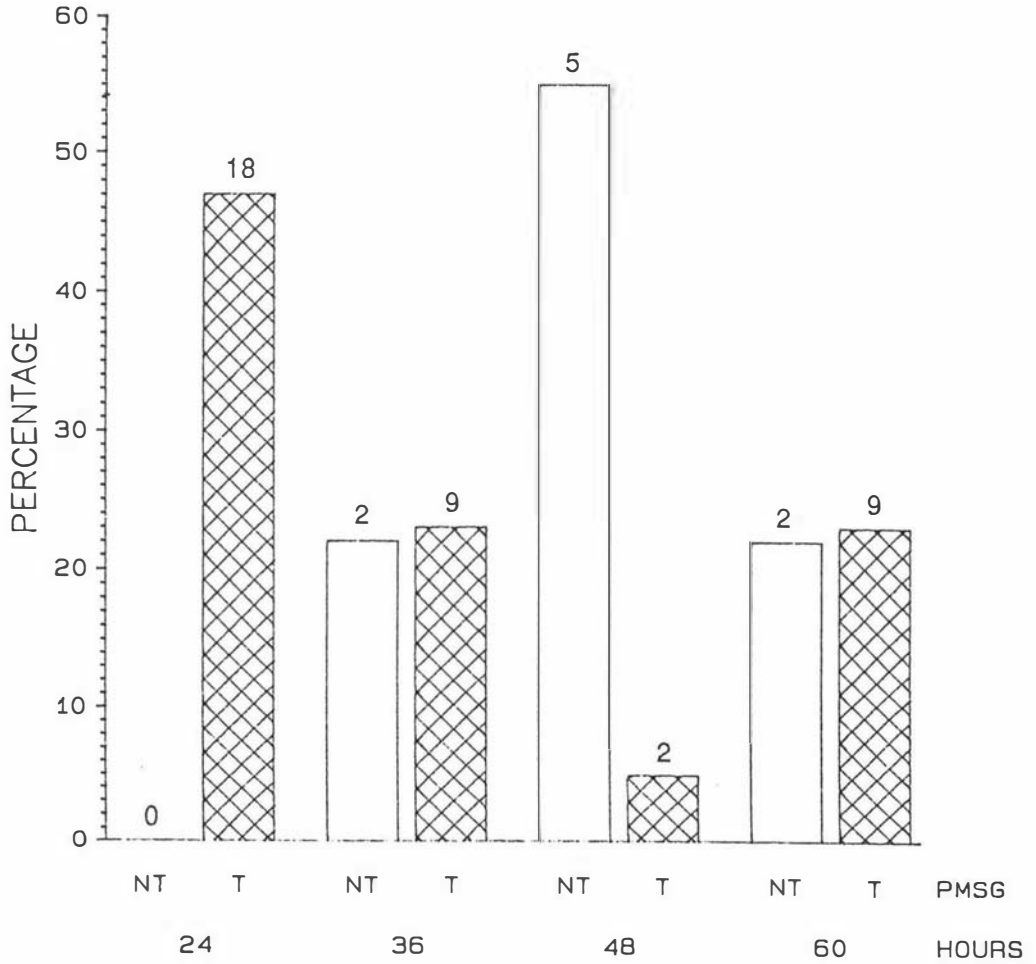

Figure 3.3 Percentage of animals showing oestrus at different times following sponge removal for animals treated with PMSG (T:crosshatched bars) and without PMSG (NT:open bars). Numbers of animals are indicated above the bars (Trial 2).

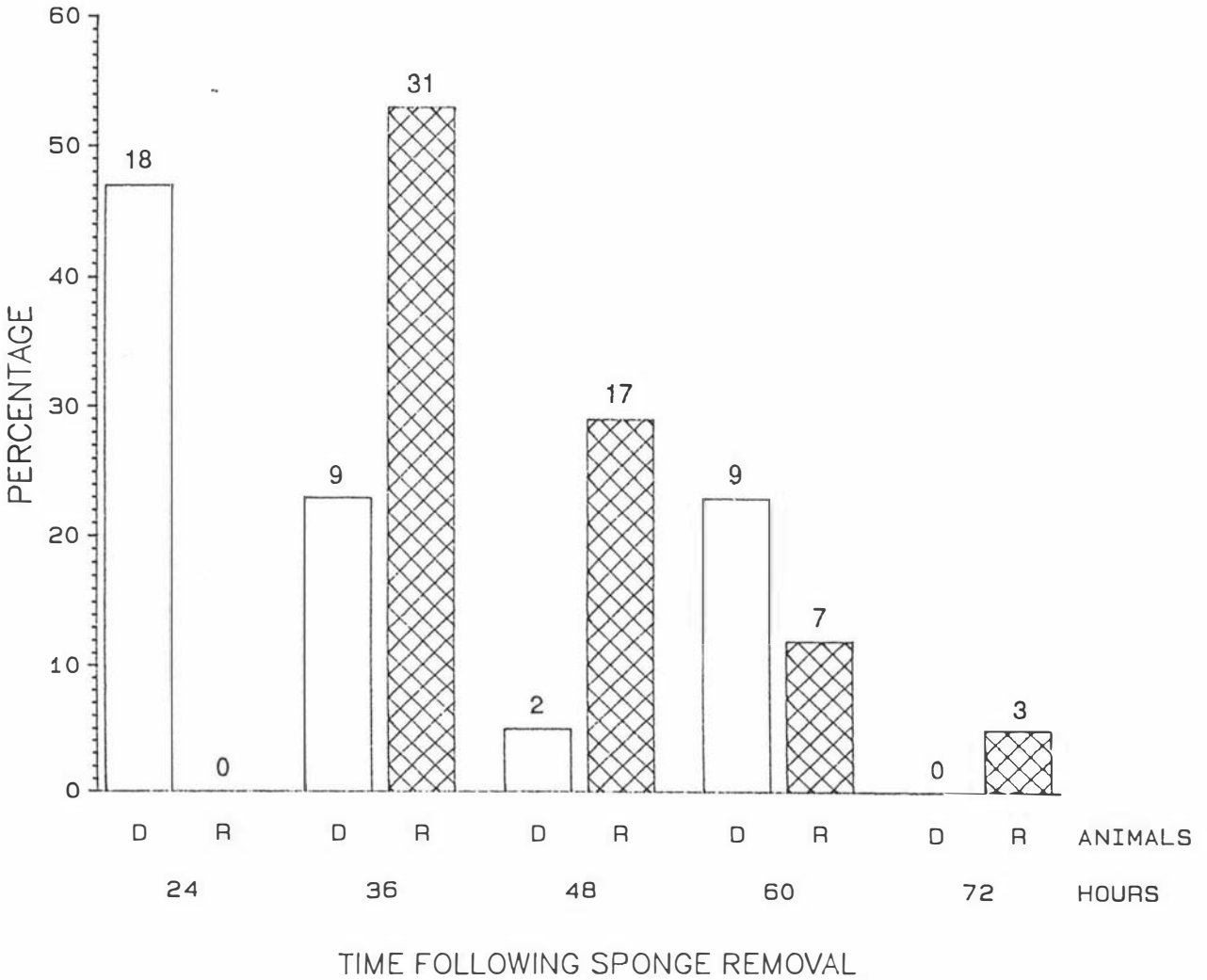

Figure 3.4 Percentage of animals showing oestrus at different times following sponge removal for animals used as donors (D:open bars) or recipients (R:crosshatched bars). Numbers of animals are indicated above the bars (Trial 2).

Trial 3.

Table 3.3 shows the incidence of oestrus following sponge withdrawal of 156 mixed-age ewes used as recipients. Two animals lost the sponge giving a 99% efficiency of sponge retention. Overall 88% of the animals were detected in heat. The statistical analysis of these data indicated a significant effect ($P<0.05$) of group of treatment on the percentage of animals detected in heat. Comparisons among the groups did not show any significant difference between groups 1 and 2 (98% vs 89%, respectively) and between groups 2 and 3 (89% vs 78%, respectively), however the percentage of animals detected in heat in group 3 was significantly lower ($P<0.01$) than the percentage of animals detected in heat in group 1 (78% vs 98%, respectively).

The distribution of onset of oestrus by group of treatment is shown in Figure 3.5. Data for 2 animals recorded in heat 24h following sponge removal were pooled with ewes detected in heat at 36h and another 2 ewes detected in heat at 96h were added to the group of animals detected in heat at 84h. Statistical analysis of these data indicated a significant effect ($P<0.01$) of group of treatment on the frequency distribution of animals showing heat. There was no significant difference in the distribution of animals detected in heat between groups 1 and 2, however their distribution was significantly different ($P<0.01$) than the distribution of group 3.

Of the 93 donors involved in the trial (73 lambs and 20 adults), 3 animals lost the sponge (2 lambs and 1 adult) giving a 97% sponge retention. Heat was detected in 81% (75/93) of the sheep, including 1 lamb and 1 adult ewe that had lost the sponge.

Figure 3.6 shows the distribution of onset of oestrus for donors and recipients. This was dependent on the type of animal. Donors showed heat on average 16h earlier than recipients. The average time of onset of oestrus for donors and recipients was 34h and 50h respectively.

Table 3.11 Effect of group of treatment on the incidence of oestrus following sponge removal (Trial 3).

Group (Period) of treatment	Total number of ewes	Number of ewes detected in heat	% of ewes detected in heat
1	51	50	98 a
2	47	42	89 a b
3	58	45	78 b
Total	156	137	88

Chi^2 = 10.76, 2 df **

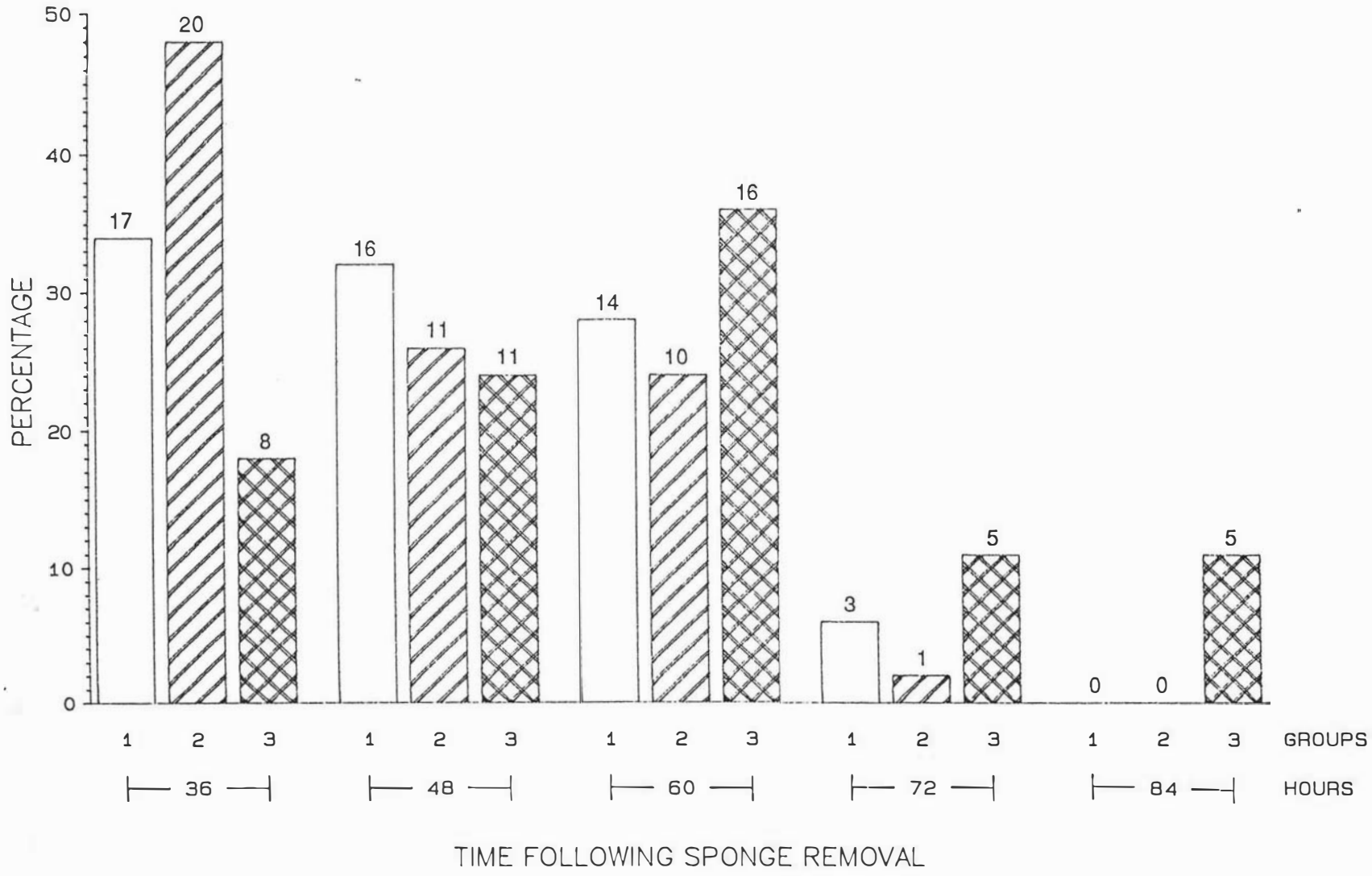

Figure 3.5 Percentage of animals showing oestrus at different times following sponge removal for groups 1 (open bars), 2 (hatched bars) and 3 (crosshatched bars). Numbers of animals are indicated above the bars (Trial 3).

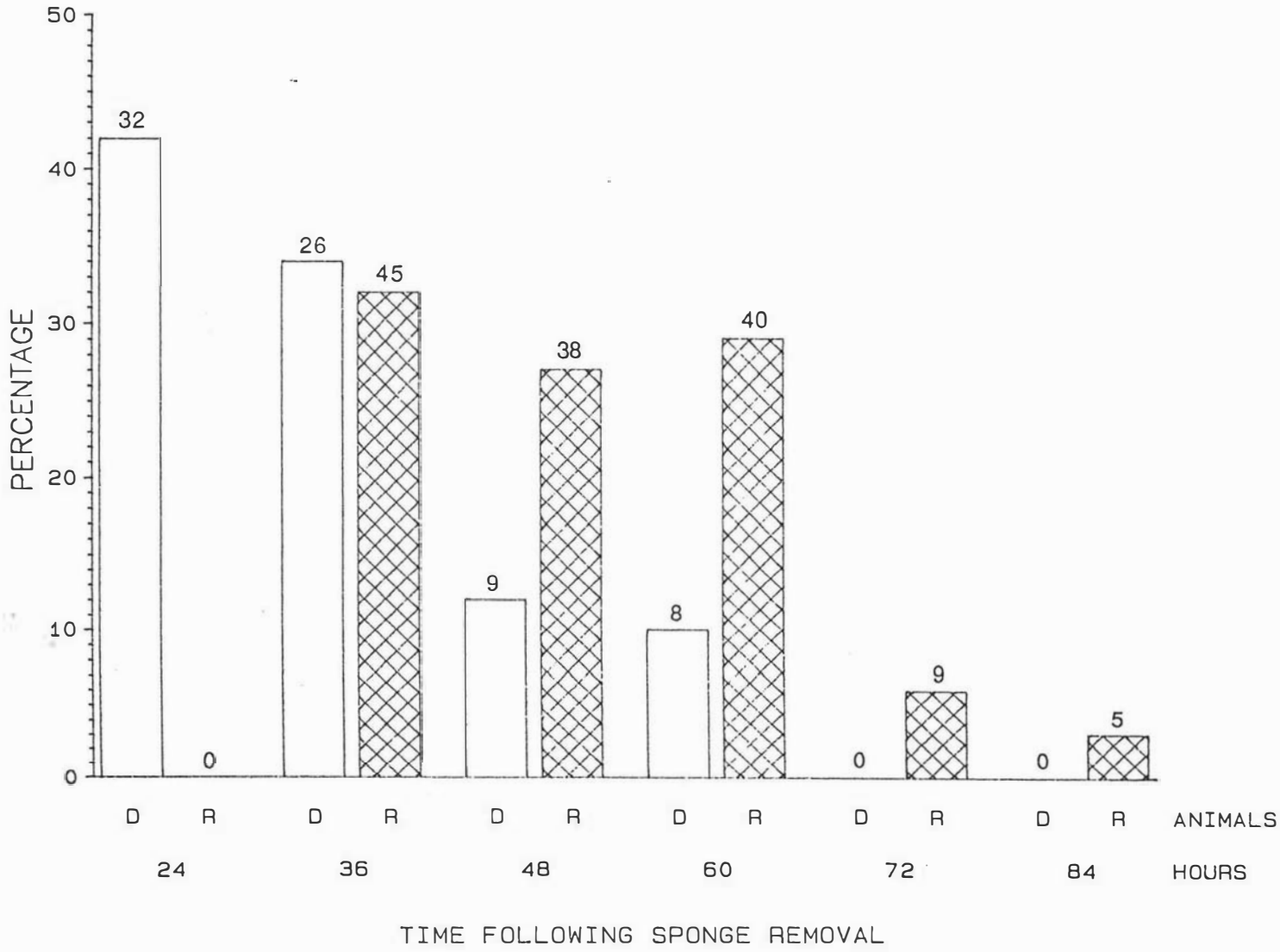

Figure 3.6 Percentage of animals showing heat at different times following sponge removal for animals used as donors (D: open bars) or recipients (R: crosshatched bars). Numbers of animals are indicated above the bars (Trial 3).

Trial 4.

Ninety donor ewe lambs were involved in the study. The data were used to evaluate the effects of immunization (Immunized vs non-immunized), source of PMSG (Massey-PMSG, Pregnecol or Folligon) and group of treatment on the percentage of animals detected in heat following sponge withdrawal. At the end of the synchronization treatment, 1 lamb was not present and its information was deleted. Sponges were not recovered from 4 of the remaining 89 lambs giving an efficiency of sponge retention of 96%. Three of the lambs that lost the sponge were not marked by the teaser ram and one lamb was recorded in heat 36h following sponge removal. Results following laparotomy conducted 7-8 days after sponge withdrawal indicated that 1 lamb was pregnant and 2 others had under-developed (infantile) reproductive tracts. Data from these animals were also eliminated leaving information from 86 animals available for analysis.

The analyses to test the effects of immunization, source of PMSG and group of treatment are shown in Tables 3.12, 3.13 and 3.14 respectively. Overall 80% of the animals were detected in heat. Analysis of the data indicated a significant effect of immunization against androstenedione ($P<0.01$) and group of treatment ($P<0.01$) on the percentage of lambs detected in heat. This was lower in immunized than non-immunized lambs (68% vs 93%, respectively Table 3.12) and in lambs treated in group 2 than group 1 (68% vs 91%, respectively Table 3.14). There was no significant effect of source of PMSG on the percentage of lambs detected in heat (89% vs 78% vs 75%, for Massey-PMSG, Pregnecol and Folligon respectively Table 3.13).

There was no significant effect of androstenedione-immunization on the distribution of onset of oestrus (Figure 3.7). Overall, 16% of the lambs were detected in heat one day after sponge removal, 80% on day 2 and 4% on day 3.

Table 3.12 Effect of immunization on the incidence of oestrus following sponge removal (Trial 4).

Immunization	Total number of lambs	Number of lambs detected in heat	% of lambs detected in heat
Immunized	44	30	68
Non-immunized	42	39	93
Total	86	69	80

Chi^2 = 7.53, 1df **

Table 3.13 Effect of source of PMSG on the incidence of oestrus following sponge removal (Trial 4).

Source of PMSG	Total number of lambs	Number of lambs detected in heat	% of lambs detected in heat
Massey-PMSG	27	24	89
Pregnecol	27	21	78
Folligon	32	24	75
Total	86	69	80

Chi^2 = 1.99, 2 df NS

Table 3.14 Effect of group of treatment on the incidence of oestrus following sponge removal (Trial 4).

Group (Period) of treatment	Total number of lambs	Number of lambs detected in heat	% of lambs detected in heat
1	46	42	91
2	40	27	68
Total	86	69	80

Chi^2 = 6.98, 1 df **

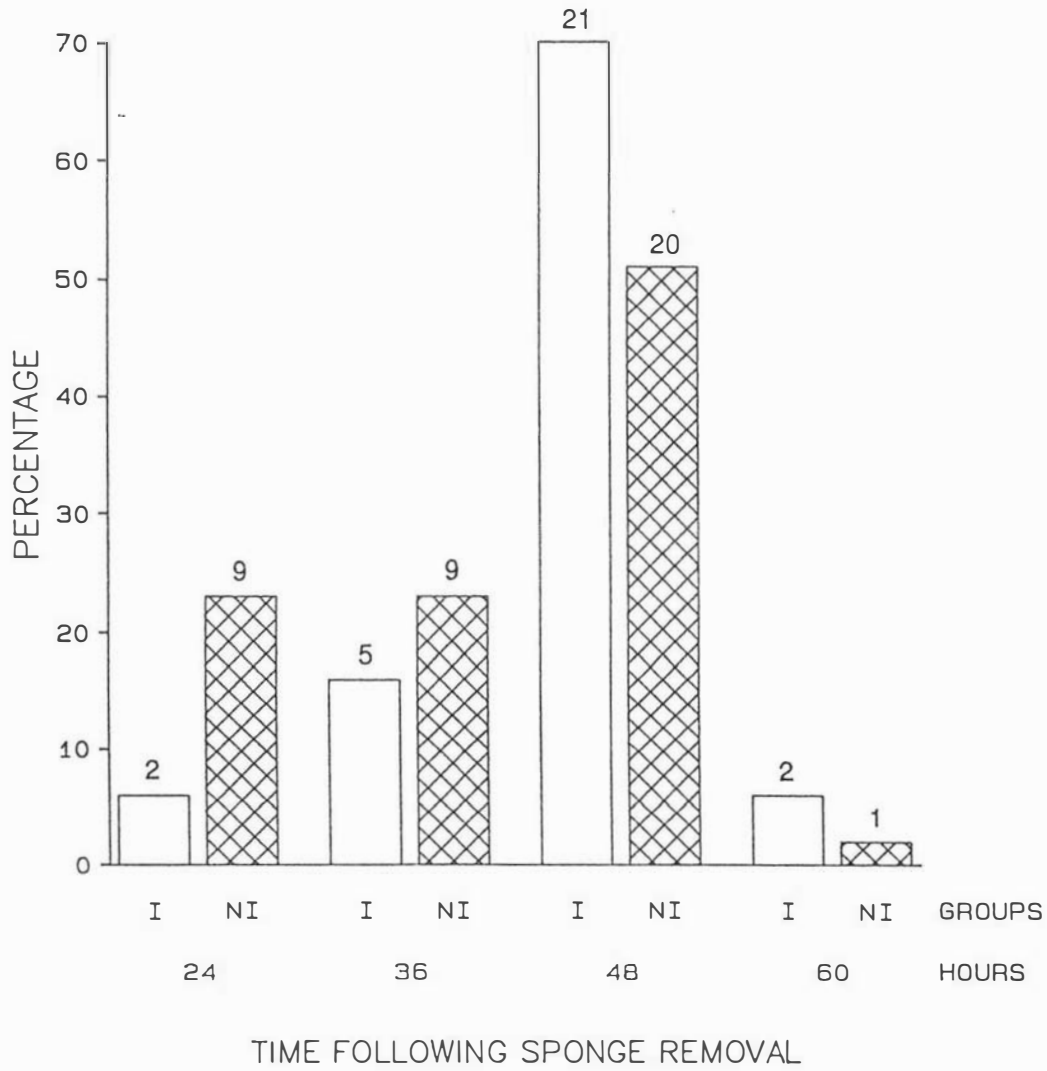

Figure 3.7 Percentage of animals showing oestrus at different times following sponge removal in immunized (I:open bars) and non–immunized (NI:crosshatched bars) ewe lambs. Numbers of animals are indicated above the bars (Trial 4).

Trial 5.

The incidence of oestrus following sponge removal from 128 Drysdale ewes used as recipients is shown in Table 3.15. Two animals lost their sponges, giving an efficiency of sponge retention of 98%. In total 90% of the ewes were detected in heat. The statistical analysis of the results indicated a significant effect ($P<0.05$) of group of treatment on the proportion of animals showing oestrus. There was a significant difference ($P<0.05$) between group 1 and group 2 (83% vs 98%, respectively), but no significant difference was found between groups 1 and 3 (83% vs 88%, respectively) and groups 2 and 3 (98% vs 88%, respectively).

The distribution of onset of oestrus of group 1 was significantly different from that of group 2 and group 3 ($P<0.01$, Figure 3.8). However, the differences were not significant between groups 2 and 3.

There were no sponge losses in the group of donors (64 lambs and 13 adults) included in the trial. Heat was detected in 86% (66/77) of the animals.

Figure 3.9 shows the distribution of onset of oestrus for donors and recipients. This was dependent on the type of animal. Donors were detected in heat on average 9h earlier than recipients. The average time of onset of oestrus for donors and recipients was 35h and 44h respectively.

Table 3.15 Effect of group of treatment on the incidence of oestrus following sponge removal (Trial 5).

Group (Period) of treatment	Total number of ewes	Number of ewes detected in heat	% of ewes detected in heat
1	48	40	83 a
2	48	47	98 b
3	32	28	88 a b
Total	128	115	90

$Chi^2 = 5.85$, 2 df *

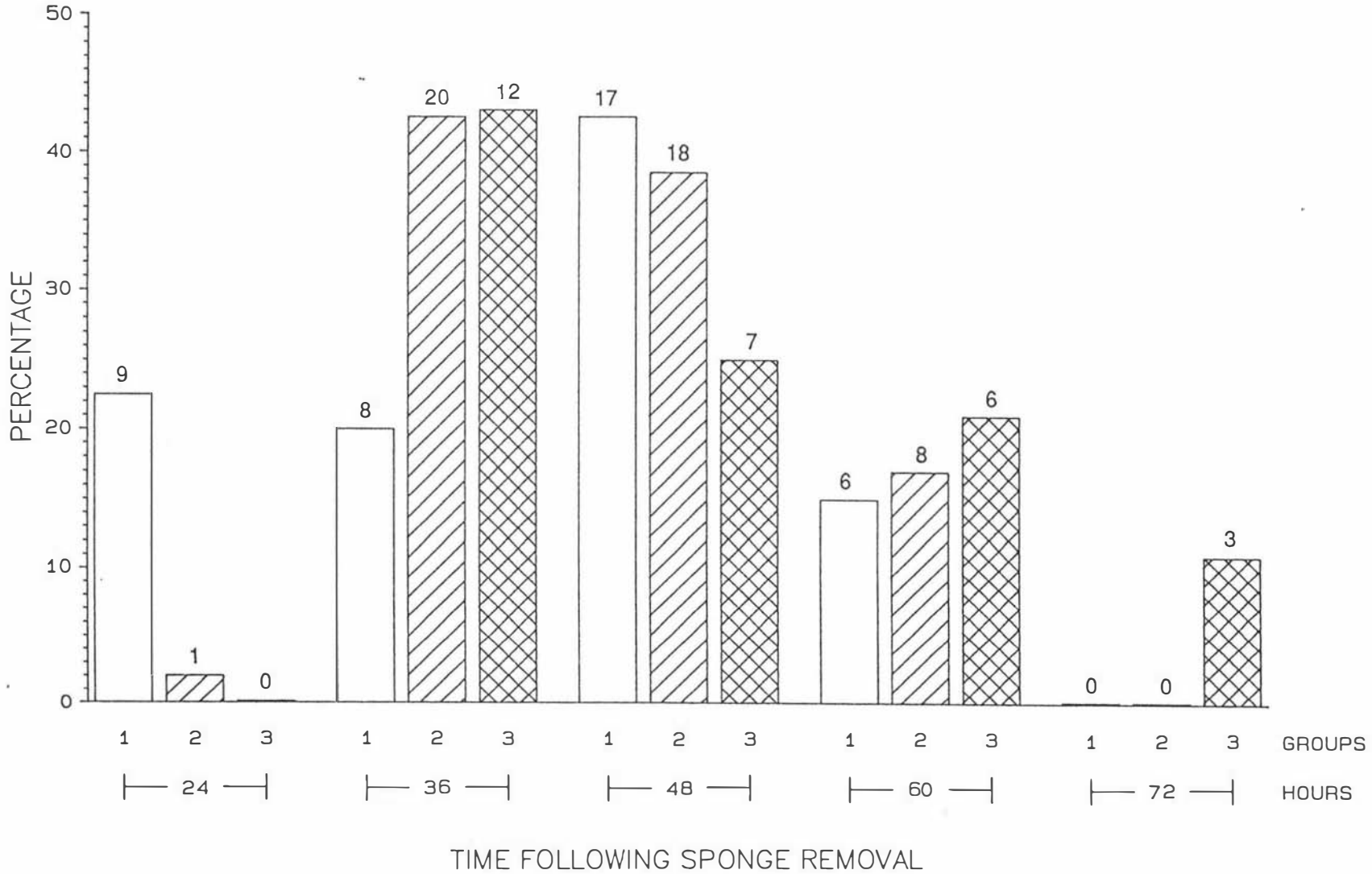

Figure 3.8 Percentage of animals showing oestrus at different times following sponge removal for groups 1 (open bars), 2 (hatched bars) and 3 (crosshatched bars). Numbers of animals are indicated above the bars (Trial 5).

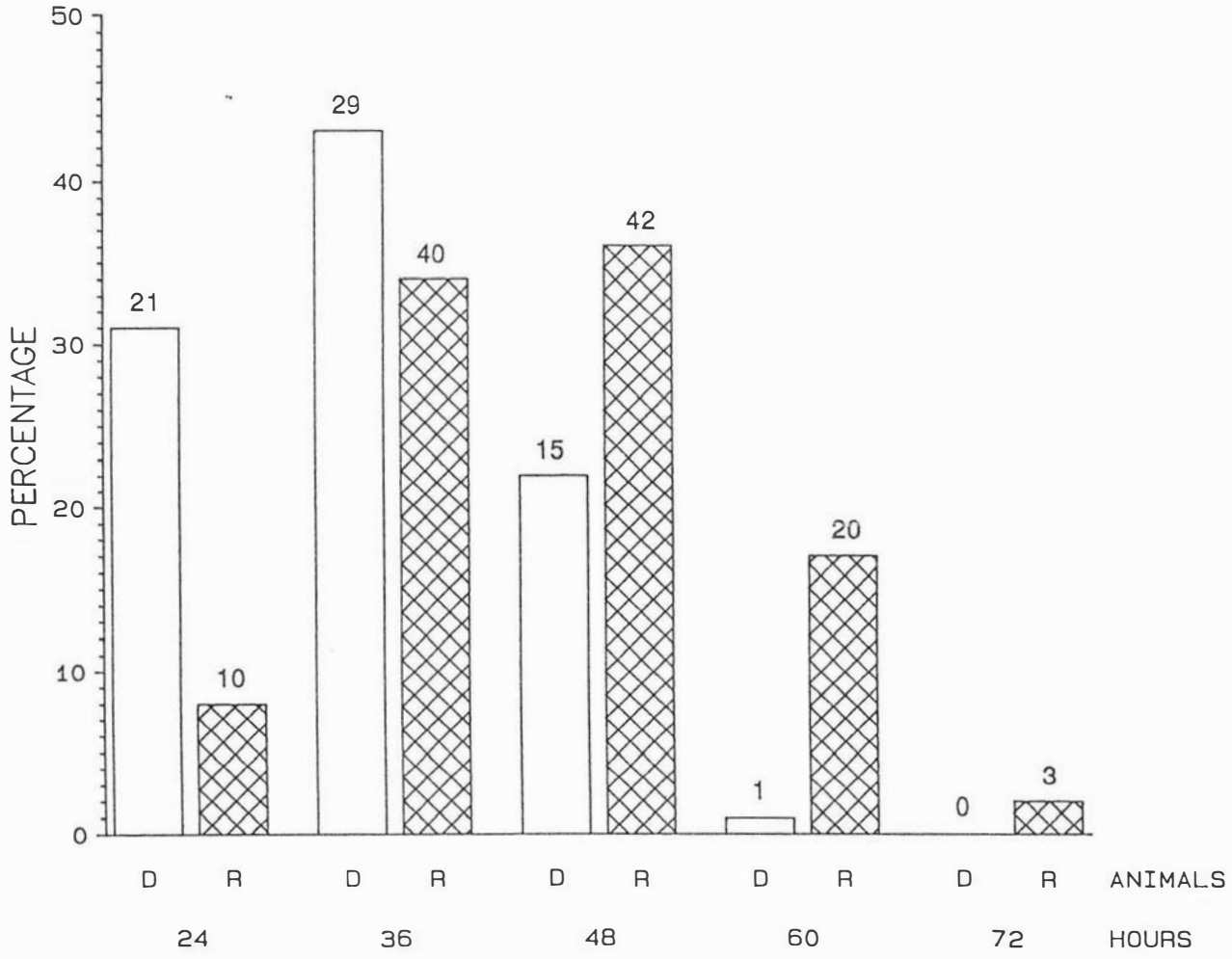

Figure 3.9 Percentage of animals showing oestrus at different times following sponge removal for animals used as donors (D: open bars) or recipients (R: crosshatched bars). Numbers of animals are indicated above the bars (Trial 5).

Trial 6.

The incidence of oestrus following sponge removal of 111 ewe lambs used in the study is shown in Table 3.16. Three sponges were not recovered from the group of animals treated at 6-7 months of age and they were not detected in heat at the expected time. No sponges were lost in the group of animals treated at 9-10 months of age. Overall 3% (3/111) of the sponges were lost.

In total 80% of the animals were detected in heat. Analysis of the data did not show significant effect of age/season on the percentage of animals detected in heat or on the distribution of onset of oestrus. The percentage of animals showing heat at the different times for each age is shown in Figure 3.10.

Table 3.16 Effect of age of treatment on the incidence of oestrus following sponge removal (Trial 6).

Age	Total number of lambs	Number of lambs detected in heat	% of lambs detected in heat
6-7 months	81	66	81
9-10 months	30	23	77
Total	111	89	80

Chi^2 = 0.32, 1df NS

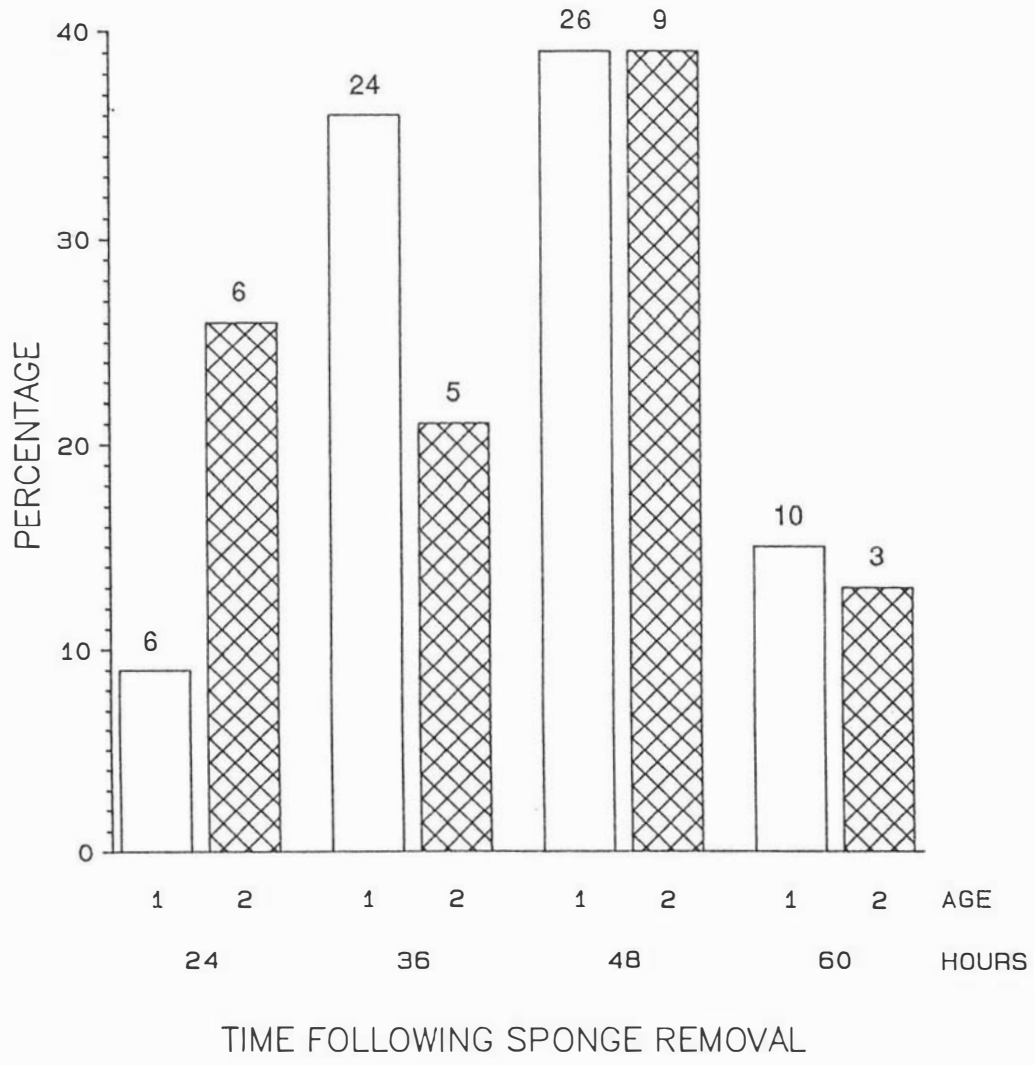

Figure 3.10 Percentage of animals showing oestrus at different times following sponge removal for animals treated at age 1 (6–7 months: open bars) and age 2 (9–10 months: crosshatched bars). Numbers of animals are indicated above the bars (Trial 6).

3.2.2 Factors affecting the ovulatory responses.

The ovulatory responses were considered as ovulation rate (Number of CL), number of large follicles (Follicles >5 mm) or total ovarian response (Ovulation rate + Large follicles).

Trial 1.

Table 3.17 shows data for the 3 measures of the ovulatory response in relation to the dose of PMSG, time when it was given and genotype of the 45 Romney ewe lambs (Analyses of variance are shown in Appendices 3.1, 3.2 and 3.3). The effect of age (lambs vs adults) on the ovulatory responses is described in Table 3.18 (Analysis of variance in Appendix 3.4).

Ovulation rate.

Initial analysis did not show any significant interactions between main effects and hence only the main effects were left in the model. There was no significant effect of any of the factors studied on ovulation rate (Appendix 3.1).

Number of large follicles.

There was a significant effect of time of injection ($P<0.001$) and genotype ($P<0.05$) (averaged across other treatments) on the number of large follicles. However, there was no significant effect of the dose of PMSG used (Appendix 3.2). Animals injected at sponge removal (0h) developed more large follicles than animals injected one day before sponge withdrawal (-24h) (2.04 vs 0.47, respectively). Similarly ewe lambs from the control line gave higher responses than fleece weight-selected ewe lambs (1.81 vs 0.75, respectively Table 3.17).

Total ovarian response.

The analyses of the results did not show any significant effect of the dose of PMSG injected or the genotype on the total ovarian response. However, there was a significant effect ($P<0.001$) of time of injection (averaged across other treatments) (Appendix 3.3). In the group of animals where an oestrous cycle was induced prior to the administration of the gonadotrophin treatment, from 11 ewe lambs induced, only 3 were detected in heat within 4 days following sponge removal. Observations at laparoscopy indicated that

only 2 lambs had ovulated, each having a single CL. The two lambs were treated with 1200 iu of Consept45 and gave 1 and 2 CL. No large follicles were observed. Induction of a natural oestrus was also attempted in 5 adult ewes. All the mature ewes ovulated and were mated. One adult ewe had 2 CL, the others had single ovulations. Following the superovulatory treatment, 3 of them gave double ovulations and the other 2 produced a single CL each. The average ovulation rate was 1.6 corpora lutea. Only one large follicle was observed in one of the ewes.

Age.

The average ovulatory responses for lambs and adults are shown in Table 3.18. There was a significant effect ($P<0.01$) of age on ovulation rate (2.00 vs 1.20 CL for adults and lambs, respectively). However there was no significant effect of age on the number of large follicles, or the total ovarian response (1.24 vs 1.00 and 2.44 vs 3.00, for lambs and adults respectively).

Table 3.17 Effect of Dose of PMSG, Time of PMSG injection and Genotype on the ovulatory responses in Romney ewe lambs (Mean ± s.e.m.) (Trial 1).

Dose of PMSG (i.u.)	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
900	22	1.27 ± 0.30	NS	0.82 ± 0.26	NS	2.09 ± 0.36	NS
1200	23	1.13 ± 0.18		1.65 ± 0.52		2.78 ± 0.55	
Time of PMSG injection							
0h	22	1.50 ± 0.30	NS	2.04 ± 0.53	***	3.54 ± 0.55	***
- 24h	23	0.91 ± 0.15		0.47 ± 0.19		1.39 ± 0.24	
Genotype							
Control	21	1.09 ± 0.18	NS	1.81 ± 0.56	*	2.90 ± 0.58	NS
Fleece weight	24	1.29 ± 0.28		0.75 ± 0.24		2.04 ± 0.35	

Table 3.18 Effect of age on the ovulatory responses (Mean ± s.e.m.) (Trial 1).

Age	No.of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Lambs	45	1.20 ± 0.17	**	1.24 ± 0.30	NS	2.44 ± 0.33	NS
Adults	18	2.00 ± 0.22		1.00 ± 0.30		3.00 ± 0.40	

Trial 2.

Ovulatory responses.

Table 3.19 shows the average ovulatory responses to PMSG treatment (Anova tables in Appendices 3.5, 3.6 and 3.7). Preliminary analyses including only lambs treated with PMSG failed to show a significant effect of source of PMSG (Consept45 vs Folligon), time when it was given (2 vs 4 days before sponge removal) or the interaction between them on any of the variables of response. In the present analyses lambs treated with PMSG were compared with lambs not treated with PMSG. Analysis of the results did not show any significant effect of gonadotrophin treatment on ovulation rate or the number of large follicles, but there was a significant effect ($P<0.05$) of gonadotrophin treatment on the total ovarian response. The total ovarian responses were not significantly different among gonadotrophin treatments (Treatments 1, 2, 3 and 4 Table 3.19). There was also no significant difference in the responses between animals not treated with PMSG (Treatment 5) and animals of treatment 1 (1.10 vs 2.40, respectively). However, the responses of animals from treatments 2, 3 and 4 were higher than the responses of animals from treatment 5 (2.80, 2.78 and 3.00 vs 1.10, respectively).

Age.

Comparisons of the ovulatory responses to PMSG were made between the 39 lambs and 11 ewes treated with PMSG (Anova in Appendix 3.8). There was a significant effect ($P<0.01$) of age on ovulation rate, with the mature animals having a higher response than ewe lambs (4.27 vs 1.97, respectively). The number of large follicles was not recorded in the group of ewes, therefore comparisons on that variable or the total ovulatory response were not conducted.

Table 3.19 Effect of gonadotrophin treatment (Trt.) on the ovulatory responses (Mean ± s.e.m.) (Trial 2).

Trt.	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
1	10	1.90 ± 0.43	NS	0.50 ± 0.31	NS	2.40 ± 0.67	ab *
2	10	1.80 ± 0.47		1.00 ± 0.36		2.80 ± 0.47	a
3	9	1.78 ± 0.60		1.00 ± 0.45		2.78 ± 0.64	a
4	10	2.40 ± 0.49		0.60 ± 0.22		3.00 ± 0.52	a
5	10	0.90 ± 0.10		0.20 ± 0.40		1.10 ± 0.10	b

Treatments (1;Consept45 given 2 days before sponge removal, 2; Consept45 given 4 days before, 3; Folligon given 2 days before, 4; Folligon given 4 days before, 5; Not treated with PMSG).

Trial 3.

Data from 71 ewe lambs were used to evaluate the effects of gonadotrophin treatment, GnRH, genotype and group (Period) of treatment on ovulation rate, the number of large follicles or the total ovarian response. The appropriate analyses of variance are shown in Appendices 3.9, 3.10 and 3.11. The effect of age on the ovulatory responses was tested using information from 17 ewe lambs and 20 adult ewes given similar gonadotrophin treatment (Anova in Appendix 3.12-3.16).

The average ovulatory responses for the three variables by treatment, GnRH, genotype and group are given in Table 3.20. Similarly the average responses for the interactions of treatment by GnRH, treatment by group and treatment by genotype are shown in Table 3.21. In these analyses data from animals treated in groups 2 and 3 were pooled (Group 2) as there were no significant differences between them and compared with those of group 1.

Ovulation rate.

Gonadotrophin treatment.

There was a significant effect ($P<0.01$) of gonadotrophin treatment (averaged across all other treatments) on ovulation rate (Appendix 3.9). Lambs treated with 1200 i.u. Folligon gave better results (4.18 CL) than any of the other gonadotrophin treatments (Table 3.20). There was no significant difference between animals treated with 24 mg FSH-P (1.78) or 1200 i.u. Folligon + 2.5 ml Ovagen (2.05) and between animals treated with 8 ml Ovagen (1.06) or 24 mg FSH-P (1.78). However, the responses were significantly lower in animals treated with 8 ml Ovagen compared to those treated with 1200 i.u. Folligon + 2.5 ml Ovagen (1.06 vs 2.05 CL, respectively).

GnRH.

The analysis of the results did not show any significant effect of GnRH administration on ovulation rate (Appendix 3.9). The average ovulation rate for ewe lambs treated with or without GnRH was 2.25 and 2.28 respectively (Table 3.20).

Genotype.

Analysis of the data did not show any significant effect of genotype on the ovulation rate (Appendix 3.9). The average ovulation rates for animals of genotype 1 (Control) and

genotype 3 (Fleece weight) were 1.75 and 2.71 respectively.

Group.

There was a significant effect (P<0.01) of group of treatment on ovulation rate (Appendix 3.9). The average ovulation rates for groups 1 and 2 were 3.72 and 1.61 respectively.

Interactions.

The analysis of the data did not show any significant interaction between gonadotrophin treatment and GnRH administration, gonadotrophin treatment and group of treatment and between gonadotrophin treatment and the genotype of the animals (Appendix 3.9). The average ovulation rates for the relevant sub-groups are shown in Table 3.21.

Number of large follicles.

The analysis of the results did not show any significant effect of gonadotrophin treatment, GnRH, genotype, group or any of the interactions between gonadotrophin treatment and GnRH, genotype or group on the number of large follicles (Appendix 3.10), although animals treated with 1200 i.u. Folligon or 1200 i.u. Folligon + 2.5 ml Ovagen tended to give higher responses. A similar tendency was observed in animals treated with GnRH or in group 2 (Table 3.20). The tendency was maintained across the interaction sub-groups (Table 3.21).

Total ovarian response.

Gonadotrophin treatment.

There was a significant effect (P<0.01) of gonadotrophin treatment (averaged across all other treatments) on the total ovarian response (Appendix 3.11). There was no significant difference between animals treated with 1200 i.u. Folligon and those treated with 1200 i.u. Folligon + 2.5 ml Ovagen and between lambs treated with 24 mg FSH-P or 8 ml Ovagen, but the total ovarian responses of lambs treated with 1200 i.u. Folligon or 1200 i.u. Folligon + 2.5 ml Ovagen were significantly different from those of lambs treated with 24 mg FSH-P or 8 ml Ovagen.

GnRH.

There was no significant effect of GnRH administration on the total ovarian response (Appendix 3.11). The average responses for animals treated with or without GnRH were

3.61 and 3.22 respectively (Table 3.20).

Genotype.

There was a significant effect ($P<0.05$) of genotype on the total ovulatory response (Appendix 3.11). Fleece weight-selected ewe lambs gave higher responses than control ewe lambs (3.92 vs 2.84, respectively).

Group.

There was a significant effect ($P<0.05$) of group of treatment on the total ovarian response (Appendix 3.11). Lambs treated in group 1 gave better responses than those treated in group 2 (4.54 vs 2.92, respectively).

Interactions.

There was a significant effect ($P<0.05$) of the interaction between gonadotrophin treatment and GnRH administration on the total ovarian response (Appendix 3.11). While the responses were higher in animals treated with 1200 i.u. Folligon or 1200 i.u. Folligon + 2.5 ml Ovagen when combined with GnRH, the responses were low when animals were treated with 24 mg FSH-P or 8 ml Ovagen when combined with GnRH. The average responses are shown in Table 3.21. Analysis of the data, however, indicated no significant effect of the interaction between gonadotrophin treatment and group of treatment or between gonadotrophin treatment and the genotype of the lambs (Appendix 3.11). The overall responses tended to be higher in group 1 than group 2 and in fleece weight-selected ewe lambs than in controls (Table 3.21).

Age.

Preliminary analysis within age did not show any significant effect of gonadotrophin treatment, group or the interaction between them on ovulation rate, number of large follicles or the total ovarian response in ewe lambs (Appendix 3.12). However, ovulation rate and the total ovarian response were significantly affected by group in the case of mature ewes (Appendix 3.13). Therefore comparisons between mature ewes and ewe lambs were made within each group of treatment.

There was no significant effect of age on ovulation rate, number of large follicles or the total ovarian response in any of the groups (Appendix 3.14 and 3.15) although adults

tended to give higher responses than lambs (Table 3.22), particularly in ovulation rate and the total ovarian response. Even after combining animals treated in the two groups (Appendix 3.16) age did not significantly affect the number of large follicles or the total ovarian response, although there was a tendency (P=0.09) for adults to give a higher ovulation rate than lambs (6.35 vs 4.18, respectively). Table 3.23 shows the average ovulatory responses for the three variables of response.

Table 3.20 Ovulatory responses (Mean ± s.e.m.) from ewe lambs by Treatment, GnRH, Genotype and Group (Trial 3).

Treatment	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Folligon (1)	17	4.18 ± 0.90	a **	1.29 ± 0.44	NS	5.47 ± 0.92	a ***
FSH-P (2)	18	1.78 ± 0.55	bc	0.61 ± 0.22		2.39 ± 0.59	b
Ovagen (3)	16	1.06 ± 0.49	c	0.62 ± 0.26		1.68 ± 0.66	b
Folligon + Ovagen (4)	20	2.05 ± 0.42	b	1.95 ± 0.61		4.00 ± 0.59	a
GnRH							
-	35	2.28 ± 0.39	NS	0.94 ± 0.26	NS	3.22 ± 0.46	NS
+	36	2.25 ± 0.52		1.36 ± 0.36		3.61 ± 0.60	
Genotype							
Control (1)	33	1.75 ± 0.48	NS	1.09 ± 0.34	NS	2.84 ± 0.58	*
Fleece weight (3)	38	2.71 ± 0.43		1.21 ± 0.29		3.92 ± 0.49	
Group							
1	22	3.72 ± 0.78	**	0.82 ± 0.19	NS	4.54 ± 0.81	*
2	49	1.61 ± 0.27		1.31 ± 0.31		2.92 ± 0.40	

Table 3.21 Effect of the interactions Treatment by GnRH (Trt x GnRH), Treatment by Group (Trt x Group) and Treatment by Genotype (Trt x Genotype) on the ovulatory responses (Mean ± s.e.m.) (Trial 3) †.

Trt x GnRH		No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
1	-	9	3.00 ± 0.78	NS	0.88 ± 0.35	NS	3.88 ± 0.82	*
1	+	8	5.50 ± 1.63		1.75 ± 0.86		7.25 ± 1.55	
2	-	9	2.00 ± 0.94		0.55 ± 0.29		2.55 ± 0.98	
2	+	9	1.55 ± 0.62		0.66 ± 0.33		2.21 ± 0.72	
3	-	8	1.87 ± 0.87		1.00 ± 0.42		2.87 ± 1.17	
3	+	8	0.25 ± 0.25		0.25 ± 0.25		0.50 ± 0.32	
4	-	9	2.22 ± 0.61		1.33 ± 0.84		3.55 ± 0.83	
4	+	11	1.90 ± 0.59		2.45 ± 0.87		4.35 ± 0.85	
Trt x Group								
1	1	6	6.16 ± 2.02	NS	0.83 ± 0.47	NS	6.99 ± 2.03	NS
1	2	11	3.09 ± 0.74		1.54 ± 0.63		4.63 ± 0.88	
2	1	5	4.40 ± 1.40		0.80 ± 0.49		5.20 ± 1.28	
2	2	13	0.76 ± 0.20		0.53 ± 0.24		1.29 ± 0.36	
3	1	6	1.50 ± 1.14		0.83 ± 0.40		2.33 ± 1.47	
3	2	10	0.80 ± 0.41		0.50 ± 0.34		1.30 ± 0.63	
4	1	5	2.80 ± 0.73		0.80 ± 0.20		3.60 ± 0.67	
4	2	15	1.80 ± 0.49		2.33 ± 0.79		4.13 ± 0.76	
Trt x Genotype								
1	1	7	3.28 ± 2.02	NS	1.14 ± 0.67	NS	4.42 ± 2.01	NS
1	3	10	4.80 ± 0.66		1.40 ± 0.61		6.20 ± 0.74	
2	1	10	0.90 ± 0.31		0.70 ± 0.30		1.60 ± 0.52	
2	3	8	2.87 ± 1.09		0.50 ± 0.32		3.37 ± 1.11	
3	1	5	1.00 ± 0.63		0.40 ± 0.40		1.40 ± 0.87	
3	3	11	1.09 ± 0.66		0.72 ± 0.33		1.81 ± 0.90	
4	1	11	1.90 ± 0.54		1.72 ± 0.89		3.62 ± 0.95	
4	3	9	2.22 ± 0.68		2.22 ± 0.86		4.44 ± 0.64	

† See Table 3.20 for treatment and genotype descriptions.

Table 3.22 Ovulatory responses (Mean ± s.e.m.) to PMSG (Folligon) administration in lambs and adults treated in groups 1 and 2 (Trial 3).

Group 1	No.of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Lambs	6	6.16 ± 2.02	NS	0.83 ± 0.47	NS	6.99 ± 2.03	NS
Adults	6	10.00 ± 2.03		1.33 ± 0.61		11.33 ± 2.21	
Group 2							
Lambs	11	3.09 ± 0.74	NS	1.54 ± 0.63	NS	4.63 ± 0.88	NS
Adults	14	4.78 ± 1.08		0.50 ± 0.20		5.28 ± 1.12	

Table 3.23 Ovulatory responses (Mean ± s.e.m.) to PMSG (Folligon) administration in lambs and adults for groups 1 and 2 combined (Trial 3).

Age	No.of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Lambs	17	4.18 ± 0.90	NS	1.29 ± 0.44	NS	5.47 ± 0.92	NS
Adults	20	6.35 ± 1.08		0.75 ± 0.23		7.10 ± 1.18	

Trial 4.

Results from 88 ewe lambs were used to evaluate the effects of three sources of PMSG (Massey-PMSG, Pregnecol and Folligon), immunization against androstenedione (Immunized vs non-immunized animals) and GnRH (with or without GnRH) on ovulation rate, number of large follicles or the total ovarian response. The appropriate analyses of variance are shown in Appendices 3.17, 3.18 and 3.19. Preliminary analyses did not show a significant effect of group (Period) of treatment and such effect was not included in the final model. The average ovulatory responses for the three variables by source of PMSG, immunization group, GnRH and the source of PMSG x GnRH interaction are shown in Table 3.24. Other interactions examined and found non-significant were eliminated from the final model. The source of Massey-PMSG used here was the one giving the highest ovulation rates from a group of 3 sources of Massey-PMSG evaluated in a previous study (Splitting; Study 1). Results on the ovulatory responses using these sources of PMSG are presented in Appendix 3.45.

Ovulation rate.

Source of PMSG.

Analysis of the results did not show any significant effect of source of PMSG on ovulation rate. The average ovulation rates for ewe lambs treated with Massey-PMSG, Pregnecol or Folligon were 1.74, 2.11 and 1.90 respectively (Table 3.24).

Immunization.

Analysis of the results indicated a significant effect ($P<0.05$) of androstenedione immunization (averaged across all the other treatments) on ovulation rate. Immunized animals' gave higher responses than non-immunized animals (2.22 vs 1.59, respectively).

GnRH.

There was no significant effect of GnRH administration on ovulation rate, although there was a tendency for animals treated with GnRH to give higher responses than non-treated animals (2.13 vs 1.68, respectively).

Source of PMSG by GnRH interaction.

There was a significant effect ($P<0.05$) of the interaction between source of PMSG and

GnRH (averaged across immunization treatment) on ovulation rate. Administration of GnRH increased the responses in animals treated with Massey-PMSG or Folligon compared with non-treated animals (2.33 vs 1.00 and 2.25 vs 1.56, for lambs treated with Massey-PMSG or Folligon with or without GnRH respectively). However, for Pregnecol-treated animals the average ovulation rates were 1.80 and 2.50 for animals with or without GnRH respectively.

Number of large follicles.

Source of PMSG.

Analysis of the data indicated a significant effect ($P<0.05$) of source of PMSG (averaged across all the other treatments) on the number of large follicles. Animals treated with Massey-PMSG or Folligon recorded higher responses than animals treated with Pregnecol (2.40 and 2.03 vs 0.81, respectively). There was no significant effect of immunization, GnRH or the interaction between source of PMSG and GnRH on the number of large follicles. The average responses by immunization group, GnRH and the interaction of source of PMSG by GnRH are shown in Table 3.24.

Total ovarian response.

Analysis of the results did not show any significant effect of source of PMSG, Immunization, GnRH or the interaction of source of PMSG and GnRH on the total ovarian response. The average responses for those main effects as well as for the source of PMSG x GnRH sub-groups are shown in Table 3.24.

Table 3.24 Effect of source of PMSG, Immunization, GnRH, and PMSG by GnRH interaction on the ovulatory responses (Mean ± s.e.m.) (Trial 4).

Source of PMSG	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Massey-PMSG (1)	27	1.74 ± 0.33	NS	2.40 ± 0.51	a *	4.14 ± 0.58	NS
Pregnecol (2)	27	2.11 ± 0.27		0.81 ± 0.20	b	2.92 ± 0.26	
Folligon (3)	32	1.90 ± 0.27		2.03 ± 0.43	a	3.93 ± 0.52	
Immunization							
Non-immunized	42	1.59 ± 0.25	*	2.14 ± 0.41	NS	3.73 ± 0.44	NS
Immunized	44	2.22 ± 0.21		1.41 ± 0.26		3.63 ± 0.35	
GnRH							
-	42	1.68 ± 0.23	NS	1.52 ± 0.35	NS	3.20 ± 0.38	NS
+	46	2.13 ± 0.23		1.97 ± 0.33		4.10 ± 0.39	
PMSGxGnRH							
1 -	12	1.00 ± 0.42	*	2.83 ± 0.96	NS	3.83 ± 1.00	NS
1 +	15	2.33 ± 0.45		2.07 ± 0.54		4.40 ± 0.69	
2 -	12	2.50 ± 0.39		0.66 ± 0.22		3.16 ± 0.42	
2 +	15	1.80 ± 0.36		0.93 ± 1.33		2.73 ± 0.34	
3 -	16	1.56 ± 0.32		1.19 ± 0.42		2.75 ± 0.53	
3 +	16	2.25 ± 0.42		2.87 ± 0.70		5.12 ± 0.80	

Trial 5.

Data from 64 ewe lambs were used to evaluate the effects of gonadotrophin treatment, genotype and group of treatment on ovulation rate, number of large follicles or the total ovarian response. The appropriate analyses of variance are presented in Appendices 3.20, 3.21 and 3.22. Preliminary analyses failed to show significant differences between lambs treated in groups 2 and 3 and their data were combined (Group 2) and compared with group 1. The effect of age on the ovulatory responses was tested using 13 ewe lambs and 13 adult ewes given similar gonadotrophin treatment (Anova in Appendix 3.23). The average ovulatory responses for the three variables by treatment, genotype and group are indicated in Table 3.25. Similarly Table 3.26 shows the average responses for the interaction of treatment by genotype.

Ovulation rate.

Gonadotrophin treatment.

There was no significant effect of the gonadotrophin treatment on ovulation rate. The average ovulation rates for lambs treated with 1200 i.u. Folligon + 100 μg GnRH, 300 i.u. Folligon + 8 ml Ovagen, 300 i.u. Folligon + 8 ml Ovagen + 100 μg GnRH, 500 i.u. Folligon + 8 ml Ovagen or 500 i.u. Folligon + 8 ml Ovagen + 100 μg GnRH were 1.61, 2.46, 2.85 1.77 and 2.75 respectively (Table 3.25).

Genotype.

There was no significant effect of genotype on ovulation rate. The average ovulation rates for control and fleece weight-selected animals were 2.77 and 1.85 respectively.

Group.

There was a significant effect ($P<0.01$) of group of treatment (averaged across all other treatments) on ovulation rate. Animals treated in group 1 gave higher responses than animals treated in group 2 (3.04 vs 1.82, respectively).

Treatment by genotype interaction.

There was a significant effect ($P<0.01$) of the interaction between treatment and genotype (averaged across groups) on ovulation rate. Ovulation rate was higher in fleece weight-selected animals (Genotype 3) than in controls (Genotype 1) when the lambs were treated with 1200 i.u. Folligon + 100 μg GnRH. However, the responses

were higher for control ewe lambs treated with Ovagen plus a small dose of Folligon (Table 3.26).

Number of large follicles.

Analysis of the data did not show any significant effect of treatment, genotype, group or the interaction of treatment by genotype on the number of large follicles (Appendix 3.21). The average responses for treatment, genotype and group are shown in Table 3.25, and for the treatment by genotype sub-groups in Table 3.26.

Total ovarian response.

Analysis of the results did not show any significant effect of gonadotrophin treatment, genotype, group or the interaction of treatment by genotype on the total ovarian response (Appendix 3.22). In Table 3.25 are indicated the average total ovarian responses for treatment, genotype and group and in Table 3.26 for the treatment by genotype interaction.

Age.

The average ovulatory responses for the three variables of response are shown in Table 3.27. Analysis of the data indicated a significant effect ($P<0.01$) of age on the total ovarian response, with adult ewes giving higher responses than ewe lambs (5.23 vs 2.84, respectively). There was no significant effect of age on ovulation rate or the number of large follicles.

Table 3.25 Effect of Treatment, Genotype and Group on the ovulatory responses in ewe lambs (Mean ± s.e.m.) (Trial 5).

Treatment	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
1200 Folligon + GnRH (1)	13	1.61 ± 0.40	NS	1.23 ± 0.42	NS	2.84 ± 0.49	NS
300 Folligon + Ovagen (2)	13	2.46 ± 0.43		1.46 ± 0.43		3.92 ± 0.66	
300 Folligon + Ovagen + GnRH (3)	13	2.85 ± 0.74		1.84 ± 0.52		4.69 ± 1.10	
500 Folligon + Ovagen (4)	13	1.77 ± 0.50		1.69 ± 0.60		3.46 ± 0.91	
500 Folligon + Ovagen + GnRH (5)	12	2.75 ± 0.50		1.50 ± 0.52		4.25 ± 0.86	
Genotype							
Control (1)	30	2.77 ± 0.39	NS	1.53 ± 0.28	NS	4.30 ± 0.53	NS
Fleece weight (3)	34	1.85 ± 0.27		1.56 ± 0.33		3.41 ± 0.50	
Group							
1	24	3.04 ± 0.38	**	1.58 ± 0.39	NS	4.62 ± 0.64	NS
2	40	1.82 ± 0.28		1.52 ± 0.26		3.35 ± 0.43	

Table 3.26 Effect of the interaction of Treatment by Genotype (Trt x Geno) on the ovulatory responses in ewe lambs (Mean ± s.e.m.) (Trial 5) †.

Trt x Geno		No. of Animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
1	1	4	0.50 ± 0.28	**	2.00 ± 0.70	NS	2.50 ± 0.50	NS
1	3	9	2.11 ± 0.48		0.89 ± 0.51		3.00 ± 0.68	
2	1	7	3.00 ± 0.65		0.86 ± 0.45		3.86 ± 0.93	
2	3	6	1.83 ± 0.47		2.17 ± 0.70		4.00 ± 1.03	
3	1	5	5.00 ± 1.14		1.80 ± 0.20		6.80 ± 1.28	
3	3	8	1.50 ± 0.62		1.87 ± 0.87		3.37 ± 1.46	
4	1	8	2.12 ± 0.74		1.50 ± 0.65		3.62 ± 1.20	
4	3	5	1.20 ± 0.58		2.00 ± 1.26		3.20 ± 1.52	
5	1	6	3.00 ± 0.51		1.83 ± 0.90		4.83 ± 1.16	
5	3	6	2.50 ± 0.19		1.17 ± 0.60		3.67 ± 1.33	

† See Table 3.25 for treatment and genotype descriptions.

Table 3.27 Effect of age on the ovulatory responses (Mean ± s.e.m.) (Trial 5).

Age	No.of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Lambs	13	1.61 ± 0.40	NS	1.23 ± 0.42	NS	2.84 ± 0.49	**
Adults	13	3.15 ± 1.07		2.08 ± 0.61		5.23 ± 1.11	

Trial 6.

Data from 111 ewe lambs were used to indicate whether the ovulatory responses might be different in a flock where the Fec^B gene was segregating. Superovulation was attempted by administering 1200 i.u. Folligon or 1200 i.u. Folligon + 100 µg GnRH given at two different ages (Age 1:6-7 months, Age 2:9-10 months). The appropriate analyses of variance to evaluate the effects of gonadotrophin treatment and age on ovulation rate, the number of large follicles and the total ovarian response are shown in Appendices 3.24, 3.25 and 3.26. The average ovulatory responses for the three variables by gonadotrophin treatment, age and their interaction are shown in Table 3.28.

Ovulation rate.

There was no significant effect of gonadotrophin treatment, age or treatment by age interaction on ovulation rate (Table 3.28) although lambs treated with 1200 i.u. Folligon + 100 µg GnRH or when treated at 9-10 months of age tended to give higher responses.

Number of large follicles.

Analysis of the data did not show significant effects of any of the variables studied or their interactions on the number of large follicles. The average responses for lambs treated with 1200 i.u. Folligon or 1200 i.u. Folligon + 100 µg GnRH were 3.82 and 3.47, similarly the average responses for animals treated at 6-7 and 9-10 months of age were 3.69 and 3.53 respectively.

Total ovarian response.

Analysis of the results did not show a significant effect of any of the variables considered or their interaction on the total ovarian response. The average responses are shown in Table 3.28.

Ovulation rate data recorded 5-10 days after the occurrence of a natural heat gave the following results. Seventy two percent (81/113) of the animals were detected in heat and ovulated. Sixty eight percent (55/81) each had a single ovulation, 22% gave twin ovulations and 10% had triple or more ovulations. The last group included one lamb with 4 corpora lutea and another with 5 corpora lutea.

The average ovulatory responses to the gonadotrophin treatment (Folligon and Folligon

+ GnRH) according to the natural ovulation rate of the animals are shown in Table 3.29. The correlation between the ovulation rate after the incidence of a natural oestrus and the ovulation rate following the gonadotrophin treatment was low and not significant (.06 and .09 for animals treated with 1200 i.u. Folligon or 1200 i.u. Folligon + 100 µg GnRH, respectively).

Table 3.28 Effect of gonadotrophin treatment, age and their interaction on the ovulatory responses (Mean ± s.e.m.) of Booroola cross lambs (Trial 6).

Treatment	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
Folligon (1)	56	3.44 ± 0.41	NS	3.82 ± 0.48	NS	7.26 ± 0.40	NS
Folligon + GnRH(2)	55	4.16 ± 0.51		3.47 ± 0.47		7.63 ± 0.47	
Age							
6-7 months (1)	81	3.65 ± 0.39	NS	3.69 ± 0.41	NS	7.34 ± 0.37	NS
9-10 months (2)	30	4.20 ± 0.60		3.53 ± 0.58		7.73 ± 0.56	
Gonadotrophin x Age							
1 1	40	3.22 ± 0.46	NS	3.87 ± 0.62	NS	7.09 ± 0.47	NS
1 2	16	4.00 ± 0.85		3.68 ± 0.68		7.68 ± 0.78	
2 1	41	4.07 ± 0.62		3.51 ± 0.54		7.58 ± 0.56	
2 2	14	4.43 ± 0.86		3.35 ± 1.00		7.78 ± 0.83	

Table 3.29 Average ovulatory responses following gonadotrophin treatment according to natural ovulation rate in Booroola cross lambs (Mean ± s.e.m.) (Trial 6).

Natural Ovulation Rate	No. of Animals	Ovulation Rate	Large Follicles	Total Response
1	55	3.82 ± 0.47	3.49 ± 0.50	7.31 ± 0.47
2	18	4.50 ± 0.78	4.11 ± 0.85	8.61 ± 0.66
3	8	3.87 ± 1.40	5.13 ± 1.30	9.00 ± 0.50
Total	81	3.97 ± 0.38	3.79 ± 0.41	7.76 ± 0.36

3.2.3 Factors affecting egg recovery, fertilization rate and percentage of transferable embryos.

Trial 1.

Recovery rate.

Data from 25 ewe lambs were used to evaluate the effects of dose of PMSG (900 i.u. vs 1200 i.u.) and time of gonadotrophin administration (24h before sponge removal vs at sponge removal) on the recovery rate of eggs. The effect of age on the recovery rate of eggs was evaluated including data from 25 lambs and 15 adult ewes treated in the same way as the ewe lambs. The percentage data in the present and subsequent trials have been calculated on two bases: (A) considering total numbers of corpora lutea, eggs recovered, eggs fertilized or embryos transferable; and (B) on a per ewe basis. However, statistical analyses were performed on data generated on a per ewe basis only.

Dose and time of PMSG administration.

The appropriate analysis of variance to examine the effect of dose and time of PMSG injection on recovery rate is given in Appendix 3.27. Similarly the average recovery rates for these factors are shown in Table 3.30.

The analysis of the data did not show a significant effect of any of the factors considered on the recovery rate of eggs. The recovery rate of animals injected with 900 or 1200 i.u. of PMSG was 56 and 55% respectively. The recovery rate of eggs tended to be higher in lambs treated 24h before sponge withdrawal than in those treated at sponge removal (63% vs 45%, respectively), but the differences were not significant.

Age.

The analysis of variance to evaluate the effect of age on the recovery rate of eggs is given in Appendix 3.28 and the recovery rates for ewe lambs and adult ewes are shown in Table 3.31. The rate of recovery of eggs was higher in adults than in lambs (91% vs 55%, respectively; $P<0.01$).

Table 3.30 Effect of dose and time of PMSG injection on the recovery rate of eggs (Trial 1).

Dose of PMSG	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
900 i.u.	12	22	11	50	56	NS
1200 i.u.	13	19	12	63	55	
Time of PMSG injection						
- 24h	14	28	16	57	63	NS
0h	11	13	7	54	45	

◊ = % calculated on the total number of eggs recovered and total corpora lutea.
△ = % calculated on a per ewe basis.

Table 3.31 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable (Trial 1).

A) Recovery rate						
	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				$A^{\lozenge}$	B^{Δ}	
Lambs	25	41	23	56	55	**
Adults	15	32	28	88	91	
B) Fertilization rate						
	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		
				$A^{\lozenge}$	B^{Δ}	
Lambs	15	23	11	48	50	*
Adults	15	28	23	82	82	
C) % of embryos transferable						
	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		
				$A^{\lozenge}$	B^{Δ}	
Lambs	9	11	6	55	50	NS
Adults	14	23	14	61	53	

$^{\lozenge}$ = % calculated on total numbers.
$^{\Delta}$ = % calculated on a per ewe basis.

Fertilization rate.

Results from 15 lambs were used to evaluate the effects of dose and time of PMSG injection on fertilization rate. The effect of age on fertilization rate was evaluated using data from 15 lambs and 15 adults.

Dose and time of PMSG injection.

There was no significant effect of dose or time of PMSG injection on fertilization rate (Table 3.32, Anova in Appendix 3.29). The average fertilization rates tended to be higher in animals receiving 1200 i.u. of PMSG compared to those receiving 900 i.u. of PMSG (62% vs 36%, respectively), but the differences were not significant. A similar trend was

observed in the rate of fertilization when PMSG was given at sponge removal compared with when it was given 24h before sponge withdrawal (80% vs 35%, respectively).

Age.

The analysis of variance evaluating the effect of age on fertilization rate is presented in Appendix 3.28, and average fertilization rates for lambs and adults are indicated in Table 3.31. Adult ewes gave higher responses than ewe lambs (82% vs 50%, respectively, $P<0.05$).

Table 3.32 Effect of dose and time of PMSG injection on fertilization rate (Trial 1).

Dose of PMSG	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		Sig.
				A◊	B⌂	
900 i.u.	7	11	4	36	36	NS
1200 i.u.	8	12	7	58	62	
Time of PMSG injection						
- 24h	10	16	6	38	35	NS
0h	5	7	5	71	80	

◊ = % calculated on the total number of eggs recovered and fertilized.
⌂ = % calculated on a per ewe basis.

Percentage of embryos transferable.

Data from 9 lambs were available to evaluate the effects of dose and time of PMSG injection on the percentage of embryos transferable. The effect of age on the percentage of embryos transferable was evaluated using data from 9 ewe lambs and 14 adult ewes.

Dose and time of PMSG injection.

The effects of dose and time of PMSG injection on the percentage of embryos transferable are shown in Table 3.33 (Anova in Appendix 3.30). Neither was significant.

Age.

There was no significant effect of age on the percentage of embryos transferable (Table 3.31, Anova in Appendix 3.28). The average percentages of transferable embryos for lambs and adults were 53 and 50% respectively.

Table 3.33 Effect of dose and time of PMSG injection on the percentage of embryos transferable (Trial 1).

Dose of PMSG	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		Sig.
				A◊	B⌂	
900 i.u.	3	4	3	75	67	NS
1200 i.u.	6	7	3	43	42	
Time of PMSG injection						
- 24h	5	6	2	33	30	NS
0h	4	5	4	80	75	

◊ = % calculated on the total number of eggs fertilized and transferable embryos.
⌂ = % calculated on a per ewe basis.

Trial 2.

Recovery rate.

Data from 32 lambs were used to evaluate the effects of source of PMSG (Source 1; Consept45 vs Source 2; Folligon) and time of PMSG administration (2 vs 4 days before sponge withdrawal) on the recovery rate of eggs. The average recovery rates for these factors and their interaction are indicated in Table 3.34 (Anova in Appendix 3.31). These were not significantly affected by source of PMSG or time of PMSG administration, however, the effect of the interaction was significant ($P<0.001$). The average recovery rate of animals treated with Consept45 or Folligon was 53 and 57%, respectively. The responses for lambs receiving PMSG 2 or 4 days before sponge removal were 47 and 65%, respectively. Higher recovery rates were recorded in animals treated with Consept45 when it was given 4 days before sponge withdrawal than in those treated with Folligon given at the same time (88% vs 41%, respectively). In animals receiving the same gonadotrophin treatments administered 2 days before sponge removal, Consept45 was associated with a recovery rate of 22% vs 67% for Folligon.

Table 3.34 Effect of source of PMSG, time of PMSG injection and their interaction on the recovery rate of eggs (Trial 2).

Source of PMSG	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
Consept45 (1)	15	33	20	61	53	NS
Folligon (2)	17	40	24	60	57	
Time of PMSG injection						
- 2 days	18	39	20	51	47	NS
- 4 days	14	34	24	71	65	
Source x Time						
1 x - 2 days	8	15	5	33	22	***
1 x - 4 days	7	18	15	83	88	
2 x - 2 days	10	24	15	63	67	
2 x - 4 days	7	16	9	56	41	

◊ = % calculated on the total number of eggs recovered and total corpora lutea.
△ = % calculated on a per ewe basis.

Fertilization rate.

Data from 22 animals were available to evaluate the effects of source of PMSG and time of its administration on fertilization rate. The average fertilization rates for these factors and their interaction are indicated in Table 3.35 (Anova in Appendix 3.32). There was no significant effect of any of the factors studied or their interaction on the rate of fertilization. The average fertilization rate in lambs treated with Consept45 or Folligon was 70 and 42%, respectively. Similarly the average fertilization rate in animals receiving the gonadotrophin treatment 2 or 4 days before sponge withdrawal was 54 and 54%, respectively. Fertilization rates tended to be higher in animals treated with Consept45 regardless of the time of administration.

Table 3.35 Effect of source of PMSG, time of PMSG injection and their interaction on fertilization rate (Trial 2).

Source of PMSG	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		Sig.
				A◊	B△	
Consept45 (1)	10	20	15	75	70	NS
Folligon (2)	12	24	12	50	42	
Time of PMSG injection						
- 2 days	11	20	12	60	54	NS
- 4 days	11	24	15	63	54	
Source x Time						
1 x - 2 days	3	5	3	60	67	NS
1 x - 4 days	7	15	12	80	71	
2 x - 2 days	8	15	9	60	50	
2 x - 4 days	4	9	3	33	25	

◊ = % calculated on the total number of eggs recovered and fertilized.
△ = % calculated on a per ewe basis.

Percentage of embryos transferable.

Due to the small number of observations only main effects were included in the model. Table 3.36 (Anova in Appendix 3.33) shows the average percentage of transferable embryos from these sheep according to the source of PMSG and its time of administration. The percentage of transferable embryos was not significantly affected by any of the factors considered.

Table 3.36 Effect of source of PMSG and time of its injection on the percentage of embryos transferable (Trial 2).

Source of PMSG	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		Sig.
				A◊	B△	
Consept45	7	15	12	80	64	NS
Folligon	6	12	6	50	55	
Time of PMSG injection						
- 2 days	7	12	7	58	57	NS
- 4 days	6	15	11	73	64	

◊ = % calculated on the total number of eggs fertilized and embryos transferable.
△ = % calculated on a per ewe basis.

Trial 3.

Recovery rate.

Data from 47 ewe lambs were used to evaluate the effects of gonadotrophin treatment (Treatment 1; 1200 i.u. of Folligon vs Treatment 2; 24 mg of FSH-P vs Treatment 3; 8 ml of Ovagen vs Treatment 4; 1200 i.u. of Folligon + 2.5 ml of Ovagen), GnRH (Treated vs not treated) and group (1 vs 2) on the recovery rate of eggs. The effect of age on the recovery rate of eggs was evaluated including data from 47 ewe lambs and 20 adult ewes. Mature ewes were treated with 1200 i.u. of Folligon, with or without GnRH. However, for the purpose of this comparison data from all the adult ewes were combined.

Gonadotrophin treatment, GnRH and group.

There was no significant effect of any of the factors considered on the recovery rate of eggs (Table 3.37, Anova in Appendix 3.34). The average recovery rates for animals treated with 1200 i.u. Folligon, 24 mg FSH-P, 8 ml Ovagen or 1200 i.u. Folligon + 2.5 ml Ovagen were 58, 42, 32 and 44%, respectively. The recovery rate of eggs tended to be higher in lambs treated with GnRH than in not treated lambs (52% vs 42%, respectively). Similarly, lambs treated in group 1 showed a slightly higher recovery rate than those of group 2 (57% vs 41%, respectively), but the difference was not significant.

Table 3.37 Effect of gonadotrophin treatment, GnRH and group on the recovery rate of eggs (Trial 3).

Gonadotrophin treatment	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B⌂	
Folligon	15	71	52	73	58	NS
FSH-P	12	32	19	59	42	
Ovagen	5	17	6	35	32	
Folligon+Ovagen	15	41	20	49	44	
GnRH						
-	25	80	44	55	42	NS
+	22	81	53	65	52	
Group						
1	17	82	54	66	57	NS
2	30	79	43	54	41	

◊ = % calculated on the total number of eggs recovered and total corpora lutea.
⌂ = % calculated on a per ewe basis.

Age.

The average recovery rates for ewe lambs and adult ewes are shown in Table 3.38 (Anova in Appendix 3.35). Analysis of the data indicated a significant effect ($P<0.01$) of age on the recovery rate of eggs. Recovery rates were higher in adults than in lambs (72% vs 47%, respectively).

Table 3.38 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable (Trial 3).

A) Recovery rate						
	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
Lambs	47	161	97	60	47	**
Adults	20	127	94	74	72	
B) Fertilization rate						
	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		
				A◊	B△	
Lambs	31	97	66	68	74	NS
Adults	17	94	63	67	73	
C) % of embryos transferable						
	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		
				A◊	B△	
Lambs	26	66	36	55	49	NS
Adults	13	63	49	78	73	

◊ = % calculated on total numbers.
△ = % calculated on a per ewe basis.

Fertilization rate.

Data from 31 ewe lambs were used to evaluate the effects of gonadotrophin treatment, GnRH and group on fertilization rate. The effect of age on fertilization rate was

evaluated using data from 31 lambs and 17 adults.

Gonadotrophin treatment, GnRH and group.

The average fertilization rates for these factors are indicated in Table 3.39 (Anova in Appendix 3.36). There was no significant effect of any of the factors studied on the rate of fertilization. The average fertilization rates for lambs treated with 1200 i.u. Folligon, 24 mg FSH-P, 8 ml Ovagen or 1200 i.u. Folligon + 2.5 ml Ovagen were 78, 62, 100 and 70%, respectively. The rate of fertilization was slightly lower in lambs treated with GnRH than in not treated lambs (69% vs 80%, respectively). Similarly fertilization rates were marginally lower in lambs treated in group 1 than in group 2 (69% vs 78%, respectively).

Age.

The average responses for adult ewes and ewe lambs shown in Table 3.38 were 73 and 74%, respectively (Anova in Appendix 3.35). These were not significantly different.

Table 3.39 Effect of gonadotrophin treatment, GnRH and group on fertilization rate (Trial 3).

Gonadotrophin treatment	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		Sig.
				A◊	B△	
Folligon	12	52	37	71	78	NS
FSH-P	6	19	7	37	62	
Ovagen	3	6	6	100	100	
Folligon+Ovagen	10	20	16	80	70	
GnRH						
-	15	44	31	70	80	NS
+	16	53	35	66	69	
Group						
1	14	54	29	54	69	NS
2	17	43	37	86	78	

◊ = % calculated on the total number of eggs recovered and fertilized.
△ = % calculated on a per ewe basis.

Percentage of embryos transferable.

Data from 26 ewe lambs were available to evaluate the effects of gonadotrophin treatment, GnRH and group on the percentage of embryos transferable. The effect of age on the percentage of embryos transferable was evaluated using data from 26 lambs and 13 adults.

Gonadotrophin treatment, GnRH and group.

The effects of gonadotrophin treatment, GnRH and group on the percentage of embryos transferable are shown in Table 3.40 (Anova in Appendix 3.37). There was no significant effect of GnRH on the percentage of embryos transferable, although lambs treated with GnRH tended to give a higher percentage of low quality embryos than not treated lambs (37% vs 62%, respectively). Embryos transferable were significantly affected by gonadotrophin treatment ($P<0.01$) and group ($P<0.01$). The few embryos produced by Ovagen-treated lambs tended to be of higher quality than those from other treatments (100% vs 51, 20 and 46% for lambs treated with 8 ml Ovagen, 1200 i.u. Folligon, 24 mg FSH-P and 1200 i.u. Folligon + 2.5 ml Ovagen, respectively). The differences were not significant between treatments 1, 2 and 4. The percentage of embryos transferable was higher in lambs treated in group 1 than in those treated in group 2 (69% vs 33%, respectively).

Table 3.40 Effect of gonadotrophin treatment, GnRH and group on the percentage of embryos transferable (Trial 3).

Gonadotrophin treatment	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		Sig.
				A◊	B⌂	
Folligon	11	37	23	62	51 b	**
FSH-P	5	7	1	14	20 b	
Ovagen	3	6	6	100	100 a	
Folligon+Ovagen	7	16	6	38	46 b	
GnRH						
-	13	31	21	68	62	NS
+	13	35	15	43	37	
Group						
1	12	29	21	72	69	**
2	14	37	15	41	33	

◊ = % calculated on the total number of eggs fertilized and transferable embryos.
⌂ = % calculated on a per ewe basis.

Age.

The percentage of embryos transferable for lambs and adults is shown in Table 3.38 (Anova in Appendix 3.35). Age did not show a significant effect on the percentage of embryos transferable, although adults tended to give higher quality embryos than lambs (73% vs' 49%, respectively).

Trial 4.

Recovery rate.

Data from 70 ewe lambs were used to evaluate the effects of three sources of PMSG (Source 1; Massey-PMSG vs Source 2; Pregnecol vs Source 3; Folligon), immunization against androstenedione (Immunized vs non-immunized) and GnRH (Treated vs not treated) on the recovery rate of eggs. The average recovery rate for sources 1, 2 and 3 of PMSG were 28, 46 and 29%, respectively (Table 3.41, Anova in Appendix 3.38). The recovery rate tended to be higher for non-immunized than for immunized ewe lambs (37% vs 33%, respectively) and for animals not treated with GnRH than for those receiving GnRH (37% vs 33%, respectively), but the differences were not significant.

Table 3.41 Effect of source of PMSG, immunization and GnRH on the recovery rate of eggs (Trial 4).

Source of PMSG	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
Massey-PMSG	19	47	17	36	28	NS
Pregnecol	25	57	29	51	46	
Folligon	26	61	14	23	29	
Immunization						
Non-immunized	30	67	25	37	37	NS
Immunized	40	98	35	36	33	
GnRH						
-	30	67	27	40	37	NS
+	40	98	33	34	33	

◊ = % calculated on the total number of eggs recovered and total corpora lutea.
△ = % calculated on a per ewe basis.

Fertilization rate.

Data from 36 animals were available to evaluate the effects of source of PMSG, androstenedione immunization and GnRH on fertilization rate. The average fertilization rates by source of PMSG, immunization and GnRH as well as for the source of PMSG x GnRH interaction are shown in Table 3.42 (Anova in Appendix 3.39).

There was no significant effect of source of PMSG or immunization. Overall, fertilization rates were higher in lambs without GnRH than in those treated with GnRH (74% vs 55%, respectively, $P<0.05$). However, the effect of GnRH was dependent on the source of PMSG administered ($P<0.01$). While the fertilization rate was lower in animals treated with Massey-PMSG or Folligon plus GnRH, higher responses were observed in lambs treated with Pregnecol plus GnRH than in animals given Pregnecol but not receiving GnRH. The average responses for animals treated with Massey-PMSG, Pregnecol or Folligon were 71, 71 and 50%, respectively. Similarly the average fertilization rate for immunized and non-immunized ewe lambs was 61 and 69%, respectively.

Table 3.42 Effect of source of PMSG, immunization, GnRH and the source of PMSG x GnRH interaction on fertilization rate (Trial 4).

Source of PMSG	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		Sig.
				A◊	B△	
Massey-PMSG (1)	9	17	11	65	71	NS
Pregnecol (2)	15	29	23	79	71	
Folligon (3)	12	14	8	57	50	
Immunization						
Non-immunized	14	25	20	80	69	NS
Immunized	22	35	22	63	61	
GnRH						
-	17	27	21	78	74	*
+	19	33	21	64	55	
Source x GnRH						
1 x -	2	3	3	100	100	**
1 x +	7	14	8	57	63	
2 x -	8	15	10	67	58	
2 x +	7	14	13	93	86	
3 x -	7	9	8	89	86	
3 x +	5	5	0	0	0	

◊ = % calculated on the total number of eggs recovered and fertilized.
△ = % calculated on a per ewe basis.

Percentage of embryos transferable.

No significant effects of source of PMSG, immunization or GnRH could be found on the percentage of embryos transferable (Table 3.43, Anova in Appendix 3.40). The average percentage of transferable embryos for lambs treated with Massey-PMSG, Pregnecol or Folligon were 43, 39 and 50%, respectively. The percentage of embryos transferable tended to be higher in immunized than in non-immunized ewe lambs (50% vs 33%) and in animals treated with GnRH than in those not receiving GnRH (46% vs 39%).

Table 3.43 Effect of source of PMSG, immunization and GnRH on the percentage of embryos transferable (Trial 4).

Source of PMSG	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		Sig.
				A◊	B⌂	
Massey-PMSG	7	11	5	45	43	NS
Pregnecol	11	23	10	43	39	
Folligon	6	8	4	50	50	
Immunization						
Non-immunized	10	20	8	40	33	NS
Immunized	14	22	11	50	50	
GnRH						
-	11	21	9	43	46	NS
+	13	21	10	48	39	

◊ = % calculated on the total number of eggs fertilized and transferable.
⌂ = % calculated on a per ewe basis.

Trial 5.

Recovery rate.

Data from 49 ewe lambs were used to evaluate the effect of gonadotrophin treatment (Treatment 1; 1200 i.u. of Folligon vs Treatment 2; 300 i.u. of Folligon + 8 ml of Ovagen vs Treatment 3; 500 i.u. of Folligon + 8 ml of Ovagen) on the recovery rate of eggs. The effect of age on the recovery rate of eggs was evaluated including data from 49 ewe lambs and 11 adult ewes. Mature ewes were treated with 1200 i.u. of Folligon + 100 µg of GnRH.

Gonadotrophin treatment.

The average recovery rates by gonadotrophin treatment are indicated in Table 3.44 (Anova in Appendix 3.41). There was no significant effect of gonadotrophin treatment on the recovery rate of eggs. The average responses for lambs treated with 1200 i.u. Folligon (Treatment 1), 300 i.u. Folligon + 8 ml Ovagen (Treatment 2) or 500 i.u. Folligon + 8 ml Ovagen (Treatment 3) were 33, 46 and 43%, respectively.

Age.

The average recovery rate of eggs from lambs and adults was 36 and 41%, respectively but the difference was not statistically significant (Table 3.45, Anova in Appendix 3.42).

Table 3.44 Effect of gonadotrophin treatment on the recovery rate of eggs (Trial 5).

Treatment	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
1	9	18	5	28	33	NS
2	22	69	26	38	46	
3	18	56	21	38	43	

◊ = % calculated on the total number of eggs recovered and total corpora lutea.
△ = % calculated on a per ewe basis.

Table 3.45 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable (Trial 5).

A) Recovery rate						
	No. of animals	No. of ovulations	No. of eggs recovered	% of eggs recovered		Sig.
				A◊	B△	
Lambs	49	143	52	36	43	NS
Adults	11	41	17	41	34	
B) Fertilization rate						
	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		
				A◊	B△	
Lambs	31	52	35	67	65	NS
Adults	6	17	11	65	64	
C) % of embryos transferable						
	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		
				A◊	B△	
Lambs	21	35	19	54	48	NS
Adults	5	11	6	55	60	

◊ = % calculated on total numbers.
△ = % calculated on a per ewe basis.

Fertilization rate.

Data from 31 lambs were used to evaluate the effects of gonadotrophin treatment, inseminator and ram on fertilization rate and together with 6 adults the effect of age.

Gonadotrophin treatment, inseminator and ram.

The analysis of variance examining the effect of gonadotrophin treatment, inseminator and ram on fertilization rate is given in Appendix 3.43. The average fertilization rates for these factors are indicated in Table 3.46. None of the factors significantly affected the rate of fertilization.

Age.

The average fertilization rate for lambs and adults was 65 and 64%, respectively as shown in Table 3.45 (Anova in Appendix 3.42), the difference was not significant.

Table 3.46 Effect of gonadotrophin treatment, inseminator and ram on fertilization rate (Trial 5).

Treatment	No. of animals	No. of eggs recovered	No. of eggs fertilized	% of eggs fertilized		Sig.
				A◊	BΔ	
1	3	5	5	100	100	NS
2	15	26	19	73	88	
3	13	21	11	52	69	
Inseminator						
1	25	42	29	69	64	NS
2	6	10	6	60	67	
Ram						
1	15	24	18	75	73	NS
2	16	28	17	61	56	

◊ = % calculated on the total number of eggs recovered and fertilized.
Δ = % calculated on a per ewe basis.

Treatments (1; 1200 i.u. Folligon, 2; 300 i.u. Folligon + 8 ml Ovagen, 3; 500 i.u. Folligon + 8 ml Ovagen).

Percentage of embryos transferable.

Data from 20 lambs were used to evaluate the effects of gonadotrophin treatment, inseminator and ram on the percentage of embryos transferable. The effect of age on the percentage of embryos transferable was evaluated including data from 21 lambs and 5 adults.

Gonadotrophin treatment, Inseminator and ram.

The percentage of embryos transferable for these factors is shown in Table 3.47 (Anova in Appendix 3.44). The analysis did not show significant effects of gonadotrophin treatment, inseminator or ram on the percentage of embryos transferable. The

percentage of embryos transferable for lambs treated with 1200 i.u. Folligon, 300 i.u. Folligon + 8 ml Ovagen or 500 i.u. Folligon + 8 ml Ovagen were 33, 50 and 50%, respectively.

Table 3.47 Effect of gonadotrophin treatment, inseminator and ram on the percentage of embryos transferable (Trial 5).

Treatment	Total No. of animals	No. of eggs fertilized	No. of embryos transferable	% of embryos transferable		Sig.
				A◊	B△	
1	3	5	1	20	33	NS
2	11	19	11	58	50	
3	7	11	7	64	50	
Inseminator						
1	17	29	15	52	44	NS
2	4	6	4	67	62	
Ram						
1	11	18	12	67	59	NS
2	10	17	7	41	35	

◊ = % calculated on the total number of eggs fertilized and transferable embryos.
△ = % calculated on a per ewe basis.

Treatments (1; 1200 i.u. Folligon, 2; 300 i.u. Folligon + 8 ml Ovagen, 3; 500 i.u. Folligon + 8 ml Ovagen).

Age.

The analysis of variance showing the effect of age on the percentage of embryos transferable is given in Appendix 3.42. The average percentage of embryos transferable for lambs and adults is indicated in Table 3.45. The data did not show a significant effect of age on the percentage of embryos transferable. The average responses for lambs and adults were 48 and 60%, respectively.

3.2.4 Factors affecting the survival of transplanted embryos.

Trial 1.

Five recipient ewes were implanted with embryos from ewe lambs of which only one became pregnant. Similarly 18 recipients were implanted with embryos from adult ewes of which 9 became pregnant, giving a 50% pregnancy rate. No statistical analysis was conducted due to the small number of observations.

Trial 3.

Maximising the number of transplanted embryos being born was one of the objectives of this investigation and factors in the availability of embryos and recipients led to a degree of confounding of the number of embryos transferred with estimated embryo quality.

Embryos from ewe lambs.

Data from 42 recipient ewes were used to evaluate the effects of embryo quality (good vs fair vs poor), number of embryos implanted (1 vs 2) and ovulation rate of the recipient (1 vs 2 CL) on their ability to develop pregnancy. Pregnancy rate was not significantly affected by any of the factors considered (Table 3.48). However, a consistent decrease in pregnancy rate was observed as microscopically-assessed embryo quality declined, and it was lower when 1 rather than 2 embryos were implanted.

Table 3.48 Effect of embryo quality, number of embryos implanted and ovulation rate of the recipient ewe on pregnancy rate following the transfer of embryos from ewe lambs (Trial 3).

Embryo quality[a]	Total number of ewes	Number of ewes pregnant	% of ewes pregnant	Sig.
Good	12	7	58	NS
Fair	12	5	42	
Poor	18	4	22	
Number of embryos implanted[b]				
1	26	8	31	NS
2	16	8	50	
Number of corpora lutea[c]				
1	25	10	40	NS
2	17	6	35	
Total	42	16	38	

[a] Chi^2 = 0.01, 2 df NS
[b] Chi^2 = 2.32, 1 df NS
[c] Chi^2 = 0.01, 1 df NS

Embryos from adult ewes.

Data from 34 recipient ewes were used to examine the effects of embryo quality (good vs fair vs poor), number of embryos implanted (1 vs 2) and ovulation rate of the recipient (1 vs 2 CL) on their ability to develop pregnancy.

There were no significant differences in pregnancy rate between recipients that were implanted with embryos of good, fair or poor qualities (50% vs 44% vs 20%), between recipients implanted with 1 or 2 embryos (25% vs 50%) or between recipients recording 1 or 2 corpora lutea (37% vs 60%) respectively at the time of transfer (Table 3.49).

Table 3.49 Effect of embryo quality, number of embryos implanted and ovulation rate of the recipient ewe on pregnancy rate following the transfer of embryos from adult ewes (Trial 3).

Embryo quality[a]	Total number of ewes	Number of ewes pregnant	% of ewes pregnant	Sig.
Good	20	10	50	NS
Fair	9	4	44	
Poor	5	1	20	
Number of embryos implanted[b]				
1	8	2	25	NS
2	26	13	50	
Number of corpora lutea[c]				
1	24	9	38	NS
2	10	6	60	
Total	34	15	44	

[a] Chi^2 = 1.02, 2 df NS
[b] Chi^2 = 1.76, 1 df NS
[c] Chi^2 = 1.29, 1 df NS

Effect of age of the donor.

The average responses for lambs and adults were 38 and 44%, respectively (Table 3.80), but the difference was not statistically significant. Similarly, survival of embryos from adult ewes and ewe lambs was not significantly different (35% vs 33%, respectively).

Table 3.50 Effect of age of the donor on pregnancy rate (Trial 3).

Age of the donor	Total number of ewes	Number of ewes pregnant	% of ewes pregnant
Ewe lambs	42	16	38
Adult ewes	34	15	44
Total	76	31	41

Chi^2 = 0.28, 1 df NS

Trial 5.

Embryos from ewe lambs.

Data from 24 recipient ewes were utilized to evaluate the effect of embryo quality (good vs fair) on their ability to develop pregnancy (Table 3.81). Five recipients implanted with poor quality embryos showing some degree of degeneration were not included in the analysis. Good quality embryos gave higher ($P<0.01$) pregnancy rates than fair quality embryos (55 vs 8%, respectively). None of the recipients implanted with poor quality embryos carried a pregnancy. The results should be taken with caution as a high proportion (73%) of good quality embryos were implanted into recipients having 2 corpora lutea.

Table 3.51 Effect of embryo quality on pregnancy rate (Trial 5).

Embryo quality	Total number of ewes	Number of ewes pregnant	% of ewes pregnant
Good	11	6	55
Fair	13	1	8
Poor	5	0	0
Total	29	7	24

Chi^2 good vs fair = 6.33, 1 df **

Embryos from adult ewes.

Ten recipient ewes were utilized. No statistical analysis was conducted on these data due to the small number of observations. Nine recipients received single embryos, 7 of them were of good quality, 1 was fair and the other one was of poor quality. One recipient received 2 fair quality embryos. At scanning only the recipient implanted with a single fair quality embryo was found not pregnant. The ewe in which 2 fair quality embryos had been implanted was found carrying twins. Overall 90% of the recipients were found pregnant.

Effect of age of the donor.

The effect of age of the donor on pregnancy rate was evaluated including data from 24 ewe lambs and 10 adult ewes (Table 3.82). Adults showed higher pregnancy rates than

lambs (90% vs 29%, respectively, P<0.01). When a further analysis was conducted including only good quality embryos for both ages, pregnancy rate was higher for adults than lambs (100% vs 54%, respectively;P<0.05).

Table 3.52 Effect of age of the donor on pregnancy rate (Trial 5).

Age of the donor	Total number of ewes	Number of ewes pregnant	% of ewes pregnant
Ewe lambs	24	7	29
Adult ewes	10	9	90
Total	34	16	47

Chi^2 = 9.42, 1 df **

Lambing data.

At lambing all lambs were weighed, their sex recorded and the gestation length calculated. Due to the small number of observations no statistical analyses were performed for any of the following comparisons.

Trial 1.

Only one ram lamb was born from ewe lambs after a gestation period of 148 days. It weighed 2.9 kg at birth, but died 3 days later. From the adults, eight lambs were born of which 5 were females and 3 were males. Their average body weight at birth was 3.36 kg, males being heavier than females (3.63 and 3.20 kg, respectively). The average gestation period was 149 days.

Trial 3.

Nineteen lambs were born from ewe lambs of which 12 were females and 7 were males. Their average birth weight was 3.83 kg (4.01 kg for males and 3.72 kg for females). Adult ewes generated 21 lambs of which 9 were females and 12 were males. Their average birth weight was 3.88 kg (3.84 kg for females and 3.94 kg for males). The overall average gestation length was 148 days.

Trial 5.

In this trial ewe lambs produced five lambs of which 2 were females and 3 were males. Two ewes that were pregnant at scanning did not lamb, but the time of the losses was not determined. The average lamb birth weight was 3.68 kg (females,3.52 kg; males 3.85 kg). Eight lambs were obtained from adult ewes (3 females and 5 males). One ewe aborted the foetus near the end of the pregnancy. Their average birth weight was 3.81 kg (3.96 kg for males and 3.70 kg for females). The average gestation period for all these pregnancies was 149 days.

3.3 Discussion.

Factors affecting the incidence and distribution of onset of oestrus.

General comments.

A total of 432 ewe lambs and 565 adult ewes were used in these trials. The overall sponge retention was 97 and 98% for ewe lambs and adult ewes. The incidence of heat for donors and recipients was 81 and 87%, respectively. These results compare favourably with reports from the literature for ewe lambs and mature ewes. Normally the incidence of oestrus following progestagen-gonadotrophin (PMSG or FSH) treatment has been shown to be around 90% or more (Quirke, 1979, 1981; Quirke et al., 1981; Chesne et al., 1987; Alwan et al., 1988; Dattena, 1989). The overall incidence of heat in the recipients (87%) used in these trials confirms the efficacy of the synchronization system used based on the criteria indicated by Lamond (1964). Although in the present investigation only progestagen (MAP) containing sponges were used, the availability of progesterone-containing CIDRs for oestrous synchronization in ewes (Welch et al., 1984) and in lambbs (McMillan, 1986; Chun, 1987) should also be recognized.

a) Effect of gonadotrophin treatment.

This discussion focuses on comparisons between donors and recipients. The group of donors included both ewe lambs and adult ewes which were treated with gonadotrophins. The distribution of onset of oestrus of sheep utilized as donors or recipients was different. This was consistently observed in trials 1, 2, 3 and 5. On the basis of these results it is concluded that the difference was due to the gonadotrophin stimulation in the donors. A similar effect has been reported in several studies (Wallace et al., 1954; Averill, 1958; Cumming and McDonald, 1967; Bradford et al., 1971) following PMSG administration given between days 10 to 14 of the oestrous cycle. In those studies an effect of PMSG was measured on the length of the oestrous cycle. Similarly, Cognie et al. (1970) reported a reduction in the time of onset of oestrus in animals treated with PMSG during (400 i.u.) or out of (800 i.u.) the breeding season. This is an important aspect to consider if a high degree of synchrony of oestrus between donors and recipients is to be achieved. In the present trials this was accomplished by removing the sponges 12-15 h earlier in recipients than in donors. A similar practice has been adopted even when recipient ewes were treated with 500-600 i.u. of PMSG at the time of sponge removal (Gatica et al., 1984; Chesne et al., 1987; Torres et al., 1987).

In another study (Quirke and Hanrahan, 1977), where donors were treated with PMSG at sponge removal, a closer synchronization of onset of oestrus was obtained by removing the sponges 4h earlier in recipients than in donors. In summary, results from our trials have shown that gonadotrophin-stimulated sheep (adults and lambs) will show heat on average 8-10 h earlier than non-stimulated sheep. In the application of embryo transfer programmes, therefore, sponges should ideally be removed 8-10 h before removal in recipients not treated with gonadotrophins. However, for practical convenience devices used for synchronizing oestrus in donors are commonly removed in the morning (between 8-10 a.m.) of the last day of the synchronization treatment, and in recipients the synchronizing devices are usually withdrawn late in the afternoon of the day before, which means that the time of device removal in recipients will be 12-15 h before that of the donors.

b) Effect of source of PMSG.

The effect of source of PMSG on the incidence of heat was examined in trials 2 and 4. Results from those trials did not show significant differences in the percentage of animals detected in heat between Consept45 and Folligon (Trial 2) or between Massey-PMSG, Pregnecol and Folligon (Trial 4). The results of these studies are in agreement with those reported by Robinson and Scaramuzzi (1986) in which three different sources of PMSG were used.

c) Effect of time of PMSG administration.

Administration of PMSG (Consept45 or Folligon) 2 or 4 days before sponge withdrawal did not affect the percentage of animals detected in heat. These results are in agreement with those reported by Roche (1968) in adult ewes injected with PMSG at the same times relative to sponge removal. However, Holst (1969) reported a lower incidence of heat when PMSG was administered 1 day before sponge removal than when it was given at the time of sponge withdrawal; the response being lower when the animals were treated during the non-breeding season.

d) Effect of group of treatment.

Due to practical limitations in collecting and transferring embryos, animals from trials 1, 3 and 5 were divided into 3 groups and each group treated in the same manner two weeks apart. Data from the recipient ewes were used to evaluate the effect of group of

treatment on the incidence of heat after sponge removal. Group of treatment affected significantly the percentage of animals showing heat in trials 3 and 5 only. Although the trials were conducted in different years, they were always carried out during the breeding season. Therefore a significant effect of group of treatment on the incidence of heat was not expected and did not occur in trial 1. The unexpected results of trial 3 may be due in part to an outbreak of facial eczema just before the sponges were withdrawn from the second group of animals. Animals treated in group 2 were not affected. The percentage of animals detected in heat apparently declined after onset of the eczema (-9% and -12% for groups 2 and 3, respectively). The differences observed between the groups in trial 5, may show the influence of unknown environmental factors on the percentage of animals detected in heat.

Group of treatment affected the distribution of onset of oestrus in a similar way to effects on the incidence of heat. A similar distribution of onset of oestrus was observed between groups of trial 1, but there were differences between the groups in trials 3 and 5. In trial 3, groups 1 and 2 exhibited a similar distribution of onset of oestrus but this was later by on average 9 hrs in group 3 and was associated with the occurrence of facial eczema. The difference in the distribution of onset of oestrus found in trial 5 is more difficult to explain. In this case no differences were observed between groups 1 and 2, but their distribution was different from that of sheep treated in group 3. On average sheep treated in group 3 were detected in heat 3h later than sheep treated in the other groups.

e) Effect of immunization against androstenedione.

Results from trial 4 showed that androstenedione immunization decreased the percentage of animals detected in heat, but no significant effect was observed on the time of onset of oestrus, although immunized sheep were detected in heat on average 6h later than non-immunized animals. A reduction in the percentage of animals showing heat associated with androstenedione immunization was observed by Scaramuzzi et al. (1987) in three different studies. However, a significant difference was observed only in a study conducted out of the breeding season in which the interval between booster immunization and sponge removal was 15 days. A delay in the time of onset of oestrus in androstenedione-immunized ewes has also been observed when PMSG (250-1000 i.u.) was given at sponge removal in a study conducted out of the breeding season

using Border Leicester x Merino ewes (Robinson and Scaramuzzi, 1986). A similar tendency was reported by Scaramuzzi et al. (1987) in Merino ewes treated with FSH-P given around the end of the synchronization treatment, although the difference was not significant. The last authors, in a second study conducted during the non-breeding season using Border Leicester x Merino ewes, reported opposite results. In that study the interval between booster immunization and sponge removal was 15 days, however, such an effect was not observed if the above interval was extended to 23 days. Overall, the results from the present study confirm the reports of Scaramuzzi et al. (1987). Thus, it seems that a decrease in the incidence of heat can be expected following androstenedione immunization in both adult ewes and ewe lambs.

Factors affecting the ovulatory responses.

a) Effect of dose-level of PMSG.

The effect of dose-level of PMSG (Consept45) on the ovulatory responses was examined in trial 1. Increasing the dose-level from 900 to 1200 i.u. did not increase ovulation rate, the number of large follicles or the total ovarian response (ovulation rate + number of large follicles). Overall the responses in this study were low and do not agree with reports from the literature where studies conducted on adult ewes have reported a significant increase in ovulation rate when the dose-level of PMSG was increased from 500 to 1000 i.u. (Robinson, 1951; Wallace, 1954), 600 to 1000 i.u. (Allison, 1975) and 600 to 1200 i.u. (Eastwood and McDonald, 1975). A similar tendency was also indicated when the dose of PMSG was increased from 750 to 1000 to 1250 i.u. in mature ewes treated during and out of the breeding season (Holst, 1969). Similarly, Rangel-Santos (1986) reported an increase in ovulation rate when PMSG was increased from 700 to 1050 i.u., but this was not significant. Although in those studies PMSG was given between days 11 to 14 of the normal oestrous cycle, studies in which 1200 i.u. of PMSG were administered around the end of the synchronization treatment (Jabbour and Evans, 1987; Jabbour et al., 1988) have also induced considerably higher ovulatory responses compared to those in the present work. Despite the fact that no strict comparisons can be made between results from the literature and those from the present trial, it appears that the difference in dose-level of PMSG used in our study (300 i.u.) was not big enough to give significantly different results. The levels of PMSG in this

trial were chosen on the basis that, in adult ewes, approximately 750 i.u. will be the minimum dose to induce multiple ovulations and a maximum of 1200 to 1500 i.u. has been recommended to avoid hyperstimulation, although 2000 i.u. has been regarded as the highest permissible dose (Gordon, 1983). Furthermore the results from the literature are from studies conducted on adult ewes and a wide variety of sources of PMSG was involved. Undoubtedly all these factors could contribute to the differences in the results.

Data from one of the present studies (Splitting; study 1) indicated a significant increase in ovulation rate of adult ewes when the dose-level of PMSG was increased from 700 to 1000 i.u., but no further improvement in ovulation rate was observed when the dose was increased up to 1300 i.u.. These findings, in general, agree with those reported in the literature for adult ewes.

b) Effect of time of PMSG administration.

Ovulation rate was not affected by the time of PMSG administration. The number of large follicles was higher when PMSG (Consept45) was given at sponge removal compared with 24h before sponge removal (Trial 1). In trial 2, it was not significantly different when PMSG (Consept45 or Folligon) was administered 2 or 4 days before sponge removal. The total ovarian response was higher for trial 1 ewe lambs injected at sponge removal. Holst (1969) reported a higher number of large follicles (1.43 vs 0.79, respectively) but no significant difference in ovulation rate (3.97 vs 4.05, respectively) or total ovarian response for animals treated with PMSG at sponge removal or 24h before. There are two studies where administration of PMSG given 2 or 4 days before sponge removal was examined. Cognie (1990) reported a higher ovulation rate in Merino ewes injected with 800 i.u. of PMSG 2 days before sponge removal than when it was injected at sponge removal. Roche (1968) compared the effect of administering PMSG (5 i.u./lb of body weight) at sponge removal and 2 or 4 days before sponge removal. The proportion of ewes showing multiple ovulations (≥2 CL) was higher following PMSG administration 2 or 4 days before sponge removal than when it was given at sponge removal. It appears that PMSG given at sponge removal is not going to improve ovulation rate but may increase the number of large follicles which is undesirable since large follicles produce high levels of oestrogens (Evans and Robinson, 1980; Armstrong et al., 1982) which have been associated with low levels of fertility in animals superovulated with high levels of PMSG (Booth et al., 1975; Evans and

Robinson, 1980; Bindon and Piper, 1982a). In agreement with results of Roche (1968) there were no significant differences in trial 2 when PMSG was given 2 or 4 days before sponge removal, but no animals were treated at sponge removal (Trial 2).

The present results with adult ewes (Splitting; study 1) indicated a higher ovulation rate from ewes treated with a raw preparation of PMSG (Massey-PMSG) 2 days before sponge removal compared to that in ewes which were given PMSG at sponge withdrawal. A similar response was recorded when PMSG was injected 2 or 4 days before sponge removal. Administering a "half dose" at either 2 or 4 days before sponge removal and the other half at sponge removal did not improve the response compared to that when PMSG was administered at sponge removal or 4 days before sponge removal. The results clearly demonstrate the importance of the time of PMSG administration in determining ovulation rate in PMSG-treated adult ewes. The alternative of splitting the dose-level of PMSG to improve the ovulation rate does not seem justified as the responses are not improved and additional handling of the animals is required.

c) Effect of source of PMSG.

There was no significant effect of the source of PMSG used on ovulation rate or the total ovarian response (Trials 2 and 4), but the number of large follicles was significantly affected by the source of PMSG in trial 4. No significant difference in the ovulatory responses were observed when Consept45 or Folligon were given 2 or 4 days before sponge removal (Trial 2). Ovulation rate and the total ovarian response were similar between ewe lambs treated with Massey-PMSG, Pregnecol or Folligon. However, the number of large follicles was lower when Pregnecol was used. Ewe lambs treated with Massey-PMSG tended to exhibit a high number of large follicles. This could be due to the lack of purity of the Massey preparation compared with Pregnecol. However, this would not explain the lack of difference when compared to Folligon. Although no significant differences in ovulation rate were observed between gonadotrophin-treated sheep, there was a tendency for lambs treated with Folligon to give a higher ovulation rate, particularly when it was administered 2 days before sponge removal. This was reflected in a similar tendency for the total ovarian response. Results from trial 4 showed the efficacy of Massey-PMSG as a raw source of PMSG inducing ovarian stimulation in a comparable way to more purified sources of PMSG.

Beehan and Sreenan (1977) with cows and Robinson and Scaramuzzi (1986) with mature ewes observed variable responses when different sources of PMSG were used. Ovulatory responses from ewe lambs in these trials were considerably lower than those reported in the literature following administration of the same or even lower levels of PMSG. This indicates that PMSG is not as effective in inducing ovarian activity in ewe lambs as in mature ewes. It may be that some sources of PMSG do have potency for inducing single ovulation but may not be potent for inducing superovulation. This suggests the need for examining the potency of each batch to induce multiple ovulations before the decision to use it in an embryo transfer programme is taken.

When the effect of source of PMSG (effect of mare) was estimated in adult ewes (Splitting; study 1), the results showed significant differences in ovulation rate induced by serum from different mares. One of the mares consistently recorded the lowest responses regardless of the dose-level used or the time of its administration. That mare was found to have the lowest concentration of PMSG/ml (40 i.u.), but this does not explain the difference in response as the volume of serum from each mare administered was adjusted to take account of the differences in concentration of PMSG. Different responses have also been reported using highly purified sources of PMSG in sheep and cattle. Thus, it appears that there are other unknown reasons why different sources of PMSG induce variable responses in addition to the likely effect of differences in the purification procedures of the gonadotrophin.

d) Effect of gonadotrophin treatment.

Ovulation rate and total ovarian response were higher when PMSG (Folligon) was used compared to FSH-P and Ovagen, which are purified pituitary sources of FSH. The number of large follicles was not significantly different between the three gonadotrophin preparations. These results do not agree with those from the literature where either no significant difference has been indicated when cattle were treated with PMSG or FSH-P (Crister et al., 1980) or a superior ovulation rate was achieved following FSH-P administration (Elsden et al., 1978; Armstrong et al., 1983; Monniaux et al., 1983; Almeida, 1987). In addition to the higher ovulation rate, sheep induced to superovulate with FSH-P have better sperm transport (Evans and Armstrong, 1984a) and fertilization rates following cervical insemination (Evans et al., 1984). Similarly, sheep (Torres et al., 1987), goats (Armstrong et al., 1983) and cattle (Elsden et al., 1978) treated with FSH-P

produced more transferable embryos than animals treated with PMSG. Analysis of these data led us to conclude that PMSG (Folligon) was the best hormone to induce superovulation in these prepubertal Romney sheep. The higher ovulation rate in PMSG-treated ewe lambs could be due to its high LH content and longer half-life (McIntosh et al., 1975) compared to the other gonadotrophins. A generally higher LH content in FSH-P preparations, although with a wide variation between batches (Murphy et al., 1984; Lindsell et al., 1986; Xu et al., 1988), compared to Ovagen (<0.2%; I.C.P., New Zealand) could explain the slight though not significant increase in ovulation rate and total ovarian response in ewe lambs treated with FSH-P compared with Ovagen.

e) Effect of FSH priming.

Priming with 2.5 ml of Ovagen on day 4 after sponge insertion followed by PMSG injection 2 days before sponge removal failed to increase ovulation rate compared to the administration of PMSG alone 2 days before sponge withdrawal (4.18 vs 2.05 CL, respectively). In fact the response was reduced significantly by the former treatment. Ewe lambs receiving Ovagen priming gave similar ovulation rates to those of animals treated with FSH-P (2.05 vs 1.78 CL, respectively), but was higher compared to the ovulation rate of ewe lambs receiving only Ovagen (2.05 vs 1.06 CL, respectively).

The results showed that priming with Ovagen on day 4 after sponge insertion had detrimental effects on the ovulatory response to PMSG given 6 days later when compared to the administration of PMSG alone. Studies conducted on mature ewes have given variable results in response to FSH-P priming early in the cycle. While Ware et al. (1988) reported similar ovulation rates between primed and non-primed ewes, Barry et al. (1988) found a higher ovulation rate in Merino ewes primed on day 2 following a previous synchronized oestrus. In cattle results have also been variable. A significant increase in ovulation rate and number of transferable embryos per cow was reported by Ware et al. (1988). In this case priming using 10 mg FSH-P was given on days 2 or 3 of the oestrous cycle. Hunton et al. (1989) also failed to show any significant effect of FSH priming early in the oestrous cycle on ovulation rate in superovulated cows using two different sources of FSH (FSH-P or Ovagen). However, Guilbault et al. (1988) reported a lower ovulation rate and embryo recovery in heifers receiving the same amount of FSH-P as priming on day 3. The diversity in the results achieved in both species highlights the need for more research in this area.

f) Effect of combining PMSG and FSH.

Combining Ovagen and PMSG (Folligon) in a single superovulatory treatment did not increase the ovulatory responses significantly compared to the use of PMSG plus GnRH (Trial 5). The lack of a significant effect was apparent not only when a low dose-level of Folligon (300 i.u.) was used, but even when the dose-level was increased up to 500 i.u.. Administration of GnRH tended to improve the ovulation rate further, but the differences were not significant. These results do not agree with reports in which mature Romney (McMillan and Hall, 1991) and Merino ewes (Ryan et al., 1984) gave higher ovulatory responses when a source of FSH was given in combination with PMSG (Folligon). McMillan and Hall although using the same gonadotrophins, gave higher levels of Folligon and Ovagen (100 i.u. and 2.6 ml more, respectively) than the present highest dose levels used. Moreover, Ovagen was given over 3 days instead of 4 days as in our case. Although there is little doubt that those factors could have been involved in the difference in response, even more important may be the fact that the present observations were on prepubertal sheep. Tervit et al. (1989) did not find significant differences in the number of ova recovered from ewe lambs (approximately 12 months old) compared to ewes when treated with 200 i.u. of PMSG plus 20 mg of FSH-P. The differences with the study reported by Ryan et al. are even more obscure, a different source of FSH (FSH-P), a higher dose-level of Folligon (800 and 1600 i.u.) and a different breed of sheep were used. On the basis of the present data the ovulatory responses from prepubertal Romney sheep to a combined gonadotrophin treatment (PMSG+Ovagen) appeared lower than those of mature ewes, but the reasons are unknown.

g) Effect of GnRH administration.

Administration of GnRH failed to improve the ovulatory responses in most cases. This was demonstrated in trial 3, in which ewe lambs were treated with GnRH (Fertagyl) following injections of Folligon, FSH-P, Ovagen or a combination of PMSG plus Ovagen, and again in trial 5 when animals were injected with Ovagen plus a low or a high dose-level of PMSG (Folligon). In the last study a significant increase in the total ovarian response was observed following GnRH administration. These observations do not support those reported by Nancarrow et al. (1984) in which two injections of GnRH (25μg) spaced 90 minutes apart and given around the time of insemination significantly increased ovulation rate, fertilization rate and the number of embryos per ewe (from 1.4

to 4.9). A similar increase in embryo yield was observed by Walker and Warnes (unpublished study cited by Walker et al., 1986) and Walker et al. (1989). In the latter study the best results were achieved when GnRH was administered at 24 and 36h after sponge removal in sheep treated with PMSG and FSH-P respectively. Administration of GnRH has been shown to synchronize the time of ovulation in sheep superovulated with FSH-P or PMSG by reducing the interval from the first to the last ovulation as well as the time between sponge removal and the median time of ovulation (Walker et al., 1986). In agreement with these studies, Jabbour and Evans (1986) reported higher ovulation rate and total follicular development in Merino ewes treated with PMSG given 2 days before sponge removal and injected with GnRH 24h following sponge withdrawal. The number of large follicles (>5mm) was lower in GnRH-treated ewes. Thus, it appears that an improvement in embryo yield can be expected due to both an increased ovulation rate and an enhanced fertilization rate resulting from an improvement in the synchrony of the time of ovulation. However, the effect of administration of GnRH has not always been positive. Quirke and Hanrahan (1975) did not find any improvement in ovulation rate or the total ovarian response in ewes injected with 50 μg GnRH 24h after sponge removal. Walker et al. (1986) reported a decrease in ovulation rate in GnRH-treated animals. The reasons for the reduction were unknown. In the present studies GnRH was always given after detection of oestrus or at 60h following sponge removal if oestrus had not been detected by that time. In this way it was expected that GnRH would increase ovulation rate by inducing the rupture of large follicles. On this basis a reduction in ovulation rate following GnRH was unexpected. It appears likely that the lower ovulation rate was a chance effect and not due to a negative effect of GnRH. Evidence to support this suggestion was obtained in trial 3 in which the highest ovulation rate was recorded when PMSG plus GnRH were used and a similar tendency was also observed in ewe lambs treated with PMSG plus Ovagen. These data collectively suggest that an increase in ovulation rate following GnRH administration as indicated here is likely provided that there are preovulatory follicles at the time of its administration.

h) Effect of genotype of the donor.

There is no clear indication that ovulatory responses might differ among genotypes. While control ewe lambs recorded higher number of large follicles in trial 1, fleece weight-selected ewe lambs recorded higher total ovarian response in trial 3. No significant differences in the ovulatory responses were observed in trial 5. A previous

study conducted on the same flock (McClelland, 1990) reported a higher percentage of fleece weight-selected ewe lambs showing heat during their first autumn than the controls. They also tended to show more oestrous cycles and display oestrus over a longer period than control ewe lambs. Although these data suggest differences in reproductive activity between the 2 Romney genotypes, one cannot draw any definite conclusions as to ovulatory responses following hormonal stimulation.

i) Effect of age of the donor.

There was a significant effect of age of the donor on the ovulatory responses (Trials 1, 2, 3 and 5). Mature ewes recorded higher ovulation rates than ewe lambs in trials 1, 2 and 5 and a similar tendency was observed in trial 3, although the difference did not reach significance. The number of large follicles was higher for adults than for lambs in trial 4 and so was the total ovarian response in trials 3 and 4. These results are consistent with the observation of Robinson (1951) who noted a considerably lower ovulation rate from ewe lambs than adults treated in a similar way. Low ovulation rate and embryo yield were reported by Armstrong and Evans (1983) following the induction of superovulation using FSH-P in sheep less than one year of age. A study that failed to find a significant difference in ovulation rate between adult ewes and ewe lambs receiving similar treatment was reported by Bradford et al. (1971) working with Finnish Landrace sheep. The average ovulation rates of ewe lambs in the last two studies were 13.4 and 10.4 respectively, which are considerably higher than the ovulation rate recorded in the present studies. Tervit et al. (1989) who superovulated Coopworth ewe lambs and adult ewes did not find a significant difference in the number of ova recovered, however, because of a lower fertilization rate in ewe lambs they gave less embryos than the adults. The use of older ewe lambs of different breeds to the Romneys now reported could in part explain the difference in the results (e.g. Tervit et al. treated the lambs in September at approximately 1 year of age). Ewe lambs in trial 2 were approximately 8-9 months old and 90% of the group not treated with gonadotrophins were detected in heat within 72h of sponge removal and were found to have ovulated when laparoscoped 7 days after the onset of oestrus.

j) Effect of group of treatment.

The effect of group of treatment on the ovulatory responses was examined in trials 3 and 5. Ovulation rate was significantly affected by group of treatment in both trials, but the total ovarian response was significantly affected by group only in trial 3. Ovulation rate and the total ovarian response in trial 3 were higher in animals treated in group 1 than in group 2 (Groups 2 and 3). As indicated previously, there was a severe outbreak of facial eczema a few days before sponge removal in group 2 which affected quite drastically the health status of the mob. This is probably the reason for the overall reduction in the ovulatory responses in that group. Although no attempt was made to specifically evaluate the influence of such an unexpected factor, it was clear by looking at the condition of the animals that they were not healthy. The higher ovulation rate in group 1 of trial 5 was unexpected, but no explanation can be offered. Results from these trials highlight how much environmental factors (known and unknown) can affect the responses to the treatments imposed.

k) Effect of immunization against androstenedione.

Ovulation rate following PMSG (Folligon) administration was higher in androstenedione-immunized ewe lambs compared to non-immunized animals. The number of large follicles and the total ovarian response were similar between immunized and non-immunized ewe lambs. In agreement with these results Robinson and Scaramuzzi (1986) reported a higher ovulation rate in androstenedione-immunized mature ewes following PMSG administration.

l) Effect of the Booroola gene.

The study was not designed to examine specific differences in the ovulatory responses between ewe lambs with or without the Booroola high fecundity gene, but to get information on the possibility of implementing a juvenile MOET scheme using a mob of animals with natural high fecundity. Overall, the ovulatory responses from this trial were considerably higher than those observed in Romney ewe lambs treated with the same (Trials 3 and 5) or similar (Trials 1 and 2) superovulatory treatments. Bearing in mind the limitations involved when comparisons are made between trials conducted in different years, results of this trial showed the potential of using this particular breed to implement a juvenile MOET scheme.

Factors affecting egg recovery, fertilization rate and percentage of embryos transferable.

a) Effect of dose of PMSG.

There was no significant difference in the recovery rate of eggs, fertilization rate or the percentage of transferable embryos between ewe lambs treated with 900 or 1200 i.u. of PMSG (Trial 1). Overall 55% of the eggs were recovered. The rate of recovery from flushing ewe lambs, although it compared favourably with the 51% recovery rate reported by Quirke and Hanrahan (1977) in which ewe lambs were treated with PMSG at sponge removal, was considerably lower than the 82% reported by McMillan and McDonald (1985) following a natural oestrus. It appears that embryo recovery was affected by oestrous synchronization and/or PMSG treatment. In the present study, the lambs were flushed late compared to that in the other 2 studies, thus a lower recover rate could be expected, but late flushing would not account for all the difference. Furthermore in both other trials the lambs were flushed at similar times yet the recovery rates were different.

The average fertilization rate in this trial (50%) was considerably lower than the 85% reported by Quirke and Hanrahan (1977) and the 91% reported by McMillan and McDonald (1985). The fact that only 500 i.u. of PMSG were used in the trial conducted by Quirke and Hanrahan could partly explain the difference in the results. Low fertilization rates have commonly been reported in animals superovulated with high levels of PMSG (Booth et al., 1975; Evans and Robinson, 1980). Matings conducted following a natural oestrus in the latter trial could explain the difference with the present trial.

The percentage of transferable embryos in this trial (50%) was low compared to the 75% reported by McMillan and McDonald (1985). Such differences could have been due to the gonadotrophin stimulation in the present study and to the fact that embryos were collected on day 5 to 6. No comparable data were reported by Quirke and Hanrahan (1977).

b) Effect of time of PMSG administration.

There was no significant difference in recovery rate, fertilization rate or the percentage of transferable embryos between lambs treated with PMSG at sponge removal or one

day before sponge withdrawal (Trial 1) and between those in which PMSG was given 2 or 4 days before sponge removal (Trial 2). The overall recovery rate, fertilization rate and percentage of transferable embryos for trials 1 and 2 were 55% vs 55%, 50% vs 54% and 50% vs 60%, respectively. The average recovery rates from these trials compare favourably with the 51% recovery rate reported by Quirke and Hanrahan (1977). However, those researchers reported 85% fertilization rate and more than 94% of the ova were at the appropiate stage of development.

c) Effect of source of PMSG.

There was no significant effect of the source of PMSG used on recovery rate, fertilization rate or the percentage of transferable embryos. This was shown first in trial 2, in which ewe lambs were treated with Consept45 or Folligon, and it was confirmed in trial 4, in which Massey-PMSG, Pregnecol or Folligon were used. No evidence to favour any gonadotrophin source can be found in the egg recovery, fertilization rate and the percentage of transferable embryos data from these Romney ewe lambs.

d) Effect of FSH vs PMSG.

The effect of gonadotrophin treatment on recovery rate, fertilization rate and the percentage of transferable embryos was examined in trial 3. Recovery rate and fertilization rate were not significantly different between lambs treated with PMSG, FSH-P or Ovagen (58, 42, 32 and 78, 62 and 100%, respectively). Overall 47% of the eggs were recovered and 74% of them were fertilized. The percentage of transferable embryos was higher in lambs treated with Ovagen compared to those treated with PMSG or FSH-P (100% vs 51 and 20%, respectively), but there were very few embryos from Ovagen-treated lambs. The higher percentage of transferable embryos in lambs treated with Ovagen agrees with observations from other studies in sheep (Armstrong and Evans, 1984a) and cattle (Chupin et al., 1984; Murphy et al., 1984; Donaldson and Ward, 1985) in which FSH preparations containing low amounts of LH produced better quality embryos than FSH preparations with high LH contamination.

e) Effect of FSH priming.

FSH priming (2.5 ml of Ovagen) on day 4 after sponge insertion followed by PMSG injection 2 days before sponge removal did not significantly affect the rate of embryo recovery, fertilization rate or the percentage of transferable embryos compared to the

use of PMSG alone. However, the percentage of transferable embryos was lower when compared to the response of ewe lambs treated with Ovagen alone. Ware et al. (1988) also found no significant effect of FSH priming on fertilization rate in cattle. However, Guilbault et al. (1988) reported a significant decrease in fertilization rate in FSH-primed compared with non-primed cows.

f) Effect of combining PMSG and Ovagen.

Combining PMSG and Ovagen in a single superovulatory treatment did not significantly increase the recovery rate, fertilization rate or the percentage of transferable embryos. Similar results were reported by Ryan et al. (1984) when mature ewes were superovulated with a combination of PMSG and FSH-P, although lower recovery rates were recorded when 1600 i.u. of PMSG were administered together with FHS-P. This was associated with more large persistent follicles which produce high levels of oestrogens (Quirke and Hanrahan, 1975; Du Mesnil Du Buisson et al., 1977). It has been suggested that this increases the rate of transport of ova through the oviducts, thus decreasing the recovery rates (Quirke and Hanrahan, 1975). The data therefore suggest that PMSG and FSH can be administered as a combined treatment without any detrimental effect on the variables considered, provided that the dose-level of PMSG will not induce the development of a high number of large persistent follicles.

g) Effect of GnRH administration.

Administration of GnRH within 6h of onset of oestrus did not affect significantly the recovery rate, fertilization rate or the percentage of transferable embryos (Trial 3). Nancarrow et al. (1984) failed to find any significant increase in recovery rate and fertilization rate in mature ewes superovulated with PMSG and injected with GnRH 24h after sponge removal. However, the number of transferable embryos was significantly increased when GnRH was used, due to a higher ovulation rate and a tendency to increase fertilization rate. In a series of studies conducted by Walker et al. (1989) the effect of GnRH administration on recovery rate was variable but there was a consistent significant increase in fertilization rate when GnRH was used in superovulated sheep that were intrauterine inseminated or naturally mated. A lower recovery rate in GnRH-treated sheep was reported by Quirke and Hanrahan (1975), but fertilization rate was not affected. In that study 2000 i.u. of PMSG were given at sponge removal inducing a significant increase in the total ovarian response, mainly due to an increase in the

number of large follicles. A high output of oestrogens from these follicles could have increased the rate of transport of ova through the oviducts, so decreasing the recovery rate. Taken together, these data suggest that although recovery rate seems not to be affected by GnRH administration, fertilization rate could be increased in naturally mated and intrauterine inseminated animals. The time of GnRH injection can affect the responses particularly when fixed-time intrauterine inseminations are performed. In the present studies GnRH was injected following detection of heat in most of the animals to ensure a surge of LH and ovulation. The lack of effect of GnRH, particularly in fertilization rate, may have been due to the low ovarian responses achieved.

h) Effect of age of the donor.

The effect of age of the donor on recovery rate, fertilization rate and the percentage of transferable embryos was examined in trials 1, 3 and 5. Higher recovery rates in adults than in lambs were found in trials 1 and 3 (91% vs 58% and 72% vs 42%, respectively) but there was no marked difference in trial 5. The higher recovery rates in adults during trials 1 and 3 are difficult to explain since the flushing procedures, the amount of media used and the time when the animals were flushed were kept constant for adults and lambs. The possibility of an effect of the size of the uterine horns can be discarded because of the small reproductive tracts of the lambs. Although ease of flushing was not objectively evaluated in this study, ewe lambs seemed easier and quicker to flush compared to adult ewes. There is the possibility that synchronization and superovulation affected egg transport more drastically in lambs than in adults and, as a consequence, their recovery rates were lower. Similar recovery rates between adults and lambs treated with PMSG were reported by Quirke and Hanrahan (1977). Their overall responses were 45 and 51%, respectively; which are comparable to the present results in trial 5 and in which no significant difference between lambs and adults was found. Nevertheless, the recovery rate from adult ewes was considerably lower compared to the rate of recovery from adults in trials 1 and 3. Tervit et al. (1989) also failed to show any significant difference in the number of ova recovered between ewe lambs (approximately 12 months old) and adult ewes treated with a combination of PMSG plus FSH-P. In another study, McMillan and McDonald (1985) reported a similar recovery rate for adults and lambs (80% vs 82%, respectively). In that study, however, recoveries were attempted by flushing the oviducts on day 3 after detection of oestrus, and a general decrease in the rate of recovery has been reported as the interval from oestrus to collection is

increased. This may result from the death of genetically nonviable embryos, the increased difficulty in removing the embryo from a relatively voluminous uterus or premature luteal regression and expulsion of the embryo (Bondurant, 1986). McMillan and McDonald flushed the animals following a natural oestrus and this may be involved in the different results from those reported here.

Fertilization rate was higher in adults than in lambs in trial 1 (82% vs 50%, respectively), but was similar between ages in trials 3 and 5 (73% vs 74% and 64% vs 65%, respectively). The differences observed in trial 1 could have been due to difficulty of the rams mating ewe lambs. Although intensity of oestrous behaviour was not recorded, it was noticed that ewe lambs did not show oestrous behaviour as clearly as the adult ewes and they also did not show as much interest for the rams as the mature ewes. Furthermore, on the basis of the tupping marks, it appeared that they had been mated fewer times than the adults. The lower fertilization rate observed in the lambs of this study is in agreement with the report by Tervit et al. (1989). Those researchers reported a lower fertilization rate in lambs than in adults following intrauterine insemination (43% vs 100%, respectively). Consequently, lambs gave fewer embryos than adults (1.6 vs 4.1 respectively). However, Quirke and Hanrahan (1977) found similar fertilization rates in adults and lambs (78% vs 85%, respectively) that had been synchronized and treated with PMSG. No significant difference in fertilization rate between adults and lambs was reported by McMillan and McDonald (1985), but in their study, sheep were mated following a natural heat and PMSG was not given. Major differences in techniques between experiments make it difficult to compare results.

There was a considerable increase in fertilization rate in trials 3 and 5 compared to trials 1 and 2, probably due to the use of intrauterine insemination in groups 2 and 3 of trial 3 and during trial 5. The use of this technique has consistently produced higher fertilization rates compared to the use of natural mating in superovulated mature ewes (Killeen and Moore, 1971; Trounson and Moore, 1974a; Nancarrow et al., 1984; Walker et al., 1989). Results of these trials showed the possibility of achieving similar fertilization rates in synchronized, gonadotrophin-treated lambs and adults. However, the present results are considerably lower than those from the literature in which fertilization rates around 90% have been commonly achieved (Killeen and Moore, 1971; Trounson and Moore, 1974a; Boland and Gordon, 1978; Dattena, 1989). Limited experience in the

use of the technique of intrauterine insemination may well be a factor, but further research is needed to identify the reasons for the low fertilization rate and to identify key factors in improving it.

The percentage of transferable embryos was similar between adult ewes and ewe lambs in trials 1 and 5 (53% vs 50% and 60% vs 48%, respectively) but it was higher in adults than in lambs in trial 3 (73% vs 49%, respectively). The fact that the only time when the percentage of transferable embryos was different between lambs and adults was in trial 3, despite the fact that no difference was found in fertilization rate, is interesting but difficult to explain. It might suggest that the quality of the embryos from ewe lambs was more drastically affected during the outbreak of facial eczema than that of embryos from adult ewes.

In agreement with results from trials 1 and 5, no significant differences in the percentage of transferable embryos were reported in the studies by Quirke and Hanrahan (1977) and McMillan and McDonald (1985). These data collectively suggest that once the eggs have been fertilized, regardless of their source (adults or lambs), a similar percentage of them will be suitable for embryo transfer.

Overall, the results from the present trials suggest that low recovery rates of embryos can be expected when prepubertal ewe lambs are flushed on day 5 to 6 after detection of oestrus. Attempts to increase the recovery rate by flushing at an earlier time when the eggs are still in the oviducts might be suggested but this would increase the likelihood of adhesion between the ovary and Fallopian tube and consequent reduction in successful repeated embryo recoveries.

Low fertilization rates are likely to occur if prepubertal lambs are naturally mated. This can be partly overcome by the use of intrauterine insemination. The percentages of transferable embryos appear to be very similar between adults and lambs. However, if fertilization rates are higher in adults, then embryo yield will also be higher for them compared to the yield of embryos from lambs.

i) Effect of immunization against Androstenedione.

Immunization against androstenedione did not significantly affect egg recovery,

fertilization rate or the percentage of transferable embryos (Trial 4). The average responses for immunized and non-immunized ewe lambs were 32% vs 33%, 61% vs 69% and 50% vs 33%, respectively. A significant reduction in recovery rate from androstenedione-immunized sheep has been reported in sheep not treated with exogenous gonadotrophins (Boland et al., 1986; Murray et al., 1985) as well as in sheep treated with FHS-P (Scaramuzzi et al., 1987). There is evidence suggesting that the decrease in recovery rate is associated with the magnitude of the androstenedione-antibody response, since the effect on recovery rate has been shown to diminish as the antibody titre declines (Boland et al., 1986). Lower fertilization rates in immunized animals were also reported. Thus Scaramuzzi et al. reported a lower percentage of viable embryos in androstenedione-immunized sheep compared to non-immunized sheep. Furthermore, Murray et al. (1985) observed retarded embryo development at days 13 and 25 to 29 of gestation in immunized sheep. The apparent negative effect of immunization against androstenedione has not always been found. A study conducted by Cognie (1987) (unpublished study cited by Scaramuzzi et al., 1987), in which crossbred Lacaune immunized ewes were treated with FSH-P, gave a higher number of viable embryos compared to non-immunized ewes. Difference in the age of the animals, the gonadotrophin source and breed are only some of the factors that are involved in the results, thus making the interpretation difficult. The potential use of immunization against androstenedione prior to the superovulation treatment in embryo transfer programmes with young sheep thus appears limited at the moment.

Factors affecting the survival of transplanted embryos.

a) Effect of quality of the embryo.

There was a consistent decrease in pregnancy rate as the quality of the embryos declined. This tendency was observed following the transfer of embryos obtained from adult ewes and ewe lambs (Trial 3) and reached significance when embryos obtained from ewe lambs were transferred in trial 5 (54% vs 8% for embryos judged to be of good and fair quality, respectively). However, the trial 5 results are confounded as 73% of the good quality embryos were implanted into recipients showing 2 corpora lutea. The present results agree with reports from the literature in which highest pregnancy rates were recorded after the transfer of good quality embryos, rather than embryos of only

medium or fair quality. The transfer of poor quality embryos resulted in very low pregnancy rates.

b) Effect of number of embryos transferred.

Results from trial 3 indicated a consistent tendency to increase pregnancy rate when the number of embryos transferred was increased from 1 to 2, although the difference was not significant. The effect was observed following the transfer of embryos from both ewe lambs and adult ewes (31% vs 50% and 25% vs 50%, respectively). Despite the major differences in percentage terms, with the small number of observations involved statistical significance was not reached. These results agree with a report by Armstrong and Evans (1983) in which it was stated that differences in pregnancy rate following the transfer of 1 or 2 embryos are usually not significant. Pregnancy rates around 75% have been reported following the transfer of 1 embryo (Moore, 1968) or 2 embryos per recipient (Torres et al., 1987), but pregnancy rate was increased to 90% (Larsen, 1971) when 3 embryos were transferred per recipient. Similarly, Moore and Shelton (1962) reported a slight increase in pregnancy rate following the implantation of 2 or 3 embryos per recipient rather than a single one. A decrease in embryo survival can be expected as the number of embryos transferred increases (Larsen, 1971). Accordingly evidence from several studies (Moore et al., 1960; Moore, 1968; Cumming and McDonald, 1970) demonstrated an increase in prenatal mortality as the number of embryos transferred was increased.

c) Effect of age of the donor.

Mature ewes tended to give higher pregnancy rates than ewe lambs in trials 1 and 3 (50% vs'20% and 44% vs 38%, respectively) and the effect of age was significant in trial 5 (89% vs 29%, respectively). Similarly when pregnancy rates were compared for animals given only good quality embryos, it was higher in mature ewes than in ewe lambs. These results confirm findings from previous reports in which a low capacity to develop in vitro (Wright et al., 1976), as well as in vivo (Quirke and Hanrahan, 1979; McMillan and McDonald, 1985), was found in embryos obtained from ewe lambs. The advantage in favour of embryos from adults was greater when the donors were treated with a progestagen and PMSG (40% more; Quirke and Hanrahan, 1979) than when the transfers were conducted following a natural oestrus (27% more; McMillan and McDonald, 1985). Results of these trials therefore indicate that embryos from ewe lambs

are less viable than those from adult ewes. The already low viability may be further reduced when lambs are treated with PMSG. Evidence to support this observation has been provided in sheep (Moor et al., 1985) and goats (Kumar et al., 1989) treated with gonadotrophins. A common finding from those studies was an early egg maturation consisting of a premature condensation of chromatin, an event that normally occurs following the preovulatory LH surge. The effect was more marked when PMSG rather than FSH was used. Clearly more research is required to identify the reasons for the low viability of embryos generated from immature sheep.

d) Effect of ovulation rate of the recipient.

There was no significant difference in pregnancy rate between recipient ewes with 1 or 2 ovulations at the time of transfer (Trial 3). Several other studies (Moore et al., 1960; Cumming, 1965; Cumming and McDonald, 1970; Bradford et al., 1971) have also failed to demonstrate a relationship between the number of corpora lutea in the recipient ewes and the survival of the transferred ova. Nevertheless there is at least one study (Averill and Rowson, 1958) in which a consistent, but non-significant increase in pregnancy rate was observed when ovulation rate in the recipient was increased from 1 to 3. The lack of significance in the present embryo transfer trial as well as in those reported in the literature might well be due to the modest number of observations involved and the power of statistical tests being so low that only large differences would have been detected. This seems likely as from the results of a New Zealand study (Kelly and Johnston, 1983) involving several thousands of naturally mated ewes there was a significantly higher conception rate in ewes with 2 CL compared to those having only 1 CL.

CHAPTER IV:

EMBRYO SPLITTING

CHAPTER IV:

EMBRYO SPLITTING

4.1 Materials and methods.

4.1.1 General management.

The same general management procedures described in Chapter 3 were also applied in these studies.

4.1.2 Experimental procedures.

Holding pipette.

This was manufactured from a piece of 75 mm length capillary tube (Propper Manufacturing Co Inc, USA) which was hand-pulled and cut in the middle into 2 pipettes. One of these was mounted in the microforge and observed through the attached dissecting microscope. With the help of an eyepiece reticle the point at which the pipette diameter was about 120 μ was determined and the filament wire brought touching that point. The device was switched on and the intensity of the heat increased slowly until the filament wire turned orange and the glass started to melt. At this point the power was switched off and as the filament cooled, it contracted to its original position causing the glass to break at the point of contact. The excess of melted glass was removed from the filament and the pipette was repositioned facing the filament evenly. The power was turned on until the filament appeared clearly orange. At this time the pipette was brought close to the filament slowly and evenly until the edges melted, leaving a narrow lumen. The pipette was held with small forceps and bent using a gas microburner flame produced by mounting an 18 gauge blunt needle on the microburner. The pipette was bent twice at an angle slightly greater than 90° each time. The first bend was made as close as possible to the finishing end and the second bend approximately 1 cm from the first.

Suction pipette.

The same procedure as above was used to make the suction pipette, but in this case the tip was not fire polished and the diameter was about 70 μ.

Microsurgical blade.

Several types of blades were used during these studies. The blades used in studies 1 and 2 were made as follows: A capillary tube was hand-pulled as before but leaving the narrow end greater. A common razor blade was cut with sharp scissors to produce a small piece along the cutting edge, approximately 2 mm wide. A drop of glue containing 85% cyanoacrylic acid esters was applied to the narrow edge of the pipette to seal its lumen and also to allow the microblade to be attached. Once set with the microblade being parallel to the pipette more glue was added to the joining point to increase the strength.

The microblades used in studies 3, 4 and 5 were also made from a common razor blade but the cut was made at an angle of approximately 30° in relation to the sharp edge of the blade. In studies 3 and 4 the blade was attached to a piece of plastic tube by melting a small portion of it from one of the ends and inserting the blade when the plastic started melting. A hand made microblade holder was used in study 5. The blade used in study 6 was obtained from A.B. Technology Pty Ltd, Australia.

Microsurgery.

All microsurgery and transfer of demi-embryos was done at room temperature. Embryo splitting was performed using a Leitz micromanipulator, an inverted or stereoscopic microscope, a microsurgical blade and a holding and suction pipette.

Flushing media.

Embryos were flushed using modified Dulbecco's Phosphate Buffered Saline (PBS) enriched with glucose (1 g/l) and sodium pyruvate (0.036 g/l) plus 10% sheep serum (enriched PBS) in studies 1, 3 and 6. On the other hand only modified PBS (plain PBS) plus 5% sheep serum was used for embryo flushing in studies 2, 4 and 5. After searching, the embryos were evaluated and those selected for splitting were kept separate.

Splitting media.

The media used while splitting was plain PBS without any glucose, sodium pyruvate or sheep serum added. Just before splitting, the embryos were placed for approximately 30 seconds in a small petri dish containing freshly prepared flushing media and 20%

sheep serum. Then they were transferred to a drop of splitting media previously placed in a petri dish.

The embryo was first located using low magnification which was then changed to a higher magnification before splitting was attempted. Micromanipulation of the embryos was always necessary, to find the most appropriate position to make the cut.

Preparation of micromanipulators.

All instruments were cleaned and rinsed with distilled water. Glass instruments (holding and suction pipettes) were sterilized using dry-heat or by washing them with distilled or highly purified boiling water. The blade was cleaned by soaking in distilled or highly purified water and sterilized in absolute alcohol.

Embryo splitting procedures.

Two embryo splitting techniques were used throughout these studies.

Technique 1: (Studies 1 and 2).

In this technique the holding pipette was placed on the left unit of the micromanipulator and the suction pipette and the blade on the right unit. The working parts of the microtools were lowered into the media and positioned with their axes parallel to the bottom of the dish and with their tips seen in the microscopic field. Then the embryo was held in position with the help of a holding pipette and it was cut through with a forward smooth movement. Sometimes the blade had to be repeatedly pushed through the embryonic tissue to obtain a complete cut. Initial attempts were made to transfer one of the demi-embryos into an evacuated zona pellucida but due to lack of experience in the technique the idea was soon abandoned. Thus 2 demi-embryos were left inside the zona pellucida, or one demi-embryo inside and another one outside the zona. Ocasionally both demi-embryos were left outside the zona pellucida due to its destruction during manipulation.

Technique 2: (Studies 3, 4, 5 and 6).

For this method the microblade was mounted on the left unit of the micromanipulator. The microblade was in position when its horizontal plane was almost parallel to the horizontal plane of the petri dish and its tip was touching the bottom of the dish, leaving

a mark of approximately 100 μ from its tip backwards. The blade was manoeuvred above the embryo until the best cutting position was achieved. With a vertical and smooth downward movement against the bottom of the plastic petri dish the embryonic tissue was divided. Most of the embryos that were divided using this technique were expanded and hatched blastocysts.

All demi-embryos were transferred to small petri dishes containing fresh splitting media until transplantation. In studies 1, 3 and 6 the time from flushing to splitting was less than 90 minutes but it was extended up to 180 minutes in studies 2, 4 and 5.

Transfer of bisected embryos.

Bisected embryos were transferred within 15 minutes of microsurgery in studies 1, 3 and 6 and within 30 minutes in studies 2, 4 and 5. Transfers were by mid-ventral laparotomy using standard surgical procedures, exposing the whole reproductive tract in studies 1, 2, 3, 4 and 5. Only a small part of the uterine horn was exposed during embryo transplantation in study 6. Oestrus in recipient ewes throughout the trials was within ± 24h of oestrus of the donors. Approximately 50 days of progesterone supplementation through the insertion of two Controlled Internal Drug Release devices (CIDR-G: AHI Plastic Moulding Co, New Zealand) was given to recipients implanted in study 6. The first device was inserted at the time of the transplantation and replaced by the second device on day 20 (13-14 days after the transfer) and left in place until the day of scanning when it was withdrawn. Recipient ewes in which embryos were transplanted were scanned by ultrasound for diagnosis of pregnancy at approximately 60 days of gestation in studies 1, 3, 4, 5 and 6.

Study 1.

The study was conducted during August-September 1988 on a secondary quarantine farm of LambXL at Cheltenham, Manawatu. A group of mixed-age commercial Romney ewes was used to generate the embryos. Superovulation was induced by administering one of the following gonadotrophins: a) FSH-P (24mg) given as 8 injections (3 mg each) twice daily every 12h, b) Folligon (1300 i.u.) or c) Massey-PMSG (1000 or 1300 i.u.) given at one of the following times in relation to sponge removal: at removal; two days before; four days before; half dose 2 days before and the other half at sponge removal; or half dose 4 days before and the other half at sponge removal. The distribution of

animals treated with Massey-PMSG to the different experimental groups is shown in Table 4.1. Only 5 ewes of those treated with 1000 or 1300 i.u. of Massey-PMSG were used as donors. The animals were bred by intrauterine A.I. with fresh semen from Danish Texel and Gotland Pelt rams 6-14h after oestrous detection. Late morulae and early blastocysts were collected nonsurgically (Dattena, 1989) from superovulated donors 5-6 days after onset of oestrus. On the basis of their morphological appearance, good and excellent quality embryos were selected for splitting. Recipient ewes were injected with Folligon (750 i.u.) two days before sponge removal or Massey-PMSG (700, 1000 or 1300 i.u.) as indicated for donors to ensure occurrence of heat and ovulation.

Study 2.

The study was carried out at the S.B.C.R.U. during April-May of 1989. A group of mixed-age commercial Romney ewes was used to generate the embryos. They were superovulated by injecting 1200 i.u. of Folligon or Massey-PMSG 2 days before sponge removal. GnRH (100 μg) was given i.v. within 3h of onset of oestrus to all the donors. The animals were bred by intrauterine A.I. with fresh semen from 2 rams from the F.W. flock 6-14h following heat detection. Late morulae and early blastocysts were collected surgically from superovulated donors 5-6 days after onset of oestrus. It was intended to select only good quality embryos for splitting but, since the numbers were low, some embryos that were not ideal for splitting were also included. Like the donors, recipient ewes received 1200 i.u. of PMSG (Massey-PMSG) to ensure heat and ovulation.

Study 3.

The trial was conducted during November 1989 on a secondary quarantine farm of LambXL'at Kiwitea, Manawatu. Finnish Texel, Gotland Pelt and Oxford Down ewes were used to generate the embryos. Superovulation was induced using FSH-P and ewes inseminated as in study 1. Early and expanded blastocysts were collected nonsurgically from superovulated donors 6-7 days after the onset of oestrus. Only good or excellent quality embryos were used. Folligon (500 i.u.) was injected at sponge removal in recipient ewes to ensure heat and ovulation.

Table 3.9 Distribution of animals to their respective treatments and groups (Splitting; Trial 1).

	TIME OF PMSG INJECTION					
DOSE OF PMSG	AT SPONGE REMOVAL	2 DAYS BEFORE SPONGE REMOVAL	4 DAYS BEFORE SPONGE REMOVAL	HALF 2 DAYS BEFORE, HALF AT SPONGE REMOVAL	HALF 4 DAYS BEFORE, HALF AT SPONGE REMOVAL	TOTAL
700 i.u.	10	10	10	10	10	50
1000 i.u.	10	10	10	10	10	50
1300 i.u.	10	10	10	10	10	50
TOTAL	30	30	30	30	30	150

Study 4.

The study was carried out during December 1989 at the S.B.C.R.U.. Commercial Romney ewes were used for embryo production. Superovulation was induced using one of the following treatments:

1) 9 mg FSH-P given as 6 injections (1.5 mg each) twice daily every 12h plus 800 i.u. of Folligon immediately before the first FSH-P injection.
2) 12 mg of FSH-P given as 6 injections (2 mg each) twice daily every 12h plus 800 i.u. of Folligon immediately before the first FSH-P injection.
3) 1200 i.u. of Folligon.
4) 1500 i.u. of Folligon.

All the treatments started 2 days before sponge removal. The animals were bred in the same way as in study 2, with semen from F.W.-rams. Expanded and hatched blastocysts were collected surgically from superovulated donors 6-7 days after onset of oestrus. Most of the embryos available were used even though some of them were not of the quality required for splitting. Recipients received 800 i.u. of Folligon given at sponge removal or 2 days before, or the same dose of Massey-PMSG given at sponge removal to ensure heat and ovulation.

Study 5.

The study was conducted at the S.B.C.R.U. during April 1990. Two groups of animals were used to generate the embryos. One group comprised 20 mixed-age Drysdale ewes and the other group 16 eight to nine month-old Drysdale ewe lambs. Ewes were superovulated with 1200 or 1500 i.u. of Folligon and ewe lambs using 1200 i.u. of Folligon or 8 ml of Ovagen in 8 injections as a constant dose (1 ml each) twice daily every 12h plus 700 i.u. of Folligon immediately before the first Ovagen injection. Administration of gonadotrophins was started 2 days before sponge removal. Animals were bred by intrauterine A.I. with fresh semen from 2 rams of the F.W. flock 6-14h following detection of oestrus. Morulae and blastocysts were collected surgically from superovulated donors 5.5 - 6.5 days after oestrous detection. Most of the embryos selected for splitting were of good quality, but some fair quality embryos were also included.

Study 6.

The study was conducted during the 1990 breeding season on a quarantine farm of LambXL at Cheltenham, Manawatu. Embryos to be bisected were brought from another LambXL quarantine farm located 5 km away from Cheltenham. Mixed-age Danish Texel and Finnish Texel ewes were used to supply the embryos. Superovulation was induced with 28-32 mg of FSH-P given as 6 injections in a descending dose (8,8,4,4,2,2 or 8,8,5,5,3,3 mg, respectively) twice daily at 8 am and 4 pm plus 200 i.u. of Pregnecol immediately before the first FSH-P injection. Animals were bred in a similar way to those in studies 1 and 3. Early, expanded and hatched blastocysts were collected surgically or nonsurgically from superovulated donors 6.0 - 7.5 days after onset of oestrus. Only good or excellent quality embryos were used.

4.2 Results.

4.2.1 Factors affecting the survival of demi-embryos.

Study 1.

One hundred and seventeen embryos were bisected in this trial. Only 65% (76/117) of them were successfully bisected to give 2 demi-embryos, 25% produced only 1 demi-embryo and 10% embryos were destroyed during the bisection process. The net efficiency of splitting (Total demi-embryos obtained / total demi-embryos expected) was 77% (181/234). Only 17% (13/76) of the recipients transplanted with 2 demi-embryos became pregnant. Fifteen foetuses were found at scanning resulting in 20% demi-embryo survival as a whole and 10% survival as demi-embryo. Two sets of identical twins were produced. Only one ewe out of 29 carrying single demi-embryos was found pregnant at scanning. Pregnancy rates for ewes transplanted with 2 whole embryos was 33% (5/15). No statistical analyses were carried out on this data.

Study 2.

Nine morulae were bisected in this trial with the 18 demi-embryos transplanted into nine recipients. Two ewes were pregnant and lambed at normal time giving a 22% pregnancy rate. Three lambs were born, 17% of the number of demi-embryos and 33% of the original number of embryos. One set of twins was generated from the study.

Study 3.

Data from 28 blastocysts bisected in this study were used to evaluate the effects of breed of the donor and technician performing the micromanipulation on demi-embryo survival. One demi-embryo was lost during the micromanipulation and one of the recipients used was not present at scanning, therefore information from only 53 demi-embryos was available for analysis.

The effects of breed of the donor on demi-embryo survival are shown in Table 4.2.
The statistical analysis included data for Gotland and Finnish Texel ewes; only 4 embryos from Oxford Down ewes were bisected making any comparisons meaningless. There were no significant differences in pregnancy rates and demi-embryo survival between embryos from Gotland and Finnish Texel ewes (69% vs 50% and 42% vs 26%, respectively). Seventy five percent (3/4) of the recipients transferred with demi-embryos

from Oxford Down ewes became pregnant, four foetuses were indentified at scanning giving a 50% demi-embryo survival. One of the recipients was found carrying twins.

Table 4.2 Effect of breed of the donor on demi-embryo survival (Study 3).

	Breed of donor		
	Gotland	Finnish Texel	Total
No. of embryos bisected	13	10	27
No. of demi-embryos	26	19	53
No. of recipients	13	10	27
No. of pregnancies (%)	9(69)	5(50)	17(63)
No. of foetuses	11	5	20
Foetuses/original embryo (%)	85	50	74
Foetuses/demi-embryo (%)	42	26	38
No. of single foetuses	7	5	14
No. of pairs of twins foetuses	2	0	3
Twins/original embryo (%)	15	0	11
Twins/pregnancy (%)	22	0	18

Embryo survival Chi^2 = 1.22, 1 df NS
Pregnancy rate Chi^2 = 0.88, 1 df NS

The effects of technician on demi-embryo survival are shown in Table 4.3. Forty five demi-embryos produced from the bisection of 23 whole embryos were included in the statistical analysis. There were no significant differences in pregnancy rates and demi-embryo survival between technician 1 and technician 2 (50% vs 69% and 30% vs 40%, respectively). Overall 11% of the bisected embryos produced identical twins.

Table 4.3 Effect of technician on demi-embryo survival (Study 3).

	Technician		
	1	2	Total
No. of embryos bisected	10	13	23
No. of demi-embryos	20	25	45
No. of recipients	10	13	23
No. of pregnancies (%)	5(50)	9(69)	14(61)
No. of foetuses	6	10	16
Foetuses/original embryo (%)	60	77	69
Foetuses/demi-embryo (%)	30	40	35
No. of single foetuses	4	8	12
No. of pairs of twins foetuses	1	1	2
Twins/original embryo (%)	10	8	9
Twins/pregnancy (%)	20	11	14

Embryo survival Chi^2 = 0.48, 1 df NS
Pregnancy rate Chi^2 = 0.88, 1 df NS

Study 4.

The results from 30 embryos bisected in this study were used to evaluate the effects of embryo quality and stage of embryo development on demi-embryo survival. Three recipients were not present at scanning and lambing. Therefore the analyses were conducted on data from 27 embryos bisected.

The effects of quality of the embryo on demi-embryo survival are shown in Table 4.4. There were no significant differences in pregnancy rates and demi-embryo survival between good and fair quality embryos (54% vs 40% and 36% vs 40%, respectively). Overall there was an incidence of 22% of identical twins.

Table 4.4 Effect of embryo quality on demi-embryo survival (Study 4).

	Embryo quality		
	Good	Fair	Total
No. of embryos bisected	22	5	27
No. of demi-embryos	44	10	54
No. of recipients	22	5	27
No. of pregnancies (%)	12(54)	2(40)	14(52)
No. of foetuses	16	4	20
Foetuses/original embryo (%)	73	80	74
Foetuses/demi-embryo (%)	36	40	37
No. of single foetuses	8	0	8
No. of pairs of twins foetuses	4	2	6
Twins/original embryo (%)	18	40	22
Twins/pregnancy (%)	33	100	43

Embryo survival $Chi^2 = 0.05$, 1 df NS
Pregnancy rate $Chi^2 = 0.34$, 1 df NS

The effects of stage of embryo development on demi-embryo survival are shown in Table 4.5. There were no significant differences in pregnancy rates and demi-embryo survival between expanded and hatched blastocysts (50% vs 53% and 37% vs 37%, respectively).

Table 4.5 Effect of stage of embryo development on demi-embryo survival (Study 4).

	Stage of embryo development		
	Expanded Blastocyst	Hatched Blastocyst	Total
No. of embryos bisected	8	19	27
No. of demi-embryos	16	38	54
No. of recipients	8	19	27
No. of pregnancies (%)	4(50)	10(53)	14(52)
No. of foetuses	6	14	20
Foetuses/original embryo (%)	75	74	74
Foetuses/demi-embryo (%)	37	37	37
No. of single foetuses	2	6	8
No. of pairs of twins foetuses	2	4	6
Twins/original embryo (%)	25	21	22
Twins/pregnancy (%)	50	40	43

Embryo survival Chi^2 = 0.016, 1 df NS
Pregnancy rate Chi^2 = 0.002, 1 df NS

Study 5.

The results from 34 embryos bisected in this study were used to evaluate the effects of embryo quality and stage of embryo development on demi-embryo survival. The effects of embryo quality on demi-embryo survival are shown in Table 4.6. There were no significant differences in pregnancy rates and demi-embryo survival between good and fair quality embryos (56% vs 33% and 28% vs 17%, respectively). No twins were produced in this study.

The effects of stage of embryo development on demi-embryo survival are shown in Table 4.7. Pregnancy rate and demi-embryo survival were significantly higher ($P<0.05$) in blastocysts compared to morulae (67% vs 23% and 33% vs 11%, respectively).

Table 4.6 Effect of embryo quality on demi-embryo survival (Study 5).

	Embryo quality		
	Good	Fair	Total
No. of embryos bisected	25	9	34
No. of demi-embryos	50	18	68
No. of recipients	25	9	34
No. of pregnancies (%)	14(56)	3(33)	17(50)
No. of foetuses	14	3	17
Foetuses/original embryo (%)	56	33	50
Foetuses/demi-embryo (%)	28	17	25
No. of single foetuses	14	3	17

Embryo survival Chi^2 = 0.91, 1 df NS
Pregnancy rate Chi^2 = 1.36, 1 df NS

Table 4.7 Effect of stage of embryo development on demi-embryo survival (Study 5).

	Stage of embryo development		
	Morula	Blastocyst	Total
No. of embryos bisected	13	21	34
No. of demi-embryos	26	42	68
No. of recipients	13	21	34
No. of pregnancies (%)	3(23)	14(67)	17(50)
No. of foetuses	3	14	17
Foetuses/original embryo (%)	23	67	50
Foetuses/demi-embryo (%)	11	33	25
No. of single foetuses	3	14	17

Embryo survival Chi^2 = 4.07, 1 df *
Pregnancy rate Chi^2 = 6.10, 1 df *

Study 6.

Results from 911 embryos judged to be of quality 1 or quality 2 were included for splitting in this study. The data were used to evaluate commercially the embryo splitting technique developed in the previous studies. Only information from whole embryos giving two demi-embryos was included in the analyses. The effects evaluated were embryo quality, stage of embryo development, age of the donor, breed of donor and technician performing the micromanipulation.

The effects of embryo quality on demi-embryo survival are shown in Table 4.8. There were no significant differences in pregnancy rates and demi-embryo survival between quality 1 and quality 2 embryos (74% vs 71% and 51% vs 45%, respectively). There was also no significant difference in the percentage of identical twins/pregnancy produced between quality 1 and quality 2 embryos (36% vs 28%, respectively).

Table 4.8 Effect of embryo quality on demi-embryo survival (Study 6).

	Embryo quality		
	1	2	Total
No. of embryos bisected	794	117	911
No. of demi-embryos	1588	234	1822
No. of recipients	794	117	911
No. of pregnancies (%)	591(74)	83(71)	674(74)
No. of foetuses	805	106	911
Foetuses/original embryo (%)	101	90	100
Foetuses/demi-embryo (%)	51	45	50
No. of single foetuses	377	60	437
No. of pairs of twins foetuses	214	23	237
Twins/original embryo (%)	27	20	26
Twins/pregnancy (%)	36	28	35

Embryo survival $Chi^2 = 2.51$, 1 df NS
Pregnancy rate $Chi^2 = 1.04$, 1 df NS
Incidence of twins $Chi^2 = 2.02$, 1 df NS

The effects of stage of embryo development on demi-embryo survival are shown in Table 4.9. There were no significant differences in pregnancy rates and demi-embryo survival between expanded blastocysts and hatched blastocysts (73% vs 78% and 49% vs 54%, respectively). The percentage of identical twins/pregnancy was also not significantly different between expanded and hatched blastocysts (34% vs 39%, respectively).

Table 4.9 Effect of stage of embryo development on demi-embryo survival (Study 6).

	Stage of embryo development		
	Expanded Blastocyst	Hatched Blastocyst	Total
No. of embryos bisected	766	145	911
No. of demi-embryos	1532	290	1822
No. of recipients	766	145	911
No. of pregnancies (%)	561(73)	113(78)	674(74)
No. of foetuses	754	157	911
Foetuses/original embryo (%)	98	108	100
Foetuses/demi-embryo (%)	49	54	50
No. of single foetuses	368	69	437
No. of pairs of twins foetuses	193	44	237
Twins/original embryo (%)	25	30	26
Twins/pregnancy (%)	34	39	35

Embryo survival Chi^2 = 2.38, 1 df NS
Pregnancy rate Chi^2 = 0.26, 1 df NS
Incidence of twins Chi^2 = 1.65, 1 df NS

The effects of age of the donor on demi-embryo survival are shown in Table 4.10. There were no significant differences in pregnancy rates between embryos generated from

mature ewes (24 months of age) and embryos generated from ewe lambs (approximately 10 months of age) (74% vs 74%, respectively). However demi-embryo survival (51 vs 47, $P<0.05$) and the percentage of identical twins/pregnancy (38% vs 27%, $P<0.01$) were higher in 24 month-old animals compared to 10 month-old animals.

Table 4.10 Effect of age of the donor (months) on demi-embryo survival (Study 6).

	Age of the donor		
	24	10	Total
No. of embryos bisected	661	250	911
No. of demi-embryos	1322	500	1822
No. of recipients	661	250	911
No. of pregnancies (%)	488(74)	186(74)	674(74)
No. of foetuses	675	236	911
Foetuses/original embryo (%)	102	94	100
Foetuses/demi-embryo (%)	51	47	50
No. of single foetuses	301	136	437
No. of pairs of twins foetuses	187	50	237
Twins/original embryo (%)	28	20	26
Twins/pregnancy (%)	38	27	35

Embryo survival $Chi^2 = 4.51$, 1 df *
Pregnancy rate $Chi^2 = 0.22$, 1 df NS
Incidence of twins $Chi^2 = 8.99$, 1 df **

The effects of breed of the donor on demi-embryo survival are shown in Table 4.11. There were no significant differences in pregnancy rates and demi-embryo survival between embryos generated from Danish Texel and Finnish Texel ewes (74% vs 74% and 50% vs 50%, respectively). There was also no significant difference in the percentage of identical twins/pregnancy between Danish Texel and Finnish Texel ewes (36% vs 34%, respectively).

The effects of technician on demi-embryo survival are shown in Table 4.12. There was a significant effect of the technician performing the micromanipulation on pregnancy rates ($P<0.05$) and demi-embryo survival ($P<0.01$). Technician 2 recorded higher pregnancy rate and demi-embryo survival than technician 1 (75% vs 66% and 51% vs 44%, respectively). However, there was no significant difference between technician 2 and technician 1 in the percentage of identical twins/pregnancy produced (35% vs 34%, respectively).

Table 4.11 Effect of breed of the donor on demi-embryo survival (Study 6).

	Breed of the donor		
	Danish Texel	Finnish Texel	Total
No. of embryos bisected	436	475	911
No. of demi-embryos	872	950	1822
No. of recipients	436	475	911
No. of pregnancies (%)	322(74)	352(74)	674(74)
No. of foetuses	439	472	911
Foetuses/original embryo (%)	101	99	100
Foetuses/demi-embryo (%)	50	50	50
No. of single foetuses	205	232	437
No. of pairs of twins foetuses	117	120	237
Twins/original embryo (%)	27	25	26
Twins/pregnancy (%)	36	34	35

Embryo survival $Chi^2 = 0.31$, 1 df NS
Pregnancy rate $Chi^2 = 0.14$, 1 df NS
Incidence of twins $Chi^2 = 0.21$, 1 df NS

Table 4.12 Effect of technician on demi-embryo survival (Study 6).

	Technician		
	1	2	Total
No. of embryos bisected	124	787	911
No. of demi-embryos	248	1574	1822
No. of recipients	124	787	911
No. of pregnancies (%)	82(66)	592(75)	674(74)
No. of foetuses	110	801	911
Foetuses/original embryo (%)	89	102	100
Foetuses/demi-embryo (%)	44	51	50
No. of single foetuses	54	383	437
No. of pairs of twins foetuses	28	209	237
Twins/original embryo (%)	22	26	26
Twins/pregnancy (%)	34	35	35

Embryo survival Chi^2 = 6.49, 1 df **
Pregnancy rate Chi^2 = 5.41, 1 df *
Incidence of twins Chi^2 = 1.21, 1 df NS

Overall demi-embryo survival was 50% or an average of one foetus for each whole embryo. This represents a significant increase ($P<0.001$) in the total number of foetuses compared to the embryo survival of whole embryos transplanted in pairs (100% vs 70%). However the increase in the number of foetuses due to splitting was not significant when compared to the survival of whole embryos transplanted as singles (100% vs 80%). When the efficiency of the embryo splitting technique was compared to the overall survival of quality 1 whole embryos (transplanted as singles or twins), there was a significant increase ($P<0.05$) in the total number of foetuses (28%) generated.

4.3 Discussion.

Factors affecting demi-embryo survival.

a) Effect of the splitting technique.

Two splitting techniques were used throughout these studies. Results when technique 1 was used (Studies 1 and 2) in which the embryos were bisected by horizontal incision using a small piece of a razor blade and a micropipette to hold the embryo were generally poor. In studies 1 and 2 pregnancy rates were 17 and 22%, demi-embryo survival was 10 and 17% and only two and one set of identical twins were obtained in each of those studies respectively. Considerably higher pregnancy rates (53 to 78%) have been reported when sheep (Gatica et al., 1984) and cattle (Lambeth et al., 1983; Ozil, 1983; Picard et al., 1986) embryos were bisected using similar techniques. Pregnancy rates in those studies when demi-embryos were transplanted individually into each recipient was slightly reduced (17 to 48%), but our results were near the lower end. These studies were the first conducted and the overall low results reflect the lack of experience with the technique. One particular reason that could have contributed to these results was the fact that it was not possible to place all the demi-embryos into an evacuated zonae pellucidae, thus decreasing their likelihood of survival. Moreover, all the embryos bisected in these studies were compact morulae and placing the bisected embryos back into empty zonae pellucidae could perhaps have increased their ability to survive. Shelton and Szell (1988) demonstrated that sheep demi-embryos generated from morulae survive better in vitro and in vivo when they were transferred within a zona pellucida. The results could have been affected by a tendency of denuded embryos to adhere to the transfer catheter. This situation was also found in the present studies in which a pasteur pipette was used to implant the demi-embryos. Despite the poor results achieved we were able to demonstrate the feasibility of the procedures used through the development of pregnancies and the production of identical twins. The experience gained in these first two studies was fundamental to the improvement of the procedures in those conducted subsequently.

Technique 2 was used in studies 3, 4, 5 and 6. The main modifications in the technique were: a) The use of the holding pipette was avoided and b) Bisection of the embryos was done by vertical incision as described by Picard et al. (1985) and Williams and Moore (1988). Because the techniques were used at different times, no strict

comparisons can be made between them, however, results obtained when technique 2 was used were considerably higher than those achieved when technique 1 was utilized. Some factors that could have been contributing to the improvement of the results when technique 2 was used include the changes indicated above, the change in the stage of development of the embryo to be bisected and an increase in the expertise of the technicians performing the bisections. Overall, the results obtained in these studies compare favourably with results from the literature in which sheep and cattle embryos have been bisected using similar procedures.

b) Effect of quality of the embryo.

Results from studies 4, 5 and 6 failed to show a significant effect of the quality of the embryos on pregnancy rate, demi-embryo survival or the incidence of twins. The effect of embryo quality on the incidence of twins was evaluated only in study 6. In studies where there was a reasonable number of embryos there was a non-significant tendency to get more foetuses from embryos judged to be of better quality. The pregnancy rates of demi-embryos obtained from quality 1 and quality 2 whole embryos for studies 4, 5 and 6 were 54% vs 40%, 56% vs 33% and 74% vs 71%, respectively. Perhaps some of the morphological differences considered when allocating embryos into the 2 quality groups were not related to differences in the capacity of the demi-embryos to survive. The majority of the embryos bisected in these studies were either expanded or hatched blastocysts, and the main criteria used to classify an embryo as being of quality 1 (good) or quality 2 (fair) was the presence or absence of cellular debris (pyknotic cells) in the blastocoele, respectively. The presence of pyknotic cells in whole embryos may not significantly interfere with the ability of the bisected embryos to develop pregnancy.

The considerably higher pregnancy rate in study 6 is difficult to explain as the same technician performed the bisections in all the studies and the quality classification and embryo spitting procedure was the same. Recipients in study 6 received progesterone supplementation on the day of transfer. This practice was shown (Dattena, 1989) to increase pregnancy rate in recipients transplanted with whole embryos and perhaps a similar effect was induced following the transfer of demi-embryos. The small number of observations in studies 4 and 5 make the observed trends difficult to interpret.

Reports in the literature clearly indicate the importance of a careful selection of the

embryos used for bisection. Good quality whole embryos produce good quality demi-embryos which consequently give higher pregnancy and embryo survival rates than results obtained from lower quality whole embryos (Seidel, 1982; Lambeth et al., 1983; Ozil, 1983; Picard et al., 1986; Arave et al., 1987; McEvoy and Sreenan, 1990). This was observed regardless of the stage of development of the embryo but it was more marked when embryos at early stages of development (compact morula and early blastocyst) were bisected. There is a general agreement among researchers that only good quality whole embryos should be bisected if successful results are to be achieved.

c) Effect of stage of embryo development.

Pregnancy rate and survival rate of embryos were similar for demi-embryos obtained from expanded or hatched blastocysts (Studies 4 and 6). However, the responses were higher when demi-embryos were obtained from blastocysts compared to morulae (Study 5). Pregnancy rate and demi-embryo survival for demi-embryos generated from blastocysts or morulae in study 5 were 67% vs 23% and 33% vs 11%, respectively. No difference in pregnancy rate between demi-embryos generated from blastocysts and expanded blastocysts was found by Kippax et al. (1991) in cattle. Similarly Chesne et al. (1987) did not find significant differences in pregnancy rate of demi-embryos obtained from whole embryos flushed on days 8, 9 or 10 after detection of oestrus. Although the stage of embryo development was not indicated in that study it can be assumed that the embryos were mainly expanded and hatched blastocysts. In support of our results of study 5, evidence that lower pregnancy and demi-embryo survival rates are obtained from morulae-derived than from blastocyst-derived demi-embryos was also reported by McEvoy and Sreenan (1990). Similarly, Williams et al. (1984) demonstrated that pregnancy rate increases with embryo age between days 5.5 and 7.5. Maurer (1988) reported the same tendency following the implantation of 2 to 4 demi-embryos per recipient. In another study, although a similar tendency was observed the difference was not significant (Kippax et al., 1991). In the present study all demi-embryos were transferred without zona pellucida and this could have contributed to the lower survival of morula-derived demi-embryos. Similar findings have also been reported in sheep (Shelton and Szell, 1988) and cattle (Warfield et al., 1987) when demi-embryos generated from morulae were transferred with or without zona pellucida. Collectively these data confirm the hypothesis that higher demi-embryo survival is achieved when blastocysts instead of morulae are bisected. On the other hand, when only blastocysts

are utilized, there appears to be an improvement in their survival when expanded or hatched blastocysts were bisected. Our results also support the observation that the zona pellucida is not important for the survival of bisected embryos obtained from expanded blastocysts (Willadsen and Godke, 1984; Warfield et al., 1987).

d) Effect of age of the donor.

The effect of age of the donor on demi-embryo survival was examined in study 6. To our knowledge this is the first time that demi-embryo survival from ewe lambs and adult ewes have been compared. Although pregnancy rate was the same (74%) for demi-embryos generated from donors 10 or 24 months old, demi-embryo survival and the incidence of twins were higher for demi-embryos generated from adult ewes than from those obtained from ewe lambs (28 and 38% vs 20 and 27%, respectively). There are two possible explanations for these results. Firstly, it could be that the bisection process decreased the ability of demi-embryos obtained from ewe lambs to develop in vivo. A second possibility is that demi-embryos from ewe lambs have a lower capacity to survive than demi-embryos from adult ewes. The second possibility seems more likely. Pregnancy rate (93% vs 83%) and survival (71 vs 60%) of whole embryos from 24 month-old sheep were higher compared to that of 10 month-old animals (data on the survival of whole embryos was generated in the same study including 36 and 227 recipients each implanted with two quality 1 embryos obtained from 10 or 24 month-old donors, respectively). Also studies in vitro (Wright et al., 1976) and in vivo (Quirke and Hanrahan, 1977; McMillan and McDonald, 1985) have shown lower developmental capacity of whole embryos obtained from ewe lambs compared to that of whole embryos obtained from adult ewes. Eggs obtained from superovulated calves (Seidel et al., 1971) and prepubertal gilts (Pinkert et al., 1989) have also shown lower capacity to develop in vitro. Collectively these data suggest that the bisection process affected in a similar way the in-vivo survival of demi-embryos from sheep of these two different ages and the lower demi-embryo survival from 10 month-old ewe lambs was due to an inherently low potential of those embryos for continued development.

e) Effect of breed of the donor.

Pregnancy rate and demi-embryo survival were not affected by the breeds of donor studied. This was shown in study 3 in which demi-embryos from Gotland and Finnish

Texel ewes were transferred. It was confirmed in study 6, in which demi-embryos from Danish Texel and Finnish Texel ewes were implanted. Although in study 3 the number of observations was low, the same results were observed when a large number of sheep were examined in study 6. On the basis of these results it is concluded that the breeds of the donors utilized in these studies did not differ in demi-embryo survival. The pregnancy rate and the survival of whole embryos from Texels of Danish origin and Texels of Finnish origin was similar (91% vs 92% and 72% vs 69%, respectively).

f) Effect of technician.

The influence of the technician performing the bisection on the efficiency of the splitting technique was shown in both studies in which this effect was examined (Studies 3 and 6), although the effect was found to be significant only in study 6. Pregnancy rate and demi-embryo survival for technician 1 and technician 2 in study 3 were 50% vs 69% and 30% vs 40%, respectively. Similarly the responses in study 6 were 66% vs 75% and 44% vs 51%, respectively. The best results were obtained by the same technician in every study. The differences reflected different degrees of experience between technicians since all the other aspects were maintained constant. This is supported by the fact that technician 2 performed 86% of the bisections in study 6. In study 3 each technician performed approximately the same number of bisections.

CHAPTER V:

ANALYSIS OF THE POSSIBLE USE OF A JUVENILE MOET SCHEME TO INCREASE THE RATE OF ANNUAL GENETIC PROGRESS IN A SHEEP FLOCK

CHAPTER V:

ANALYSIS OF THE POSSIBLE USE OF A JUVENILE MOET SCHEME TO INCREASE THE RATE OF ANNUAL GENETIC PROGRESS IN A SHEEP FLOCK

An attempt was made to study how the implementation of a juvenile MOET scheme might affect the annual genetic gain in a Romney flock. For convenience a flock of 100 breeding ewes was considered. The structure of the flock using normal reproduction is shown in Table 5.1.

Table 5.1 Structure of the flock using normal reproduction.

Age of ewe at birth of lamb(yr)	No. of Ewes	No. of Lambs/ewe	No. of Lambs born
2	29	0.9	26.1
3	27	1.1	29.7
4	23	1.3	29.9
5	21	1.4	29.4
All	100		115.1

Table 5.2 gives the parameters of the efficiency of juvenile MOET. These are based on results from the experiments discussed earlier.

Table 5.2 Efficiency of the juvenile MOET scheme.

Parameter	Response
Lambs responding to the treatment (%)	70
Ovulation rate (CL)	4
Recovery rate (%)	50
Fertilization rate (%)	70
Transferable embryos (%)	50
Embryo survival (%)	50

Evidence from the literature and from the present work suggests that further improvements can be achieved in some of the parameters indicated. The effects of the improvement in some of those parameters have also been assessed.

A) The model.

The annual genetic gain in fleece weight was calculated using the following formula:

$$\Delta Ga = \frac{\frac{MLf}{TLf}(r_{TI} i \sigma g) + \frac{ALf}{TLf}(r_{TI} i \sigma g)_{2,3,4\ yrs} + \frac{MLm}{TLm}(r_{TI} i \sigma g) + \frac{ALm}{TLm}(r_{TI} i \sigma g)}{\frac{MLf}{TLf}(L, lambs) + \frac{ALf}{TLf}(L, 2,3,4\ yrs) + \frac{MLm}{TLm}(L, lambs) + \frac{ALm}{TLm}(L, rams)}$$

where

ΔGa = annual genetic gain.

r_{TI} = the correlation between the genotype of the animal for fleece weight and the selection criteria.

i = standardized selection differential.

σg = genetic standard deviation.

L = generation interval.

MLf, MLm = number of female and male lambs generated from MOET.

ALf, ALm = number of female and male lambs generated from adult ewes.

TL = total number of lambs produced.

Two r_{TI} values were calculated. It was assumed older sheep would be selected on observations of hogget fleece weight. A heritability of 0.3 was assumed. Thus the r_{TI} used was 0.55 for adults. In the case of lambs no assumption was made on how selection was going to be carried out. It would probably be on a combination of hogget fleece weights of related animals and the lamb's own fleece weight. Some simple calculations suggested that an r_{TI} of 0.45 should be achievable. Similarly, a σg of 0.25 was assumed for adults and lambs. The standard deviation considered for fleece weight was 0.45 kg. All the ewe lambs can be programmed for embryo transfer and are mated using 4 rams. All 5-year-old adult ewes can be used as recipients and 2 possibilities are

considered when an extra supply of embryos is generated: a) Additional recipients can be obtained from outside the farm, or b) they can be obtained from the different age groups of adult ewes of the selected flock, selecting progressively by age group as required. No attempt was made to select the poorest ewes within each age group as recipients. Fifty percent of the embryos are transferred as pairs and fifty percent as singles. Adult ewes utilized as recipients and not getting pregnant are not re-mated. It is assumed 13 percent of lambs are lost from lambing to the time when they are submitted to the MOET programme.

B) Specific studies.

1) Consequences of conducting a MOET scheme on 6 month-old ewe lambs.

The annual genetic gain (ΔGa), the generation interval (L) and the number of lambs available at the time of the normal breeding season for a flock using a traditional breeding scheme or a juvenile MOET scheme are shown in Table 5.3. In this example only 5-year-old ewes are used as recipients. The effects of implementing this scheme when all the recipients are obtained from the selected flock are shown in Table 5.4. The consequences of increasing ovulation rate, fertilization rate, the percentage of transferable embryos, embryo survival and recovery rate are also examined in both cases. The likely effects of each improvement were evaluated additively after each improvement had been included in the model. In this way the full potential of conducting MOET on ewe lambs is more clearly visualized. Four two-year-old rams were used for mating. At the time of selection the same proportion of animals is selected from those generated from the MOET scheme or from those obtained using normal reproduction. The present calculations correspond to the 5th year of implementation of the scheme. By this time the flock population has stabilized and the first offspring generated from the scheme have been included in the flock for the last time. Forty-two ewe lambs are submitted to the MOET programme at this stage.

Overall, there is a considerable decrease in L when using MOET compared to the use of normal reproduction. There is also a consistent increase in ΔGa in both cases, when only some (Table 5.3) or all (Table 5.4) of the recipients were obtained from the selected flock. Higher rates of genetic progress are achieved using MOET despite a considerable

decrease in the number of lambs produced, particularly in the case in which all the recipients are obtained from the selected flock. When only data from animals submitted to the MOET scheme are considered, the following is concluded:

Implementation of the MOET scheme tends to decrease the generation interval as the ovulation rate increased, regardless of whether only some (Table 5.3) or all the recipients (Table 5.4) are obtained from the selected flock. The decrease in the generation interval is due to the fact that more lambs are generated from the MOET scheme with the improvement in each of the parameters considered. The effect is greater when all the recipients are obtained from the selected flock and is due to the fact that more older adult ewes are utilized as recipients.

The annual genetic gain consistently increases as ovulation rate increases or after each parameter is improved in both circumstances, when only some of the recipients or all of them are obtained from the selected flock. The rates of genetic progress are higher when only some of the recipients are obtained from the selected flock compared to the case in which all the recipients are chosen from the flock. This is due to a higher selection intensity as the total number of lambs to select from is higher.

If all the recipients are obtained from the selected flock there will generally be a reduction in the total number of lambs produced, which is partly due to the fact that recipient ewes not becoming pregnant are not remated. The effect is more marked at the highest ovulation rate (10CL). This is due to the low efficiency of generating pregnancies by the embryo transfer programme and to the need for using a higher proportion of the adult ewes as recipients. Using MOET, a comparable number of lambs to those observed following natural mating will be obtained only in the case in which some of the recipients are obtained from outside the farm and if the ovulation rate is 7 CL combined with 85% of fertilization rate and 60% of transferable embryos. Another possibility would be to increase the ovulation rate up to 10 corpora lutea. The results indicate that a higher ΔGa will be achieved when ewes from within the flock that are not younger than 5-year-old are not used as recipients. Although slightly lower rates of annual genetic are indicated when all the recipients are obtained from the selected flock, these are still higher than the rates of genetic progress achieved when using normal reproduction.

Although, the calculations indicate the possibility of increasing the rate of genetic progress if the efficiency of the MOET programme is improved, results from chapter 3 show some of the difficulties implementing the scheme in the practice. In the best of the cases an ovulation rate of 4 CL can be obtained. Likewise the improvements in fertilization rate, the percentage of transferable embryos, survival rate and recovery rate are also achievable. However, the improvement in the annual genetic gain is marginal and this has to be balance against the costs involved conducting the embryo transfer programme.

Table 5.3 Effect of implementing a MOET scheme in ewe lambs when extra outside ewes are used as recipients when there are not sufficient 5-year olds within the selected flock.

	Breeding scheme			
	Normal reproduction	MOET scheme		
		4CL	7CL	10CL
ΔGa(a)	0.0616	0.0630	0.0676	0.0721
L(b)	3.54	2.83	2.69	2.57
Lambs from MOET	0	9	16	22
Lambs from adults	100	74	74	75
Total	100	83	90	97
a) Effects of increasing fertilization rate up to 85%.				
ΔG(a)		0.0646	0.0707	0.0750
L(b)		2.78	2.63	2.50
Lambs from MOET		11	19	27
Lambs from adults		74	75	75
Total		85	94	102
b) Effects of increasing the percentage of transferable embryos up to 60%.				
ΔG(a)		0.0664	0.0722	0.0780
L(b)		2.74	2.57	2.42
Lambs from MOET		13	23	33
Lambs from adults		75	74	74
Total		88	97	107
c) Effects of increasing embryo survival up to 60%.				
ΔG(a)		0.0676	0.0744	0.0810
L(b)		2.69	2.50	2.34
Lambs from MOET		16	27	39
Lambs from adults		74	75	75
Total		90	102	114
d) Effects of increasing recovery rate up to 60%.				
ΔG(a)		0.0702	0.0780	0.0856
L(b)		2.63	2.42	2.25
Lambs from MOET		19	33	47
Lambs from adults		74	74	74
Total		93	107	121

(a): annual genetic gain in kg.
(b): generation interval in years.

Table 5.4 Effect of implementing a MOET scheme in ewe lambs when all the recipients are obtained from the selected flock.

	Breeding scheme			
	Normal	MOET scheme		
	reproduction	4CL	7CL	10CL
ΔGa(a)	0.0616	0.0630	0.0650	0.0670
L(b)	3.54	2.83	2.58	2.19
Lambs from MOET	0	9	16	22
Lambs from adults	100	74	67	54
Total	100	83	83	76
a) Effects of increasing fertilization rate up to 85%.				
ΔG(a)		0.0646	0.0660	0.0672
L(b)		2.78	2.40	1.94
Lambs from MOET		11	19	27
Lambs from adults		74	61	46
Total		85	80	73
b) Effects of increasing the percentage of transferable embryos up to 60%.				
ΔG(a)		0.0660	0.0671	0.0688
L(b)		2.72	2.19	1.74
Lambs from MOET		13	23	33
Lambs from adults		73	54	37
Total		86	77	70
c) Effects of increasing embryo survival up to 60%.				
ΔG(a)		0.0670	0.0689	0.0731
L(b)		2.67	2.12	1.67
Lambs from MOET		16	27	39
Lambs from adults		73	54	37
Total		89	81	76
d) Effects of increasing recovery rate up to 60%.				
ΔG(a)		0.0680	0.0713	0.0739
L(b)		2.53	1.87	1.41
Lambs from MOET		19	33	47
Lambs from adults		67	45	26
Total		86	78	73

(a): annual genetic gain in kg.
(b): generation interval in years.

2) Effects of change in the percentage of ewe lambs selected.

The effects are presented in Table 5.5. Calculations were conducted for the case in which only 5-year-old adults from the selected flock were used as recipients. There was a need to obtain extra recipient ewes from outside the flock in every case considered. The effects were determined assuming that fertilization rate was 85%, the percentage of transferable embryos 60%, embryo survival 60% and recovery rate 60%. We considered increasing selection intensities in the ewe lambs generated from the MOET programme when three different ovulation rates are obtained (4, 7 or 10 CL). In general an increase in selection intensity leads to a larger genetic superiority, which is even higher as ovulation rate increases.

Table 5.5 Effect of decreasing the percentage of ewe lambs selected on the annual genetic gain (ΔGa) considering three ovulation rate responses (4, 7 and 10 CL).

	Ovulation rate	Percentage selected			
		100	70	50	30
ΔGa(a)	4 CL	0.0693	0.0717	0.0732	0.0750
ΔGa(a)	7 CL	0.0752	0.0791	0.0814	0.0842
ΔGa(a)	10 CL	0.0806	0.0856	0.0887	0.0924

(a): annual genetic gain in kg.

3) Effect of increasing the accuracy of selection.

The effect of increasing the accuracy of selection (r_{TI}) in ewe lambs when different percentages of them are selected is shown in Table 5.6. Only data for the case in which 5-year-old ewes were utilized as recipients is presented. An ovulation rate of 4 CL was assumed. The results indicate that the annual genetic gain is increased either by increasing the accuracy of selection or through higher selection intensities. Slightly faster rates of genetic progress are obtained for each 10% increase in the proportion of ewe

lambs selected compared to those when accuracy of selection is increased by 5%. The proportion of ewe lambs selected will be partly determined by the number of lambs generated and this number has been shown to decrease considerably when many lambs are used as donors, particularly when all the recipients are obtained from the selected flock. Improving the accuracy of selection for fleece weight in lambs will demand the use of methods which can use information from the parents and other close relatives.

Table 5.6 Effect of increasing the degree of accuracy of selection (r_{TI}) when different proportions of ewe lambs are selected on the annual genetic gain (ΔGa).

Percentage selected	Accuracy of selection				
	0.35	0.40	0.45	0.50	0.55
90	0.0700	0.0701	0.0702	0.0704	0.0705
80	0.0706	0.0708	0.0710	0.0712	0.0714
70	0.0712	0.0715	0.0717	0.0720	0.0723
60	0.0717	0.0721	0.0724	0.0728	0.0731
50	0.0723	0.0728	0.0732	0.0736	0.0741
40	0.0730	0.0735	0.0740	0.0745	0.0751
30	0.0737	0.0743	0.0750	0.0756	0.0762

4) Effect of changing the age of the rams used for breeding on the annual genetic gain.

The effect of age of the ram on the annual genetic gain was evaluated considering different percentages of ewe lambs selected. It was assumed that all the improvements indicated previously are achieved and that only some of the recipients are obtained from the selected flock. The ovulation rate considered was 4 CL. Four rams from each age group are assumed to be used for mating. The results are presented in Table 5.7.

Overall, higher rates of genetic progress are achieved using ram lambs compared to the use of 2- or 3-year-old rams. This is due to the considerable reduction in the generation

interval (not shown here) which compensates the effect of their low r_{TI} (0.45). Improvement in any of these parameters or both of them will result in higher rates of annual genetic progress. Analysis of the data within each age group indicated a slight but consistent increase in ΔGa as selection intensity of ewe lambs increases. In summary, the results show the potential of using young rams for mating compared to the use of older rams.

Table 5.7 Effect of change in the age of the rams on the annual genetic gain (ΔGa) when different percentages of ewe lambs are selected.

Ewe lambs selected (%)	Age of the ram (years)		
	1	2	3
100	0.0756	0.0693	0.0570
70	0.0787	0.0717	0.0590
50	0.0806	0.0732	0.0602
30	0.0828	0.0750	0.0616

The effect of improving the degree of accuracy of selection of the ram lambs used for breeding is shown in Table 5.8. All the assumptions indicated above also apply here. The results clearly show that, if increases in the degree of accuracy of selection in the young rams can be achieved, this increases ΔGa considerably.

Table 5.8 Effect of increasing the degree of accuracy of selection (r_{TI}) of the ram lambs used for breeding on the annual genetic gain (ΔGa) considering different percentages of ewe lambs selected.

Ewe lambs selected (%)	Accuracy of selection				
	0.35	0.40	0.45	0.50	0.55
100	0.0628	0.0692	0.0756	0.0820	0.0884
70	0.0659	0.0723	0.0787	0.0851	0.0915
50	0.0678	0.0742	0.0806	0.0870	0.0934
30	0.0700	0.0764	0.0828	0.0892	0.0956

5) Generating all the replacements by MOET.

In this case it is assumed that the number of lambs generated from MOET should be equal to the number of lambs obtained when normal reproduction is used and consequently all the replacements are chosen from them. In order to generate all the replacements by MOET, assuming that all the improvements are achieved, the ovulation rate had to be at least 18 corpora lutea per ewe lamb responding to the gonadotrophin treatment. From the practical point of view it is very unlikely that this can be achieved.

In summary, results from the present simulation show that, with the degree of efficiency in the embryo transfer programme achieved so far, it is possible to reduce the generation interval, however this is not reflected in great increases in genetic progress. The ΔGa that can be achieved assuming all the different improvements indicated are obtained and only some of the recipients come from the selected flock, for an ovulation rate of 4, 7 or 10 CL will be 8.4, 16.4 or 24.0 g/year more, respectively compared to the annual genetic gain obtained using normal methods of reproduction. Considerable improvements in the rates of annual genetic gain can be expected if ram lambs are used for mating, particularly when combined with some degree of selection in the ewe lambs as well. This possibility is worthy of further consideration since, from the practical point of view it can be achieved, although the selection of ram lambs can present some limitations. From the present calculations it is clear that the success of the scheme depends primarily on the number of lambs available for selection. The emphasis of most of the research in this thesis has been on increasing ovulation rate, but results in table 5.3 show that recovery rate, fertilization rate and survival rate are important in determining the number of lambs and the genetic gain. The number of lambs generated could be increased even further if high quality embryos are divided, as demonstrated in adult ewes (see chapter 4), thus increasing further the rates of genetic progress by increasing the accuracy of selection (from identical twins). In conclusion to obtain the highest rates of genetic progress it will be necessary to integrate the most efficient elements of the scheme.

CHAPTER VI:

GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER VI:

GENERAL DISCUSSION AND CONCLUSIONS

The main objective was to evaluate the feasibility of implementing a MOET scheme using 6 to 7 month-old Romney ewe lambs. This could reduce the generation interval to approximately 1 year and be reflected in considerable annual rates of genetic progress. A key point in the viability of the programme was to successfully superovulate ewe lambs. Thus attempts were made to develop a system of superovulating ewe lambs which would give consistent responses and at the same time be cheap enough to be applied in practice. Since PMSG is the cheapest source of gonadotrophin available, this was used throughout the work. Ovulation rates in ewe lambs were not significantly different when PMSG from different sources was compared within a trial. However, considerable variation in the responses was observed among different trials. This suggests that environmental factors are also affecting the responses. Because different sources of PMSG could have different potency for inducing superovulation it is recommended that the batch of PMSG be evaluated before the decision is made to use it widely. Thus for example PMSG from 3 individual mares showed considerable variation in response even when given at a standard dose-level. Evidence in cattle (Humphrey et al., 1979) suggests that higher ovulation rates are obtained using sources of PMSG with high FSH/LH ratio, but such information is not provided by the supplier of the commercial preparations of PMSG. Thus it is clear that there is a lack of basic information on the characteristics of the sources of PMSG, so that a better interpretation of the results can be achieved.

Evidence from the literature (e.g. Elsden et al., 1978; Armstrong et al., 1983) suggests that pituitary gland preparations induce higher ovulation rates than PMSG. However, their high cost often limits their use. Two pituitary preparations (FSH-P and Ovagen) were evaluated in trial 3. The administration of the more expensive gonadotrophin preparations failed to improve the ovulatory responses of the ewe lambs compared to the use of the cheaper PMSG. The lowest responses were recorded using the source of FSH containing the lowest amount of LH (Ovagen). The improvement in the responses when PMSG was used could be due to its high LH content and longer half life (McIntosh et al., 1975). However, evidence with mature animals (sheep: Armstrong

and Evans, 1984; cattle: Donaldson and Ward, 1985, 1986) reported lower responses using gonadotrophin preparations with high LH content. Although FSH has been identified as the key hormone for follicle recruitment in adult ewes (Driancourt, 1991), basal levels of LH are also involved in recruitment (McNeilly et al., 1990; cited by Driancourt, 1991). Basal levels of LH are probably low in ewe lambs, thus when superovulation is attempted using pituitary preparations with low LH content the number of follicles recruited is low and as a consequence the ovulation rates will be low.

Significant improvements in ovulation rate in adult ewes have been obtained when pituitary gland preparations and PMSG were combined in a single superovulatory treatment (Ryan et al., 1984; McMillan and Hall, 1991). However, the ewe lambs in the present work that received the two gonadotrophins did not show a statistically significant improvement although they tended to have the highest ovulatory responses. The present trials involved only small dose of PMSG (300 or 500 i.u.), so there is the possibility that increasing the dose-level of PMSG injected could improve the ovulatory responses even further.

Administration of GnRH has been shown to improve ovulation rates in superovulated mature ewes (Nancarrow et al., 1984) but the present results using ewe lambs (Trials 3, 5 and 6) do not always support that conclusion, although, ewe lambs treated with PMSG plus GnRH (Trials 3 and 6) or Ovagen plus PMSG plus GnRH (Trial 5) tended to give higher ovulation rates. The purpose of administering GnRH is to induce ovulation of preovulatory follicles, however, the ovarian stimulation was poor particularly when FSH-P or Ovagen was used. This could explain the lack of response to GnRH administration. Although it has been shown that the time of GnRH administration could be critical when given at a fixed time following the removal of the synchronizing device, this was not a concern in the present trials as GnRH was given shortly after oestrus had been detected in most of the cases.

Higher ovulation rates were obtained in superovulated ewe lambs that had previously been immunized against androstenedione, however, the degree of improvement (0.63 extra CL) was not great enough to make the MOET scheme viable. Whether greater responses to immunization could be obtained using ewe lambs from prolific breeds making the scheme viable needs to be determined but there are 2 reports (Quirke and

Gosling, 1980; Kyle and Smith, 1984) which have shown that non-superovulated mature ewes from prolific breeds gave higher ovulation rates following androstenedione immunization compared to non-immunized ewes.

In these trials adult ewes consistently recorded higher ovulation rates than ewe lambs, even though their responses were generally lower than those reported in the literature. The lower ovulation rates from ewe lambs could be due to a reduced number of recruitable follicles at the time the superovulatory treatment was initiated. Reports of adult sheep (McMillan and Hall, 1991; McMillan et al., 1991) have demonstrated the association of follicle populations at the start of the synchronization and superovulatory treatments with ovulation rate. The possibility of improving the ovulation rates following superovulation by treating the lambs at 8 to 9 months of age seems unlikely (Trial 2), although comparable responses to those obtained in adults have been indicated treating ewe lambs at approximately 1 year of age (Tervit et al., 1989; W.H. Vivanco, personal communication). This approach of delaying the use of MOET until 1 year of age was not considered feasible for this investigation; instead the main investigations were in animals before or close to puberty at 6 to 7 months of age.

There were also other effects of age on the responses noted in donor ewes. Thus recovery rates of eggs were higher in adults than in lambs, but the reasons are not clear as flushing procedures were the same. Problems of egg transport through the oviducts or degeneration and expulsion from the uterus before the time of flushing may be involved. Further the donor ewe lambs should be artificially inseminated as natural mating in the paddock was found to produce low fertilization rates. Although in most of the present studies intrauterine insemination was used, this might present difficulties to some technicians due to the small reproductive tract of the lambs. However, the use of multiple cervical inseminations might also give acceptable results.

There is no conclusive evidence that fleece weight-selected ewe lambs and controls differ in their capacity to respond to the superovulatory treatments. Nevertheless, the tendency for lambs from the fleece weight-selected flock to show greater responses is in agreement with findings by McClelland (1990). It is possible that the Romney animals being studied are less sensitive to gonadotrophins than other genotypes. This was not investigated as such but there was the opportunity to examine the applicability of MOET

in a Booroola-Romney x Perendale flock. Overall, the ovulatory responses were considerably higher compared to those obtained using Romney ewe lambs. Although only ovulation rate data were recorded in this trial (Trial 6), further studies need to be conducted involving collection and transfer of embryos to fully evaluate such possibility. However, it should be remembered that a higher sensitivity in prolific genotypes could be restricted to the use of PMSG (Oldham et al., 1984) and not to pituitary preparations such as FSH-P (Bindon and Piper, 1986; unpublished study cited by Bindon et al., 1986).

Environmental effects although always present are difficult to identify and even more difficult to control. In this investigation ovulation rates were significantly affected by time (period) when the animals were treated in trials 3 and 5. Although in trial 3 the decrease in ovulation rate was associated with an outbreak of facial eczema, no explanation was found for the differences between the groups in trial 5. The adverse effects of facial eczema on ewe reproduction, as shown by reduced lambing percentage and incidence of multiples and therefore indicators of lower ovulation rates, have been reported by Smeaton et al. (1985).

Ewe lambs superovulated with Ovagen produced better quality embryos. These findings agree with reports from other researchers (Armstrong and Evans, 1984a; Donaldson and Ward, 1985; Mapletoft & Murphy, 1989) in which higher embryo quality was obtained using pituitary gland preparations with low LH contamination. However, because of the poor ovulation rates in the ewe lambs less embryos were obtained using Ovagen compared with PMSG.

A consistent increase in pregnancy rate was found after transferring embryos judged to be of higher quality or when 2 embryos were transferred with embryos both from lambs and adult ewes. Such a tendency has been well documented in the literature. Higher pregnancy rates were consistently recorded when transferring embryos from adult ewes. Lower developmental capacity of embryos obtained from young animals has been reported not only in sheep (McMillan and McDonald, 1985) but also in calves (Seidel et al., 1971) and prepubertal gilts (Pinkert et al., 1989). Thus eggs obtained from prepubertal animals apparently have an inherent low capacity for development. The reason(s) for this have yet to be identified but the availability of in-vitro procedures for

egg maturation, fertilization and culture will be helpful in finding out answers to some of these questions.

The results from splitting sheep embryos and their transfer to recipients (Studies 3, 4, 5 and 6) showed that satisfactory pregnancy rates and embryo survival rates could be achieved. The highest pregnancy rates and embryo survival rates were obtained by splitting good quality expanded or hatched blastocysts. The zona pellucida was not required for continuous development when blastocysts were bisected. Embryo survival and the incidence of twins were lower when demi-embryos were generated from 10-month-old lambs compared with demi-embryos derived from ewes 24 months of age or older. These results are in the same direction to that for "whole embryos" after transfer and generated from adult ewes vs ewe lambs (McMillan and McDonald, 1985).

Calculations of the results on the implementation of the juvenile MOET scheme showed that faster rates of annual genetic progress are achievable using such a scheme compared with the use of normal reproduction. A rather-simple deterministic model was used in these calculations. A more-complex stochastic model could give more accurate estimates and this technique is likely to indicate slightly higher rates of genetic gain associated with the juvenile MOET. Higher rates of progress were indicated when only the oldest ewes were used as recipients from within the selected flock. This is due to either a decrease in the generation interval or an increase in the selection differential or both. The annual rates of genetic progress are also likely to be improved by re-mating recipients not getting pregnant by MOET and by selecting, as recipients, ewes with the lowest breeding values across the different age groups but these factors were not included in calculations of the rate of genetic progress. A key point in the implementation of the scheme is the utilization of methods of selection which can make use of information from the lambs, the parents and other close relatives to improve the accuracy of the selection criteria.

Although from the present results it was not possible to clearly identify a hormonal treatment consistently able to induce superovulatory responses, our evidence suggests that administration of 1200 i.u. of PMSG (a dose with enough potency to induce superovulation) 2 days before sponge removal plus 100 μg GnRH within 6h of oestrous detection is the most appropriate superovulatory treatment in prepubertal Romney ewe

lambs. Higher fertility rates in donor ewe lambs are likely to be achieved using intrauterine insemination. The efficiency of the MOET scheme (number of lambs born) could be further improved if high quality embryos are split and transferred into appropriate recipients.

Looking at the efficiency of the MOET scheme, low ovulation rate after gonadotrophin stimulation remains the primary factor limiting the implementation of the scheme under practical conditions. However when ovulation rate rises above 4 the low survival rate of embryos from the ewe lambs also becomes a major factor. A valid criticism of the present work is that some conclusions have been drawn from results of several treatments and trials applied over several years. Collectively the results in the ewe lambs were poor although the adult animals responded better. The approach of trying a range of treatments has been examined and found not to be particularly informative in identifying reasons for poor responses. It is clear that a better understanding of the factors affecting the growth of ovulatory follicles including their recruitment, selection and dominance in the ovary in the prepubertal lamb is required. If it can be described what mechanisms are influencing the potential ovulation rate then the development of successful ways of stimulation may be more likely.

The conclusion from this investigation is that successful and repeatable methods for superovulation in 6 to 7 month-old lambs have yet to be described. Improvements will come from manipulation of the mechanisms resulting in ovulatory activity and a range of techniques might have a role. Some increase in efficiency can result from splitting blastocysts and by transferring 2 demi-embryos rather than a single zygote. Improvements in fertility and in embryo recovery and transfer would all add to the overall level of success. Finally, the investigation also showed the genetic benefits of the juvenile MOET scheme when compared to the use of normal reproduction methods. These benefits will be available only if improvements in the superovulation and embryo transfer procedures are achieved.

APPENDICES

APPENDICES

Appendix 3.1 Factors affecting the ovulation rate of PMSG (Consept45) treated ewe lambs: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.0042	NS
Time of PMSG injection	1	0.1095	NS
Genotype	1	0.0054	NS
Error	41	1.4889	
Total	44	1.6071	

(A) = Transformed data.

Appendix 3.2 Factors affecting the number of large follicles of PMSG (Consept45) treated ewe lambs: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.0941	NS
Time of PMSG injection	1	0.7807	***
Genotype	1	0.2824	*
Error	41	2.2411	
Total	44	3.4253	

(A) = Transformed data.

Appendix 3.3 Factors affecting the total ovarian response of PMSG (Consept45) treated ewe lambs: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.0210	NS
Time of PMSG injection	1	0.8180	***
Genotype	1	0.1329	NS
Error	41	1.6745	
Total	44	2.6555	

(A) = Transformed data.

Appendix 3.4 Effect of age on the ovulatory responses of PMSG (Consept45) treated animals: Analyses of variance (Trial 1).

A) Ovulation rate			
Source of variation	D.F.	S.S.(B)	Sig.
Age	1	8.2285	**
Error	61	75.2000	
Total	62	83.4285	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.0082	NS
Error	61	4.6218	
Total	62	4.6300	
C) Total ovarian response			
Source of variation	D.F.	S.S.(B)	Sig.
Age	1	3.9682	NS
Error	61	273.1111	
Total	62	277.0793	

(A) = Transformed data, (B) = Untransformed data.

Appendix 3.5 Effect of gonadotrophin treatment on ovulation rate of ewe lambs: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	4	0.2707	NS
Error	44	1.8406	
Total	48	2.1114	

(A) = Transformed data.

Appendix 3.6 Effect of gonadotrophin treatment on the number of large follicles of ewe lambs: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	4	4.6020	NS
Error	44	46.5000	
Total	48	51.1020	

(B) = Untransformed data.

Appendix 3.7 Effect of gonadotrophin treatment on the total ovarian response of ewe lambs: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	4	0.4014	*
Error	44	1.4084	
Total	48	1.8098	

(A) = Transformed data.

Appendix 3.8 Effect of age on ovulation rate: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.3930	**
Error	48	2.5974	
Total	49	2.9904	

(A) = Transformed data.

Appendix 3.9 Factors affecting the ovulation rate of gonadotrophin treated ewe lambs: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	1.2825	**
GnRH	1	0.0690	NS
Genotype	1	0.2653	NS
Group	1	0.7205	**
Treatment x GnRH	3	0.3704	NS
Treatment x Group	3	0.2318	NS
Treatment x Genotype	3	0.1970	NS
Error	55	4.1304	
Total	70	7.6750	

(A) = Transformed data.

Appendix 3.10 Factors affecting the number of large follicles of gonadotrophin treated ewe lambs: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	0.3003	NS
GnRH	1	0.0164	NS
Genotype	1	0.0726	NS
Group	1	0.0010	NS
Treatment x GnRH	3	0.3442	NS
Treatment x Group	3	0.1494	NS
Treatment x Genotype	3	0.1465	NS
Error	55	4.2553	
Total	70	5.4155	

(A) = Transformed data.

Appendix 3.11 Factors affecting the total ovarian response of gonadotrophin treated ewe lambs: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	1.7931	***
GnRH	1	0.0047	NS
Genotype	1	0.3615	*
Group	1	0.4472	*
Treatment x GnRH	3	0.7304	*
Treatment x Group	3	0.2962	NS
Treatment x Genotype	3	0.0155	NS
Error	55	3.9816	
Total	70	8.1226	

(A) = Transformed data.

Appendix 3.12 Effect of Treatment, Group and their interaction on the ovulatory responses of gonadotrophin treated ewe lambs:Analyses of variance (Trial 3).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.1515	NS
Group	1	0.2255	NS
Treatment x Group	1	0.0445	NS
Error	13	1.3172	
Total	16	2.7132	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.0015	NS
Group	1	0.0381	NS
Treatment x Group	1	0.1881	NS
Error	13	1.1214	
Total	16	1.3695	
C) Total ovarian response			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.1892	NS
Group	1	0.0666	NS
Treatment x Group	1	0.0044	NS
Error	13	0.7335	
Total	16	1.0427	

(A) = Transformed data.

Appendix 3.13 Effect of Treatment, Group and their interaction on the ovulatory responses of gonadotrophin treated adult ewes: Analyses of variance (Trial 3).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.0006	NS
Group	1	0.3797	*
Treatment x Group	1	0.0124	NS
Error	16	1.0084	
Total	19	1.4604	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.0001	NS
Group	1	0.1209	NS
Treatment x Group	1	0.0288	NS
Error	16	0.8122	
Total	19	0.9637	
C) Total ovarian response			
Source of variation	D.F.	S.S.(A)	Sig.
Treatment	1	0.0005	NS
Group	1	0.4093	*
Treatment x Group	1	0.0030	NS
Error	16	1.0618	
Total	19	1.5285	

(A) = Transformed data.

Appendix 3.14 Effect of age on the ovulatory responses of gonadotrophin treated animals; Group 1: Analyses of variance (Trial 3).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1713	NS
Error	10	0.6576	
Total	11	0.8289	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.0279	NS
Error	10	0.6725	
Total	11	0.6999	
C) Total ovarian response			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1450	NS
Error	10	0.5478	
Total	11	0.6928	

(A) = Transformed data.

Appendix 3.15 Effect of age on the ovulatory responses of gonadotrophin treated animals; Group 2: Analyses of variance (Trial 3).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1647	NS
Error	23	1.8494	
Total	24	2.0141	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1506	NS
Error	23	1.5179	
Total	24	1.6685	
C) Total ovarian response			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.0057	NS
Error	23	1.4806	
Total	24	1.4863	

(A) = Transformed data.

Appendix 3.16 Effect of age on the ovulatory responses of gonadotrophin treated animals: Analyses of variance (Trial 3).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.2755	NS
Error	35	3.1737	
Total	36	3.4492	
B) Number of large follicles			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.0532	NS
Error	35	2.3333	
Total	36	2.3865	
C) Total ovarian response			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.0583	NS
Error	35	2.5713	
Total	36	2.6297	

(A) = Transformed data.

Appendix 3.17 Effect of PMSG source, Immunization and GnRH on ovulation rate of ewe lambs: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(B)	Sig.
PMSG source	2	3.1162	NS
Immunization	1	8.4062	*
GnRH	1	3.8478	NS
PMSG x GnRH	2	14.5483	*
Error	79	177.2645	
Total	85	206.4302	

(B) = Untransformed data.

Appendix 3.18 Effect of PMSG source, Immunization and GnRH on the number of large follicles of ewe lambs: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(A)	Sig.
PMSG source	2	0.6590	*
Immunization	1	0.1132	NS
GnRH	1	0.1399	NS
PMSG x GnRH	2	0.2598	NS
Error	79	7.0374	
Total	85	8.2384	

(A) = Transformed data.

Appendixe 3.19 Effect of PMSG source, Immunization and GnRH on the total ovarian response of ewe lambs: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	2	0.0385	NS
Immunization	1	0.0053	NS
GnRH	1	0.1861	NS
PMSG x GnRH	2	0.1256	NS
Error	79	5.0809	
Total	85	5.6085	

(A) = Transformed data.

Appendix 3.20 Factors affecting the ovulation rate of gonadotrophin treated ewe lambs: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	4	25.2806	NS
Genotype	1	8.3869	NS
Group	1	25.5884	**
Treatment x Genotype	4	47.2921	**
Error	53	138.3087	
Total	63	230.9375	

(B) = Untransformed data.

Appendix 3.21 Factors affecting the number of large follicles of gonadotrophin treated ewe lambs: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	4	1.4487	NS
Genotype	1	0.0085	NS
Group	1	0.0333	NS
Treatment x Genotype	4	2.7506	NS
Error	53	183.8877	
Total	63	197.8593	

(B) = Transformed data.

Appendix 3.22 Factors affecting the total ovarian response of gonadotrophin treated ewe lambs: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	4	38.0153	NS
Genotype	1	9.7992	NS
Group	1	25.3943	NS
Treatment x Genotype	4	31.4473	NS
Error	53	455.9794	
Total	63	549.1093	

(B) = Transformed data.

Appendix 3.23 Effect of age on the ovulatory responses of gonadotrophin treated animals: Analyses of variance (Trial 5).

A) Ovulation rate			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1432	NS
Error	24	1.9737	
Total	25	2.1170	
B) Number of large follicles			
Source of variation	D.F.	S.S.(B)	Sig.
Age	1	4.6538	NS
Error	24	87.2307	
Total	25	91.8846	
C) Total ovarian response			
Source of variation	D.F.	S.S.(B)	Sig.
Age	1	44.4615	**
Error	24	231.3846	
Total	25	275.8461	

(A) = Transformed data, (B) = Untransformed data.

Appendix 3.24 Factors affecting the ovulation rate of gonadotrophin treated ewe lambs: Analysis of variance (Trial 6).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	1	8.8919	NS
Age	1	6.9704	NS
Treatment x Age	1	0.9604	NS
Error	107	1293.1840	
Total	110	1315.6396	

(B)= Untransformed data.

Appendix 3.25 Factors affecting the number of large follicles of gonadotrophin treated ewe lambs: Analysis of variance (Trial 6).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	1	2.6209	NS
Age	1	0.6400	NS
Treatment x Age	1	0.0057	NS
Error	107	1391.2706	
Total	110	1395.2972	

(B)= Untransformed data.

Appendixe 3.26 Factors affecting the total ovarian response of gonadotrophin treated ewe lambs: Analysis of variance (Trial 6).

Source of variation	D.F.	S.S.(B)	Sig.
Treatment	1	1.8577	NS
Age	1	3.3859	NS
Treatment x Age	1	0.8176	NS
Error	107	1157.3458	
Total	110	1165.4774	

(B)= Untransformed data.

Appendix 3.27 Factors affecting the recovery rate of eggs: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.0120	NS
Time of PMSG injection	1	0.2776	NS
Error	22	13.1747	
Total	24	13.4547	

(A) = Transformed data.

Appendix 3.28 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable. Analyses of variance (Trial 1).

A) Recovery rate of eggs			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	2.7191	**
Error	38	15.8991	
Total	39	18.6183	
B) Fertilization rate			
Age	1	1.7371	*
Error	28	11.7358	
Total	29	13.4730	
C) % of embryos transferable			
Age	1	0.0242	NS
Error	21	13.0637	
Total	22	13.0879	

(A) = Transformed data.

Appendix 3.29 Factors affecting fertilization rate: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.3919	NS
Time of PMSG injection	1	1.8724	NS
Error	12	5.1185	
Total	14	7.5666	

(A) = Transformed data.

Appendix 3.30 Factors affecting the percentage of embryos transferable: Analysis of variance (Trial 1).

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	1	0.6498	NS
Time of PMSG injection	1	1.5500	NS
Error	6	3.0649	
Total	8	4.9957	

(A) = Transformed data.

Appendix 3.31 Factors affecting the recovery rate of eggs: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	1	0.0399	NS
Time of PMSG injection	1	0.7275	NS
Source x Time	1	3.9037	***
Error	28	8.7018	
Total	31	13.1788	

(A) = Transformed data.

Appendix 3.32 Factors affecting fertilization rate: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	1	1.3114	NS
Time of PMSG injection	1	0.0748	NS
Source x Time	1	0.1899	NS
Error	18	10.8242	
Total	21	12.4242	

(A) = Transformed data.

Appendix 3.33 Factors affecting the percentage of transferable embryos: Analysis of variance (Trial 2).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	1	0.0589	NS
Time of PMSG injection	1	0.0072	NS
Error	10	6.7811	
Total	12	6.8432	

(A) = Transformed data.

Appendix 3.34 Factors affecting the recovery rate of eggs: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	0.4281	NS
GnRH	1	0.2790	NS
Group	1	0.2636	NS
Error	41	13.2177	
Total	46	14.2564	

(A) = Transformed data.

Appendix 3.35 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable. Analyses of variance (Trial 3).

A) Recovery rate of eggs			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	2.4813	**
Error	65	21.1820	
Total	66	23.6634	
B) Fertilization rate			
Age	1	0.0033	NS
Error	46	19.2795	
Total	47	19.2829	
C) % of embryos transferable			
Age	1	1.0439	**
Error	37	16.8530	
Total	38	17.8970	

(A) = Transformed data.

Appendix 3.36 Factors affecting fertilization rate: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	0.8137	NS
GnRH	1	0.2999	NS
Group	1	0.1661	NS
Error	25	10.5458	
Total	30	12.0815	

(A) = Transformed data.

Appendix 3.37 Factors affecting the percentage of embryos transferable: Analysis of variance (Trial 3).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	3	3.4292	**
GnRH	1	0.4624	NS
Group	1	3.1107	**
Error	20	5.4224	
Total	25	12.1436	

(A) = Transformed data.

Appendix 3.38 Factors affecting the recovery rate of eggs: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	2	1.3902	NS
Immunization	1	0.1979	NS
GnRH	1	0.0250	NS
Error	65	22.5475	
Total	69	24.2258	

(A) = Transformed data.

Appendix 3.39 Factors affecting fertilization rate: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	2	2.3584	NS
Immunization	1	0.8759	NS
GnRH	1	2.1439	*
PMSG x GnRH	2	6.0250	**
Error	29	11.2446	
Total	35	19.5846	

(A) = Transformed data.

Appendix 3.40 Factors affecting the percentage of embryos transferable: Analysis of variance (Trial 4).

Source of variation	D.F.	S.S.(A)	Sig.
Source of PMSG	2	0.1145	NS
Immunization	1	0.5912	NS
GnRH	1	0.0359	NS
Error	19	9.7678	
Total	23	10.6925	

(A) = Transformed data.

Appendix 3.41 Effect of gonadotrophin treatment on the recovery rate of eggs: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	2	0.1245	NS
Error	46	18.5918	
Total	48	18.7164	

(A) = Transformed data.

Appendix 3.42 Effect of age on the recovery rate of eggs, fertilization rate and the percentage of embryos transferable. Analyses of variance (Trial 5).

A) Recovery rate of eggs			
Source of variation	D.F.	S.S.(A)	Sig.
Age	1	0.1519	NS
Error	58	22.2657	
Total	59	22.4176	
B) Fertilization rate			
Age	1	0.0227	NS
Error	35	19.2541	
Total	36	19.2768	
C) % of embryos transferable			
Age	1	0.2411	NS
Error	24	13.5615	
Total	25	13.8026	

(A) = Transformed data.

Appendix 3.43 Factors affecting fertilization rate: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	2	1.9850	NS
Inseminator	1	0.1151	NS
Ram	1	0.7912	NS
Error	26	13.9415	
Total	30	16.6439	

(A) = Transformed data.

Appendix 3.44 Factors affecting the percentage of embryos transferable: Analysis of variance (Trial 5).

Source of variation	D.F.	S.S.(A)	Sig.
Treatment	2	0.0350	NS
Inseminator	1	0.0938	NS
Ram	1	0.4131	NS
Error	16	9.8782	
Total	20	10.6006	

(A) = Transformed data.

Appendix 3.45 Ovulatory responses of mature ewes treated with different sources of Massey-PMSG (Splitting; Study 1).

The study, conducted during August-September of 1988, included a group of 150 mixed-age sheep. The study evaluated the effects of three dose levels of Massey-PMSG (700 vs 1000 vs 1300 i.u.), five times of PMSG injection (1) at sponge removal; (2) 2 days before sponge removal; (3) 4 days before sponge removal; (4) half dose 2 days before sponge removal and half at sponge withdrawal and (5) half dose 4 days before sponge removal and half at sponge withdrawal and mare (source of Massey-PMSG) (1 vs 2 vs 3) on ovulation rate, the number of large follicles and total ovarian response. The average ovulatory responses for the three variables of response by dose-level of PMSG, time of PMSG injection and mare are given in Appendix 3.45a and for the interactions dose of PMSG by time of PMSG injection and time of PMSG injection by mare in Appendix 3.45b (appropriate analyses of variance are presented in Appendix 3.45c, 3.45d and 3.45e).

Ovulation rate.

Dose of PMSG.

Ovulation rate (averaged across other treatments) was significantly affected ($P<0.001$) by the dose of PMSG administered (Appendix 3.45c). Higher responses were observed following the injection of 1000 or 1300 i.u. of PMSG compared to the administration of 700 i.u. (3.36, 3.27 vs 1.20, respectively) (Appendix 3.45a). There was no significant difference in ovulation rate between sheep treated with 1000 or 1300 i.u. of PMSG.

Time of PMSG injection.

There was a significant effect ($P<0.01$) of time of PMSG injection (averaged across other treatments) on ovulation rate (Appendix 3.45c). There was no significant difference between sheep treated with a single injection of PMSG given 2 (Time 2) or 4 (Time 3) days before sponge removal (4.50 vs 3.42, respectively) and between those receiving PMSG at sponge removal (Time 1), 4 days before sponge removal (Time 3), a split dose half given 2 days before sponge removal and half at removal of the sponge (Time 4) or a divided dose half given 4 days before sponge removal and half at sponge removal (Time 5) (1.89 vs 3.42 vs 1.73 vs 1.74, respectively) (Appendix 3.45a). However, the ovulation rate was higher for sheep receiving a single injection of PMSG 2 days before

sponge removal compared to animals receiving a single PMSG injection at sponge removal, a split dose half given 2 days before sponge removal and the other half at sponge removal or a split dose half given 4 days before sponge removal and the other half at sponge removal (4.50 vs 1.89, 1.73, 1.74, respectively).

Mare.

Ovulation rate (averaged across other treatments) was significantly affected ($P<0.01$) according to which mare the PMSG come from. No significant difference was found between mares 1 and 2 (2.69 vs 3.54, respectively) and between mares 1 and 3 (2.69 vs 1.68, respectively). However, the responses were higher for mare 2 than mare 3 (3.54 vs 1.68, respectively).

Interactions.

Ovulation rate was significantly affected ($P<0.05$) by the interaction between dose of PMSG and time of PMSG injection (Appendix 3.45c). The time of PMSG injection did not significantly affect the ovulation rate when 700 i.u. of PMSG were administered. However, when 1000 i.u. were injected the responses were higher when PMSG was given at sponge removal, or 2 or 4 days before sponge removal, compared to when the dose levels of PMSG were divided. When the dose of PMSG was increased to 1300 i.u., higher responses were recorded when administering PMSG 2 days before sponge removal. No significant differences were observed between the other times of injection considered. In general, the highest responses were recorded following the administration of PMSG 2 or 4 days before sponge withdrawal.

Number of large follicles.

Dose of PMSG.

In the general analysis of variance, the effect of dose of PMSG was significant at $P=0.06$ (Appendix 3.45d), and the test of multiple comparison of means indicated a significant difference between them (averaged across other treatments). No significant difference in the number of large follicles was found when 700 or 1300 i.u. of PMSG were injected (0.17 vs 0.56, respectively) or between the administration of 700 and 1000 i.u. (0.17 vs 0.09, respectively) (Appendix 3.45a). However, the number of large follicles was higher when 1300 i.u. were administered compared to that when 1000 i.u. were used (0.56 vs 0.09, respectively).

Time of PMSG injection.

There was no significant effect of the time when PMSG was administered on the number of large follicles (Appendix 3.45d).

Mare.

The number of large follicles was significantly affected ($P<0.01$) by mare (averaged across other treatments) (Appendix 3.45d). Responses were not significantly different between mare 1 and mare 2 (0.31 vs 0.50, respectively) and between mares 1 and 3 (0.31 vs 0.05, respectively) (Appendix 3.45a). The responses were higher for mare 2 than for mare 3 (0.50 vs 0.05, respectively).

Interactions.

The time of PMSG injection x mare interaction affected the number of large follicles ($P<0.05$, Appendix 3.45d). The highest incidence of large follicles was recorded following the administration of PMSG from mare number 2 given 2 days before sponge removal (1.50). A similar response to that of mare 2 was observed when PMSG from mare number 1 was divided and half administered 2 days before sponge removal and the other half at sponge withdrawal (1.50 vs 1.14, respectively). Overall, administration of PMSG from mare number 3 tended to give the lowest incidence of large follicles.

Total ovarian response.

Dose of PMSG.

There was a significant effect ($P<0.001$) of dose of PMSG (averaged across other treatments) on the total ovarian response (Appendix 3.45e). The responses were higher when sheep were injected with 1000 or 1300 i.u. compared to the responses when they received 700 i.u. (3.45, 3.83 vs 1.37, respectively) (Appendix 3.45a). There was no significant difference in the response between animals receiving 1000 or 1300 i.u. of PMSG.

Time of PMSG injection.

Analysis of the data indicated a significant effect ($P<0.01$) of time of PMSG injection (averaged across other treatments) on the total ovarian response (Appendix 3.45e). Similar responses were recorded between animals that received a single injection of PMSG 2 (Time 2) or 4 (Time 3) days before sponge removal (4.96 vs 3.72, respectively)

and between those in which PMSG was given as a single injection at sponge removal (Time 1), 4 days before sponge removal (Time 3) or as a divided dose, half given 2 days before sponge removal and half at sponge withdrawal (Time 4) or when half of the dose was given 4 days before sponge removal and half at the time of sponge withdrawal (Time 5) (2.00 vs 3.72 vs 2.13 vs 1.96, respectively). The total ovarian response was higher when PMSG was given as a single injection 2 days before sponge removal compared to when it was given as a single injection at sponge removal, as a divided dose half given 2 days before sponge removal and the other half at sponge removal or when the dose was divided and half given 4 days before sponge removal and the other half at sponge removal (4.96 vs 2.00, 2.13, 1.96, respectively).

Mare.

Analysis of the results indicated a significant effect ($P<0.001$) of mare (averaged across other treatments) on the total ovarian response (Appendix 3.45e). Similar responses were recorded for mares 1 and 2, but their responses were higher when compared to the responses of mare 3 (3.00, 4.04 vs 1.73, respectively) (Appendix 3.45a).

Interactions.

Dose of PMSG by time of PMSG injection was the only significant interaction affecting the total ovarian response ($P<0.05$; Appendix 3.45e). The total ovarian response was not significantly affected by the time of PMSG injection when 700 i.u. of PMSG were administered. When 1000 i.u. were injected, the lower responses were recorded when the dose of PMSG was divided and these were not significantly different to the responses recorded when PMSG was given at sponge removal. The highest responses were observed when administering PMSG 2 or 4 days before sponge removal. The total ovarian responses following the administration of 1300 i.u. of PMSG were higher when given 2 days before sponge removal compared to those when PMSG was given at sponge withdrawal. Similar responses were recorded when PMSG was given 2 or 4 days before sponge withdrawal or when a divided dose of PMSG was given. The responses were also similar when PMSG was given at sponge removal, 4 days before sponge withdrawal or when the dose of PMSG was divided. Overall, there was a tendency for the responses to be higher when PMSG was given 2 days before sponge withdrawal followed by its administration 4 days before sponge removal.

Appendix 3.45a Ovulatory responses (Mean ± s.e.m.) from adult ewes by Dose of PMSG, Time of injection and Mare.

Dose of PMSG	No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
700	40	1.20 ± 0.13	a ***	0.17 ± 0.09	NS	1.37 ± 0.18	a ***
1000	42	3.36 ± 0.65	b	0.09 ± 0.07		3.45 ± 0.69	b
1300	48	3.27 ± 0.39	b	0.56 ± 0.19		3.83 ± 0.45	b
Time of injection							
1	28	1.89 ± 0.40	a **	0.11 ± 0.07	NS	2.00 ± 0.43	a **
2	26	4.50 ± 0.81	b	0.46 ± 0.22		4.96 ± 0.95	b
3	26	3.42 ± 0.76	ab	0.30 ± 0.19		3.72 ± 0.76	ab
4	23	1.73 ± 0.36	a	0.39 ± 0.30		2.13 ± 0.43	a
5	27	1.74 ± 0.29	a	0.22 ± 0.11		1.96 ± 0.35	a
Mare							
1	45	2.69 ± 0.41	ab **	0.31 ± 0.17	ab **	3.00 ± 0.42	a ***
2	44	3.54 ± 0.60	a	0.50 ± 0.16	a	4.04 ± 0.68	a
3	41	1.68 ± 0.27	b	0.05 ± 0.04	b	1.73 ± 0.27	b

Times of injection (1; at sponge removal, 2; 2 days before sponge removal, 3; 4 days before sponge removal, 4; half dose 2 days before and half at sponge removal, 5; half dose 4 days before and half at sponge removal).

Appendix 3.45b Effect of the interactions Dose of PMSG by Time of injection (Dose x Time), and Time of injection by Mare (Time x Mare) on the ovulatory responses (Mean ± s.e.m.) †.

Dose x Time		No. of animals	Ovulation Rate	Sig.	Large Follicles	Sig.	Total Response	Sig.
700	1	10	1.00 ± 0.14	*	0.00 ± 0.00	NS	1.00 ± 0.14	*
700	2	8	1.75 ± 0.52		0.25 ± 0.25		2.00 ± 0.70	
700	3	6	0.83 ± 0.16		0.66 ± 0.49		1.50 ± 0.56	
700	4	8	1.00 ± 0.18		0.00 ± 0.00		1.00 ± 0.18	
700	5	8	1.38 ± 0.26		0.12 ± 0.12		1.50 ± 1.15	
1000	1	8	3.12 ± 1.15		0.00 ± 0.00		3.12 ± 0.32	
1000	2	8	6.00 ± 1.97		0.37 ± 0.37		6.37 ± 2.30	
1000	3	10	5.20 ± 1.64		0.00 ± 0.00		5.20 ± 1.64	
1000	4	6	1.00 ± 0.44		0.00 ± 0.00		1.00 ± 0.44	
1000	5	10	1.00 ± 0.29		0.10 ± 0.10		1.10 ± 0.31	
1300	1	10	1.80 ± 0.57		0.30 ± 0.21		2.10 ± 0.69	
1300	2	10	5.50 ± 1.08		0.70 ± 0.47		6.20 ± 1.37	
1300	3	10	3.20 ± 0.85		0.40 ± 0.40		3.60 ± 0.93	
1300	4	9	2.88 ± 0.75		1.00 ± 0.76		3.88 ± 0.75	
1300	5	9	2.88 ± 0.65		0.44 ± 0.29		3.32 ± 0.83	
1	1	9	2.11 ± 1.05	NS	0.00 ± 0.00	*	2.11 ± 1.05	NS
1	2	10	2.30 ± 0.65		0.10 ± 0.10		2.40 ± 0.71	
1	3	9	1.22 ± 0.14		0.22 ± 0.22		1.44 ± 0.33	
2	1	10	3.40 ± 0.79		0.00 ± 0.00		3.40 ± 0.79	
2	2	8	7.62 ± 1.93		1.50 ± 0.59		9.12 ± 2.27	
2	3	8	2.75 ± 0.92		0.00 ± 0.00		2.75 ± 0.92	
3	1	9	4.67 ± 1.22		0.33 ± 0.33		5.00 ± 1.14	
3	2	8	4.25 ± 1.90		0.62 ± 0.49		4.87 ± 1.89	
3	3	9	1.44 ± 0.47		0.00 ± 0.00		1.44 ± 0.47	
4	1	7	1.42 ± 0.64		1.14 ± 0.98		2.56 ± 1.04	
4	2	9	1.66 ± 0.47		0.11 ± 0.11		1.77 ± 0.43	
4	3	7	2.14 ± 0.88		0.00 ± 0.00		2.14 ± 0.88	
5	1	10	1.60 ± 0.33		0.30 ± 0.21		1.90 ± 0.45	
5	2	9	2.55 ± 0.68		0.33 ± 0.23		2.88 ± 0.82	
5	3	8	1.00 ± 0.32		0.00 ± 0.00		1.00 ± 0.32	

† See Appendix 3.45a for description of the times of injection.

Appendix 3.45c. Factors affecting the ovulation rate of PMSG treated (Massey-PMSG) adult ewes: Analysis of variance.

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	2	1.1145	***
Time of injection	4	1.1111	**
Mare	2	0.7455	**
Dose x Time	8	1.1700	*
Dose x Mare	4	0.1135	NS
Time x Mare	8	0.7083	NS
Error	101	7.0131	
Total	129	12.0173	

(A) = Transformed data.

Appendix 3.45d Factors affecting the number of large follicles of PMSG treated (Massey-PMSG) adult ewes: Analysis of variance.

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	2	0.1495	NS (b)
Time of injection	4	0.0746	NS
Mare	2	0.2170	**
Dose x Time	8	0.1673	NS
Dose x Mare	4	0.0681	NS
Time x Mare	8	0.4844	*
Error	101	2.6913	
Total	129	3.8637	

(A) = Transformed data.
(b) = P = 0.06.

Appendix 3.45e Factors affecting the total ovarian response of PMSG treated (Massey-PMSG) adult ewes: Analysis of variance.

Source of variation	D.F.	S.S.(A)	Sig.
Dose of PMSG	2	1.3123	***
Time of injection	4	1.0716	**
Mare	2	1.0190	***
Dose x Time	8	1.2283	*
Dose x Mare	4	0.0780	NS
Time x Mare	8	0.8961	NS
Error	101	7.1434	
Total	129	12.7713	

(A) = Transformed data.

REFERENCES

REFERENCES †

Acritopoulou, S. & Haresign, W. (1980). Response of ewes to a single injection of an analogue of PGF-2∝ given at different stages of the oestrous cycle. *J. Reprod. Fert.*, **58**:219-223.

Akbar, A.M., Nett, T.M. & Niswender, G.D. (1974). Metabolic clearance and secretion rates of gonadotropins at different stages of the oestrous cycle in ewes. *Endocrinology*, **94**:1318-1324.

Allen, W.R. (1969). The immunological measurement of pregnant mare serum gonadotropin. *J. Endocr.*, **43**:593-598.

Allen, W.R. (1982). Immunological aspects of the endometrial cup reaction and the effect of xenogeneic pregnancy in horses and donkeys. *J. Reprod. Fert. Suppl.*, **31**:57-94.

Allen, W.R. & Moor, R.M. (1972). The origin of equine endometrial cups. I. Production of PMSG by fetal trophoblast cells. *J. Reprod. Fert.*, **29**:313-316.

Allison, A.J. (1967). *The Effect of Liveweight on Ewe Fertility*. Master of Agricultural Science thesis. Lincoln College, New Zealand, 137pp.

Allison, A.J. (1975). Effect of nutritionally induced live-weight differences on the ovarian response and fertility in ewes treated with pregnant mare's serum gonadotrophin. *N. Z. J. Agric. Res.*, **18**:101-107.

Allison, A.J. & Robinson, T.J. (1970). The effect of dose level of intravaginal progestagen on sperm transport, fertilization and lambing in the cyclic Merino ewe. *J. Reprod. Fert.*, **22**:515-531.

Almeida, A.P. (1987). Superovulation in cattle: A combined treatment using syncromate B with either pregnant mare serum gonadotrophin or follicle stimulating hormone. *Theriogenology*, **27**:329-335.

Alwan, S.F., Boland, M.P. & Gordon, I. (1988). Superovulation and oocyte recovery in the ewe. *Theriogenology*, **29**:1143-1148.

Arave, C.W., Bunch, T.D., Mickelsen, C.H. & Warnick, K. (1987). Factors affecting survivability of transferred whole and demi-embryos in a commercial dairy herd. *Theriogenology*, **28**:373-382.

Armstrong, D.T., Miller, B.G., Walton, E.A., Pfitzner, A.P. & Warnes, G.M. (1982). Endocrine responses and factors which limit the response of follicles to PMSG and FSH. In: *Embryo Transfer in Cattle, Sheep and Goats*, pp.8-15. Eds. J.N. Shelton, A.O. Trounson, N.W. Moore & J.W. James. Aust. Soc. Reprod. Biol. Publication.

† An alphabetical list of the abbreviated journals is given after the references.

Armstrong, D.T. & Evans, G. (1983). Factors influencing success of embryo transfer in sheep and goats. *Theriogenology*, **19**:31-42.

Armstrong, D.T., Pfitzner, A.P., Warnes, G.M. & Seamark, R.F. (1983). Superovulation treatments and embryo transfer in Angora goats. *J. Reprod. Fert.*, **67**:403-410.

Armstrong, D.T. & Evans, G. (1984a). Hormonal regulation of reproduction : Induction of ovulation in sheep and goats with FSH preparations. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination*, Illinois, **4**:VII8-VII15.

Armstrong, D.T. & Evans, G. (1984b). Intrauterine insemination enhances fertility of frozen semen in superovulated ewes. *J. Reprod. Fert.*, **71**:89-94.

Ashworth, C.J., Sales, D.I. & Wilmut, I. (1984). Patterns of progesterone secretion and embryonic survival during repeated pregnancies in Damline ewes. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination*, Illinois, **2**:74 Abstr.

Averill, R.L.W. (1956). The transfer and storage of sheep ova. *Proceedings of the 3rd International Congress on Animal Reproduction and Artificial Insemination*, Cambridge, **3**:7-9.

Averill, R.L.W. (1958). The production of living sheep eggs. *J. Agric. Sci. Camb.*, **50**:17-33.

Averill, R.L.W. & Rowson, L.E.A. (1958). Ovum transfer in the sheep. *J. Endocr.*, **16**:326-336.

Baker, R.D. & Shea, B.E. (1985). Commercial splitting of bovine embryos. *Theriogenology*, **23**:3-12.

Barry, D.M., Van Niekerk, C.H., Coetzer, W.A. & Robertson, M.S. (1988). Superovulatory response and time of ovulation in sheep treated with FSH-p or PMSG followed by GnRH or hCG. *Theriogenology*, **29**:217 Abstr.

Beehan, D. & Sreenan, J.M. (1977). Factors affecting superovulation response to PMSG in the cow. Proc. Soc. Study Fert. 11-15. Dublin. (cited by Sreenan et al. 1978).

Betteridge, K.J. & Moore, N.W. (1977). Techniques and results in sheep and goats. In: *Embryo Transfer in Farm Animals*, pp. 37-37. Ed. K.J. Betteridge. Canada Department of Agriculture, Monograph 16, Ottawa, Canada.

Betteridge, K.J., Sugden, E.A., Eaglesome, M.D., Randall, G.C.B. & Mitchell, D. (1981/82). Plasma progesterone levels in superovulated cattle in relation to hatched embryo sizes, ovulation rates, and premature regression of corpora lutea. *Anim. Reprod. Sci.*, **4**:207-212.

Bindon, B.M. (1984). Reproductive biology of the Booroola Merino sheep. *Aust. J. Biol. Sci.*, **37**:163-189.

Bindon, B.M., Chang, T.S. & Turner, H.N. (1971). Ovarian response to gonadotrophin by Merino ewes selected for fecundity. *Aust. J. Agric. Res.*, **22**:809-820.

Bindon, B.M. & Pennycuik, P.R. (1974). Differences in ovarian sensitivity of mice selected for fecundity. *J. Reprod. Fert.*, **36**:221-224.

Bindon, B.M. & Turner, H.N. (1974). Plasma LH of the pre-pubertal lamb: A possible early indicator of fertility. *J. Reprod. Fert.*, **39**:85-88.

Bindon, B.M. & Piper, L.R. (1982a). Physiological basis of the ovarian response to PMSG in sheep and cattle. In: *Embryo Transfer in Cattle, Sheep and Goats*, pp. 1-15. Eds. J.N. Shelton, A.O. Trounson, N.W. Moore & J.W. James. Australian Society for Reproductive Biology Publication.

Bindon, B.M. & Piper, L.R. (1982b). Physiological characteristcs of high fecundity sheep and cattle. *Proceedings of the World Congress on Sheep and Beef Cattle Breeding. Volume I, Technical*, pp. 315-331. Eds. R. A. Barton & W. C. Smith. The Dunmore Press, Palmerston North, New Zealand.

Bindon, B.M. & Piper, L.R. (1986). Unpublished study (cited by Bindon et al., 1986).

Bindon, B.M, Piper, L.R., Cahill, I.P., Driancourt, M.A. & O'shea, T. (1986). Genetic and hormonal factors affecting superovulation. *Theriogenology*, **25**:53-70.

Boland, M.P. (1973). *Studies Related to Egg Transfer in Sheep*. Ph. D. thesis. N.U.I. Dublin. (cited by I. Gordon, 1975).

Boland, M.P. & Gordon, I. (1978). Recovery and fertilization of eggs following natural service and uterine insemination in the Galway ewe. *Ir. Vet. J.*, **32**:123-125.

Boland, M.P., Murray, J.D., Scaramuzzi, R.J., Sutton, R., Hoskinson, R.M., Hazelton, I.G. & Nancarrow, C.D. (1984). Reproductive wastage and chromosomal abnormalities in early embryos from androstenedione-immune and control Merino ewes. In : *Reproduction in Sheep*, pp. 137-139. Eds. D.R. Lindsay & D.T. Pearce. Cambridge University Press.

Boland, M.P., Murray, J.D., Hoskinson, R.M., Hazelton, I.G., Sutton, R. & Nancarrow, C.D. (1985). Ovarian response to PMSG treatment in ewes immunized against Oestradiol-17β. *Aust. J. Biol. Sci.*, **38**:339-345.

Boland, M.P., Nancarrow, C.D., Murray, J.D., Scaramuzzi, R.J., Sutton, R., Hoskinson, R.M. & Hazelton, I.G. (1986). Fertilization and early embryonic development in androstenedione-immune Merino ewes. *J. Reprod. Fert.*, **78**:423-431.

Bondurant, R.H. (1986). Embryo transfer in sheep and goats. In: *Current Therapy in Theriogenology*, pp. 63-66. Ed. D. A. Morrow. Saunders Company.

Booth, W.D., Newcomb, R., Strange, H., Rowson, L.E.A. & Sacher, H.B. (1975). Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. *Vet. Rec.*, **97**:366-369.

Braden, A.W.H. (1964). The incidence of morphologically abnormal ova in sheep. *Aust. J. Biol. Sci.*, **17**:499-503.

Braden, A.W.H., Lamond, D.R. & Radford, H.M. (1960). The control of the time of ovulation in sheep. *Aust. J. Agric. Res.*, **11**:389-401.

Bradford, G.E., Quirke, J.F. & Hart, R. (1971). Natural and induced ovulation rate of Finnish Landrace and other breeds of sheep. *Anim. Prod.*, **13**:627-635.

Bronson, R.A. & McLaren, A. (1970). Transfer to the mouse oviduct of eggs with and without the zona pellucida. *J. Reprod. Fert.*, **22**:129-137.

Buckrell, B.C., Gartley, C.J., Mehren, K.G. & Goodrowe, K.L. (1990). Superovulation in Dall's sheep (*Ovis dalli dalli*). *Theriogenology*, **33**:201 Abstr.

Cahill, L.P. (1982). Factors influencing the follicular response of animals to PMSG. In: *Embryo Transfer in Cattle, Sheep and Goats*, pp. 5-7. Ed. The Australian Society for Reproductive Biology.

Campbell, B.K., Scaramuzzi, R.J., & Downing, J.A. (1987). FSH, LH and progesterone levels in androstenedione-immune ewes. *Proc. Endocr. Soc. Aust.*, **30**:37 Abstr.

Callesen, H., Greve, T. & Hyttel, P. (1986). Preovulatory endocrinology and oocyte maturation in superovulated cattle. *Theriogenology*, **25**:71-86.

Chesne, P., Colas, G., Cognie, Y., Guerin, Y. & Sevellec, C. (1987). Lamb production using superovulation, embryo bisection and transfer. *Theriogenology*, **27**:751-757.

Chun, B.S. (1987). *A Study on the Breeding Performance of Romney and Border Leicester cross Romney Ewe Lambs after CIDR Treatment*. Master of Agricultural Science thesis. Massey University, Palmerston North, New Zealand, 123pp.

Chupin, D., Combarnous, Y. & Procureur, R. (1984). Antagonistic effect of LH on FSH-induced superovulation in cattle. *Theriogenology*, **21**:229 Abstr.

Christie, W., Newcomb, R. & Rowson, L.E.A. (1979). Embryo survival in heifers after transfer of an egg to the uterine horn contralateral to the corpus luteum and the effect of treatments with progesterone or hCG on pregnancy rates. *J. Reprod. Fert.*, **56**:701-706.

Christie, W., Newcomb, R. & Rowson, L.E.A. (1980). Non-surgical transfer of bovine eggs: investigation of some factors affecting embryo survival. *Vet. Rec.*, **106**:190-193.

Clarke, I.J. (1973). *A study of the ovarian response of New Zealand Romney ewes sequentially superovulated with Pregnant Mare Serum Gonadotrophin*. Master of Agricultural Science thesis, Massey University, Palmerston North, New Zealand, 106pp.

Clarke, J.N., Roberts, E.M., Carter, A.H. & Kirton, A.H. (1966). Hormonal synchronization of oestrus in Romney ewes during the breeding season. *Proc. N. Z. Soc. Anim. Prod.*, **26**:107-121.

Clarke, I.J. & Cummins, J.T. (1982). The temporal relationship between gonadotrophin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology*, **111**:1737-1739.

Clegg, M.T., Cole, H.H., Howard, C.B. & Pigon, H. (1962). Influence of fetal genotype on equine chorionic gonadotrophin secretion. *J. Endocr.*, **15**:245-248.

Cognie, Y. (1987). Unpublished study (cited by Scaramuzzi et al., 1987).

Cognie, Y. (1990). Current techniques for synchronization and artificial insemination of sheep. In: *Reproductive Physiology of Merino Sheep*, pp. 207-216. Ed. The University of Western Australia, Nedlands.

Cognie, Y., Mariana, J-C. & Thimonier, J. (1970). Etude du moment d'ovulation chez la brebis normale ou traitee par un progestagene associe ou non a une injection de PMSG. *Ann. Biol. Anim. Bioch. Biophys.*, **10**:15-24.

Cognie, Y. & Mauleon, P. (1983). Control of reproduction in the ewe. In: *Sheep Reproduction*, pp. 381-392. Ed. W. Haresign. Butterworths, London.

Cognie, Y., Chupin, D. & Saumande, J. (1986). The effect of modifying the FSH/LH ratio during the superovulatory treatment in ewes. *Theriogenology*, **25**:148 Abstr.

Cole, H.H. & Hart, G.H. (1930). The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturity of the immature rat. *Am. J. Physiol.*, **93**;57-68.

Cox, R.I., Wilson, P.A., Scaramuzzi, R.J., Hoskinson, R.M., George, J.M., & Bindon, B.M. (1982). The active immunization of sheep against oestrone, androstenedione or testosterone to increase twinning. *Proc. Aust. Soc. Anim. Prod.*, **14**:511-514.

Crister, J.K., Rowe, R.F., Del Campo, M.R. & Ginther, O.J. (1980). Embryo transfer in cattle: Factors affecting superovulatory response, number of transferable embryos, and length of post-treatment estrous cycles. *Theriogenology*, **13**:397-406.

Crosby, F.F., Boland, M.P., El-Kamali, A.A. & Gordon, I. (1980). Superovulation in the ewe using HAP. *Theriogenology.*, **13**:92 Abstr.

Cumming, I.A. (1965). *A Study of Ovulation and Early Prenatal Mortality in the New Zealand Romney Ewes.* Master of Agricultural Science thesis, Massey University, Palmerston North, New Zealand, 97pp.

Cumming, I.A. & McDonald, M.F. (1967). The production of ova by New Zealand Romney ewes following hormonal stimulation. *N. Z. J. Agric. Res.*, **10**:226-236.

Cumming, I.A. & McDonald, M.F. (1970). Embryo survival in mature Romney ewes relative to live weight and face cover. *N. Z. J. Agri. Res.*, **13**:372-384.

Dattena, M. (1989). *Multiplication of Five Breeds of "Exotic" Sheep in New Zealand Using the Technique of Embryo Transplantation.* Master of Philosophy thesis, Massey University, Palmerton North, New Zealand, 99pp.

Day, F.T. & Rowlands, I.W. (1945). Serum gonadotrophin in Welsh and Shetland ponies. *J. Endocr.*, **5**:1-8.

De Armas, R., Solano, R., Caral, J., Alcala, J. & Padlal, C. (1986). Cultivo in vitro de embriones micromanipulados. *Rvta. Cub. Cienc. Vet.*, **17(3&4)**:103-108.

Del Campo, M.R., Rowe, R.F. & Ginther, O.J. (1979). Relationship between side of embryo and transfer and pregnancy rate in cattle. *Proceedings of the 71st Annual Meeting of the American Society of Animal Science.* p.290 Abstr.

Dieleman, S.J., Bevers, M.M. & Gielen, J.T. (1987). Increase of the number of ovulations in PMSG/PG treated cows by administration of monoclonal anti-PMSG shortly after the endogenous LH peak. *Theriogenology*, **27**:222 Abstr.

Domanski, E., Przekop, F. & Polkowska, J. (1980). Hypothalamic centres involved in the control of gonadotropin secretion. *J. Reprod. Fert.*, **58**:493-499.

Donaldson, L.E. (1985a). Effect of insemination regimen on embryo production in superovulated cows. *Vet. Rec.*, **117**:35-36.

Donaldson, L.E. (1985b). LH and FSH profiles at superovulation and embryo production in the cow. *Theriogenology*, **23**:441 Abstr.

Donaldson, L.E. (1990). FSH-P batch variation. *Theriogenology*, **33**:215 Abstr.

Donaldson, L.E. & Ward, D.N. (1985). Superovulation in cattle: dose response to FSH-W with and without LH contamination. *Theriogenology*, **23**:189 Abstr.

Donaldson, L.E. & Ward, D.N. (1986). Effect of luteinizing hormone on embryo production in superovulated cows. *Vet. Rec.*, 119:625-626.

Douglas, R.H. & Ginther, O. (1973). Luteolysis following a single injection of prostaglandin $F_{2\alpha}$ in sheep. *J. Anim. Sci.*, **37**:990-993.

Driancourt, M.A. (1991). Follicular dynamics in sheep and cattle. *Theriogenology*, **35**:56-79.

Driancourt, M.A., Jego, Y., Cahill,L.P. & Bindon, B.M. (1985). Follicle population dynamics in sheep with different ovulation rate potentials. *Livest. Prod. Sci.*, **13**:21-33.

Driancourt, M.A. & Fry, R.C. (1988). Differentiation of ovulatory follicles in sheep. *J. Anim. Sci. Suppl.*2, **66**:9-20.

Du Mesnil Du Buisson, F., Renard, J.P. & Levasseur, M.C. (1977). Factors influencing the quality of ova and embryos. In: *Embryo Transfer in Farm Animals*, pp.24-26. Ed. K.J. Betteridge. Canada Department of Agriculture, Monograph 16, Ottawa, Canada.

Dutt, R.H. (1954). Fertility rate and embryonic death loss in ewes early in the breeding season. *J. Anim. Sci.*, **13**:464-473.

Dyrmundsson, O.R. (1972). *Studies on the Attainment of Puberty and Reproductive Performance in Clun Forest Ewe and Ram Lambs.* Ph. D. thesis, University of Wales.

Dyrmundsson, O.R. (1973). Puberty and early reproductive performance in sheep. 1. Ewe lambs. *Anim. Breed. Abstr.*,41:273-289.

Dyrmundsson, O.R. & Lees, J.L. (1982). Attainment of puberty and reproductive performance in Clun Forest ewe lambs. *J. Agric. Sci. Camb.*, **78**:39-45.

Eastwood, K.C. & McDonald, M.F. (1975). Reproductive differences in two- year-old Romney and Border Leicester x Romney ewes after gonadotrophin treatment. *N. Z. J. Agric. Res.*, **18**:109-117.

Echternkamp, S.E. & Laster, D.B. (1976). Plasma LH concentrations for prepubertal, postpubertal anestrous, and cyclic ewes of varying fecundity. *J. Anim. Sci.*, **42**:444-447.

Elsden, R.P., Nelson, L.D. & Seidel, G.E.Jr. (1978). Superovulating cows with follicle stimulating hormone and pregnant mare's serum gonadotrophin. *Theriogenology*, **9**:17-26.

Eppleston, J., Nancarrow, C.D., Battye, K., Hoskinson, R.M. & Roberts, E.M. (1988). Effect of Anti-oestradiol-17β antibodies on the reproductive response of ewes superovulated with PMSG. *Aust. J. Biol. Sci.*, **41**:441-446.

Evans, G. & Robinson, T.J. (1980). The control of fertility in sheep: Endocrine and ovarian responses to progestagen-PMSG treatment in the breeding season and in anoestrus. *J. Agric. Sci. Camb.*, **94**:69-88.

Evans, G. & Armstrong, D.T. (1984a). Reduction of sperm transport in ewes by superovulation treatments. *J. Reprod. Fert.*, **70**:47-53.

Evans, G., Holland, M.K., Nottle, H.B., Sharpe, P.H. & Armstrong, D.T. (1984). Production of embryo in sheep using FSH preparations and laparoscopic intrauterine insemination. In: *Reproduction in Sheep*, pp. 313-315. Eds. D.R. Lindsay & D.T. Pearce. Cambridge University Press.

Finnie, A.D., Lamming, G.E., Brooks, A.N. & Haynes, N.B. (1985). Opioid and dopamine involvement in LH suppression in prepubertal lambs. Program of the 1985 Winter Meeting of the Society for the Study of Fertility, December 1985, London (abstract 53).

Fitzgerald, J. & Butler, W.R. (1982). Seasonal effects and hormonal patterns related to puberty in ewe lambs. *Biol. Reprod.*, **27**:853-863.

Fitzgerald, J., Michel, F. & Butler, W.R. (1982). Growth and sexual maturation in ewes: Dietary and seasonal effects modulating luteinizing hormone secretion and first ovulation. *Biol. Reprod.*, **27**:864-870.

Fitzgerald, J.A. (1984). The effect of castration, estradiol, and LHRH on LH secretion of lambs fed different levels of dietary energy. *J. Anim. Sci.*, **59**:460-469.

Foster, D.L. (1981). Mechanism for delay of first ovulation in lambs born in the wrong season (fall). *Biol. Reprod.*, **25**:85-92.

Foster, D.L. (1988). Puberty in the female sheep. In : *The Physiology of Reproduction*, Volume 2, pp. 1739-1762. Eds. E. Knobil & J.D. Neill. Raven Press Ltd, New York.

Foster, D.L. & Ryan, K.D. (1979). Endocrine mechanisms governing transition into adulthood: A marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in the lamb during puberty. *Endocrinology*, **105**:896-904.

Foster, D.L., Jaffe, R.B. & Niswender, G.D. (1975a). Sequential patterns of circulating LH and FSH in female sheep during the early postnatal period: Effect of gonadectomy. *Endocrinology*, **96**:15-22.

Foster, D.L., Lemons, J.A., Jaffe, R.B. & Niswender, G.D. (1975b). Sequential patterns of circulating luteinizing hormone and follicle-stimulating hormone in female sheep from early post-natal life through the first estrous cycles. *Endocrinology*, **97**:985-994.

Foster, D.L. & Olster, D.H. (1985). Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. *Endocrinology*, **116**:375-381.

Foster, D.L. & Yellon, S.M. (1985). Photoperiodic time measurement is maintained in undernourished lambs with delayed puberty. *J. Reprod. Fert.*, **75**:203-208.

Foster, D.L., Yellon, S.M. & Olster, D.H. (1985). Internal and external determinants of the timing of puberty in the female. *J. Reprod. Fert.*, **75**:327-344.

Foster, D.L., Karsch, F.J., Olster, D.H., Ryan, K.D. & Yellon, S.M. (1986). Determinants of puberty in a seasonal breeder. *Recent Prog. Horm. Res.*, **42**:331-384.

Foster, D.L. & Ryan, K.D. (1987). Puberty in the lamb: Sexual maturation of a seasonal breeder in a changing environment. In: *The Control of the Onset of Puberty*, II. Eds. P.C. Sizonenko, M.L. Aubert, & M.M. Grumbach. Williams & Wilkins, Baltimore. (cited by D.L. Foster, 1988).

Gatica, R., Boland, M.P., Crosby, T.F. & Gordon, I. (1984). Micromanipulation of sheep morulae to produce monozygotic twins. *Theriogenology*, **21**:555-560.

Gherardi, P.B. & Martin, G.B. (1978). The effect of multiple injections of Pregnant Mare's Serum on the ovarian activity of Merino ewes. *Proc. Aust. Soc. Anim. Prod.*, **12**:260 Abstr.

Gherardi, P.B. & Lindsay, D.R. (1980). The effect of season on the ovulatory response of Merino ewes to serum from pregnant mares. *J. Reprod. Fert.*, **60**:425-429.

Gibb, M., Thurley, D.C., McNatty, K.P. (1981). Active immunization of Romney ewes with an androstenedione-protein conjugate. *N. Z. J. Agric. Res.*, **24**:5-9.

Gilmour, A.R. (1985). REG: A Generalized Linear Models Programme. Miscellaneous Bulletin No. 1, Division of Agricultural Services, NSW Department of Agriculture, NSW, Australia.

Gomez, K.A. & Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research.* Second edition. Wiley, New York. 680pp.

Gonzalez-Menclo, F., Manns, J. & Murphy, D.B. (1978). FSH and LH activity of PMSG from mares at different stages of gestation. *Anim. Reprod. Sci.*, **1**:137-144.

Gordon, I. (1958). The use of progesterone and serum gonadotrophin (P.M.S.) in the control of fertility of the sheep. I. The hormonal augmentation of fertility in the ewe during the breeding season. *J. Agric. Sci. Camb.*, **50**:123-151.

Gordon, I. (1983). Embryo transfer in sheep. In: *Controlled Breeding in Farm Animals*, pp. 265-268. Ed. I. Gordon. Pergamon Press, U.K.

Gordon, I., Williams, G., & Edwards, J. (1962). The use of serum gonadotrophin (P.M.S.) in the induction of twin-pregnancy in the cow. *J. Agric. Sci Camb.*, **59**:143-198.

Greve, T., Callesen, H. & Hyttel, P. (1983). Endocrine profiles and egg quality in the superovulated cow. *Nord. Vet. Med.*, **35**:408-421.

Greve, T., Callesen, H. & Hyttel, P. (1984a). Characterization of plasma LH-profiles in superovulated dairy cows. *Theriogenology*, **21**:237 Abstr.

Greve, T., Callesen, H. & Hyttel, P. (1984b). Plasma progesterone profiles and embryo quality in superovulated dairy cows. *Theriogenology*, **21**:238 Abstr.

Guilbault, L.A., Roy, G.L., Grasso, F., Menard, D.P. & Bousquet, D. (1988). Ovarian follicular dynamics in superovulated heifers pretreated with FSH-P at the beginning of the estrous cycle. An ultrasonographic approach. *Theriogenology*, **29**:257 Abstr.

Hafez, E.S.E. (1952). Studies on the breeding season and reproduction of the ewe. *J. Agric. Sci.Camb.*, **42**:189-265.

Hafez, E.S.E. (1953). Puberty in female farm animals. *Emp. J. Exp. Agric.*, **21**:217-225.

Hammond, J.Jr. (1944). On the breeding season in the sheep. *J. Agric. Sci. Camb.*, **34**:97-105.

Hammond, J.Jr., Hammond, J. & Parkes, A.S. (1942). Hormonal augmentation of fertility in sheep. 1. Induction of ovulation and superovulation and heat in sheep. *J. Agric. Sci. Camb.*, **32**:308-323.

Hancock, J.L. & Hovell, G.J.R. (1961). Transfer of sheep ova. *J. Reprod. Fert.*, **2**:295-306.

Hancock, J.L. (1962). Fertilization in farm animals. *Anim. Breed. Abstr.*, **30**:285-310.

Hanrahan, J.P. (1980). Animal production in Australia. *Proc. Aust. Soc. Anim. Prod.*, **13**:405-408.

Hanrahan, J.P. & Quirke, J.F. (1982). Selection on ovulation rate in sheep aided by the use of superovulation and egg transfer. *Proceedings of the 3rd World Conference on Sheep and Beef Cattle Breeding*, **2**:329-335.

Hart, D.S. (1956). Sources of loss in the unfertilised and fertilised sheep's ova. *Proc. N. Z. Soc. Anim. Prod.*, **16**:101-104.

Harvey, T.G., Johnson, D.L., Tervit, H.R. & Welch, R.A.S. (1984). Synchronization and artificial insemination of ewes - techniques which have possible commercial application. *Proc. N. Z. Soc. Anim. Prod.*, **44**:7-9.

Heyman, Y. (1985). Factors affecting the survival of whole and half-embryos transferred in cattle. *Theriogenology*, **23**:63-65.

Heyman, Y. & Renard, J.P. (1978). Production de jumeaux par transplantation d'embryons chez les bovins de races a viande. *Annls. Med. Vet.*, **122**:157-163.

Holst, P.J. (1969). The ovarian response to pregnant mare serum (PMS) administered after intravaginal progestagen treatment of cyclic and anoestrous ewes. *Aust. J. Agric. Res.*, **20**:1143-1150.

Hope, R.W. & Bavister, B.D. (1983). Effect of removing the zona pellucida on development of hamster and bovine embryos *in vitro* and *in vivo*. *Theriogenology*, **19**:391 Abstr.

Hoskinson, R.M., Hinks, N.T. & Scaramuzzi, R.J. (1982). Effect of PMSG on ovulation rate in the oestrone immunized ewe. *Proc. Aust. Soc. Reprod. Biol.*, **14**:93 Abstr.

Hsu, Y. & Gonda, M.A. (1980). Monozygotic twin formation in mouse embryos in vitro. *Science*, **209**:605-606.

Huffman, L.J. & Goodman, R.L. (1985). LH pulse patterns leading to puberty in the ewe lamb. *Biol. Reprod.32 Suppl.*, **1**:342 Abstr.

Humphrey, W.D., Murphy, B.D., Reiger, D., Mapletoft, R.J., Manns,J.G. & Fretz, P.G. (1979). Effects of FSH/LH ratio of PMSG on ovulatory responses. *Theriogenology*, **11**:101 Abstr.

Hunter, G.L., Adams, C.E., & Rowson, L.E. (1955). Inter-breed ovum transfer in sheep. *J. Agric. Sci. Camb.*, **46**:143-149.

Hunton, J.R., Carric, M., Sawyer, G. & Donnelly, P. (1989). Does FSH source and priming influence cattle embryo yield? *Proc. Aust. Soc. Reprod. Biol.*, **21**:49 Abstr.

Jabbour, H.N. & Evans, G. (1987). Superovulatory response of Merino ewes treated with PMSG and GnRH or PMSG antiserum. *Proc. Aust. Soc. Reprod. Biol.*, **20**:36 Abstr.

Jabbour, H.N., Evans, G. & Moore, N.W. (1988). Fertilization in superovulated ewes following insemination with different doses of fresh or frozen-thawed semen. *Proc. Aust. Soc. Reprod. Biol.*, **20**:92 Abstr.

Jackson, G.L., Kuehl, D., McDowell, K. & Zaleski, A. (1978). Effect of hypothalamic deafferentation on secretion of luteinizing hormone in the ewe. *Biol. Reprod.*, **17**:808-819.

Karsch, F.J., Bittman, E.L., Foster, D.L., Goodman, R.L., Legan, S.J. & Robinson, J.E. (1984). Neuroendocrine basis of seasonal reproduction. *Recent Prog. Horm. Res.*, **40**:185-232.

Keane, M.G. (1973). Effects of body condition and level of nutrition on the response of ewes to hormone treatment in the non-breeding season. *Ir. J. Agric. Res.*, **12**:229-237.

Keeling, B.J., King, M.E. & Gunn, G.J. (1986). Active immunization against androstenedione in a synchronized early-lambing flock. *Proc. Br. Soc. Anim. Prod.*, **42**:443 Abstr.

Keisler, D.H., Inskeep, E.K. & Dailey, R.A. (1985). Roles of pattern of secretion of luteinizing hormone and the ovary in attainment of puberty in ewe lambs. *Domestic Anim. Endocr.*, **2**:123-132.

Kelly, R.W. & Johnstone, P.D. (1983). Influence of site of ovulation on the reproductive performance of ewes with 1 or 2 ovulations. *N. Z. J. Agric. Res.*, **26**:433-435.

Kelly, R.W., Owens, J.L., Crosbie, S.F., McNatty, K.P. & Hudson, N. (1983/84). Influence of Booroola Merino genotype on the responsiveness of ewes to pregnant mares serum gonadotrophin, luteal tissue weights and peripheral progesterone concentrations. *Anim. Reprod. Sci.*, **6**:199-207.

Kennaway, D.J. & Gilmore, T.A. (1984). Effects of melatonin implants in ewe lambs. *J. Reprod. Fert.*, **70**:39-45.

Kennedy, J.P., Worthington, C.A. & Cole, E.R. (1974). The postnatal development of the ovary and the uterus of the Merino lamb. *J. Reprod. Fert.*, **36**:275-282.

Killeen, I.D. & Moore, N.W. (1970). Fertilization and survival of fertilized eggs in the ewe following surgical insemination at various times after the onset of oestrus. *Aust. J. Biol. Sci.*, **23**:1279-1287.

Killeen, I.D. & Moore, N.W. (1971). The morphological appearance and development of sheep ova fertilized by surgical insemination. *J. Reprod. Fert.*, **24**:63-70.

Killeen, I.D. & Caffery, G.J. (1982). Uterine insemination of ewes with the aid of a laparoscope. *Aust. Vet. J.*, **59**:95.

Kippax, I.S., Christie, W.B. & Rowan, T.G. (1991). Effects of method of splitting, stage of development and presence or absence of zona pellucida on foetal survival in commercial bovine embryo transfer of bisected embryos. *Theriogenology*, **35**:25-35.

Kumar, J., Osborn, J.C., Cameron, A.W.N., Batt, P.A. & Trounson, A.O. (1989). PMSG induces premature condensation of chromatin in goat oocytes. *Aust. Soc. Reprod. Biol.*, **21**:24 Abstr.

Kunkel, R.N. & Stricklin, W.R. (1978). Donor-recipient asynchrony, stage of embryo development and the post-transfer survival of bovine embryos. *Theriogenology*, **9**:96 Abstr.

Kyle, S.E. & Smith, J.F. (1984). Effect of immunization against steroid hormones on ewes of different Booroola genotypes. *New Zealand Ministry of Agriculture and Fisheries, Agricultural Research Division, Annual Report* 1983/1984.

Lambeth, V.A., Looney, C.R., Voelkel, S.A., Jackson, D.A., Hill, K.G. & Godke, R.A. (1983). Microsurgery of bovine embryos at the morula stage to produce monozygotic twin calves. *Theriogenology*, **20**:85-95.

Lamond, D.R. (1964). Synchronisation of ovarian cycles in sheep and cattle. *Anim. Breed. Abstr.*, **32**:269-285.

Lamond, D.R. & Urquhart, E.F. (1961). Sheep laparotomy cradle. *Aust. Vet. J.*, **37**:430-431.

Land, R.B. & McGovern, P.T. (1968). Ovulation and fertilization in the lamb. *J. Reprod. Fert.*, **15**:325-327.

Land,R.B. & Hill,W.G. (1975). The possible use of superovulation and embryo transfer in cattle to increase response to selection. *Anim. Prod.*, **21**:1-12.

Land, R.B. & Wilmut, I. (1977). The survival of embryos transferred in large groups to sheep of breeds with different ovulation rates. *Anim. Prod.*, **24**:183-187.

Land, R.B., Morris, B.A., Baxter, G., Fordyce, M. & Forster, J. (1982). Improvement of sheep fecundity by treatment with antisera to gonadal steroids. *J. Reprod. Fert.*, **66**:625-634.

Larsen, W.A. (1971). *A Study of Some Factors Affecting Reproductive Performance in New Zealand Romney and Border-Leicester x Romney Two-tooth Ewes.* Master of Agricultural Science thesis, Massey University, Palmerton North, New Zealand, 100pp.

Lawson, R.A.S. & Cahill, L.P. (1983). Modification of the embryo-maternal relationship in the ewe by progesterone treatment early in the oestrous cycle. *J. Reprod. Fert.*, **67**:473-475.

Lee, C.N. & Ax, R.L. (1984). Milk progesterone of dairy cows injected gonadotrophin releasing hormone at the first postpartum breeding. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination*, Illinois, **2**:401 Abstr.

Lehman, M.N., Robinson, J.E., Karsch, F.J. & Silverman, A.J. (1986). Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) pathways in the sheep brain during anestrus and the mid-luteal phase of the estrous cycle. *J. Comp. Neurol.*, **244**:19-35.

Levine, J.E., Pau, K.Y.F., Ramirez, V.D. & Jackson, G.L. (1982). Simultaneous measurement of luteinizing-hormone releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology*, **111**:1449-1455.

Linares, T. & King, W.A. (1980). Morphological study of the bovine blastocyst with phase contrast microscopy. *Theriogenology*, **14**:123-133.

Linares, T. & Ploen, L. (1981). On the ultrastructure of seven day old normal (Blastocysts) and abnormal bovine embryos. *Anat. Histol. Embryol.*, **10**:212-226.

Lincoln, G.A. & Ebling, F.J.P. (1985). Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion, and moulting in rams. *J. Reprod. Fert.*, **73**:241-253.

Linder, G.M. & Wright, R.W.Jr. (1983). Bovine embryo morphology and evaluation. *Theriogenology*, **20**:407-416.

Lindsell, C.E., Rajkumar, K., Manning, A.W., Emery, S.K., Mapletoft, R.J. & Murphy, B.D. (1986). Variability in FSH:LH ratios among batches of commercially available gonadotrophins. *Theriogenology*, **25**:167 Abstr.

Long, S.E. & Williams, C.V. (1980). Frequency of chromosomal abnormalities in early embryos of the domestic sheep (*Ovis aries*). *J. Reprod. Fert.*, **58**:197-201.

Lopyrin, A.I. (1938). Multifoetation in sheep. Moscow. (cited by T.J. Robinson, 1951).

Mallampati, R.S., Pope, A.L. & Casida, L.E. (1971). Breeding pattern in Targhee ewes and ewe lambs throughout the year. *J. Anim. Sci.*, **33**:1278-1281.

Mansour, A.M. (1959). The hormonal control of ovulation in the immature lamb. *J. Agric. Sci. Camb.*, **52**:87-93.

Martensz, N.D. & Scaramuzzi, R.J. (1979). The concentrations of luteinizing hormone, stimulating hormone and progesterone during the breeding season in ewes immunized against androstenedione or testosterone. *J. Endocr.*, **81**:249-259.

Martensz, N.D., Baird, D.T., Scaramuzzi, R.J. & Van Look, P.F.A. (1976). Androstenedione and the control of luteinizing hormone in the ewe during anoestrus. *J. Endocr.*, **69**:227-237.

Martin, G.B. (1984). Factors affecting the secretion of luteinizing hormone in the ewe. *Biol. Rev.*, **59**:1-87.

Mapletoft, R.J. & Murphy, B.D. (1989). Superovulation in the cow: The effect of reduced LH activity in gonadotrophin preparations. *Proceedings of the 2nd Annual Meeting of the Australian Association of Animal Artificial Breeders.* Monash University, Melbourne.

Mapletoft, R.J., Pawlyshyn, V., Garcia, A., Bo, G.A., Willmott, N., Saunders, J. & Schmutz, S. (1990). Comparison of four different gonadotropin treatments for inducing superovulation in cows with 1;29 translocation. *Theriogenology*, **33**:282 Abstr.

Massey, J.M., Anderson, J.G., Ellis, W.C., Sorensen, A.M. Jr., & Kraemer, D.C. (1982). Development of bovine embryos following enzymatic removal of the zona pellucida. *Theriogenology*, **17**:99 Abstr.

Massip, A., Van Der Zwalmen, P., Mulnard, J. & Zwijsen, W. (1983). Atypical hatching of cow blastocyst leading to complete twin half blastocysts. *Vet. Rec.*, **112**:301.

Mathews, M.V. & Murdoch, W.J. (1984). Increased secretion of luteinizing hormone in prepubertal ewes treated with an antagonist of opiates. *Domestic Anim. Endocr.*, **1**:167-173.

Mauleon, P. (1969). Oogenesis and folliculogenesis. In: *Reproduction in Domestic Animals*, 2nd edition, pp. 187-215. Eds. H.H. Cole & P.T. Cupps. Academic Press, New York.

Maurer, R.P. (1988). Embryo splitting and transfer in sheep. *Theriogenology*, **29**:276 Abstr.

McEvoy, T.G. & Sreenan, J.M. (1990). Effect of embryo quality and stage of development on the survival of zona pellucidae-free cattle demi-embryos. *Theriogenology*, **33**:1245-1253.

McClelland, A.L. (1990). *A Study of Physiological and Productive Differences Between Fleece Weight Selected and Control Romney Sheep*. Ph. D. thesis, Massey University, Palmerton North, New Zealand, 249pp.

McDonald, M.F. & Ch'ang, T.S. (1966). Variation in ovarian activity of Romney Marsh ewes. *Proc. N. Z. Soc. Anim. Prod.*, **26**:98-106.

McFeely, R.A. (1967). Chromosome abnormalities in early embryos of the pig. *J. Reprod. Fert.*, **13**:579-581.

McFeely, R.A. & Rajakoski, E. (1968). Chromosome studies on early embryos of the cow. *Proceedings of the 6th International Congress on Animal Reproduction and Artificial Insemination*, Paris, **2**:905-907.

McGaugh, J.W., Olds, D. & Kratzer, D.D. (1974). Ovum recovery in superovulated cows and cleavage rates in the fertilized ova. *Theriogenology*, **1**:213-217.

McGowan, M.R., Braithwaite, M., Jochle, W. & Mapletoft, R.J. (1985). Superovulation of beef cattle with Pergonal (hMG): A dose response trial. *Theriogenology*, **24**:173-184.

McIntosh, J.E., Moor, R.M. & Allen, W.R. (1975). Pregnant mare serum Gonadotrophin : rate of clearance from the circulation of sheep. *J. Reprod. Fert.*, **44**:95-100.

McKelvey, W.A.C. & Robinson, J.J. (1984). Normal lambs born following transfer of embryos by laparoscopy. *Vet. Rec.*, **115**: 230 Abstr.

McKelvey, W.A.C., Robinson, J.J. & Aitken, R.P. (1985). A simplified technique for the transfer of ovine embryos by laparoscopy. *Vet. Rec.*, **117**:492-494.

McKelvey, W.A.C., Robinson, J.J., Aitken, R.P. & Robertson, I.S. (1986). Repeated recoveries of embryos from ewes by laparoscopy. *Theriogenology*, **25**:255-265.

McMillan, W.H. (1986). Hogget oestrus synchronization: a comparison of CIDR and sponge. *Proc. N. Z. Soc. Anim. Prod.*, **46**:225-228.

McMillan, W.H. & McDonald, M.F. (1985). Survival of fertilized ova from ewe lambs and adult ewes in the uteri of ewe lambs. *Anim. Reprod. Sci.*, **8**:235-240.

McMillan, W.H. & Hall, D.R.H. (1990). Superovulation in the ewe: Effects of stage of cycle and PMSG in oFSH (Ovagen) treated ewes. *Proc. Aust. Soc. Reprod. Biol.*, **22**:38 Abstr.

McMillan, W. H. & Hall, D.R.H. (1991). Superovulation in the ewe : Are follicle numbers a useful predictor of super-ovulation rate? *Proc. N. Z. Soc. Anim. Prod.*, In Press.

McMillan, W.H., Hall, D.R.H. & Evans, P.H. (1991). Are follicle numbers a source of variation in superovulation rate in sheep? *Proc. Aust. Soc. Reprod. Biol.*, **23**:110 Abstr.

McNatty, K.P., Hudson, N.L., Gibb, M. & Collins, F.L. (1988). Plasma concentrations of FSH, LH and progesterone in sheep immunized against androstenedione-human serum albumin conjugate. *J. Reprod. Fert.*, **82**:63-69.

McNeilly, A.S., Picton, H.M., Campbell, B.K. & Baird, D.T. (1990). Gonadotrophic control of follicle growth in the ewe. *Proceedings of the 3rd Ruminant Reproduction Symposium*, Nice. (cited by M.A. Driancourt, 1991)

Mertes, P.C. & Bondioli, K.R. (1985). Effect of splitting technique on pregnancy rate from half embryos. *Theriogenology*, **23**:209 Abstr.

Mikel-Jenson, A., Greve, T., Madej, A. & Edqvist, L-E. (1982). Endocrine profiles and embryo quality in the PMSG-$PGF_{2\alpha}$ treated cow. *Theriogenology*, **18**:33-34.

Modlinski, J.A. (1970). The role of the zona pellucida in the development of mouse eggs in vivo. *J. Embryol. Exp. Morph.*, **23**:539-547.

Monniaux, D., Chupin, D. & Saumande, J. (1983). Superovulatory responses of cattle. *Theriogenology*, **19**:55-82.

Moor, R.M. (1965). *The Corpus Luteum of the Sheep with Special Reference to the Mechanism Controlling its Life-span.* Ph.D. thesis. University of Cambridge. England (cited by R. Newcomb, 1977).

Moor, R.M. & Cragle, R.G. (1971). The sheep egg: enzymatic removal of the zona pellucida and culture of eggs in vitro. *J. Reprod. Fert.*, **27**:401-409.

Moor, R.M., Krulp, T.A.M. & Green, D. (1984). Intraovarian control of folliculogenesis: Limits to superovulation? *Theriogenology*, **21**:103-116.

Moor, R.M., Osborn, J.C. & Crosby, I.M. (1985). Gonadotrophin-induced abnormalities in sheep oocytes after superovulation. *J. Reprod. Fert.*, **74**:167-172.

Moore, N.W. (1968). The survival and development of fertilized eggs transferred between Border Leicester and Merino ewes. *Aust. J. Agric. Res.*, **19**:295-302.

Moore, N.W. (1974). Multiple ovulation and ovum transfer in the goat. *Proc. Aust. Soc. Anim. Prod.*, **10**:246-249.

Moore, N.W. (1975). The control of time of oestrus and ovulation and the induction of superovulation in cattle. *Aust. J. Agric. Res.*, **26**:295-304.

Moore, N.W. (1977). Egg and embryo collection methods. In: *Embryo Transfer in Farm Animals*, pp. 38. Ed. K.J. Betteridge. Canada Department of Agriculture, Monograph 16, Ottawa, Canada.

Moore, N.W. (1982). Egg transfer in the sheep and goat. In: *Mammalian Egg Transfer*, pp. 119-133. Ed. C.E. Adams. CRC Press, Boca Raton.

Moore, N.W., Rowson, L.E.A. & Short, R.V. (1960). Egg transfer in sheep. Factors affecting the survival and development of transferred eggs. *J. Reprod. Fert.*, **1**:332-349.

Moore, N.W. & Shelton, J.N. (1962). The application of the technique of egg transfer to sheep breeding. *Aust. J. Agric. Res.*, **13**:718-724.

Moore, N.W. & Shelton, J.N. (1964a). Response of the ewe to a horse anterior pituitary extract. *J. Reprod. Fert.*, **7**:79-87.

Moore, N.W. & Shelton, J.N. (1964b). The application of the technique of egg transfer to sheep breeding. *Aust. J. Agric. Res.*, **13**:718-724.

Murphy, B.D., Mapletoft, R.J., Manns, J. & Humphrey, W.D. (1984). Variability in gonadotrophin preparations as a factor in the superovulatory response. *Theriogenology*, **21**:117-125.

Murphy, B.D., Rajkumar, K., Bristol, F. & Flood, P.F. (1985). Effecto del genotypo del semental y la edad de la yegua sobre los niveles de gonadotrofina corionica equina. *Rev. Med. Vet. (Bs. As.)*., **66**:72-76.

Murray, J.D., Boland, M.P., Moran, C., Nancarrow, C.D., Scaramuzzi, R.J., Sutton, R., Hoskinson, R.M. & Hazelton, I.G. (1985). Embryonic chromosomal abnormalities and reproductive wastage in Fecundin treated and control Merino ewes. *Theriogenology*, **23**:211 Abstr.

Mutiga, E.R. & Baker, A.A. (1982). Superovulatory response in Merino ewes to three PMSG dose levels. *Theriogenology*, **17**:100 Abstr.

Mutiga, E.R. & Baker, A.A. (1984). Transfer of sheep embryos through a laparoscope. *Vet. Rec.*, **114**:401-402.

Nancarrow, C.D., Murray, J.D., Boland, M.P., Sutton, R. & Hazelton, I.G. (1984). Effect of gonadotrophin releasing hormone in the production of single-cell embryos for pronuclear injection of foreign genes. In: *Reproduction in Sheep*, pp. 286-288. Eds. D.R. Lindsay & D.T. Pearce. Cambridge University Press.

Nancarrow, C.D., Murray, I.D., Scaramuzzi, R.J., Marshall, J.T., Hazelton, I.G., Hoskinson, R.M. & Boland, M.P. (1985). Fertilization and embryo development in androstenedione-immune sheep. *Proc. Aust. Soc. Reprod. Biol.*, **17**:6 Abstr.

Newcomb, R. (1977). Techniques and results in sheep and goats. In : *Embryo Transfer in Farm Animals*, pp. 40-41. Ed. K.J. Betteridge. Canada Department of Agriculture, Monograph 16, Ottawa, Canada.

Newcomb, R. (1979). Surgical and nonsurgical transfer of bovine embryos. *Vet. Rec.*, **105**:432-434.

Newcomb, R & Rowson, L.E.A. (1976). Aspects of nonsurgical transfer of bovine eggs. *Proceedings of the 8th International Congress on Animal Reproduction and Artificial Insemination*, Krakow, **3**:262-265.

Newcomb, R., Christie, W.B. & Rowson, L.E.A. (1978). Comparison of the fetal survival rate in heifers after the transfer of an embryo surgically to one uterine horn and nonsurgically to the other. *J. Reprod. Fert.*, **52**:395-397.

Newcomb, R. & Rowson, L.E.A. (1980). Investigation of physiological factors affecting non-surgical transfer. *Theriogenology*, **13**:41-49.

Newcomb, R., Christie, W. & Rowson, L.E.A. (1980). Foetal survival rate after surgical transfer of two bovine embryos. *J. Reprod. Fert.*, **59**:31-36.

Nibart, M., Sripongpun, S., Cedden, F., Mechekour, B., Le Guine, B. & Thibier, M. (1988). Histological study of bovine intact and demi-embryos. *Theriogenology*, **29**:283 Abstr.

Nicholas, F.W. & Smith, C. (1983). Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Anim. Prod.*, **36**:341-353.

Nowak, R. & Rodway, R.G. (1985). Effect of intravaginal implants on the onset of ovarian activity in adult and prepubertal ewes. *J. Reprod. Fert.*, **74**:287-293.

Oldham, C.M., Gray, S.J. & Poindron, P. (1984). Progeny testing for the 'F' gene using prepubertal ewe lambs. In: *Reproduction in Sheep*, pp. 260-261. Eds. D.R. Lindsay & D.T. Pearce. Cambridge University Press.

Ozil, J.P. (1983). Production of identical twins by bisection of blastocysts in the cow. *J. Reprod. Fert.*, **69**:463-468.

Ozil, J.P., Heyman, Y. & Renard, J.P. (1982). Production of monozygotic twins by micromanipulation and cervical transfer in the cow. *Vet. Rec.*, **110**:126-127.

Pathiraja, N., Carr, W.R., Fordyce, M., Foster, J., Land, R.B. & Morris, B.A. (1984). Concentration of gonadotropins in the plasma of sheep given gonadal steroid antisera to raise ovulation rate. *J. Reprod. Fert.*, **72**:93-100.

Pau, K.Y., Kuehl, D.E. & Jackson, D.L. (1982). Effect of frontal hypothalamic deafferentation on luteinizing hormone secretion and seasonal breeding in the ewe. *Biol. Reprod.*, **27**:999-1009.

Philipon, P. & Driancourt, M.A. (1987). Potential of active immunization against androstenedione to improve fecundity in sheep. *Anim. Reprod. Sci.*, **15**:101-112.

Picard, L., King, W.A. & Betteridge, K.J. (1985). Production of sexed calves from frozen thawed embryos. *Vet. Rec.*, **117**:603-608.

Picard, L., Greve, T., King, W.A., Betteridge, K.J. & Jorgensen, P.H. (1986). Bisection of post-compaction bovine embryos: The difference in viability between the two monozygotic halves. *Acta Vet. Scand.*, **27**:33-48.

Pinkert, C.A., Kooyman, D.L., Baumgartner, A. & Keisler, D.H. (1989). In-vitro development of zygotes from superovulated prepubertal and mature gilts. *J. Reprod. Fert.*, **87**:63-66.

Piper, L.R., Bindon, B.M., Curtis, Y.M., Cheers, M.A. & Nethery, R.D. (1982). Response to PMSG in Merino crosses. *Proc. Aust. Soc. Reprod. Biol.*, **14**:82 Abstr.

Pope, W.F., Lawyer, M.S., Nara, B.S. & First, N.L. (1986). Effect of asynchronous superinduction on embryo survival and range of blastocyst development in swine. *Biol. Reprod.*, **35**: 133-137.

Quinlivan, T.D. & Robinson, T.J. (1967). The number of spermatozoa in the fallopian tube of ewes at intervals after artificial insemination following withdrawal of SC-9880-impregnated intravaginal sponges. In : *The Control of the Ovarian Cycle in the Sheep*, pp. 177-194. Ed. T.J. Robinson. Sydney University Press.

Quinlivan, T.D. & Robinson, T.J. (1969). Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. *J. Reprod. Fert.*, **19**:73-86.

Quirke, J.F. (1979). Oestrus, ovulation, fertilization and early embryo mortality in progestagen-PMSG treated Galway ewe lambs. *Ir. J. Agric. Res.*, **18**:1-11.

Quirke, J.F. (1981). Regulation of puberty and reproduction in female lambs; a review. *Livest. Prod. Sci.*, **8**:37-53.

Quirke, J.F. & Hanrahan, J.P. (1975). Effect of Gonadotrophin-releasing hormone and Human Chorionic Gonadotrophin on the response of the ewe to Pregnant Mare Serum Gonadotrophin. *J. Reprod. Fert.*, **43**:167-170.

Quirke, J.F. & Hanrahan, J.P. (1977). Comparison of the survival in the uteri of adult ewes of cleaved ova from adult ewes and ewe lambs. *J. Reprod. Fert.*, **51**:487-489.

Quirke, J.F. & Gosling, J.P. (1980). Effect of immunization against androstenedione on ovulation rate in Galway and Finnish Landrace ewes. An Foras Taluntais, Animal Production Research Report, pp. 94-95.

Quirke, J.F., Hanrahan, J.P. & Gosling, J.P. (1981). Duration of oestrus, ovulation rate, time of ovulation and plasma LH, total oestrogen and progesterone in Galway adult ewes and ewe lambs. *J. Reprod. Fert.*, **61**:265-272.

Quirke, J.F., Hanrahan, J.P., Kilpatrick, M.J. & Tait, A. (1986). Effects of active immunization on ovulation rate and fecundity in Galway and Finnish landrace sheep. *Livest. Prod. Sci.*, **15**:27-32.

Ramirez, V.D. & McCann, S.M. (1963). Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology*, **72**:452-464.

Rainio, V. (1991). PMSG-dose in Finnsheep embryo production. *Theriogenology*, **35**:261 Abstr.

Rangel-Santos, R. (1987). *Studies on Embryo Transplantation Procedures Using Booroola-Merino x Perendale Ewes*. Master of Agricultural Science thesis, Massey University, Palmerton North, New Zealand, 178pp.

Renard, J.P., Heyman, Y. & Ozil, J.P. (1980). Importance of gestation losses after non-surgical transfer of cultured and non-cultured bovine blastocysts. *Vet. Rec.*, **107**:152-153.

Rhodes, L. & Nathanielsz, P.W. (1988). Comparison of a controlled internal drug release device containing progesterone with intravaginal medroxyprogesterone sponges for estrus synchronization in ewes. *Theriogenology*, **30**:831-836.

Robinson, T.J. (1951). The control of fertility in sheep. II. The augmentation of fertility by gonadotrophin treatment of the ewe in the normal breeding season. *J. Agric. Sci. Camb.*, **41**:6-63.

Robinson, T.J. (1967). Conclusions. In: *The Control of the Ovarian Cycle in the Sheep*, pp. 237-244. Ed. T.J. Robinson. Sydney University Press.

Robinson, T.J., Salamon, S., Moore, N.W. & Smith, T.F. (1967). The evaluation of SC9880-impregnated intravaginal sponges for the synchronisation of oestrus for large scale artificial insemination of Merino ewes in Summer and Autumn. In : *The Control of the Ovarian Cycle in the Sheep*, pp. 208-236. Ed. T.J. Robinson. Sydney University Press.

Robinson, J.J., Wallace, J.M. & Aitken, R.P. (1989). Fertilisation and ovum recovery rates in superovulated ewes following cervical insemination or laparoscopic intrauterine insemination at different times after progestagen withdrawal and in one or both horns. *J. Reprod. Fert.*, **87**:771-782.

Robinson, T.J. & Scaramuzzi, R.J. (1986). Immunization against androstenedione and out-of season breeding in sheep. *Proc. Aust. Soc. Anim. Prod.*, **16**:323-326.

Roche, J.F. (1968). Effect of the interval between withdrawal of progestagen pessaries and time of PMSG administration on oestrus and ovulation in cyclic ewes. *Ir. J. Agric. Res.*, **7**:1-6.

Rowlands, I.W. (1963). Levels of gonadotropins in tissues and fluids, with emphasis on domestic animals. In: *Gonadotropins*, pp. 74-107. Ed. H.H. Cole. W.H. Freeman & Company, San Francisco.

Rowson, L.E.A. & Adams, I.E. (1957). An egg transfer experiment in sheep. *Vet.Rec.*, **69**:849-850.

Rowson, L.E.A. & Moor, R.M. (1966a). Development of the sheep conceptus during the first fourteen days. *J. Anatomy*, 100:777-785.

Rowson, L.E.A. & Moor, R.M. (1966b). Embryo transfer in the sheep: The significance of synchronising oestrus in the donor and recipient animals. *J. Reprod. Fert.*, **11**:207-212.

Ryan, J.P, Bilton, R.J., Hunton, J.R. & Maxwell, W.M.C. (1984). Superovulation of ewes with a combination of PMSG and FSH-P. In: *Reproduction in Sheep*, pp. 338-341. Eds. D.R. Lindsay & D.T. Pearce. Cambridge University Press.

Ryan, K.D. & Foster, D.L. (1988). Unpublished study (cited by D.L. Foster, 1988).

SAS Institute, Inc. (1988). SAS/STAT User's Guide, Release 6.03 Edition, Cary, NC: SAS Institute Inc.

Saumande, J., Chupin, D., Mariana, J.C., Ortavant, R. & Mauleon, P. (1978). Factors affecting the variability of ovulation rates after PMSG stimulation. In : *Control of Reproduction in the Cow*, pp. 195-224. Ed. J.M. Sreenan. Martinus Nijhoff, The Hague.

Scaramuzzi, R.J. (1976). Physiological effects of immunizing sheep against oestradiol-17β. In : *Physiological Effects of Immunity Against Reproductive Hormones*, pp. 67-90. Eds. R. Edwards & W.M. Johnson. Cambridge University Press.

Scaramuzzi, R.J. (1979). Antibodies to androgens, and ovulation rate in the ewe. *J. Steroid Biochem.*, **11**:957-961.

Scaramuzzi, R.J. (1984). Changes in pituitary-ovarian functions in ewes immune to steroid hormones. *Anim. Prod. Aust.*, **15**:191-194.

Scaramuzzi, R.J., Davidson, W.G. & Van Look, P.F.A. (1977). Increasing ovulation rate in sheep by active immunization against an ovarian steroid androstenedione. *Nature, London*, **269**:817-818.

Scaramuzzi, R.J., Martensz, N.D. & Van Look, P.F.A. (1980). Ovarian morphology and the concentration of steroids, and of gonadotrophins during the breeding season in ewes actively immunized against oestradiol-17β or oestrone. *J. Reprod. Fert.*, **59**:303-310.

Scaramuzzi, R.J., Martin, G.B., Hoskinson, R.M., Downing, J.A., Gow, C.B., Turnbull, R.E. & Hinks, N.T. (1982). Studies of the periovulatory period in Merino ewes immunized against androstenedione or oestrone. *Proc. Aust. Soc. Reprod. Biol.*, **14**:94 Abstr.

Scaramuzzi, R.J. & Hoskinson, R.M. (1984). Active immunization against steroid hormones for increasing fecundity. In : *Immunological Aspects of Reproduction in Mammals*, pp. 445-474. Ed. D.B. Crighton. Butterworths, London.

Scaramuzzi, R.J., Campbell, B.K., Cognie, Y. & Downing, J.A. (1987). Increasing prolificacy of ewes by means of gonadotrophin therapy and treatment with Fecundin. In : *New Techniques in Sheep Production*, pp. 47-56. Eds. I. Fayez, M. Marai & J.B. Owen. Butterworths, London.

Schiewe, M.C., Bush, M., Stuart, L.S. & Wildt, D.E. (1984). Laparoscopic embryo transfer in domestic sheep: A preliminary study. *Theriogenology*, **22**:675-682.

Scudamore, C.L., Robinson, J.J. & Aitken, R.P. (1991). The effect of timing of laparoscopic insemination in superovulated ewes, with or without sedation, on the recovery of embryos, their stage of development and subsequent viability. *Theriogenology*, **35**:907-914.

Seidel, G.E.Jr., Larson, L.L., Spilman, C.H., Hahn, J. & Foote, R.H. (1971). Culture and transfer of calf ova. *J. Dairy Sci.*, **54**:923-926.

Seidel, G.E.Jr. (1981). Critical review of embryo transfer procedures with cattle. In: *Fertilization and Embryonic Development In Vitro*, pp. 323-353. Eds. L. Mastroianni & J.D. Biggers. Plenum Press, New York.

Seidel, G.E. (1982). Applications of microsurgery to mammalian embryos. *Theriogenology*, **17**:23-34.

Seike, N., Saeki, K., Utaka, K., Sakai, M., Takaruka, R., Nagao, Y. & Kanagawa, H. (1989). Production of bovine identical twins via transfer of demi-embryos without zonae pellucidae. *Theriogenology*, **32**:211-220.

Shackell, G.H. (1991). The timing of oestrus, LH surge and ovulation in ewes following synchronization with MAP sponges FGA sponges or CIDR'S. *Proc. N. Z. Soc. Anim. Prod.*, 51:73-78.

Shea, B.F. (1981). Evaluating the bovine embryo. *Theriogenology*, **15**:31-42.

Shea, B.F., Hines, D.J., Lightfoot, D.E., Ollis, G.W. & Olson, S.M. (1976). The transfer of bovine embryos. In: *Egg Transfer in Cattle*, pp. 145-152. Ed. L.E.A. Rowson. Commission of the European Communities, Luxemburg.

Shelton, J.N. & Szell, A. (1988). Survival of sheep demi-embryos in vivo and in vitro. *Theriogenology*, **30**:855-863.

Shelton, J.N. & Moore, N.W. (1966). Survival of fertilized eggs transferred to ewes after progesterone treatment. *J. Reprod. Fert.*, **11**:149-151.

Shelton, J.N. & Moore, N.W. (1967). The response of the ewe to pregnant mare serum and the horse anterior pituitary extract. *J. Reprod. Fert.*, **14**:175-177.

Skrzyszowska, M. & Smorag, Z. (1989). Cell loss in bisected mouse, sheep and cow embryos. *Theriogenology*, **32**:115-122.

Smeaton, D.C., Hockey, H-U.P. & Towers, N.R. (1985). Effects of facial eczema on ewe reproduction and ewe and lamb live weights. *Proc. N. Z. Soc. Anim. Prod.*, **45**:133-135.

Smith, C. (1986) Use of embryo transfer in genetic improvement of sheep. *Anim. Prod.*, **42**:81-88.

Smith, J.F. (1976). Selection for fertility and response to PMSG in Romney ewes. *Proc. N. Z. Soc. Anim. Prod.*, **36**:247-251.

Smith, J.F. & Allison, A.J. (1971). The effect of exogenous progestagen on the production of cervical mucus in the ewe. *J. Reprod. Fert.*, **24**:279-282.

Smith, J.F., Cox, R.I., McGowan, L.T., Wilson, P.A. & Hoskinson, R.M. (1981). Increasing the ovulation rate in ewes by immunization. *Proc. N. Z. Soc. Anim. Prod.*, **41**:193-197.

Smith, J.F., Tervit, H.R., McGowan, L.T. & Cox, R.I. (1983). Effect of oestrone immunization on the ovulation rate and fertilisation of Coopworth and Romney ewes treated with PMSG. *Proc. Endocr. Soc. Aust.*, **26**:7 Abstr.

Sokal, R.R. & Rohlf, F.J. (1981). *Biometry: The principles and Practice of Statistics in Biological Research.* Second edition. W.H. Freeman and Company, New York. 859pp.

SPSS^x, Inc. (1985). Advanced Statistics Guide. SPSS^x Inc., Chicago, Ill., 505pp.

Sreenan, J.M. (1976). Egg transfer in the cow: Effect of site of transfer. *Proceedings of the 8th International Congress on Animal Reproduction and Artificial Insemination*, Krakow, **3**:269-272.

Sreenan, J.M., Beehan, D. & Gosling, J.P. (1978). Ovarian responses in relation to endocrine status following PMSG stimulation in the cow. In: *Control of Reproduction in the Cow*, pp. 144-158. Ed. J.M. Sreenan. Martinus Nijhoff, The Hague.

Tervit, H.R. (1967). *Studies on the in vivo Cleavage and the in vitro Culture of New Zealand Romney Sheep ova.* Master of Agricultural Science thesis, Massey University, Palmerton North, New Zealand, 96pp.

Tervit, H.R. & Havik, P.G. (1976). A modified technique for flushing ova from the sheep uterus. *N. Z. Vet. J.*, **24**:138-140.

Tervit, H.R. & McDonald, M.F. (1969). Abnormalities and dimensions of ova from New Zealand Romney ewes. *N. Z.J. Agric. Res.*, **12**:21-30.

Tervit, H.R., Allison, A.J., Smith, J.F., Harvey, T.M. & Havik, P.G. (1976). Egg transfers in exotic sheep. *Proc. N. Z. Soc. Anim. Prod.*, **36**:67-75.

Tervit, H.R., Havik, O.G. & Smith, J.F. (1977). Egg transfer in cattle: Pregnancy rate following transfer to the uterine horn ipsilateral or contralateral to the functional corpus luteum. *Theriogenology*, **7**:3-10.

Tervit, H.R., Cooper, M.W., Goold, P.G. & Haszard, G.M. (1980). Nonsurgical embryo transfer in cattle. *Theriogenology*, **13**:63-71.

Tervit, H.R., Thompson, J., McMillan, W., James, R. & Parton, G. (1989). Superovulation of anoestrous mature and hogget ewes. *Proc. Aust. Soc. Reprod. Biol.*, **21**:127 Abstr.

Thimonier, J.T., Bindon, B.M. & Piper, L.R. (1979). Ovarian response to PMSG in cattle selected for twinning. *Proc. Aust. Soc. Reprod. Biol.*, **11**:5 Abstr.

Thompson, J.G.E. & Smith, J.F. (1988). Effect of nutrition on the ovulatory response of Coopworth ewes to varying doses of two FSH preparations. *Proc. N. Z. Soc. Anim. Prod.*, **48**:81-85.

Toro, M.A., Sillo, L., Rodriganez, J. & Dobao, M.T. (1988). Embryo transfer and genetic improvement of wool yield in sheep. *Proceedings of the 3rd World Congress of Sheep and Beef Cattle Breeding*, **1**:214-216.

Torres, S. & Cognie, Y. (1984). Super-ovulation and egg transfer in the ewe. *Reprod. Nutr. Develop.*, **24**:623-631.

Torres, S., Cognie, Y. & Colas, C. (1987). Transfer of superovulated sheep embryos obtained with different FSH-P. *Theriogenology*, **27**:407-419.

Torres, S. & Sevellec, C. (1987). Repeated super-ovulation and surgical recovery of embryos in the ewe. *Reprod. Nutr. Develop.*, **27**:859-863.

Trounson, A.O. & Moore, N.W. (1974a). Fertilisation in the ewe following multiple ovulation and uterine insemination. *Aust. J. Biol. Sci.*, 27:301-304.

Trounson, A.O. & Moore, N.W. (1974b). Attempts to produce identical offspring in sheep by mechanical division of the ovum. *Aust. J. Biol. Sci.*, **27**:505-510.

Trounson, A.O. & Moore, N.W. (1972). Ovulation rate and survival of fertilized ova in Merino ewes selected for and against multiple births. *Aust. J. Agric. Res.*, **23**:851-858.

Trounson, A.O., Willadsen, S.M. & Moor, R.M. (1976). Effect of prostaglandin analogue Cloprostenol on oestrus, ovulation and embryonic viability in sheep. *J. Agric. Sci. Camb.*, **86**:609-611.

Wallace, L.R. (1954). Studies on the augmentation of fertility of Romney ewes with Pregnant-Mare Serum. *J. Agric. Sci. Camb.*, **45**:60-79.

Walker, D. (1977). Laboratory methods of equine pregnancy diagnosis. *Vet. Rec.*, **100**:396-399.

Walker, S.K., Warnes, G.M., Quinn, P., Seamark, R.F. & Smith, D.H. (1985). Laparoscopic technique for the transfer of embryos in sheep. *Aust. Vet. J.*, **62**:105-106.

Walker, S.K. & Warnes, G.M. (1986). Unpublished study (cited by Walker et al., 1986).

Walker, S.K., Smith, D.H., Ashman, R.J. & Seamark, R.F. (1986). The use of synthetic Gonadotrophin releasing hormone treatment in the collection of sheep embryos. *J. Reprod. Fert.*, **77**:135-142.

Walker, S.K., Smith, D.H., Frensham, A., Ashman, R.J. & Seamark, R.F. (1989). Timing of multiple ovulations in the ewe after treatment with FSH or PMSG with and without GnRH. *Theriogenology*, **31**:741-752.

Wang, H., Wu, M., Xu, K., Hagele, W.C. & Mapletoft, R.J. (1987). Control of superovulation in the cow with a PMSG antiserum. *Theriogenology*, **27**:291.

Wang, H., Wu, M., Patt, D., Murphy, B.D. & Mapletoft, R.J. (1988). Superovulation in beef heifers with PMSG: Effect of dose and monoclonal antibodies to PMSG. *Theriogenology*, **29**:323 Abstr.

Ware, C.B., Northey, D.L., Boland, M.P. & First, N.L. (1988). Early cycle FSH-p priming as a prelude to superovulating gonadotropin administration in ewes and heifers. *Anim. Reprod. Sci.*, **16**:97-105.

Warfield, S.J., Seidel, G.E. Jr. & Elsden, R.P. (1987). Transfer of bovine demi-embryos with and without the zona pellucida. *J. Anim. Sci.*, **65**:756-761.

Watson, R.H. & Gamble, L.C. (1961). Puberty in the Merino ewe with special reference to the influence of season of birth upon its occurrence. *Aust. J. Agric. Res.*, **12**:124-138.

Welch, R.A.S., Andrewes, W.D., Barnes, D.R., Bremner, K. & Harvey, T.G. (1984). CIDR dispensers for oestrous and ovulation control in sheep. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination*, Illinois, **1**:354-355 Abstr.

Wiggins, E.L., Barker, H.B. & Miller, W.W. (1970). Estrual activity in Ramboullet ewes. *J. Anim. Sci.*, **30**:405-408.

Willadsen, S.M. (1979a). Embryo transplantation in sheep. In : *The Management and Diseases of Sheep*, pp. 69-85. Ed. The British Council and Commonwealth Agricultural Bureaux.

Willadsen, S.M. (1979b). A method for culture of micromanipulated sheep embryos and its use to produce monozygotic twins. *Nature, London*, **277**:298-300.

Willadsen, S.M. (1980). The viability of early cleavage stages containing half the normal number of blastomeres in sheep. *J. Reprod. Fert.*, **59**:357-362.

Willadsen, S.M. & Godke, R.A. (1984). A simple procedure for the production of identical sheep twins. *Vet. Rec.*, **114**:240-243.

Willadsen, S.M. & Fehilly, C.B. (1983). The developmental potential and regulatory capacity of blastomeres from two, four and eight cell sheep embryos. In: *Fertilization of the Human Egg in vitro*, pp. 353-357. Eds. H.M. Beier & H.R. Lindner. Springer-Verlag, Berlin.

Willadsen, S.M. & Polge, C. (1981). Attempts to produce monozygotic quadruplets in cattle by blastomere separation. *Vet. Rec.*, **108**:211-213.

Willadsen, S.M., Lehn-Jensen, H., Fehilly, C.B. & Newcomb, R. (1981). The production of monozygotic twins of preselected parentage by micromanipulation of non-surgically collected cow embryos. *Theriogenology*, **15**:23-29.

Williams, T.J., Elsden, R.P. & Seidel, G.E. Jr. (1984). Pregnancy rates with bisected bovine embryos. *Theriogenology*, **22**:521-531.

Williams, T.J. & Moore, L. (1988). Quick splitting of bovine embryos. *Theriogenology*, **29**:477-484.

Wilmut, I. & Sales, D.I. (1981). Effect of an asynchronous environment on embryonic development in sheep. *J. Reprod. Fert.*, **61**:179-184.

Wilmut, I., Sales, D.I. & Ashworth, C.J. (1985). The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. *Theriogenology*, **23**:107-119.

Worthington, C.A. & Kennedy, J.P. (1979). Ovarian response to exogenous hormones in six-week-old lambs. *Aust. J. Biol. Sci.*, **32**:91-95.

Wright, J.M. (1981). Nonsurgical embryo transfer in cattle: Embryo-recipient interactions. *Theriogenology*, **15**:43-56.

Wright, R.W.Jr., Anderson, G.B., Cupps, P.T., Drost, M. & Bradford, G.E. (1976). In vitro culture of embryos from adult and prepuberal ewes. *J. Anim. Sci.*, **42**:912-917.

Xu, K., Wu, M., Wang, H. & Mapletoft, R.J. (1988). FSH-P lot number and superovulation response in beef heifers. *Theriogenology*, **29**:335 Abstr.

Yellon, S.M. & Foster, D.L. (1986). Melatonin rhythms time photoperiod-induced puberty in the female lamb. *Endocrinology*, **119**:44-49.

Zar, J.H. (1984). *Biostatistical Analysis*. Second edition. Prentice-Hall, Inc., New York. 718pp.

LIST OF ABBREVIATED JOURNAL NAMES

Acta Vet. Scand.	Acta Veterinaria Scandinavica
Am. J. Physiol.	American Journal of Physiology
Anim. Breed. Abstr.	Animal Breeding Abstracts
Anim. Prod.	Animal Production
Anim. Reprod. Sci.	Animal Reproduction Science
Ann. Biol. Anim. Bioch. Biophys.	Annals de Biologie Animale Biochimie Biophysique
Ann. Med. Vet.	Annals de Medecine Veterinaire
Aust. J. Agric. Res.	Australian Journal of Agricultural Research
Aust. J. Biol. Sci.	Australian Journal of Biological Sciences
Aust. J. Exp. Agric. Anim. Husb.	Australian Journal of Experimental Agriculture and Animal Husbandry
Aust. Vet. J.	Australian Veterinary Joumal
Biol. Reprod.	Biology of Reproduction
Biol. Rev.	Biological Reviews
Can. J. Anim. Sci.	Canadian Journal of Animal Science
Domest. Anim. Endocr.	Domestic Animal Endocrinology
Emp. J. Exp. Agric.	Empire Journal of Experimental Agriculture
Ir. J. Agric. Res.	Irish Journal of Agricultural Research
Ir. Vet. J.	Irish Veterinary Journal
J. Agric. Sci. Camb.	Journal of Agricultural Science
J. Anatomy.	Journal of Anatomy
J. Anim. Sci.	Journal of Animal Science
J. Comp. Neurol.	Journal of Comparative Neurology
J. Endocr.	Journal of Endocrinology
J. Reprod. Fert.	Journal of Reproduction and Fertility
J. Steroid. Biochem.	Journal of Steroid Biochemistry
Livest. Prod. Sci.	Livestock Production Science
Mol. Cell. Endocr.	Molecular and Cell Endocrinology
Nord. Vet. Med.	Nordisk Veterinaer Medicin
N.Z. Farmer.	New Zealand Farmer
N.Z. J. Agric. Res.	New Zealand of Agricultural Research
N.Z. Vet. J.	New Zealand Veterinary Journal
Proc. Aust. Soc. Anim. Prod.	Proceedings of the Australian Society of Animal Production

Proc. Aust. Soc. Reprod. Biol.	Proceeding of the Australian Society for Reproductive Biology
Proc Br. Soc. Anim. Prod.	Proceedings of the British Society of Animal Production
Proc. N.Z. Soc. Anim. Prod.	Proceedings of the New Zealand Society of Animal Production
Proc. Ruakura Farmers' Conf.	Proceedings of the Ruakura Farmers' Conference
Recent Prog. Horm. Res.	Recent Progress in Hormone Research
Reprod. Nutr. Develop.	Reproduction, Nutrition, Development
Rev. Cub. Cienc. Vet.	Revista Cubana de Ciencias Veterinarias
Rev. Med. Vet.	Revista de Medicina Veterinaria (Buenos Aires)
Vet. Rec.	Veterinary Record