A STUDY OF COMMERCIAL EMBRYO TRANSFER PROGRAMMES CONDUCTED WITH TEXEL AND AWASSI SHEEP

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Abstract

The results of two commercial embryo transfer (ET) programmes conducted in Central Hawkes Bay, comprising 93 Texel and 78 Awassi donor ewes, were analysed to identify variables that affect the success of commercial ET programmes. The production of high quality embryos for export was the primary objective in the Texel programme, while the rapid multiplication of the Awassi was the sole purpose of the Awassi programme. Reproductive parameters such as; ovarian response to exogenous gonadotrophins, recovery and fertilisation rate of ova, yield of good quality embryos and embryo survival rate to scanning and birth were evaluated. The influence of age, ovulatory response, repeated flushing, the number of corpora lutea in recipient ewes and donor surgeon, on the reproductive parameters, were assessed.

The Texel ewes were all purebred consisting of two-tooth and four-tooth animals. The Awassi ewes were either three-quarter or purebred, and included both ewe hoggets and mixed age ewes.

Synchronisation of oestrus was attempted using a double CIDR-G™ regime. The super ovulatory programme differed in each breed but consisted of a series of FSHp injections in combination with PMSG. The ewes were inseminated *intra-uterine* with fresh diluted semen from a ram of the same breed after detection of oestrus. Embryo recovery was attempted 6-6.5 days after insemination using a standard flushing technique in which the oviducts and uterus were exposed by mid-ventral laparotomy. Two or three embryos were transferred into each recipient ewe within 1.5 hours (Texel) and 4 hours (Awassi) of recovery.

Ovarian response to superovulatory treatments was not significantly affected by any of the variables recorded in this study, although the Texel programme provided a higher ovulatory response than the Awassi programme (8.89 CL vs 7.08 CL). The embryo recovery rates were 71.8% for the Texel and 78.1% for the Awassi sheep. Age of the donor ewes significantly affected ($p=0.006$) recovery rate of ova in the Awassi sheep, adult ewes and ewe hoggets recorded recovery rates of 90.7% and 65.2%. Fertilisation

rate was not affected by any of the factors studied with 94.6% and 77.6%, of the Texel and Awassi ova, being fertilised. The overall yield of good quality embryos was 91.6% for the Texel and 80.1% for the Awassi. The yield of good quality embryos was significantly affected (p=0.03) by age in the Awassi, 87.5% of adult embryos and 64.4% of ewe hogget embryos were of good quality. Survival rates in the Awassi data set were significantly affected by the lower scanning and birth rates of the Awassi ewe hoggets than Awassi ewes (43.8% vs 65.9%: p=0.02) and (39.3% vs 59.4%: p=0.03) respectively. Each Texel donor ewe produced, on average, 3.89 embryos that were of sufficient quality to be preserved and therefore not transferred, resulting in 0.70 lambs being born per donor ewe in the Texel programme. The Awassi programme produced 2.10 lambs donor per ewe, however, this was significantly affected (p=0.03) by the age of the donor ewes, with adult ewes produced an average of 2.7 lambs per donor, and ewe hoggets, 1.2 lambs per donor.

This work demonstrated the variable nature of embryo transfer programmes as well as the difficulty in achieving acceptable results from ewe hoggets. The Texel and adult Awassi results compared favourably to the other published reports and illustrate that on-farm commercial embryo transfer can provide acceptable results.

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List of Abbreviations

ACP	Acetylpromazine
AI	Artificial insemination
BSA	Bovine serum albumin
°C	Degrees Celsius
CIDR	Controlled internal drug release
CL	Corpus luteum
CO_2	Carbon dioxide
DMSO	Dimethyl sulpoxide
FGA	Flurogestone acetate
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
HAP	Horse anterior pituitary extract
hMG	Human menopausal gonadotrophin
i.u	International units
LH	Luteinizing hormone
MAP	Medroxyprogesterone acetate
mg	Milligrams
ml	Millilitres
MOET	Multiple ovulation embryo transfer
No.	Number of
pFSH	Porcine follicle stimulating hormone
PMSG	Pregnant mares' serum gonadotrophin
PBS	Phosphate buffered saline
SE	Standard error

Chapter One

Introduction

Genetic improvement in the New Zealand sheep industry is an effective means to increase productivity and economic returns. The importation of new 'exotic' breeds with desirable genes that can improve animal performance are of considerable interest. Since release from quarantine in 1990, the Texel has been widely utilised as a lean meat producing breed. Following the importation of sheep and embryos, MOET technology was employed by a commercial firm and the Ministry of Agriculture to increase the number of Texel offspring, thereby allowing comparisons to be made with established breeds and their crosses. The Awassi breed, released from quarantine in 1995, has yet to play a significant role in the New Zealand sheep industry. With MOET the numbers of animals containing exotic genes have been increased and their application to New Zealand farming systems is being assessed.

A considerable amount of research has been conducted on developing techniques for superovulation, embryo recovery from donors and embryo transfer to recipients, making it possible to increase the reproductive rate of sheep to a significant degree. As well as introducing new genetic material and multiplication of genotypes, embryo transfer allows the generation of a large number of offspring from superior females which has the potential to increase the female selection differential and reduce generation intervals, thereby increasing the rate of genetic gain.

Current MOET technology provides variable responses often characterised by low embryo harvests leading to only a modest number of lambs being born per donor ewe. To effectively utilise embryo transfer technology, the number of lambs born per donor ewe needs to be increased to acceptable levels. If consistently high ovulation rates following superovulatory treatment occur, then the overall effectiveness of an embryo transfer programme would be greatly enhanced. Both recovery and fertilisation rates have the potential to reach 90-95% but such results are infrequently recorded. Currently

embryo survival rates are around 60% (Tervit 1989), but there is potential for this to be improved.

In terms of a genetic improvement programme, MOET has the potential to significantly increase the rate of genetic gain. Smith (1986) suggested genetic gain could be increased by 89-102% if ten lambs could be produced from 6-8 month old donor ewes, or 50-70% if 5 lambs were produced. Gaffney *et al.*, (1991) indicated an increase in genetic gain of 49% or 19% was possible when 4 or 2.2 lambs were produced from adult donor ewes.

Using data from a Texel programme at Glen Aros farm in Central Hawkes Bay and an Awassi programme at Mathews Station also in Central Hawkes Bay, it was possible to analyse the results being achieved in commercial farm conditions. Each are profit driven independent enterprises, whether it be for the production of embryos for export or the production of live lambs the procedure must be financially viable.

Chapter Two

Literature Review

2.1 Oestrus Synchronisation

Oestrus synchronisation is an essential part of an embryo transfer programme. It allows superovulatory treatments to be administered at the correct stage of the oestrous cycle of the donor ewes as well as ensuring a close synchrony of oestrous between donor and recipient ewes. Oestrus synchronisation also enables efficient utilisation of available labour and technical resources. The techniques of oestrus synchronisation have been reviewed by several workers (Moore, 1982; Gordon, 1983; Tempest and Minter, 1987).

There are essentially two methods by which oestrus can be controlled: 1) Prostaglandin treatments, which lead to luteolysis followed by follicular growth and ovulation: or 2) The administration of progesterone or other progestogens, which suppresses follicular growth until the cessation of treatment.

Prostaglandin treatments have proven to be an effective oestrus synchronisation method (Cumming, 1979; Bondurant, 1986), however they are not widely used with superovulatory gonadotrophins (Moore, 1982). Douglas and Ginther (1973) and Hawk (1973) observed that a single 10-15 mg intramuscular injection of $PGF_{2\alpha}$ could induce regression of the corpus luteum (CL). The CL of the ewe is only responsive to prostaglandin between day 4 and 14 of the oestrous cycle (Gordon, 1983), therefore two $PGF_{2\alpha}$ treatments 8-9 days apart (Tempest and Minter, 1987) are required to ensure that all sheep in the flock are at an appropriate stage of the oestrous cycle to respond. Most workers have detected oestrus in treated ewes within approximately 40 hours (Acritopoulou and Haresign, 1980; Gordon, 1983; Tempest and Minter, 1987).

Progesterone (and progestogen) treatments have been used extensively in conjunction with superovulatory gonadotrophin treatments in ewes (Moore, 1982). Prior to 1964, progesterone was administered through feed or by daily injection (Gordon, 1975). The injection of animals has a high labour demand which is impractical (Cumming, 1979),

whereas feeding gives a variable dose rate, and therefore a wider spread of oestrus (Tempest and Minter, 1987). The practical breakthrough came with the development of polyurethane sponges, which could be impregnated with a synthetic progesterone and implanted intravaginally to release progesterone through the vaginal wall into the bloodstream (Robinson and Lamond, 1966). The sponges, containing either flurogestone acetate (FGA) or medroxyprogesterone acetate (MAP) (Gordon, 1971), are placed in the vagina for 12-14 days (Robinson, 1979), to promote a synchronised oestrus within 36-48 hours of sponge removal (Cognie and Mauleon, 1983). More recently a controlled internal drug releasing device (CIDR) containing 30-50 mg of progesterone has been developed (Welch *et al.*, 1984).

The sponge, although cheaper than the CIDR, has the inherent problems of vaginal discharge and an unpleasant smell at removal. The CIDR has overcome both of these problems and has added advantages of being easy to insert and results in higher vaginal retention rate than sponges (Welch *et al.*, 1984).

Ewes synchronised with CIDR's exhibit oestrus earlier than ewes synchronised with a sponges (Clarke *et al.*, 1984; Shackell, 1991). The synchrony of the onset of oestrus may also be better (Welch *et al.*, 1984). However, there is no significant difference in fertilisation rate for the two devices with natural ovulation (Maxwell and Hewitt, 1986).

Several workers have attempted to increase the efficiency of oestrus synchronisation with the addition of a gonadotrophin during the later stages of progesterone treatment. Hunter (1982) and Tempest and Minter (1987) reported that pregnant mares' serum gonadotrophin (PMSG) administered either 2 days prior to, or at the cessation of progesterone treatment, gave a more precise and reliable synchronisation, with oestrus being slightly earlier and more compact (Cognie and Mauleon, 1983).

2.2 Superovulation

The success of an embryo transfer programme is largely dependent on the superovulatory response of donor ewes. The number of ovulations dictates the potential number of embryos, and consequently the number of lambs born from an embryo transfer programme. The application of reliable methods to superovulate sheep, therefore appears to have the greatest influence on the overall effectiveness of an embryo transfer scheme. Considerable variation exists in response to superovulatory treatments, particularly between animals (McMillan and Hall, 1991) and various hormonal treatments (Maxwell *et al.*, 1990).

2.2.1 *Physiology of the oestrous cycle and ovulation*

A factor of importance in relation to the regulation of ovulation rate is the hormonal basis of follicle growth and maturation. The ovary of an adult ewe contains between 12,000 and 86,000 primordial follicles and between 100 and 400 larger follicles at various stages of development (Cahill *et al.*, 1979). In the absence of superovulatory treatments, usually only one or two follicles ovulates at the end of each oestrous cycle. This is a result of a series of complex interactions governed principally by the delicate interplay between the hypothalamic-pituitary axis, the ovaries and the uterus which regulates the lifespan of the corpus luteum (Haresign *et al.*, 1983).

Differentiation of the ovulatory follicle is thought to be a two step process (Di Zerga and Hogden, 1981). Follicle recruitment is the first step, a group of follicles capable of ovulating is established, from which selection then occurs, typically, in sheep, leading to one or two follicles completing preovulatory enlargement (hence becoming dominant) while the others undergo atresia (Driancourt *et al.*, 1985). Follicular recruitment has been shown to occur approximately 48 hours prior to the pre-ovulatory luteinizing hormone (LH) surge (Haresign, 1985). This has been demonstrated by Smeaton and Robertson (1971) who showed that if a recently ruptured follicle is cauterised at the end of oestrus, a new follicle able to generate another pre-ovulatory LH peak is present within 48 hours. LH treatment during seasonal anoestrus will result in a LH peak within

30-50 hours (McNatty *et al.*, 1981). These results suggest that recruitment coincides with luteolysis.

It has been widely assumed that follicle stimulating hormone (FSH) plays a key role in the determination of ovulation rate (Baird, 1983) and the control of follicular growth (Webb and Gauld, 1987). This belief is based upon strong evidence obtained over many years showing that exogenous FSH treatments or treatments that lead to increases in endogenous FSH levels increase ovulation rate (Scaramuzzi and Campbell, 1990). FSH treatment also increases the total number of non-atretic follicles and the level of follicular oestradiol biosynthesis (McNatty *et al.*, 1985). Picton *et al.*, (1990) has shown that the growth of follicles to an ovulatory size is totally dependent on FSH and the number of follicles that develop is dependent on both the amount of FSH and the time of exposure to FSH. Despite these findings the hormonal interactions that determine ovulation rate in normal cycling ewes are unclear (Scaramuzzi and Radford, 1983; Scaramuzzi and Campbell, 1990).

The induction of aromatase activity in the follicle, during the final stages of development, represents an irrevocable commitment by the granulosa cells within a follicle, to differentiated function and cessation of granulosa cell growth (Scaramuzzi and Campbell, 1990). These follicles are referred to as recruited or committed follicles. The minimum size of recruited follicles is 2mm in diameter (Driancourt and Cahill, 1984; Webb and Gauld, 1987).

Selection of the ovulatory follicle is demonstrated by the presence of a large dominant follicle secreting oestradiol and binding LH on the theca and granulosa layers. The mechanisms for selection are unclear, but in the majority of cases (70%), the largest healthy follicles are selected (Driancourt *et al.*, 1985). There are currently two hypotheses for the mechanisms of selection (Driancourt *et al.*, 1985).

1. When a follicle is big enough or has reached a given stage of maturity, it manages to stay in a state of equilibrium, in which it does not respond to decreasing FSH levels which urge other follicles to go into atresia.

2. Selection is an active process, where mature follicles inhibits the growth of other follicles.

Although there is no evidence ruling out the first hypothesis, data suggests that the second is more likely to explain the mechanisms of selection.

Committed follicles are critically dependent on FSH for their continued survival. With adequate FSH support, they secrete oestradiol in increasing amounts, leading to the induction of LH receptors on the granulosa cells and transformation into activated follicles (Scaramuzzi and Campbell, 1990). Without adequate FSH support aromatase activity is not maintained, oestradiol secretion falls and androgen accumulates within the follicle leading to atresia (Lindsay, 1991). In activated follicles aromatase activity is independent of FSH (Scaramuzzi and Campbell, 1990). This independence allows the activated follicle to continue to develop while inhibiting the development of other committed follicles by secreting increasing amounts of oestradiol and inhibin which suppresses peripheral FSH levels, ensuring those follicles which are at a slightly less advanced stage of development are deprived of continued FSH stimulation (Baird, 1983). The theory that deprivation of FSH causes atresia in other committed follicles is supported by the fact that administration of exogenous FSH, during the follicular phase, facilitates continued oestradiol production (Lindsay, 1991). This prevents the atretic process and allows the development of more than one preovulatory follicle (McNatty *et al.*, 1985).

The role of LH in follicle selection is unclear. LH alone will not stimulate follicular development, however depending on the level of FSH stimulation, LH pulses can partially inhibit the stimulatory action of FSH on follicular growth (McNeilly *et al.*, 1990). Furthermore, complete removal of LH by passive immunisation inhibits FSH-stimulated follicular development.

In cyclic ewes, preovulatory surges of FSH and LH are provoked by a rise in oestrogen secretion in the pre-ovulatory follicle (Baird *et al.*, 1981). The mechanisms controlling the LH surge have been well documented. Increasing gonadotrophin releasing hormone

(GnRH) discharges and an increasing sensitivity of the pituitary gland to GnRH, induced by high oestradiol levels (Reeves *et al.*, 1971), result in rapid elevation of LH levels. This preovulatory surge of LH initiates a sequence of changes in the steroidogenesis of the dominant follicle and its structure, as it prepares to expel the oocyte (Driancourt *et al.*, 1985).

For ovulation to occur, the frequency of tonic LH pulses must be sufficient to produce a sustained increase in LH which is necessary to stimulate the preovulatory oestradiol rise which subsequently induces an LH surge, resulting in ovulation (McNatty *et al.*, 1981).

The cumulus cells of the growing follicle actively secrete glycoproteins which form a viscous mass enclosing the oocyte and its corona. Intra-follicular pressure rises initially in response to the LH surge and then fluctuates before ovulation (Baker, 1982). During the final phase of preovulatory maturation a small area of the mature follicle becomes thin and translucent to form a bulge on the surface of the ovary. At ovulation this bulge ruptures at the apex, releasing some of the follicular fluid and the viscous mass embedding the oocyte. This viscous mass spreads at the ovarian surface to facilitate the 'pick up' of oocytes by the mucosal cells of the fimbria. At the time of ovulation the ovum, together with the surrounding cells in a gelatinous mass, protrudes at the ovarian surface and is swept into the ostium of the oviduct by the action of the motile kinocilia of the fimbria (Holst, 1974).

Following rupture the follicle collapses and luteinization occurs in which a CL is formed. The CL grows rapidly after ovulation secreting increasing quantities of progesterone until levels plateau 7-8 days after ovulation. LH is secreted in pulses during this period and each pulse induces an increase in oestradiol and androgen concentrations (Scaramuzzi and Baird, 1976). Following conception the CL will persist throughout the duration of pregnancy, if conception does not occur regression of the CL will begin approximately 14 days after ovulation.

2.2.2 *Manipulating ovulation rate using exogenous gonadotrophins*

The ability to increase ovulation rates in domestic animals through gonadotrophin treatment originated from experiments carried out over 65 years ago (Parkes, 1929). Historically the most common method to superovulate ewes has been the use of PMSG treatments (Eastwood and McDonald, 1975; Gheradi and Lindsay, 1980; Driancourt, 1991). Superovulatory responses in ewes have also been induced with pituitary gonadotrophins of equine origin; horse anterior pituitary extract (HAP: Moore and Shelton, 1964; Boland and Gordon, 1982), porcine follicle stimulating hormone (pFSH: Robinson *et al.*, 1989; Scudamore *et al.*, 1991a; Dattena *et al.*, 1994) and human menopausal gonadotrophin (hMG: Schiewe *et al.*, 1985; Scudamore *et al.*, 1991b).

Two actions of gonadotrophin treatments, which increase ovulation rates have been identified. Firstly, administration of exogenous gonadotrophins lowers the size at which they can trigger follicle recruitment (Mariana *et al.*, 1991). All healthy follicles of >0.8mm in diameter (Driancourt, 1987) are mobilised for terminal follicular growth following gonadotrophin administration. Secondly, gonadotrophin treatments reduce atresia in medium to large follicles, thereby leaving more follicles to ovulate (Lindsay, 1991).

The biological half-life of the different gonadotrophins has traditionally determined the most effective means of administration to elicit a superovulatory response (Maxwell *et al.*, 1990). The long half-life of PMSG (21 hours in sheep: McIntosh *et al.*, 1975) has allowed a single injection to be used, while the short half-life of FSH preparations (2 hours in sheep: Akbar *et al.*, 1974) requires it to be administered as once or twice daily injections over a three to four day period.

The relatively long half-life of PMSG has been associated with a high number of non-ovulated or cystic follicles (Wallace, 1992), these follicles produce high circulatory levels of oestrogens which increase the rate of transport of ova through the oviducts and therefore decreases the rate of embryo recovery (Whyman and Moore, 1980). In

addition, the use of PMSG in sheep has been associated with premature activation of oocytes that result in aged or abnormal oocytes at the time of ovulation.

The majority of studies comparing various gonadotrophin treatments have demonstrated the superiority of FSH preparations to PMSG in terms of increased ovulation rate (Walker *et al.*, 1986; Robinson *et al.*, 1989; Scudamore *et al.*, 1991a). Further benefits of FSH treatments include high ovulatory response rates with low-moderate doses (D'Alessandro *et al.*, 1996), better sperm transport and higher fertilisation rates following cervical insemination. The need for a labour intensive multiple injection regime is the major disadvantage involved with FSH treatments (Wilmut, 1987).

The administration of PMSG in combination with FSH can overcome the disadvantages of using either gonadotrophin alone (Ryan *et al.*, 1984). A 400-800 i.u. dose of PMSG in combination with a superovulatory dose of FSH (12-20 mg) was shown to increase the number of ewes that exhibited a superovulatory response as well as improve the yield of embryos without any increase in the number of persistent follicles.

Administration of GnRH in a superovulatory regime induces an earlier than normal pre-ovulatory LH surge and is effective in increasing the proportion of developing follicles that ovulate (Jabbour and Evans, 1991). GnRH administration also improves the synchrony of ovulation by; reducing the period during which superovulation begins, the interval from first to last ovulation (Walker *et al.*, 1986), and the percentage of recovered ova that are fertilised (Walker *et al.*, 1989).

Irrespective of the gonadotrophin used to induce superovulation, there is marked individual variation in the ovulatory response from consistent doses (Maxwell *et al.*, 1990). Reasons for this variation involve the dynamics of follicular development during the oestrous cycle and differences in the FSH and LH concentrations of hormonal preparations (Moor *et al.*, 1984; Driancourt *et al.*, 1985).

2.2.3 *LH/ FSH ratios*

Variable LH concentration in FSH preparations is a likely cause of abnormal pre-ovulatory oocytes. LH appears to specifically block fertilisation, perhaps through premature stimulation of the maturing oocyte so that it becomes incapable of being fertilised (Moor *et al.*, 1984). Furthermore it has been shown that normal progesterone, LH and FSH concentrations are necessary for optimal embryo production in superovulated cattle (Donaldson, 1985). Abnormal concentrations of endogenous progesterone, LH and FSH are followed by atypical follicular maturation and reduced embryo production.

The superovulatory response has been found to be inversely proportional to the amount of LH activity in exogenous FSH preparations (Armstrong and Evans, 1984). Keeping constant the amount of FSH while reducing LH concentrations has been shown to increased ovulation rates. Similar findings were made in later work (Henderson *et al.*, 1988).

2.2.4 *Dose of gonadotrophin*

A dose-response relationship to PMSG has been reported by several workers (Bindon *et al.*, 1971; Smith, 1976; Evans and Robinson, 1980; Samartzi *et al.*, 1995). In general, an increase from low (200 i.u. PMSG) to higher (1200-1500 i.u. PMSG) doses is associated with a linear increase in ovulation rate. However, higher doses of PMSG (>2000 i.u.) decrease ovulation rate due to the failure of many follicles to ovulate. This has been attributed to the secretion of excessive levels of oestrogen from the unovulated follicles which results in an unfavourable uterine environment (Evans and Robinson, 1980). Similar dose-response relationships have been reported following HAP treatments (Moore and Shelton, 1964; Shelton and Moore, 1967). However, in contrast to PMSG, the number of large follicles remains relatively constant with an increased dose of HAP (Shelton and Moore, 1967).

Increased doses of pFSH have also been associated with increased ovulation rates in sheep during the breeding season. Armstrong and Evans (1984), Torres ***et al.***, (1987)

and Henderson *et al.*, (1988) have all reported increased ovulation rates following increased pFSH doses. Unlike PMSG, high doses of pFSH do not appear to decrease ovulation rate.

2.2.5 *Timing of administration*

Henderson *et al.*, (1988) demonstrated that continuous exposure to elevated plasma FSH concentrations is necessary to bring additional follicles to pre-ovulatory maturity. An increase in the frequency of multiple ovulations can be achieved by infusing FSH for a 24 hour period in the 48 hours before initiating luteolysis (McNatty *et al.*, 1985). Presumably this treatment is advancing the maturation of a follicle which would have normally undergone atresia, thereby allowing it to survive the pre-ovulatory fall in plasma FSH concentrations and attain maturity. This has also been illustrated by several other workers (Wright *et al.*, 1981; Armstrong and Evans, 1984; Torres and Cognie, 1984), in which ovulation rates were increased using repeated pFSH injections starting before the onset of luteolysis and continuing throughout the follicular phase. Infusion of pFSH for only the first 24 hours from the start of luteolysis or delaying the start of infusion for longer than 12 hours after the start of luteolysis, fails to increase mean ovulation rates (Henderson *et al.*, 1988).

2.2.6 *On-farm hormonal regimes*

Superovulatory regimes for on-farm embryo transfer programmes have been developed under a range of conditions with many breeds of sheep. The majority of commercial embryo transfer procedures now employ regimes using both PMSG and FSH in order to avoid the disadvantages of using either hormone alone. A variety of superovulatory regimes have been used during embryo transfer programmes in Merino flocks (van Reenan, 1990). The results are displayed on Table 2.1.

Table 2.1 Effect of superovulatory regime on the number of embryos transferred per donor ewe programmed (van Reenan, 1990).

Superovulatory regime	Mean number of embryos transferred
pFSH	4.4
PMSG alone	2.2
PMSG + FSH	2.5
Folltropin (purified pFSH)	3.2
Ovagen (purified ovine FSH)	6.5
Ovagen + PMSG	6.4
Ovagen 1 shot	3.9

From these experimental results a regime involving donors ewes being primed with 2.5ml of Ovagen on day 4 and then given seven 1ml injections of Ovagen twice daily from day 10 through day 14 has, under field conditions, been found to produce an average of 8 transferable embryos per donor ewe flushed (van Reenan 1990).

Regimes using PMSG and FSH have provided good results in field conditions (Holt, 1989; Heywood, 1993). A 400 i.u. dose of PMSG administered 48 hours before progesterone treatment removal with FSH treatment commencing simultaneously, in the form of 2 intramuscular injections per day (2mg, day 1; 1.5mg, day 2; and 0.5mg, day 3: 8mg total) for 3 successive days, was found to produce 9.8 CL per ewe, with 6.9 ova recovered and 5.8 fertilised embryos per ewe.

5.3 Embryo Recovery

The ewe normally ovulates near the end of oestrus, approximately 24-27 hours after its onset (Jainudeen and Hafez, 1993), with the first cleavage of the fertilised egg occurring 24 hours after fertilisation.

The distribution of each stage of embryonic development varies with the day of collection, as shown on Table 2.2.

Table 2.2 Embryo development rate (Torres and Sevellec, 1987).

Day of recovery after onset of oestrus	Predominant stage or number of cells
2	2 - 4 cell
3	8 cell
4	8 - 16 cell
5	24 - 32 cell
6	40 cell (compact morula)
7	60 cell (early and unhatched blastocyst)
10	hatched blastocyst
14	elongation of blastocyst

Bondurant (1986) reported that recovery rates frequently decrease as oestrus to collection intervals increase. This may result from the early death of genetically non-viable embryos, the increased difficulty in removing the microscopic embryo from a relatively voluminous uterus or the premature luteal regression and expulsion of the embryo.

When embryos collected from the same animal exhibit various stages of development, they should be considered retarded and of poor viability (Killeen, 1981). Embryos assessed as being behind their developmental age, and hence retarded, result in lower pregnancy rates than those ewes with normally developed embryos (Eldsen *et al.*, 1978). Donaldson (1985) suggested this may result from the following.

1. Not all ova are ovulated simultaneously.
2. Not all ova are fertilised simultaneously.
3. Even if two ova are fertilised at the same time the rate of cleavage for each may differ slightly.

Therefore after 5-7 days of development, these factors may produce embryos at different morphological stages of development.

2.3.1 *Day of flush*

Embryos are present in the uterine horns from around the 4th day after the onset of oestrus (Moore, 1982). Although embryos collected up to day 14 after oestrus are

capable of normal development in recipient ewes (Peterson *et al.*, 1976), collection normally occurs between days 3-8. Holt (1989) reported that recovery rates are not affected by the day of uterine flush provided it occurs between days 4-8 after oestrus. The timing of oviduct flushing also has a minimal effect on recovery rates if conducted between days 3-7 after oestrus (Moore, 1982; Wilmut, 1987). After day 6 hatched blastocysts are often difficult to distinguish among the cellular debris in the flushing media (Moore, 1982). Collection prior to day 3 is generally not attempted as Averill and Rowson (1958) found that no 2-cell and only 16% of 4-cell sheep embryos developed into viable lambs when transferred into the uterus. This is thought to be a result of 2-cell and 4-cell embryos being more sensitive to manipulation than embryos at further stages of development (Moore and Shelton, 1964b).

Commercial work by van Reenan (1990) has shown the best results are obtained when the embryos are collected by uterine flush 7-7.5 days after oestrus. Other commercial work (Holt, 1989) recommends uterine flushing 5 days after fertilisation (6 days after oestrus). These techniques should yield a recovery rate of approximately 80%.

2.3.2 *Ovulation rates*

High superovulatory responses (>16 corpora lutea) have been associated with increased embryo losses, fertilisation failure and the failure of ewes to produce embryos or lambs following embryo transfer (Betteridge and Moore, 1977; Armstrong and Evans, 1983). However, contrary to these findings, Torres and Cognie (1984) and Scudamore *et al.*, (1991b) found no decrease in recovery rate with increased ovulation rates. In addition, Torres *et al.*, (1987) found a positive correlation between the number of corpora lutea and the number of morula recovered.

2.3.3 *Repeated flushing*

Early work reported by Moore (1977), indicated that an initial collection had no effect on the recovery rate of a subsequent collection 1 year later. However, the formation of adhesions following surgical embryo recovery severely limits the number of times an animal can be flushed (Wallace, 1992). Moore (1980) suggested that if care was

exercised, 2-3 effective collections from individual donors could be possible. However, Torres and Sevellec (1987) found that repeated surgeries significantly depressed subsequent recovery rates from 88% for the first to 52% for the second and 24% for the third recovery attempts.

2.4 Embryo Survival

Numerous factors affect the survival rates of embryos, including those that influence the natural developing embryo and the additional effects imposed by embryo collection and transfer procedures.

2.4.1 *Donor-recipient oestrus synchrony*

Close synchrony between the onset of oestrus in donor and recipient ewes is one of the most important factors influencing the success of an embryo transfer programme. This requirement for synchrony can be met with the use of progesterone treatment to control the time of oestrus in recipient ewes (Wilmut, 1987). To ensure a close synchrony, progesterone treatment of the recipients should be discontinued 24 hours earlier than treatment in donor ewes (Moore, 1980), as the interval to the onset of oestrus is shorter in donor ewes given gonadotrophin. Attempts have been made to increase the efficiency of oestrus synchronisation in recipient ewes with low doses (300-500 i.u.) of PMSG after progesterone treatment (Boundurant, 1986). However, improvements using this treatment have been recorded more frequently in out-of-season transfer programmes (Moore, 1980).

Reports on the degree of asynchrony an embryo can tolerate have produced mixed results. Shelton and Moore (1964b) found optimum results, in terms of pregnancy rates and embryo survival rates, when oestrus in the recipient occurred within 12 hours of that in the donor ewes. Subsequent work by Rowson and Moor (1966) found a difference of ±48 hours was tolerable, but a variation of ±72 hours produced very poor (8%) pregnancy rates in recipient ewes. McKelvey *et al.*, (1985) found evidence of reduced pregnancy rates from as little as 8 hours of asynchrony.

The most likely reason for the death of the embryo is that the uterine environment may not be suitable for the out-of-phase embryo (Rowson and Moor, 1966). Another possibility is that the out of phase embryo is incapable of exerting a sufficient luteotrophic action on the recipient's CL, resulting in luteolysis and consequently the embryo is lost (Wilmut *et al.*, 1985). Wilmut and Sales (1981) showed that sheep embryos collected on day 4 and transferred into an advanced asynchronous recipient on day 7 failed to survive, whereas embryos collected on day 8 and transferred into a day 6 uterus were better able to survive (Wilmut, 1987). This suggests that if asynchrony is unavoidable it is preferable to select a recipient who showed oestrus behaviour after the donor.

2.4.2 *Number of embryos transferred*

In early work (Moore and Shelton, 1962), the number of embryos transferred was found to have little or no effect upon the proportion of recipients which subsequently established pregnancies. However, the survival rate of transplanted embryos varied according to the number of embryos present initially. Larsen (1971) observed a significant difference in the percentage of ewes that became pregnant when 1 or 3 embryos were transferred. Ninety percent of ewes receiving three embryos became pregnant while 66% of the ewes that received a single embryo became pregnant.

The difference between survival rates for one or two embryos transferred is usually small and not significant (35.1% vs 31.8%; Armstrong and Evans, 1983). Increasing the number of embryos transferred results in a lowering of the survival rate for one vs three embryos (66% vs 50%; Larsen, 1971) and one vs four embryos (62% vs 39%; Cumming, 1965). Greaney (1991) reported a 57.4% survival rate when two embryos were transferred. Trounson (1983) obtained a 92% survival rate when three embryos were transferred.

The number of embryos transferred in any situation is dependent on several factors including value of offspring and objective of the programme. The incidence of multiple births within the potential recipients also needs to be considered. Those breeds which

rarely have twins should receive a single embryo, while two or three could be transferred to high fecund breeds (Moore, 1980). The majority of workers transfer two embryos into each recipient ewe (Dattena, 1989; Holt, 1989; van Reenen, 1990; Greaney, 1991; and others).

2.4.3 *Site of transfer*

To achieve optimal survival rates, the section of the reproductive tract to which the embryos are transferred should be determined by the age or physiological development of the embryo. Moore (1982) and Ishwar and Memon (1996) reported that embryos collected on or before day 4 after the onset of oestrus, should be transferred into the oviducts while embryos collected after day 4 should be transferred into the uterus. While Moore and Shelton (1964b) showed that embryos collected up to 3.5 days after oestrus have higher survival rates when transferred into the oviduct rather than the uterus, other workers have reported contrasting results. Killeen (1976) reported a greater survival rate of 2 day-old embryo transferred into the uterus (71%) compared to the oviduct (60%). He also found no significant difference between survival rates of 4 day-old embryos transferred to either the oviduct or uterus. Rowson and Moore (1966) recorded survival rates of 75%, 71% and 63% for 5 day old morulae, 7 and 9 day old blastocysts, respectively, when transferred into the uterus.

Armstrong and Evans (1983) compared the oviduct and uterus as the site of transfer for embryos at a more advanced stage than 8-cell (3-3.5 days), and found no significant difference in survival rates were evident (40% vs 47.5%) for the oviduct and uterus respectively.

2.4.4 *Effect of supplementary progesterone*

The majority of the work conducted to investigate the effect of supplementary progesterone on embryo survival rates has been following natural mating or AI. Several workers have found no significant increase in embryo survival rates from progesterone supplementation following natural servicing (Murray *et al.*, 1988; Diskin and Niswender, 1989; Walsh, 1989; Sviatko *et al.*,1993; Thompson *et al.*, 1995). McMillian

(1987) found progesterone supplementation to reduce the number of return matings in hoggets only. Contrary to these studies Peterson *et al.*, (1984) showed that progesterone therapy for six days (days 10-16), after the removal of a progesterone synchronising device, increased the lambing rate from 67%-95%. This increase was thought to result from a reduction in embryo mortality.

Following embryo transfer, progesterone treatments have been shown to improve embryo survival. Parr *et al.*, (1982) observed a relationship between progesterone dose and embryo survival to day 21. Ewes given progesterone doses of 5, 10, 15, 20, or 25mg per day, had embryo survival rates of 69%, 83%, 79%, 90% and 82%, respectively. Additional work by Dattena (1989) found significantly higher embryo survival rates when recipient Romney ewes received progesterone following the transfer of embryos from three of the six donor breeds used in the trial.

2.5 Technical Factors Influencing the Success of Embryo Transfer

The method of mating is critical if high fertilisation rates are to be achieved in an embryo transfer programme. Irrespective of the type of superovulation treatment, fertilisation frequently fails particularly in ewes showing a high ovulatory response. Fertilisation failure is equally evident in ewes following vaginal or cervical insemination, particularly if large doses of gonadotrophin are used (Moore, 1982). Failure has been attributed to faulty transport of spermatozoa within the female reproductive tract, particularly the cervix (Betteridge and Moore, 1977; Boundurant, 1986; Maxwell *et al.*, 1990). However, this problem can be alleviated if semen is deposited directly into the uterus (Trounson and Moore, 1974).

2.5.1 *Artificial insemination technique*

Four artificial insemination techniques have currently been developed. Although natural mating is an option, it is rarely used in conjunction with commercial embryo transfer work.

1. *Cervical insemination*: This method is the most commonly used technique in commercial flocks (Wallace, 1992), but is rarely used with embryo transfer.

Acceptable fertilisation rates have been reported using fresh semen, but the use of frozen-thawed semen usually results in unacceptable fertilisation rates (10%-30%; Maxwell and Hewitt, 1986).

2. *Vaginal insemination*: This method is rarely used in embryo transfer work. Workers have reported results comparable with those obtained using cervical insemination (Clarke *et al.*, 1984; Maxwell and Hewitt, 1986).
3. *Transcervical insemination*: This is a technique modified from cervical insemination, in which the cervix is retracted into the vagina to allow an inseminating instrument to be introduced to the cervical canal. Literature on the use of this technique with embryo transfer is limited, but acceptable pregnancy rates have been reported with the use of frozen-thawed semen (58%; Halbert *et al.*, 1990). Using this technique to inseminate frozen semen into 1914 commercial ewes, Buckrell *et al.*, (1993) noted that semen could be deposited into the uterus of 86.6% of the ewes with 41.3% producing a lamb.
4. *Intra-uterine insemination*: This is the most commonly used AI technique in embryo transfer work. This laparoscope-aided technique has radically improved fertilisation rates as it circumvents the natural cervical barrier. The major advantage of this technique is that it uses the lowest dose of semen, whilst still yielding the highest fertilisation rates (Clarke *et al.*, 1984). However, intra-uterine AI is the most expensive and technically demanding of the techniques available. Clarke *et al.*, (1984) recorded 83% fertilisation with intra-uterine AI using fresh semen, similar results were reported by Dattena (1989), 85% and Scudamore *et al.*, (1991a), 87.3%. Smith *et al.*, (1995) recorded pregnancy rates of 63% using frozen-thawed semen.

2.5.2 *Timing of insemination*

The majority of studies suggest the optimum time for *intra-uterine* AI is 24-48 hours after the cessation of progesterone treatment. Wright *et al.*, (1981) obtained greater than 90% fertilisation using a 12 hour interval between onset of oestrus and intra-uterine insemination. Torres and Cognie (1984) achieved 94% fertilisation after a 24 hour interval. Maxwell *et al.*, (1990) reported higher fertilisation rates and embryo yield

following a 24 hour rather than a 48 hour interval, suggesting that the handling of the animals or manipulation of the reproductive tract may interfere with egg capture at the later insemination time. Delaying insemination to 60 hours significantly reduces the number of transferable quality embryos (Scudamore *et al.*, 1991b), from 100% in the 48 hour group to 35% in the 60 hour group. This suggests that delaying insemination until well beyond ovulation results in the fertilisation of aged oocytes of reduced developmental competence.

On-farm embryo transfer results have shown the highest fertilisation rates are obtained after intra-uterine insemination with fresh semen 24-28 hours (Holt, 1989) or 36 hours (Heywood, 1993), after sponge withdrawal. When frozen-thawed semen is used, insemination 48-56 hours after sponge withdrawal is recommended (Holt, 1989). van Reenen (1990) reported the effect of interval from CIDR withdrawal to AI on the number of transferable embryos obtained in on-farm embryo transfer programmes, as shown in Table 2.3.

Table 2.3 Effect of timing of insemination after CIDR withdrawal on the number of transferable embryos obtained (van Reenen 1990).

Timing of insemination after CIDR withdrawal	Mean number of transferable embryos recovered
24 hours	3.8
36 hours	5.7
48 hours	5.2
24 and 36 hours	5.5

2.5.3 *Embryo recovery technique*

The majority of embryo recoveries in MOET schemes are carried out under general anaesthesia, with the oviducts and uterus exposed by mid-ventral laparotomy as described by Hunter *et al.*, (1955). Oviductal flushing using this technique results in relatively high rates of embryo recovery, 80% (Trounson and Moore, 1974) and 78% (Tervit and Havik, 1976). In an attempt to reduce the incidence and severity of reproductive tract adhesions Moore and Shelton (1962) employed a uterine flush

technique which had an 76% recovery rate. Other workers have recorded similar results using this technique, 83% (Tervit and Havik 1976) and 76% (Torres and Cognie, 1984).

Laparoscopic embryo recovery (McKelvey *et al.*, 1986), eliminates the need for exteriorising the reproductive tract and almost totally prevents the formation of post-operative adhesions. This technique enabled ova to be recovered from superovulated ewes on three occasions over 2 months, with subsequent recovery rates of 35%, 76% and 66% . In order to avoid the high degree of manual dexterity required with the full laparoscopic technique a laparoscope-aided technique has been developed (Tervit, 1989). This technique facilitates repeated collections with recovery rates of 63%, 71% and 85% being achieved for successive flushes carried out at monthly intervals.

Methods of non-surgical embryo collection, similar to those used for transcervical insemination, have been documented in sheep (Coonrod *et al.*, 1986). The insertion of a catheter system through the retracted cervix allowed embryos to be collected in 11 of the 26 ewes studied. Because of the tortuous nature of the cervix this method is time consuming and not suitable for all ewes.

2.5.4 *Embryo transplant technique*

The majority of commercial MOET operations transfer embryos surgically into the reproductive tract exteriorised at mid-ventral laparotomy. The animals are under local anaesthesia and restrained in a laparotomy cradle as described by Lamond and Urquhart (1961).

The most common method of transfer is by a pipette attached by flexible tubing to a 2ml syringe. A smooth ended pipette inserted through the fimbria should be used for transfer into the oviduct, while uterine transfers are carried out using a sharp ended pipette inserted into the lumen 3-5 cm from the utero-tubal junction (Moore, 1982).

Successful embryo transfer by laparoscopy has been reported by McKelvey and Robinson (1984). When two embryos were transferred to each of 21 ewes using this technique, 16 established pregnancies with the production of 24 lambs.

2.6 Embryo Preservation

Numerous implications in the use of frozen embryos have been reviewed. Those relevant to embryo transfer in sheep have been described by Schneider and Mazur (1986) as follows.

1. Recipients do not have to be synchronised for the day of embryo collection. Transfer can be done whenever recipients come into the proper stage of the cycle. As a consequence, it is not necessary to keep large recipient flocks.
2. Frozen embryos can be shipped far more easily and inexpensively than live animals, however current animal health control regulations have limited the importation of frozen embryos from many countries. Frozen embryos, however, will help lessen the problem, as collected embryos can be stored in a frozen state while the parents can be examined for diseases, including those with longer incubation periods.
3. Germ plasm of rare breeds can be preserved.

2.6.1 *Freezing medium*

The medium used most often to freeze embryos has been Dulbecco's phosphate buffered saline (PBS), to which a cryoprotectant is added (Willadsen *et al.*, 1976; Maurer, 1978; Wilmut, 1987). A number of cryoprotectants have been used to freeze morula and blastocyst stage sheep embryos, including dimethyl sulpoxide (DMSO), glycerol (Tervit and Gould, 1984; Schneider and Mazur, 1986a), ethylene glycol (Tervit, 1983; McGinnis *et al.*, 1993), propanediol (Tervit, 1983) and propylene glycol (Szell *et al.*, 1990; Schiewe *et al.*, 1991). A limited number of comparative studies have been conducted on the effectiveness of these cryoprotectants, although slightly higher lambing percentages have been reported following transfer of embryos frozen in ethylene glycol compared with glycerol (Tervit and Gould, 1984).

2.6.2 *Process of freezing*

The first technique developed to preserve sheep embryos relied on slow cooling at 0.2-2.0°C/min to a temperature of -60°C before plunging directly into liquid nitrogen at-196°C (Willadsen *et al.*, 1976). Currently the most commonly used freezing protocols involve cooling embryos at -1.0 or -2.0°C/min from ambient temperature to -7°C where ice formation in the extra cellular medium is artificially induced (Maxwell *et al.*, 1990). Cooling then proceeds at -0.3°C/min to -30°C to -40°C then the containers are plunged into liquid nitrogen and stored for varying lengths of time. Post-thaw embryo viability has been shown to improve when embryos are cooled to at least -30°C before rapid cooling in liquid nitrogen (Schiewe *et al.*, 1991).

2.6.3 *Thawing process*

The required thawing rate depends on the freezing regime used. Embryos cooled slowly to temperatures between -30 and -40°C and then rapidly to -196°C require rapid thawing. Cells treated in this way may contain some intracellular ice, and thawing has to be rapid to prevent injury from the recrystallisation of that ice (Schneider and Mazur, 1986). Consequently embryos cooled slowly to temperatures below -60°C before transfer into liquid nitrogen, require a significantly slower thawing process. As rapid freezing procedures are more commonly used, fast thawing procedures are generally preferred. The most widely used method of fast thawing embryos is to place the embryos directly in a 37°C water bath until the ice melts (Tervit, 1983; Wilmut, 1987; Maxwell *et al.*, 1990).

2.6.4 *Cryoprotectant removal*

Cryoprotectants exert a large osmotic pressure on the blastomeres and if the extra- and intra-cellular gradient is too large, cellular damage can occur (Maurer, 1978). Therefore after thawing, the cryoprotectant needs to be either serially diluted or removed, thereby reducing the danger of osmotic damage to the embryos. A method to remove the cryoprotectant from the freezing solution was described by Rangel Santos (1987), which involved six gradually decreasing concentrations of glycerol in PBS.

A single step removal technique using a 0.25-0.50 M sucrose solution has also been described (Schneider and Mazur, 1986; Maxwell *et al.*, 1990). Embryos remain in contact with the medium for 5-10 minutes at room temperature. Thereafter the embryos are pipetted into isotonic PBS medium. McGinnis *et al.*, (1993) reported on a 2-step removal procedure which included a 10 minute exposure to 0.25 M sucrose solution before placement in a 1.0 M sucrose solution, however no increase in embryo survival was observed in comparison to the 1-step removal procedure.

2.7 Implementation of a MOET Programme

Among the early investigations into the advantages of implementing a MOET scheme were those by Land and Hill (1975) for beef cattle; for traits measurable in both sexes prior to puberty, it was shown that the rate of genetic improvement could be doubled. Nicholas and Smith (1983) extended the application to dairy cattle and reported similar results, thereby achieving rates of improvement substantially greater than those obtainable through large scale progeny testing schemes.

Smith (1986) was one of the first to examine the application of MOET technology to the sheep industry, his conclusions were similar to those reported in the earlier cattle investigations and the results have since become the basis of comparison for subsequent work. Smith suggested an increase in genetic gain could be achieved largely through a reduction in the generation interval, a reduction that was possible with MOET. The most effective model envisaged a large stud flock to supply rams of a particular breed and the generation interval to be a single year as all the offspring were the progeny of hogget rams and hogget donor ewes. The inability to obtain an adequate number of embryos from young ewes was noted, but if the technology could be improved through research and development significant advances could be made. If high transfer rates (10 progeny per donor) at 6-8 months of age could be achieved, then, with appropriate selection schemes, the rates of genetic change could almost be doubled compared with efficient schemes using normal reproduction. This applied to most traits including those measured before reproductive age (growth and carcass traits) as well as female reproductive and wool traits measured at 14 to 16 months of age. Even with moderate

rates of embryo transfer (5 progeny per donor) gains of 50% to 70% in genetic response could be obtained. However the difficulty in producing a reasonable number of transferable embryos in a juvenile MOET scheme will always limit the application of such models.

Subsequent work used more accurate simulations and found the benefits of MOET were not as substantial as originally thought. Responses were reduced to account for a future reduction in genetic variation due to selection (the so-called Bulmer effect) and inbreeding depression. Both effects become increasingly important as population size decreases and selection intensity increases.

The first comprehensive study into the genetic and economic application of MOET technology to sheep (Gaffney *et al.*, 1991), compared natural mating with both juvenile and adult MOET schemes at different response rates (2.2, 4 and 8 live progeny per donor ewe) over a 20 year period. The reduction in genetic variance due to the Bulmer effect was shown to have a significant effect on response, increasing with increased family size. The reduction in response due to this effect is slightly higher for the natural service scheme than some of the MOET schemes because of the higher intensity of selection in ewes, which have a longer generation interval. The accuracy of selection, as expected, is lower in the juvenile schemes and the shorter generation interval leads to higher cumulative inbreeding over the 20 year period. The advantages in genetic response over natural schemes, when 4 (or 2.2) live lambs were born per donor ewe, were found to be 49% (or 17%) in adult MOET and 89% (or 51%) in juvenile MOET schemes.

Adjusting the predicted responses for the cumulative effects of inbreeding and linkage disequilibrium (Bulmer effect) allows a more accurate assessment of the genetic merit of MOET schemes. The absence of such adjustments in earlier work (Land and Hill, 1975; Nicholas and Smith, 1983; Smith, 1986) was a significant factor in their overestimation of the genetic merit of MOET.

Wray and Goddard (1994) used a model in which a single trait was examined over a 14 year period. Comparisons were made on the basis of response depressed by the effect of inbreeding cumulated and discounted over a medium time horizon (14 years). Advantages in the response criterion of adult and juvenile schemes were 23% and 33% respectively for 'future' embryo transfer success rates using mass selection. These results being at the conservative extreme of those of Smith (1986) who predicted 30% to 80% advantages in juvenile MOET over natural mating schemes.

While the majority of studies of MOET in sheep have assumed that all matings in the flock use MOET, Brash *et al.*, (1996) suggested that it may be a mix of MOET and natural mating or artificial insemination that provides optimum results. MOET was examined in what may be its most profitable area: to breed stud rams which will disseminate genetic improvement widely through multiplier studs to commercial flocks. Embryos were collected at a rate equivalent to 3.45 live lambs per donor ewe. The benefits of MOET were then calculated from the discounted expressions of rams sold, in comparison with the costs incurred. The results showed that as MOET is introduced into the natural mating flock, the genetic gain increases rapidly at first but then tends to plateau as the proportion of MOET bred lambs reaches 100%. Indicating that the economic optimum is nearly always less than 100% MOET. The optimum number of lambs born from MOET is approximately twice the number of stud rams to be produced for stud use and sale.

At present MOET in New Zealand is an expensive procedure costing approximately $320 per donor ewe programmed and flushed and $75 per recipient prepared (NZ Breeding Service: Hamilton; personnel communication, 1996). With 6 recipients prepared per donor this represents a cost of $750 per donor, or with 3.5 lambs per donor a cost of $214 per lamb. If 0.35 rams are sold per lamb born, then to cover the costs of MOET, each ram must be sold at a premium of $612 over and above the price usually received for rams. Although many nucleus breeders may also own daughter studs and therefore the cost of a MOET scheme may be spread over a large number of rams sold, in the present economic climate it may be difficult to financially justify a MOET breeding programme.

It is evident that the cost of each lamb born is a direct function of the number of lambs that are able to be sold from each donor ewe. Therefore if research and development were able to improve the response rate of donor ewes, the premium at which the stud rams needed to be sold would be reduced, consequently reducing the financial risk the individual breeder is faced with when adopting this technology. However, in spite of this, on a national basis, the benefits may easily outweigh the cost of a MOET scheme. If the MOET flock provides rams to the daughter studs, which use them to breed rams for commercial flock, the industry as a whole could achieve substantial economic benefits.

A major problem for the sheep industry in New Zealand is the lack of development in extension services, which will inform the farmer of the new technologies and their potential benefits. Until the awareness of techniques like MOET is stimulated it is unlikely the application of such methods will be widespread.

Another area in which MOET may have considerable potential is in developing countries (Nicholas, 1996). Despite the relatively sophisticated technology required, the fact that it is undertaken at a single site, under the control of a small team of workers, makes it potentially an attractive proposition. The benefits of improvement in a MOET nucleus can then be spread through to the commercial level by the provision of natural service rams from the nucleus, or if the source is insufficient to meet demand, from one or more multipliers.

James (1988) outlined the use of MOET for the rapid multiplication of rare animals, such as exotic breeds of sheep or valuable mutants. An idea of the rate of expansion can be obtained by supposing a ewe can be treated to give 5 female offspring when she is 2 years old, and that a similar result can be obtained in each of the following 5 years, so that a ewe can produce 30 daughters in her lifetime, with an equal number of sons, and all her daughters and their daughters achieving the same results. The number of animals born of both sexes 10 years after the birth of the first 5 daughters of a single original ewe would be nearly 175,000. Thus 3 original ewes could result in over 500,000

progeny being born after their first lamb drop. While conducting such a large programme would be difficult the potential is evident. Although inbreeding would need to be monitored the goal of rapid multiplication can be achieved.

MOET will also play a vital role in the application of currently developing reproductive technologies such as transgenics, embryo sexing, cloning and marker assisted selection.

Chapter Three

Materials and Methods

Materials and methods for the Texel and Awassi MOET programmes vary in part an they will be discussed separately where appropriate.

3.1 Texel Programme

All the animals used are the property of Premier Texels Ltd and were kept year round on the Glen Aros farm near Tikokino in Central Hawkes Bay. Embryo recovery took place at Glen Aros and was conducted on 3 consecutive days during March 1995.

3.1.1 *Donor and recipient ewes*

The donor ewes were purebred Texels, consisting of two age groups (two-tooth and four-tooth). Some of the four-tooth ewes had been used for embryo collection the previous year whereas embryo recovery had not been previously attempted from the two-tooth ewes. The recipient ewes were mixed-aged Romneys. All ewes (recipients and donors) were individually identified with plastic ear tags Donor and recipient ewes were grazed on ryegrass-clover pasture and subjected to normal husbandry practice throughout the year so as to maintain satisfactory liveweights. Nutritional 'flushing' of donor ewes, on ad libitum pasture, began 3-4 weeks before the initiation of superovulatory treatments. Importance was placed on liveweight gain rather than final liveweight. Recipient ewes were offered a rising plane of nutrition over the same period, and were in, at least, forward store condition at the time of embryo transfer.

3.1.2 *Rams*

Pure-bred Texel rams were used for artificial insemination. Semen morphology tests were carried out on all rams prior to the trial, those rams with greater than 25% abnormal sperm were not used. Vasectomized Dorset and Romney rams harnessed with marker crayons were used as 'teasers' for oestrus detection among recipient and donor ewes at a ratio of 1:10.

3.1.3 *Synchronisation of oestrus*

Progesterone impregnated (9%) controlled internal drug release devices (CIDR-G™: Carter-Holt-Harvey, NZ) were implanted for 12 days to attempt oestrus synchrony in the donor ewes. The CIDR-G™ was replaced after 9 days in order to maintain a high circulating level of progesterone.

The recipient ewes were synchronised in oestrus with a single CIDR-G™ left in place for 11 days.

All ewes (donors and recipients) were put with the teaser rams immediately following CIDR-G™ removal. Donor ewes were examined 3 times a day for mating marks. Recipient ewes were examined every 12 hours. A ewe was considered as showing heat when she was well marked with crayon on her rump.

3.1.4 *Superovulatory regime*

For commercial reasons, the specific nature of the superovulatory regime employed in this trial is confidential. However, it commenced 72 hours prior to CIDR-G™ removal and involved a series of FSH-P injections (Folltropin; Vetrepharm Ltd, Canada) in combination with PMSG.

3.1.5 *Artificial insemination technique*

Semen was collected by artificial vagina. After collection the motility of the semen was microscopically evaluated and the volume and colour of the ejaculate examined to subjectively assess the semen concentration. The semen was then diluted at a ratio of 1:8 (semen:diluent) with a standard egg yolk diluent and kept for a maximum of 1 hour before insemination. Once oestrus was detected in the donor ewes they were inseminated ***intra-uterine*** approximately 8-12 hours later. Donor ewes were fasted from the time of oestrus detection to the time of artificial insemination. The ewes were tranquillised with 10mg Acetylpromazine (ACP; Ethical Agents Ltd, New Zealand) given intramuscularly and a local anaesthetic, 2% Xylocaine, was injected into the abdominal wall just anterior to the mammary glands. The ewes were restrained and

inclined in a laparotomy cradle for insemination. The technique used was the same as that described by Dattena (1989). A total of 200 million sperm (100 million sperm each horn) were inseminated. Antibiotic (Streptopen) injections were given to all ewes after insemination.

3.1.6 *Embryo recovery*

Recovery of embryos was attempted 6.5 days after oestrus detection (6 days after AI). The donor ewes were fasted for 12 hours prior to surgery. General anaesthesia was initially induced with sodium thiopentone (Pentothal) at 20mg/kg of liveweight and maintained with halothane and oxygen.

Figure I

Laparoscopic examination of the ovaries

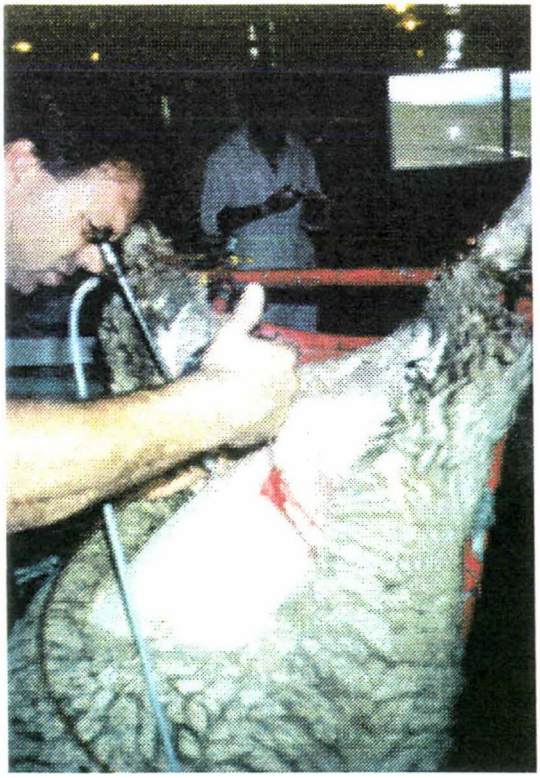
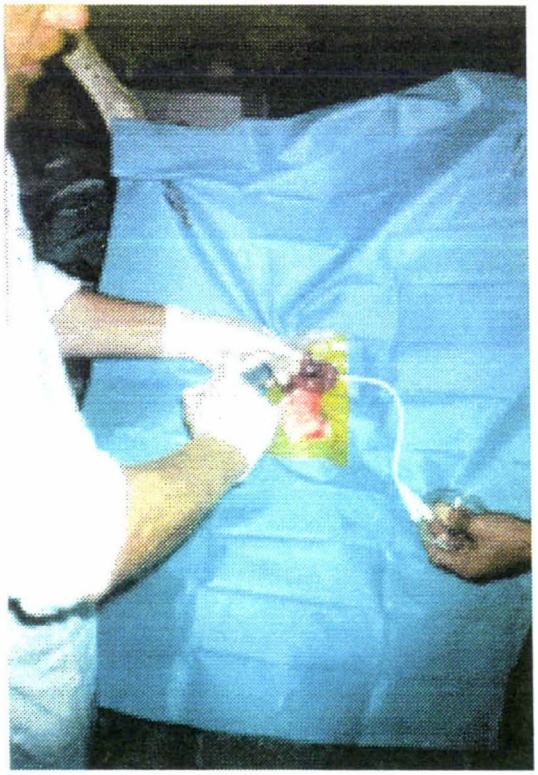


Figure II

Flushing of the uterus



Following the cleaning and removal of wool from the abdominal area, the ovulatory response of each donor ewes was assessed by laparoscopic examination. The abdominal cavity was inflated with CO_2 gas to aid in the manipulation of the reproductive tract and

visualisation of the ovaries (see Figure I). If less than 3 corpora lutea were present on the ovaries, the animal was not operated on.

A mid-ventral incision was made in the abdominal wall and the uterus was exteriorised. Each uterine horn was then flushed in the same manner as the technique described by Heywood (1993), as shown in Figure II. The flushing media was 30-35 mls of phosphate buffered saline (PBS) with 2% bovine serum albumin (BSA) stored at 37°C, which was allowed to cool to room temperature (approx. 20°C) after embryo flushing.

All donor ewes received an antibiotic injection after surgery and an injection of a prostaglandin, to prevent possible pregnancy, 24 hours later.

3.1.7 *Embryo classification*

The search for embryos was carried out immediately after recovery using a stereoscopic microscope. The embryos were examined and classified into 3 quality classes (Q1, Q2 and Q3+), according to the following criteria.

1. Degree of symmetry of the embryo.
2. Density of the cell mass.
3. Degree of cell division as would be expected for the age of the embryo.
4. The presence of irregularities or degenerative areas,

as described in Table 3.1.

Table 3.1 Embryo classifications

Classification	Description
Q1	Embryos of the appropriate stage of development with no signs of irregularities or degenerate areas.
Q2	Embryos of the appropriate stage of development but showing some irregularities and/or degenerate areas.
Q3+	Unfertilised ova or under-developed fertilised eggs of 2-8 cells, with or without degenerate areas or embryos of the appropriate stage of development, but with a high proportion of degenerate cells and/or irregularities

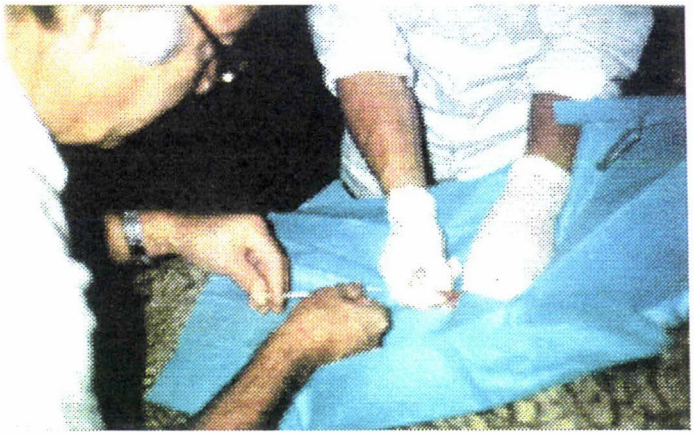
Only recovered embryos that were assessed as Q1 and had not reached the hatched blastocyst stage were frozen, the majority of the remaining Q1 and Q2 embryos were transferred into recipient ewes. Q3+ embryos were not transferred.

3.1.8 *Transfer to recipients*

Transfer of embryos was carried out with the ewes sedated under Acetylpromazine tranquilliser (10mg intramuscularly) and local anaesthesia (10 mg; 2% Xylocaine) infused subcutaneously while the ewes were restrained in a laparotomy cradle. The number of corpora lutea on each ovary was determined laparoscopically and provided a CL was present on the ovary, two embryos of either Q1 or Q2 quality were transferred into that uterine horn. All embryos were transferred within 1½ hours of recovery. The transfer technique used was the same as that described by Heywood (1993), as shown in Figure III. A CIDR-G™ was inserted and an antibiotic injection was given to all recipient ewes after the transfer was completed.

Figure III

Transfer of embryos into recipient ewes



3.1.9 *Management of recipients*

The CIDR-G™ inserted after transfer remained in place for a minimum of 21 days, after which time it was removed. The recipient ewes were fed ad libitum for the following 3 weeks.

All recipient ewes were subjected to ultra-sound scanning approximately 55 days after surgery, to determine pregnancy and the number of foetuses in order to estimate

embryo survival rates. Based on the scanning results, those recipients that were not pregnant were then removed from the flock.

3.1.10 *Embryo preservation*

Embryos selected for preservation were frozen following a standard embryo freezing procedure. The embryos were stored in individually marked 0.25 ml straws with an ovine culture medium containing 10% glycerol. A maximum of 5 embryos were sealed into each straw using small hot scissors. The embryos were then frozen.

The freezing of embryos was carried out in a programmable freezing machine. The freezing procedure was initiated at a cooling rate of -2°C/min from ambient temperatures until -5°C to -7°C was reached. The embryos were kept at this temperature for 5 minutes after which the embryos were cooled at -0.3°C/min until they reached -35°C. The embryos were then plunged directly into liquid nitrogen at -196°C. Once frozen and maintained in liquid nitrogen the embryos were able to be stored for export.

3.2 Awassi Programme

All the animals used are the property of Awassi New Zealand Ltd and were kept year round on Mathews Station near Tikokino in Central Hawkes Bay. Embryos were collected on 7 separate occasions within a 21 month period (see Table 3.2).

Table 3.2 Details of embryo collections attempted from Awassi sheep.

Date	Number of ewes flushed		
	Hoggets	Mixed age ewes	Total
12-13/6/95	25		25
12/3/96		8	8
19/3/96		9	9
26/3/96		10	10
18/4/96	5	7	13
9/5/97		6	6
27/3/97		8	8

3.2.1 *Donor and recipient ewes*

The donor ewes were either three-quarter or purebred Awassi ewes, consisting of hoggets and mixed-age ewes. The majority of the mixed age ewes were 18-22 months of age, but the flock did include several 5 year-old ewes. A number of the mixed age ewes had previously been used for embryo collection. The recipient ewes were mixed-aged Romneys. The ewes were subjected to normal husbandry practice throughout the year so as to maintain satisfactory liveweights. Nutritional 'flushing' of donor ewes began 6-8 weeks before the initiation of superovulatory treatments. Care was taken to avoid the grazing of oestrogenic pastures. Importance was placed on liveweight gain rather than final liveweight. Recipient ewes were offered a rising plane of nutrition over the same period. All ewes were individually identified with plastic ear tags.

3.2.2 *Rams*

Pure-bred Awassi rams were used for artificial insemination. Semen was collected 1-2 times a week for 4 weeks prior to artificial insemination, during which time a semen morphology test was carried out on all rams; those rams with greater than 25% abnormal sperm were not used. Vasectomized Romney and halfbred Romney Poll Dorset rams harnessed with marker crayons were used as 'teasers' for oestrus detection among recipient and donor ewes at a ratio of 1:10.

3.2.3 *Synchronisation of oestrus*

Progesterone impregnated (9%) controlled internal drug release devices (CIDR-G™; Carter-Holt-Harvey, NZ) were implanted for 12 days to attempt oestrus synchrony in donor ewes. The CIDR-G™ was replaced after 10 days in order to maintain a high circulating level of progesterone.

The recipient ewes were synchronised in oestrus with a single CIDR-G™ left in place for 12 days.

All ewes (donors and recipients) were put with 'teaser rams' immediately following CIDR-G™ removal. Donor and recipient ewes were examined 4 times a day for mating marks. A ewe was considered as showing heat when she was well marked with crayon on her rump.

3.2.4 *Superovulatory regime*

The superovulatory regime commenced 10 days after the insertion of CIDR's and consisted of a series of descending doses of FSHp (Folltropin; Vetrepharm Ltd, Canada) administered intramuscularly over a 4 day period, with an injection of Folligon (Intervet Laboratories Ltd, UK) at CIDR-G™ removal. The doses (mls) of FSHp for hoggets and ewes were given as shown in Table 3.2.

Table 3.3 Superovulatory regime for Awassi hoggets and ewes (ml of FSHp)

Day	10 am	10 pm	11 am	11 pm	12 am	12 pm	13 am	13 pm
Hoggets	2.5	2.5	1.5	1.5	1.0	0.9	0.8	0.8
Ewes	2.7	2.7	1.7	1.7	1.3	1.2	1.0	1.0

Note: CIDR-G™ insertion is day 1.

Hoggets received a total dose of 11.5 mls and ewes 13.3 mls. A 0.5ml dose of Folligon was administered in both hoggets and ewes on day 12 (pm).

3.2.5 *Artificial insemination technique*

Semen was collected by artificial vagina, the motility of the semen was assessed by microscopic evaluation and the volume and colour of the ejaculate were examined to subjectively assess semen concentration. The semen was then diluted at a ratio of 1:8 (semen:diluent) with a commercial Tris-citrate sheep semen diluent. The majority of inseminations occurred within 1-2 hours of collection. If the semen was not inseminated within a 2 hour period, a chilling process was initiated to reduce the semen to 4°C. Once oestrus was detected in the donor ewes they were inseminated *intra-uterine* approximately 8-12 hours later. Donor ewes were fasted from the time of oestrus detection to the time of artificial insemination. The ewes were tranquillised with 10mg Acetylpromazine (ACP; Ethical Agents Ltd, New Zealand) and a local anaesthetic of 5mg Xylocaine was injected anterior to the mammary glands. The ewes were restrained and inclined in a laparotomy cradle for insemination. The technique used was the same as that described by Dattena (1989). A total of 200 million sperm (100 million sperm into each horn) were inseminated.

3.2.6 *Embryo recovery*

Embryo recovery was attempted 6.5-7 days after oestrus detection (6-6.5 days after AI). The donor ewes were fasted for 12 hours prior to surgery. General anaesthesia was

initially induced with sodium thiopentone (Pentothal) at 20mg/kg of liveweight and maintained with a mixture of halothane and oxygen.

Following the cleaning and removal of wool from the abdominal area, the ovulatory response of each donor ewe was assessed laparoscopically prior to surgery. If less than 2 corpora lutea were present on the ovaries, the animal was not operated on.

Each uterine horn was then flushed with the same materials and in the same manner as described in the Texel section.

3.2.7 *Embryo classification*

The search for embryos was carried out immediately after recovery using a stereoscopic microscope. Embryos were classified in the same manner as described in the Texel materials and methods section. All Q1 and Q2 and the majority of Q3+ embryos were transferred.

3.2.8 *Transfer to recipients*

Transfer of embryos was carried out with the ewes under a Xylazine/Ketamine tranquilliser. A mixture of 2mg Xylazine and 300mg Ketamine was administered intravenously while the ewes were restrained in a laparotomy cradle. The number of corpora lutea on each ovary was determined laparoscopically and provided a CL was present on the ovary, two embryos were transferred into that uterine horn. All embryos were transferred within 4 hours of recovery. The transfer technique used was the same as that described by Heywood (1993). A CIDR-G™ was inserted in all recipient ewes after the transfer was completed.

3.2.9 *Management of recipients*

The CIDR-G™ implanted after transfer remained in place for a minimum of 14 days, after which time it was removed. Management of recipient ewes was the same as that described in the Texel materials and methods section.

3.3 Analysis of Data

All information obtained from Texel ewes at the Glen Aros farm will be defined as the Texel data set, while that obtained from Awassi ewes at Mathews station will be defined as the Awassi data set.

3.3.1 *Ovulation rate and recovery rate*

Ovulation rate was defined as the total number of fresh corpora lutea present in both ovaries, at the time of embryo recovery.

The recovery rate was defined as the total number of ova recovered/ number of corpora lutea present. Only Awassi ewes with an ovulation rate of 2 or more, and Texel ewes with an ovulation rate of 3 or greater were flushed.

For statistical analysis the data are expressed on a per ewe basis. The original data included values between 0 and 100% and was not normally distributed, therefore the data were analysed following square root transformation. The significance of factors affecting the response variable were tested using ANOVA procedures and student t-tests on the transformed data. However to facilitate the interpretation and discussion of the results, arithmetic means are also reported.

3.3.2 *Fertilisation rate, yield of good quality embryos and embryo survival to scanning and birth.*

Fertilisation rate was defined as the total number of fertile embryos recovered/ total number of ova recovered.

The percentage of good quality embryos recovered was defined as the number of good quality embryos recovered/ number of fertilised embryos recovered. Embryos classified as either Q1 or Q2 at the time of recovery were considered good quality embryos.

Embryo survival to scanning was defined as the number of foetuses present at scanning (day50-55)/ number of embryos transferred into recipient ewes.

Embryo survival to birth was defined as the number of lambs born/ number of embryos transferred into recipient ewes.

It is important to note that in the Texel data set all Q2 embryos and only Q1 embryos not of export quality were transferred. All Q1 and Q2 and the majority of Q3 embryos were transferred in the Awassi data set.

Fertilisation rate, yield of good quality embryos and survival rates to scanning and birth were analysed using chi-square tests.

The following were used to determine levels of significance

$p > 0.05$ Not significant

$p < 0.05$ Significant

$p < 0.01$ Highly significant

Due to the limited numbers of observations in some cases, it is difficult to obtain relevant levels of significance, therefore variable effects with a statistically significance of $p<0.10$ are presented in tabular form in the results section. Non-significant results are included in Appendix I.

Although general comparisons are made between the Texel and Awassi, detailed statistical comparisons between the two breeds are not included as each programme was conducted separately in differing environments.

Chapter Four

Results

4.1 Ovulation Rate

A total of 93 Texel and 78 Awassi observations were available to study the ovulatory response following their respective treatments.

The ovulation rates for the respective breeds are presented in Table 4.1.1 and the ovulation rates for each donor breed and age combination are presented in Table 4.1.2.

Table 4.1.1 Overall ovulation rate for each breed

Breed	No.Ewes	No.corpora lutea	Ovulation rate	± SE
Texel	93	827	8.89	0.38
Awassi	78	552	7.08	0.27

Table 4.1.2 Ovulation rate for each donor breed by donor age combination

Breed	Age (months)	No.ewes	Ovulation rate	± SE	p-value
Texel	18-22	55	8.49	0.46	
	30-34	38	9.47	0.34	0.22
Awassi	6-12	30	7.03	0.60	
	18+	48	7.10	0.50	0.79

There was no significant interaction between the respective age groups in the Texel data set.

No significant interaction was found between age groups in the Awassi data set.

4.2 Recovery Rate

Only Awassi ewes with 2 or more corpora lutea and Texel ewes with 3 or more corpora lutea were flushed and hence available for recovery rate analysis. A total of 92 Texel and 75 Awassi ewes were available to estimate recovery rate.

The recovery rates for the two breeds are presented in Table 4.2.1. The recovery rates for each donor breed and age combination is presented in Table 4.2.2. The effect of repeated flushing in the Awassi data set is presented in Table 4.2.3. The percentage of embryos recovered relative to ovulatory response in the Awassi data set is presented in Table 4.2.4.

Table 4.2.1 Overall recovery rate for each breed

Breed	No. ewes	No.ovulations	No.embryos recovered	Recovery rate (%)
Texel	92	824	592	71.8
Awassi	75	548	428	78.1

Table 4.2.2 Recovery rate for each donor breed by donor age combination

Breed	Age (months)	No. ewes	Recovery rate (%)	± SE	p-value
Texel	18-22	54	68.3	4.9	
	30-34	38	77.8	5.6	0.32
Awassi	6-12	30	65.2	7.6	
	18+	45	90.7	6.4	0.006

Table 4.2.3 Effect of repeated flushing on recovery rate for the Awassi data set

Previously Flushed	No.ewes	Recovery rate (%)	±SE	p-value
Yes	26	92.8	5.5	
No	52	74.7	8.9	0.06

Table 4.2.4 Effect of ovulatory response on recovery rate in the Awassi data set

Number of ovulations	No. ewes	Recovery rate (%)	±SE	p-value
1-6	31	94.0	8.7	
7-10	34	69.2	5.3	0.08
11+	10	77.1	13.0	

Examination of the Texel data showed an average recovery rate of 72.2 ± 3.7% (Appendix I: Table 7.2.1). There were no significant interactions found. There was no significant effect of age (Table 4.2.2), donor surgeon (Appendix I: Table 7.2.2) or ovulatory response group (Appendix I: Table 7.2.3).

From the analysis of the Awassi data the average recovery rate was 80.5 ±4.8% (Appendix I: Table 7.2.1). Between age groups their was a highly significant interaction (p=0.006, Table 4.2.2), while previous flushing (p=0.06, Table 4.2.3) and ovulatory response (p=0.08, Table 4.2.4) did not have statistically significant effects, there was no effect of repeated flushing of Awassi ewes only (Appendix I: Table 7.2.5) on embryo recovery rate.

4.3 Fertilisation Rate

Only ewes from which embryos had been recovered were used for fertilisation rate analysis. A total of 88 Texel and 74 Awassi ewes were therefore available to estimate fertilisation rate.

The overall fertilisation rate for the two breeds is presented in Table 4.3.1. The fertilisation rate for each donor breed and donor age combination is presented in Table 4.3.2.

Table 4.3.1 Overall fertilisation rate for each breed.

Breed	No. ewes	No.embryos recovered	No.fertile embryos	Fertilisation rate (%)
Texel	88	592	560	94.6
Awassi	74	428	332	77.6

Table 4.3.2 Fertilisation rate for each donor ewe by donor age combination

Breed	Age (months)	No. ewes	No.embryos recovered	Fertilisation rate (%)	p-value
Texel	18-22	52	312	95.4	
	30-34	36	280	95.7	0.76
Awassi	6-12	29	138	77.5	
	18+	45	290	77.6	0.99

When analysed individually the average fertilisation rate was 94.1% (± 1.7) for the Texel and 69.7% (± 4.4) for the Awassi animals respectively.

Analysis of the Texel data found no significant effect of age (Table 4.3.2), sire (Appendix I: Table 7.3.1) or ovulatory response group (Appendix I: Table 7.3.3), on fertilisation rate.

There was no significant effect of age (Table 4.3.2), sire (Appendix I: Table 7.3.2), ovulatory response group (Appendix I: Table 7.3.4) or previous flushing (Appendix I: Table 7.3.5), on fertilisation rate in the Awassi data set.

4.4 Embryo Quality

Only ewes from which fertilised embryos had been recovered were used for embryo quality analysis. A total 86 Texel and 70 Awassi ewes were therefore available to assess embryo quality.

The overall yield of good quality embryos for both breeds is presented in Table 4.4.1. The yield of good quality embryos for each donor breed and age combination is presented in Table 4.4.2.

Table 4.4.1 Yield of good quality embryos for each breed

Breed	No.fertile embryos	No.good quality embryos	good quality embryos(%)
Texel	560	513	91.6
Awassi	332	266	80.1

Table 4.4.2 Yield of good quality embryos for each donor breed and donor age combination

Breed	Age (months)	No.fertile embryos	Good quality embryos(%)	p-value
Texel	18-22	292	89.7	
	30-34	268	93.7	0.47
Awassi	6-12	107	64.4	
	18+	225	87.5	0.03

Analysis of the Texel data set found there was no significant effect of donor age (Table 4.2.2) or ovulatory response group (Appendix I: Table 7.4.1) on the yield of good quality embryos.

Donor age was the only factor to have a significant effect (p=0.03, Table 4.2.2) on embryo quality in the Awassi data set. Ovulatory response group, (Appendix I: Table 7.4.2) had no effect on the yield of good quality embryos.

4.5 Embryo Survival to Scanning

All Q2 embryos and Q1 embryos not of sufficient quality to be frozen were transfered in the Texel data set, while all Q1, Q2 and the majority of Q3 embryos were transferred in the Awassi data set. Therefore embryo survival to scanning was estimated with 125 Texel embryos and 306 Awassi embryos.

The overall survival rates for the two breeds are presented in Table 4.5.1. The survival rate for each donor breed and age combination is presented in Table 4.5.2. The effect of the number of corpora lutea in the Awassi recipient ewes is presented in Table 4.5.3.

Table 4.5.1 Embryo survival rate to scanning for each breed

Breed	No.embryos transferred	No.embryos surviving	Survival rate (%)
Texel	125	75	60.0
Awassi	306	182	59.5

Table 4.5.2 Embryo survival to scanning for each donor breed by donor age combination

Breed	Age (months)	No.embryos transferred	Survival rate (%)	p-value
Texel	18-22	84	61.9	
	30-34	41	56.1	0.66
Awassi	6-12	89	43.8	
	18+	217	65.9	0.02

Table 4.5.3 Effect of the number of corpora lutea on the survival rate to scanning in the Awassi data set

Number of CL's in recipient ewes	No.embryos transferred	No.embryos surviving	Survival rate (%)	p-value
1	214	117	54.7	
2	92	65	70.7	0.10

Analysis of the Texel data set showed donor age did not significant effect embryo survival to scanning (Table 4.5.2).

With the Awassi data, age of the ewe had a significant effect on embryo survival ($p=0.02$, Table 4.5.2), while the number of corpora lutea present in the recipient ewes did not have a statistically significant effect ($p=0.10$, Table 4.5.3).

4.6 Embryo Survival to Birth

All Q2 embryos and Q1 embryos not of sufficient quality to be frozen were transferred in the Texel data set, while all Q1, Q2 and the majority of Q3 embryos were transferred in the Awassi data set. Therefore embryo survival to birth was estimated with 125 Texel embryos and 306 Awassi embryos.

The overall survival to birth rates for the two breeds are presented in Table 4.6.1. The survival rate for each donor breed and age interaction is presented in Table 4.6.2. The effect of the number of corpora lutea in the Awassi recipient ewes is presented in Table 4.6.3.

Table 4.6.1 Embryo survival rate to birth for each breed

Breed	No.embryos transferred	No.embryos surviving	Survival rate (%)
Texel	125	65	52.0
Awassi	306	164	53.6

Table 4.6.2 Embryo survival to birth for each donor breed by donor age combination

Breed	Age (months)	No.embryos transferred	Survival rate (%)	p-value
Texel	18-22	84	54.8	
	30-34	41	46.3	0.53
Awassi	6-12	89	39.3	
	18+	217	59.4	0.03

Table 4.6.3 Effect of the number of corpora lutea on the survival rate to birth in the Awassi data set

Number of CL's in recipient ewes	No.embryos transferred	No.embryos surviving	Survival rate (%)	p-value
1	214	104	48.6	
2	92	60	65.2	0.07

There was no significant effect of donor age, on embryo survival to birth, in the Texel data set (Table 4.5.2).

With the Awassi data, age of the sheep had a significant effect on embryo survival ($p=0.03$, Table 4.6.2), The number of corpora lutea present in recipient ewes did not influence the survival of the embryos ($p=0.07$, Table 4.6.3).

The Texel programme produced 356 embryos, at an average of 3.89 per donor ewe, that were of sufficient quality to be preserved for export. In addition 65 lambs were produced at an average of 0.70 per donor ewe.

The Awassi programme produced a total a total of 164 lambs at an average of 2.1 per donor ewe programmed.

Chapter Five

Discussion and Conclusions

5.1 Ovulation Rate

Texel ewes were found to be more responsive than Awassi ewes (8.89 vs 7.08). Numerous workers have reported on superovulatory response being modified by the breed being treated. These include. Bindon *et al.*, (1971) and Smith (1976) who observed higher superovulatory responses in ewes selected for fecundity, which may indicate that the ovaries of high fecundity sheep are more sensitive to gonadotrophins.

Age did not significantly affect ovulatory response in either data set. The higher response of the older Texel ewes was in contrast to the findings of Greaney (1991) who, using similar aged Texel ewes, found younger ewes to have a significantly higher ovulation rate than older ewes. A similar trend was also found in the majority of other breeds used in that study.

Similar ovulatory responses were observed in both Awassi age groups. From the literature the effect of age on ovulatory response to superovulation is unclear. Robinson (1951) noted a considerably lower ovulation rate from ewe lambs than adult ewes treated in a similar way. Low ovulation rates as well as poor embryo yields have been reported following the induction of superovulation in ewes less than 1 year old (Armstrong and Evans 1983). Similar results were also reported using a variety of hormonal regimes (Rangel-Santos *et al.*, 1991). However, Tervit *et al.*, (1989), who superovulated Coopworth ewe lambs and adult ewes, did not find a significant difference in the ovulatory response, but as a results of the low fertilisation rate in ewe lambs they gave less embryos than the adult ewes.

Cahill and Fry (1986) suggested that factors present in the follicular fluid can have a direct effect on the ovary and therefore modulate the gonadotrophin effects, as a result local factors may play an important role in the determination of ovulation rate. It is therefore probable that the state of the ovary at the time of stimulation, in terms of the

content of the follicular fluid or the number of large follicles and their sensitivity to local factors, may influence ovulatory response.

5.2 Recovery Rate

Recovery rate of ova was not significantly affected by any of the factors studied (donor age, donor surgeon or ovulatory response) in the Texel data set. Among the Awassi data, age had a significant effect, while previous flushing and ovulatory response did not have a statistically significant effect on average recovery rate. The recovery rates for the two breeds (71.8% and 78.1%: Table 4.2.1) were consistent with other workers using the same recovery technique, 79% (Moore and Shelton, 1962), 83% (Tervit and Havik, 1976), and 76%, (Torres and Cognie, 1984).

Older ewes had a higher, but not significantly, average recovery rate in the Texel data set (77.8% vs 68.3%: Table 4.2.6), while Awassi ewe lambs showed significantly lower embryo recovery rates than adult Awassi ewes (65.2% vs 90.7%, P=0.006: Table 4.2.6). In other New Zealand work, Rangel-Santos *et al.*, (1991) reported similar findings in ewe lambs (56% vs 87%). This again highlights the difficulty in achieving acceptable results from ewe lambs.

Repeated flushing of donor ewes has often been shown to result in the development of adhesions in the reproductive tract and lead to reduced recovery rates. Results from the Awassi data show a different trend, ewes that had been previously flushed had a higher average recovery rate than those that had not (92.8% vs 74.7%: Table 4.2.7). This was due largely in part to the inclusion of hoggets, which, with their significantly lower recovery rates, reduced the recovery rate for first time flushed animals. When the Awassi hogget data were eliminated, the outcome was similar with previously flushed ewes again having a higher, but not significantly, average recovery rate (92.8% vs 88.2%). This result is consistent with that of Moore (1977) who found initial recovery rates to have no effect on a subsequent collection 1 year later. However, other workers have found significant decreases in recovery rate for subsequent flushing (Hanrahan and Quirke, 1982; Torres and Sevellec, 1987). For that reason other non-surgical, laparoscopic (McKelvey *et al.*, 1986) and laparoscopically-aided (Tervit, 1989), methods have been developed.

Differences in skill between the surgeon conducting the embryo recovery did not cause significant variation on recovery rate in the Texel data set. Average recovery rates of 71.8%, 72.1% and 69.4% (Appendix I, Table 7.2.2) indicate similar abilities in conducting the procedure.

From the literature it appears uncertain as to the effect of ovulatory response on recovery rate. There was no significant difference in either the Texel data set (Appendix I, Table 7.2.3), or the Awassi data set (Table 4.2.4), which supports the findings of other workers (Torres and Cognie, 1984; Scudamore *et al.*,1991b). However there was a large range of responses in the Awassi data with the lowest response group having an average recovery rate of 94.0% compared to 69.2% and 77.1% for the medium and high response groups. Hanrahan and Quirke (1982) also reported a decline in recovery rate when the number of corpora lutea exceeded five.

5.3 Fertilisation Rate

Fertilisation rate of ova was not significantly affected by any of the factors studied (donor age, sire or ovulatory response) in the Texel data set. Neither of these factors, as well as previous flushing, had significant effects on the fertilisation rate in the Awassi data set. Fertilisation rate for the Texel data set was considerably higher than that of the Awassi (94.6% vs 77.6%: Table 4.3.1). The majority of studies suggest that the optimum time for AI is between 8-24 hours after the onset of oestrus. Both the Texel and Awassi ewes were inseminated between 8 and 12 hours after the onset of oestrus. The Texel results are similar other findings, Wright *et al.*, (1981) obtained a greater than 90% fertilisation rate with a 12 hour interval and Torres and Cognie (1984) achieved 94% fertilisation after a 24 hour interval. The Awassi results are well below those documented, the reasons for this are unclear but could be attributed to poor ram performance.

Age of the donor ewe had no effect on fertilisation rate in either data set, this was expected in the Texel data set, but the relatively high fertilisation rate in the Awassi ewe hoggets was not. Other workers have reported very poor fertilisation rates in ewe hoggets. Rangel-Santos *et al.*, (1991) found ewe hogget fertilisation rates to be as low as 48% compared to 82% in adult ewes.

The sire used had no effect on fertilisation rate in the Texel data set, fertilisation rates of 99.5%, 94.1% and 90.0% (Appendix I, Table 7.3.1) indicate consistent results. The fertilisation rate obtained from the six Awassi rams indicated a much larger range of results (62.4%-89.7%) however, it was not significant. All of the Texel and Awassi rams underwent semen morphology testing prior to the trial and each ejaculate was microscopically evaluated for volume and motility before insemination. Only rams with good quality ejaculates that had less than 25% abnormal sperm and were used. The implementation of these procedures is likely to have ensured that fertilisation rate was not significantly affected by the ram used.

Ovulatory response did not have a significant effect on fertilisation rate, although fertilisation rate was shown to increase with ovulation rate in the Awassi data set (69.5%, 76.8% and 89.9%: Appendix I, Table 7.3.4) for low, medium and high ovulatory response groups. These results are consistent with other studies where the presence of unfertilised embryos can not be attributed to excessive superovulation (Torres and Cognie, 1984). However, Wright *et al.*, (1981), Dattena (1989) and Greaney (1991) have failed to observed a decline in fertilisation rate as ovulation rate increased.

Contrary to other findings, repeated flushing did not decrease fertilisation rate. Awassi ewes that had been flushed previously had a slightly higher fertilisation rate than those which had not (81.1% vs 75.4%: Appendix I, Table 7.3.5), but the difference was not statistically significant. Hanrahan and Quirke (1982) recorded an average drop of 8% in fertilisation rate for each subsequent flushing. McKelvey *et al.*, (1986) suggested the decrease was due to the adhesions which develop in the ewes as a result of previous embryo recovery attempts.

5.4 Embryo Quality

The yield of good quality embryos was not influenced by any of the factors studied (donor age or ovulatory response) in the Texel data set; age was the only factor to have a significant effect in the Awassi data set. The overall yield of good quality embryos was 91.6% for the Texel data set and 80.1% for the Awassi data set (Table 4.4.1). The lower yield in the Awassi is due in part to the significantly lower yield in Awassi ewe hoggets than in Awassi ewes (64.4% vs 87.5%: p=0.03, Table 4.4.2). This is consistent with other workers who have reported a decline in embryo quality in younger donors (Dingwall *et al.*, 1993; Wolf and Mylne, 1994). The reasons for the variation in the quality of embryos are unknown, but events leading to fertilisation are thought to be involved. Donaldson (1984) indicated that an LH imbalance may result in the disturbance of normal oocyte and follicle maturation which subsequently results in poor ovum quality and reduced fertilisation rates. Reference has been made of varying ratios of FSH and LH in gonadotrophin preparations and the effect they may have on oocyte development and the formation of the embryos (Moor *et al.*, 1984). Therefore better control of LH levels may yield a higher percentage of good quality embryos. However, various breeds and age groups may have differing LH or FSH requirements (Dattena 1989).

5.5 Embryo Survival to Scanning and Birth

Survival rate to scanning and birth was not significantly affected by age in Texel data set. While age of the donor ewe had a significant effect, previous flushing and the number of corpora lutea in the recipients did not have a statistically significant effect in the Awassi data set. The survival rates to scanning (60.0% vs 59.5%) and birth (52.0% vs 53.6%) were similar for the Texel and Awassi respectively. This was not predicted as the top quality embryos collected from the Texel ewes were frozen and not transferred.

The survival rates in the Awassi data set were significantly affected by the lower scanning ewes (43.8% vs 65.9%; p=0.02, Table 4.5.1) and birth rates (39.3% vs 59.4%: p=0.03, Table 4.5.2) of the Awassi ewe hoggets than the Awassi adult ewes respectively. This is consistent with other reports on the subject. Quirke and Hanrahan (1977), found 33.3 % survival to birth for ewe hoggets compared to 72.9% for adult donor ewes. McMillan and McDonald (1985) reported 52.1% for ewes and 25% for ewe lambs when transferred into the uterus of ewe lambs. These results strongly suggest some form of impairment in the development of hogget ova.

The number of corpora lutea in the recipient ewes did not have a statistically significant effect on scanning and birth rates (p=0.10, Table 4.5.3 and p=0.07, Table 4 6.3) in the Awassi data set. This supports the findings of other workers who also found no effect (Moore and Rowson, 1960; Cumming and McDonald, 1970). Ewes received supplementary progesterone in the form of a CIDR-G™ after transfer. This exogenous source of progesterone is thought to mask any effect the number of CL's in recipient ewes, and hence variation in endogenous progesterone, would have on embryo survival rates (Greaney, 1991).

5.6 Number of Lambs Produced

Of the 93 Texel ewes programmed 65 lambs were produced at an average of 0.70 lambs per donor ewe. However it must be remembered that all the un-hatched blastocysts recovered from the Texel ewes were frozen for intended export and thus not transferred This accounts for an additional 3.89 embryos per donor ewe programmed that were frozen. If the assumption was made that half of the embryos would have survived to yield lambs, then it would indicate approximately 2.65 lambs per donor ewe. From the 78 Awassi ewes treated, 164 lambs were produced at an average of 2.10 lambs per donor. This compares favourably with the reports of other workers; 2.7 lambs, Hanrahan and Quirke (1982); 1.6, Walker *et al.*, (1986); 1.5, Dattena (1989); 2.1, Tervit (1989); 1.66, Greaney (1991). When the age groups are considered separately, the results show that the younger animals are less responsive. The 48 Awassi adult ewes produced a total of 129 lambs at an average of 2.7 per donor, while the 30 Awassi ewe hoggets produced 35 lambs at an average of 1.2 per donor.

The implementation of a successful embryo transfer programme is dependant on all the variables discussed in previous sections, however both a consistent ovulatory response and a high recovery rate of fertilised eggs are important from an economic and genetic improvement view point. The theoretical benefits of MOET have been assessed by several workers including Smith (1986) who suggested that, compared to normal reproduction, the rates of genetic change could be doubled with the use of MOET technology. More conservative estimates from Gaffney *et al.*, (1991), found genetic gains could be increased by 17% and 51% for adult and juvenile MOET schemes when 2.2 live lambs were born per donor ewe. While the potential genetic benefits are evident the inherent problem of obtaining an acceptable number of live offspring remains.

To achieve widespread acceptance, MOET technology must be financially beneficial to users, and for this to occur the value of the end product must be greater than the input. It is appropriate to compare the number of lambs born (2.1 per ewe programmed: Awassi) with other less expensive breeding options that have been employed to utilise the reproductive capabilities of the ewe. Accelerated lambing systems involving three

lambings in two years have produced 1.7 (Robinson, 1980) and 2.1 lambs/ewe/year (Dawe, 1991), while a mild dose of PMSG and synchronised natural mating has produced 1.76 lambs/ewe/year (Robinson, 1980). However the advantage that MOET has over these procedures is that they are able to be repeated several times throughout the year. As well as the lambs born to surrogate mothers the donor can itself produce one or more lambs in the same season.

It is important to note that the majority of other MOET studies that have been discussed were conducted in a controlled environment for the purpose of research. The information used in this study is from on-farm commercial operations in which conditions and procedure may not be as controlled as they are in a research orientated environment. In both the Texel and Awassi programmes the majority of the animal preparation was conducted on the property by trained farm staff, with contracted veterinarians performing the surgery. The results presented here indicate that successful MOET programmes can be conducted in an on-farm environment.

Chapter Six

References

Acritipoulou, S. and Haresign, W. (1980). Response of ewes to a single injection of an analogue of $PGF_{2\alpha}$ given at different stages of the oestrous cycle. *Journal of Reproduction and Fertility* 58:219-223.

Akbar, A.M., Nett, T.M. and Niswender, G.D. (1974). Metabolic clearance and secretion rates of gonadotrophins at different stages of the oestrous cycle in ewes. *Journal of Endocrinology* 94:1318-1324.

Allison, A.J., Aspinall, J.M., Edwards, J.R., Lynch, P.R. and Vivanco, H.W. (1989). Sheep breeds exotic to New Zealand; their performance and application to industry. *2nd International Congress for Sheep Veterinarians. Sheep and Beef Cattle Society of New Zealand Veterinary Association. Proceedings of the 19th Seminar. Massey University, New Zealand.* p204-214.

Armstrong, D.T. and Evans, G. (1983). Factors influencing success of embryo transfer in sheep and goats. *Theriogenology* 19:31-42.

Armstrong, D.T. and Evans, G. (1984). Hormonal regulation of reproduction: Induction of ovulation in sheep and goats with FSH preparations. *10th International Congress on Animal Reproduction and Artificial Insemination.* p8-15.

Averill, R.L.W. and Rowson, L.E.A. (1958). Ovum transfer in the sheep. *Journal of Endocrinology* 16:326-336.

Baker, T.G. (1982). Oogenesis and ovulation. In: Austin, C.R. and Short, R.V. (eds.). *Reproduction in Mammals #1; Germ Cells and Fertilisation.* Cambridge University Press, pp. 17-45.

Baird, D.T. (1983). Factors regulating the growth of the preovulatory follicle in the sheep and human. *Journal of Reproduction and Fertility* 69:343-352.

Baird, D.T., Swanston, I.A. and McNeilly, A.S. (1981). Relationship between LH, FSH and prolactin concentration and the secretion of androgens and oestrogens by the preovulatory follicle in the ewe. *Biology of Reproduction* 24:1013-1025.

Bindon, B.M., Chang, T.S. and Turner, H.N. (1971). Ovarian response to gonadotrophins by Merino ewes selected for fecundity. *Australian Journal of Agricultural Research* 22:809-820.

Betteridge, K.J. and Moore, N.W. (1977). Techniques in sheep and goats. In: Betteridge, K.J. (ed.). *Embryo transfer in farm animals: a review of techniques and applications,* Canada Department of Agriculture, pp. 37-38.

Boland, M.P, and Gordon, I. (1982). Effect of repeated horse anterior pituitary extract treatment on ovulatory response. *Veterinary Record* 111:391-392.

Bondurant, R.H. (1986). Embryo transfer in sheep and goats. In: Morrow, D.A (ed.). *Current therapy in theriogenology #2; diagnosis, treatment and prevention of reproductive diseases in small and large animals,* W.B Saunders, pp. 63-66.

Boundy, T., Clarkson, M.J. and Winter, A.C. (1985). Embryo transfer in sheep under practise conditions. *Veterinary Record* 117:15, 379-381.

Brash, L.D., Wray, N.R. and Goddard, M. (1996). Use of MOET in Merino breeding programmes: a practical and economic appraisal. *Journal of Animal Science* 62:241-254.

Buckrell, B.C., Buschbeck, C., Gartley, C.J., Kroetsch, T., McCutcheon, W., Martin, J., Penner, W.K. and Walton, J.S. (1993). A breeding trial using a transcervical technique for artificial insemination in sheep. *Proceedings of a Refresher Course for Veterinarians, University of Sydney.* 215:89-90.

Cahill, J.N. and Fry, R.C. (1986). Folliculogenesis and egg production. *Australian Society of Reproductive Biology*: 136.

Cahill, L.P., Marina., J.C. and Mauleon, P. (1979). Total ovarian follicular populations in ewes of high and low ovulation rate. *Journal of Reproduction and Fertility* 55:27-36.

Clarke, J.N., Tervit, H.R., Welch, R.A.S. and Harvey, T.G. (1984). Artificial Insemination in the sheep industry. *Proceedings of the Ruakura Farmers Conference*, pp. 54-58.

Coonrod, S.A., Coren, B.R., McBride, B.L., Bowen, M.J. and Kramer, D.C. (1986). Successful non-surgical collection of ovine embryos. *Theriogenology* 25:147.

Cognie, Y. and Mauleon, P. (1983). Control of reproduction in the ewe. In. Haresign, W. (ed.). *Sheep Production*, Butterworths, pp. 381-392.

Crosby, T.F., Boland, M.P., Al-Kamali, A.A. and Gordon, I. (1981). Superovulation and egg transfer in the Texel ewe after various progestogen treatments. *Research Report 1978-1979; University College of Dublin, Faculty of Agriculture.* p71-73.

Cumming, I.A. (1965). *A study of ovulation and early prenatal mortality in the New Zealand Romney ewe.* Master of Agricultural Science Thesis. Massey University. New Zealand.

Cumming, I.A. (1979). Synchronisation of ovulation. In: Tomes G.J., Robertson D.E. and Lightfoot R.J. (eds.). *Sheep breeding, 2nd ed.* Butterworths, pp. 403-421.

Cumming, I.A. and McDonald, M.F. (1970). Embryo survival in mature Romney ewes relative to liveweight and face cover. *New Zealand Journal of Agricultural Research* 13:372-384.

D'Alessandro, A., Martemucci, G., Toteda, F., Gambacorta, M. and Manchisi, A. (1996). Superovulation and embryo production in ewes using commercial pFSH. *Small Ruminant Research* 9: 3, 255-261.

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. New Zealand.

Dattena, M., Vespignani, S., Branca, A., Gallus, M., Ledda, S., Naitana, S. and Cappani, P. (1994). Superovulatory response and quality of embryos recovered from anoestrus ewes after a single injection of porcine FSH dissolved in polyvinylprolidone. *Theriogenology* 42: 2, 235-239.

Dawe, S. (1991). Intensive Systems. In: Cottle, D.J. (ed). *Australian Sheep and Wool Handbook.* Intak Press. pp. 386-386.

Dingwall, W.S., McKelvey, W.A.C., Mylne, J. and Simms, G. (1993). An evaluation of MOET in Suffolk sheep. *Proceedings of the British Society of Animal Production (Winter Meeting)*, Paper No. 173.

Diskin, M.G. and Niswender, G.D. (1989). Effect of progesterone supplementation on pregnancy and embryo survival in ewes. *Journal of Animal Science* 67:6, 1559-1563.

Di Zerga, G.S. and Hogden, G.D. (1981). Folliculogenesis in the primate ovarian cycle. *Endocrine Review* 2:27-49.

Donaldson, L.E. (1984). Dose of FSHp as a source of variation in embryo production from superovulated cows. *Theriogenology* 22:205-212.

Donaldson, L.E. (1985). LH and FSH profiles at superovulation and embryo production in the cow. *Theriogenology* 23:441.

Douglas, R.H. and Ginther, O.J. (1973). Luteolysis following a single injection of $PGF_{2\alpha}$ in sheep. *Journal of Animal Science* 37:990-993.

Driancourt, M.A. (1987). Follicular dynamics and intraovarian control of follicular development in the ewe. In: Roche, J.F. and O'Callaghan, D. (eds.). *Follicular growth and ovulation rate in farm animals.* Martinus Nijhoff, pp. 87-105.

Driancourt, M.A. (1991). Follicular dynamics in sheep and cattle. *Theriogenology* 35:55-80.

Driancourt, M.A. and Cahill, L.P. (1984). Pre-ovulatory follicular events in sheep. *Journal of Reproduction and Fertility* 71:205-211.

Driancourt, M.A., Gibson, W.R. and Cahill, L.P. (1985). Follicular dynamics throughout the oestrous cycle in sheep: a review. *Reproduction, Nutrition and Development* 25:1-15.

Eastwood, K. and McDonald, M.F. (1975). Reproductive differences in two-year-old Romney and Border Leicester x Romney ewes after gonadotrophin treatment. *New Zealand Journal of Agricultural Research* 18:109-117.

Eldsen, R.P., Nelson, L.D. and Seidel, G.E.Jr. (1978). Super-ovulating cows with follicle stimulating hormone and pregnant mare serum gonadotrophin. *Theriogenology* 9:17-26.

Evans, G. and Robinson, T.J. (1980). The control of fertility in sheep: endocrine and ovarian responses to progestogen - PMSG treatments in the breeding season and in anoestrus. *Journal of Agricultural Science (Cambridge)* 94:69-88.

Gaffney, B., Wade, C.M., Goddard, M.E. and Nicholas, F.W. (1991). Genetic and economic value of multiple ovulation and embryo transfer in nucleus Merino flocks. *Proceedings of the Australian Association of Animal Breeding and Genetics* 9:101-105.

Gheradi, P.B. and Lindsay, D.R. (1980). The effect of season on the ovulatory response of Merino ewes to serum from pregnant mares. *Journal of Reproduction and Fertility* 60:425-429.

Gordon, I. (1971). Induction of early breeding in sheep by standard and modified progestogen-PMS treatments. *Journal of Agricultural Science (Cambridge)* 76:337-341.

Gordon, I. (1975). Hormonal control of reproduction in sheep. *Proceedings of the British Society of Animal Production* 4:79-93.

Gordon, I. (1983). Artificial control of oestrus and ovulation. In: Gordon, I. (ed.). *Controlled Breeding in Farm Animals*, Pergamon Press UK, pp. 181-195.

Greaney, K.B. (1991). *A study of the results of an embryo transfer programme conducted during two seasonal periods using five imported breeds of sheep*. Master of Agricultural Science Thesis. Massey University. New Zealand.

Halbert, G.W., Dobson, H., Walton, J.S., Sharpe, P. and Buckrell, B.C. (1990). Field evaluation of a technique for transcervical intra-uterine insemination of ewes. *Theriogenology* 33:1231-1243.

Hanrahan, J.P. and Quirke, J.F. (1982). Selection on ovulation rate in sheep aided by the use of super-ovulation and egg transfer. *Proceedings of the World Congress of Sheep and Cattle Breeding* 2:329-335.

Haresign, W. (1985). The physiological basis for variation in ovulation rate and litter size in sheep: a review. *Livestock Production Science* 13:3-20.

Haresign, W., McLeod, B.J. and Webster, G.M. (1983). Endocrine control of reproduction in the ewe. In. Haresign, W. (ed.). *Sheep Production*, Butterworths, pp. 353-379.

Henderson, K.M., Savage, L.C., Ellen, R.L., Ball, K. and McNatty, K.P. (1988). Consequences of increasing or decreasing plasma FSH concentrations during the pre-ovulatory period in Romney ewes. *Journal of Reproduction and Fertility* 84:187-196.

Heywood, T. (1993). ET sheep. *Proceedings of a Refresher Course for Veterinarians, University of Sydney.* 215:69-76.

Holloway, I.J., Purchas, R.W., Power, M.T. and Thomson, N.A. (1984). A comparison of the carcass and meat quality of Awassi-cross and Texel-cross ram lambs. *Proceedings of the New Zealand Society of Animal Production* 54:209-214.

Holst, P.J. (1974). The time of entry of ova into the uterus of the ewe. *Journal of Reproduction and Fertility* 36:427-428.

Holt, N. (1989). ET in sheep. *Proceedings of a Refresher Course for Veterinarians, University of Sydney.* 127:31-54.

Hunter, G.J., Adams, C.E. and Rowson, L.E.A. (1955). Inter-breed ovum transfer in sheep. *Journal of Agricultural Science. (Cambridge).* 46:143-149.

Hunter, R.H.F. (1982). *Reproduction of Farm Animals*, Longman.

Ishwar, A.K and Memon, M.A. (1996). Embryo transfer in sheep and goats: a review. *Small Ruminant Research* 19:35-43.

Jabbour, H.N. and Evans, G. (1991). Ovarian and endocrine responses of Merino ewes following treatment with PMSG and GnRH or PMSG antiserum. *Animal Reproduction Science* 24:259-270.

James, J.W. (1988). Increasing genetic gains using reproductive techniques. *Proceedings of the Australian Association of Animal Breeding and Genetics, Economics of Livestock Improvement* 7:1-20

Jainudeen, M.R. and Hafez, E.S.E. (1993). Sheep and goats. In: Hafez, E.S.E. (ed.) *Reproduction in Farm Animals, 6th ed,* Lea and Febiger, pp. 330-342.

Kassem, R., Owen, J.B., Fadel, I., Juha, H. and Whitaker, C.J. (1989). Aspects of fertility and lamb survival in Awassi sheep under semi-arid conditions. *Research and Development in Agriculture* 6:3,161-168.

Killeen, I.D. (1976). The effects of age of egg and site of transfer on survival of transferred eggs in the ewe. *Theriogenology* 6:637.

Killeen, I.D. (1981). Embryo transfer procedures in the sheep; the factors which have a major influence on success rate. In: *Embryo Transfer in Goats*, Australian Society of Reproduction and Biology, pp.38-40

Knight, T.W., Thomson, N.A., Power, M.J. and Gallen, J. (1984). Growth rates, wool production and health of Awassi and Texel crossbred ram lambs. *Proceedings of the New Zealand Society of Animal Production* 54:247-250.

Land, R.B. and Hill, W.G. (1975). The possible use of superovulation and embryo transfer in cattle to increase response to selection. *Journal of Animal Production* 21:1-12.

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. New Zealand.

Lamond, D.R. and Urquhart, E.J. (1961). Sheep laparotomy cradle. *Australian Veterinary Journal* 37:430-431.

Lindsay, D.R. (1991). Reproduction in the sheep and goat. In: Cupps, P.T. (ed.). *Reproduction in Domestic Animals, 4th ed*, Academic Press, pp. 491-515.

McGinnis, L.K., Duplontis, S.C. and Youngs, C.R. (1993). Cryopreservation of sheep embryos using ethylene glycol. *Journal of Animal Reproduction Science* 30:273-280.

McIntosh, J.E.A., Moor, R.M. and Allen, W.R. (1975). Pregnant mare serum gonadotrophin: Rate of clearance from the circulation of sheep. *Journal of Reproduction and Fertility* 44:95-100.

McKelvey, W.A.C. and Robinson, J.J. (1984). Normal lambs following transfer of embryos by laparoscopy. *Veterinary Record* 115:230.

McKelvey, W.A.C., Robinson, J.J. Aitken, R.P. (1985). The evaluation of a laparoscopic insemination technique in ewes. *Theriogenology* 24:519-535.

McKelvey, W.A.C., Robinson, J.J. Aitken, R.P. and Robertson, I.S. (1986). Repeated recoveries of embryos from ewes by laparoscopy. *Theriogenology* 25:855-865.

McMillian, W.H. (1987). Post-mating progesterone supplementation in ewes and hoggets. *Proceedings of the New Zealand Society of Animal Production* 47:151-153.

McMillan, W.H. and McDonald, M.F. (1985). Survival of fertilised ova from ewe lambs and adult ewes in the uterus of ewe lambs. *Journal of Animal Reproduction Science* 8:235-240.

McMillan, W.H. and Hall, D.R.H. (1991). Superovulation in ewes: are follicle numbers a useful predictor of ovulation rate?. *Proceedings of the New Zealand Society of Animal Production* 51:133-137.

McNatty, K.P., Gibb, M., Dodson, C. and Thurley, D.C. (1981). Evidence that changes in luteinizing hormone secretion regulate the growth of the pre-ovulatory follicle in the ewe. *Journal of Endocrinology* 90:375-389.

McNatty, K.P., Hudson, N., Gibb, M., Ball, K., Henderson, K.M., Heath, D.A., Lun, S. and Kieboom, L.E. (1985). FSH influences follicle viability, oestradiol biosynthesis and ovulation rate in Romney ewes. *Journal of Reproduction and Fertility* 75:121-131.

McNeilly, A.S., Picton, H.M., Campbell, B.K. and Baird, D.T. (1990). Gonadotrophic control of follicle growth in the ewe. *Proceedings of the 3rd Ruminant Reproduction Symposium. Nice.*

Mariana, J.C., Monniaux, D., Driancourt, M.A. and Mauleon, P. (1991). Folliculogenesis. In: Cupps, P.T. (ed.). *Reproduction in Domestic Animals, 4th ed*, Academic Press, pp. 119-167.

Maurer, R.R. (1978) Freezing mammalian embryos: a review of the techniques. *Theriogenology* 9:1,45-68.

Maxwell, W.M.C. and Hewitt, L.J. (1986). A comparison of vaginal, cervical and intra-uterine insemination of sheep. *Journal of Agricultural Science (Cambridge),* 106*:* 191-193.

Maxwell, W.M.C., Szell, A., Hunton, J.R. and Ryan, J.P. (1990). Artificial breeding: embryo transfer and cloning. In. Oldham, C.M., Martin, G.B. and Purvis, I.W. (eds.). *Reproductive Physiology of Merino Sheep; concepts and consequences.* University of Western Australia, Perth, pp. 217-237.

Moor, R.M., Kruip, T.A.M. and Green, D. (1984). Intraovarian control of folliculogenesis: limits to superovulation?. *Theriogenology* 21:103-116.

Moore, N.W. (1977). Techniques in sheep and goats. In: Betteridge, K.J. (ed.). *Embryo transfer in farm animals: a review of techniques and applications*, Canada Department of Agriculture, pp. 38.

Moore, N.W. (1980). Procedures and results obtainable in sheep and goats. In: Morrow, D.A. (ed.). *Current therapy in Theriogenology: diagnosis, treatment and prevention of reproductive diseases in animals.* W.B. Saunders, pp. 89-94.

Moore, N.W. (1982). Egg transfer in the sheep and goat. In: Adams, C.E. (ed.). *Mammalian Egg Transfer*, CRC Press, pp. 119-133.

Moore, N.W. and Rowson, L.E.A. (1960). Egg transfer in sheep. Factors effecting the survival and development of transferred eggs. *Journal of Reproduction and Fertility* 1:332-349.

Moore, N.W. and Shelton, J.N. (1962). The application of the technique of egg transfer to sheep breeding. *Australian Journal of Agricultural Research* 13:718-724.

Moore, N.W. and Shelton, J.N. (1964). Response of the ewe to a horse anterior pituitary extract. *Journal of Reproduction and Fertility* 7:79-87.

Moore, N.W. and Shelton, J.N. (1964b). Effect of degree of synchronisation between donor and recipient, age of egg and site of transfer on the survival of transferred eggs. *Journal of Reproduction and Fertility* 7:145-152.

Murray, B.F., Boland, M.P., Crosby, T.F. and Roche, J.F. (1988). The effect of progesterone supplementation on pregnancy and litter size in the ewe. *11th International Congress on Animal Production and Artificial Insemination, University College Dublin, Ireland. Volume #2*, paper No. 110.

Nicholas, F.W. (1996). Genetic improvement through reproductive technology. *Journal of Animal Reproduction Science* 42:205-214.

Nicholas, F.W. and Smith, C. (1983). Increased rates of genetic change in dairy cattle by embryo transfer. *Journal of Animal Production,* 36:341-353.

Parkes, A.S. (1929). The internal secretions of the ovary. In: Longmans, London, pp 158-163.

Parr, R.A., Cumming, I.A. and Clarke, I.J. (1982). Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of sheep embryos in early gestation. *Journal of Agricultural Science (Cambridge)* 98:39-46.

Peterson, A.Y., Barnes, D., Shanley, R. and Welsh, R.A.S. (1984). Administering progesterone after mating improves pregnancy rate in sheep. *Proceedings of the New Zealand Endocrinology Society* 21:13.

Peterson, A.J., Tervit, H.R., Faircloigh, R.J., Havik, P.G. and Smith, J.F. (1976). Jugular levels of 13, 14-dihydro-15-keto-prostaglandin F and progesterone around luteolysis and early pregnancy in the ewe. *Prostaglandins* 12:551-558.

Picton, H.M., Tsonis, C.G. and McNeilly, A.S. (1990). FSH causes a time dependent stimulation of preovulatory follicle growth in the absence of pulsatile LH secretion in ewes chronically treated with gonadotrophin releasing hormone agonist. *Journal of Endocrinology* 126:297-307.

Quirke, J.F. and Hanrahan, J.P. (1977). Comparison of the survival in the uteri of adult ewes of cleaved ova from adult and ewe lamb. *Journal of Reproduction and Fertility* 51:487-489.

Rangel-Santos, R. (1987). *Studies on embryo transplantation procedures using Booroola-Merino x Perendale ewes.* Master of Agricultural Science Thesis. Massey University. New Zealand.

Rangel-Santos, R., McDonald, M.F. and Wickham, G.A. (1991). Evaluation of the feasibility of a juvenile MOET scheme in sheep. *Proceedings of the New Zealand Society of Animal Production* 51:139-142.

Reeves, J.J., Arimura, A. and Schally, A.V. (1971) Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the oestrous cycle in sheep. *Journal of Animal Science* 31:123-126.

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. *Journal of Agricultural Science* 41:6-63.

Robinson, T.J. (1979). Controlled breeding of sheep and goats. In. Tomes, G.J., Robertson, D.E and Lightfoot, R.J. (eds.). *Sheep breeding, 2nd ed.* Butterworths, pp. 439-450.

Robinson, T.J. (1980). Programmed year-round sheep breeding. *Australian Journal of Experimental Agriculture and Animal Husbandry* 20:667-673.

Robinson, T.J. and Lamond, D.R. (1966). Control of reproduction in sheep and cattle. *Proceedings of the Australian Society of Animal Production* 6:47.

Robinson, T.J., Wallace, J.M. and Aitken, R.P. (1989). Fertilisation and ovum recovery rates in superovulated ewes following cervical insemination or laparoscopic intra-uterine insemination at different times after progestogen withdrawal and in one or both horns. *Journal of Reproduction and Fertility* 87:771-782.

Rowson, L.E.A. and Moore, R.M. (1966). Embryo transfer in sheep: the significance of synchronising oestrus in the donor and recipient animal. *Journal of Reproduction and Fertility* 11:207-212.

Ryan, J.P., Bilton, R.J., Hunton, J.R. and Maxwell, W.M.C. (1984). Super-ovulation in ewes with a combination of PMSG and FSH. In: Lindsay, D.R. and Pearce, D.T. (eds.). *Reproduction in Sheep.* Cambridge University Press, pp. 338-341.

Samartzi, F., Boscos, C., Vainas, E. and Tsakalof, P. (1995). Superovulatory response of Chios sheep to PMSG during spring and autumn. *Journal of Animal Reproduction Science* 39: 3, 215-222.

Scaramuzzi, R.J. and Baird, D.T. (1976). The oestrous cycle of the ewe after active immunisation against prostaglandin F2∝. *Journal of Reproduction and Fertility* 46:39-47.

Scaramuzzi, R.J. and Radford, H.M. (1983). Factors regulating ovulation rate in the ewe. *Journal of Reproduction and Fertility* 69:353-367.

Scaramuzzi, R.J. and Campbell, B.K. (1990). Physiological regulation of ovulation rate in the ewe: a new look at an old problem. In. Oldham, C.M., Martin, G.B. and Purvis, I.W. (eds.). *Reproductive Physiology of Merino Sheep; concepts and consequences.* University of Western Australia, Perth, pp. 71-84.

Schiewe, M.C., Howard, J.G., Stuart, L.S., Goodrowe, K.L. and Wildt, D.E. (1985). Human menopausal gonadotrophin (hMG) for superovulation of sheep. *Theriogenology* 23: 227.

Schiewe, M.C., Rall, W.F., Stuart, L.D. and Wildt, D.E. (1991). Analysis of cryoprotectant, cooling rate and *in situ* dilution using conventional freezing or vitrification for cryopreserving sheep embryos. *Theriogenology* 36:279-293.

Schneider, U and Mazur, P (1986). Implications and applications of long term preservation by freezing. In. Marrow, D.A. (ed). *Current Therapy in Theriogenology 2nd ed*, W.B. Saunders, Philadelphia. pp. 81-83.

Scudamore, C.L., Robinson, J.J., Aitken, R.P., Kennedy, D.J., Ireland, S. and Robertson, I.S. (1991a). Laparoscopy for intra-uterine insemination and embryo recovery in superovulated ewes at a commercial embryo transfer unit. *Theriogenology* 35:330-337.

Scudamore, C.L., Robinson, J.J. and Aitken, R.P. (1991b). 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.

Shelton, J.N. and Moore, N.W. (1967). The response of the ewe to pregnant mare serum and the horse anterior pituitary extract. *Journal of Reproduction and Fertility* 14:175-177.

Shackell, G.H. (1991). The timing of oestrus, LH surge and ovulation in ewes following synchronisation with MAP sponges, FGA sponges and CIDR's. *Proceedings of the New Zealand Society of Animal Production* 51:71-77.

Smeaton, T.C. and Robertson, H.A. (1971). Studies on the growth and atresia of graafian follicles in the ovary of the sheep. *Journal of Reproduction and Fertility* 25:243-252.

Smith, C. (1986). Use embryo transfer in genetic improvement of sheep. *Journal of Animal Production* 42:81-88.

Smith, J.F. (1976). Selection for fertility and response to PMSG in Romney ewes. *Proceedings of the New Zealand Society of Animal Production* 36:247-251.

Smith, J.F., Parr, J., Beaumont, S., Oliver, J.E. and Upreti, G.C. (1995). Comparison of cervical, transcervical and laparoscopic insemination of ewes with chilled stored and frozen ram semen. *Proceedings of the New Zealand Society of Animal Production* 55: 248-250.

Sviatko, M.B., Cardenas, H., McClure, K.E. and Pope, W.F. (1993). The effect of small doses of progesterone on blastocyst morphology in sheep. *Sheep Research Journal* 9:3,119-124.

Szell, A., Zhang, J. and Hudson, R. (1990). Rapid cryopreservation of sheep embryos by direct transfer into liquid nitrogen vapour at -180° C. *Journal of Reproduction, Fertility and Development* 2: 613-618.

Tempest, W.M. and Minter, C.M. (1987). Synchronised breeding and lambing. In: Fayez, I., Marai, I. And Owen, J.B. (eds.). *New Techniques in Sheep Production*, Butterworths, pp. 221-237.

Tervit, H.R. (1983). Export of animals as frozen embryos. *Proceedings of the New Zealand Society of Animal Production* 43:179-181.

Tervit, H.R. (1989). Embryo transfer and sperm sexing. *2nd International Congress for Sheep Veterinarians. Proceedings of the Society's 19th Seminar*, pp. 144-157.

Tervit, H.R. and Havik, P.G. (1976). A modified technique for flushing ova from the sheep uterus. *New Zealand Veterinary Journal* 24:138-140.

Tervit, H.R. and Gould, P.G. (1984). Deep-freezing sheep embryos. *Theriogenology* 9:268.

Tervit, H.R., Thompson, J., McMillan, W., James, R. and Parton, G. (1989). Superovulation of anoestrous mature and ewe hoggets. *Proceedings of the Australian Society of Reproductive Biology* 21:127 Abstr.

Thompson, J.G., Bell, A.C.S., McMillan, W.H., Peterson, A.J. and Tervit, H.R. (1995). Donor and recipient ewe factors affecting in vitro development and post-transfer survival of cultured sheep embryos. *Journal of Animal Reproduction Science* 40: 4, 269-279.

Torres, S. and Cognie, Y. (1984). Super-ovulation and egg transfer in the ewe. *Nutrition, Reproduction and Development* 24:(5A) 623-631.

Torres, S., Cognie, Y. and Colas, C. (1987). Transfer of superovulated sheep embryos with different FSH-p. *Theriogenology* 28: 407-419.

Torres, S. and Sevellec, C. (1987). Repeated superovulation and surgical recovery of embryos in the ewe. *Reproduction, Nutrition and Development* 27:859-863.

Trounson, A. (1983). Comparative embryo transfer in Australia. *Theriogenology* 19:17-29.

Trounson, A. and Moore, N.W. (1974). Fertilisation in the ewe following multiple ovulation and uterine insemination. *Australian Journal of Biological Sciences* 27:301-301.

van Reenen, G. (1990). Development of a commercial E.T. technique for Merino sheep in Central Otago. *Proceedings of the Australian Association of Animal Breeding and Genetics; Technology Prospects and Transfer* 8:551-552.

Walker, S.K., Smith, D.H, and Seamark, R.F. (1986). Timing of multiple ovulations in the ewe after treatment with FSH or PMSG with and without GnRH. *Journal of Reproduction and Fertility* 77:135-142.

Walker, S.K., Smith, D.H., Frensham, A., Ashman, R.J. and Seamark, R.F. (1989). The use of synthetic gonadotrophin releasing hormone treatment in the collection of sheep embryos. *Theriogenology* 31:4,741-753.

Wallace, J.M. (1992). Artificial insemination and embryo transfer. In: Speedy, A.W. (ed.). *Progress in Sheep and Goat Research*, Redwood Press, pp. 1-24.

Walsh, P.A. (1989).*A study of the effects of post-mating progesterone supplementation on the reproductive performance in the ewe.* Master of Agricultural Science. Massey University Thesis. New Zealand.

Webb, R. and Gauld, I.K. (1987). Endocrine control of follicular growth in the ewe. In: Roche, J.F. and O'Callaghan, D. (eds.*). Follicular growth and ovulation rate in farm animals.* Martinus Nijhoff, pp. 107-118.

Welch, R.A.S., Andrews, W.D., Barnes, D.R., Bremner, K. and Harvey, T.G. (1984). CIDR dispensers for oestrus and ovulation control in sheep., *10th International Congress on Animal Reproduction and Artificial Insemination* 1:354.

Whyman , D. and Moore R.W. (1980). Effects of PMSG and the prostaglandin F-2∝ analogue, cloprostenol, on superovulation, fertilisation and egg transfer in the ewe. *Journal of Reproduction and Fertility* 60:257.

Willadsen, S,M., Polge, C., Rowson, L.E.A. and Moor, R.M. (1976). Deep freezing of sheep embryos. *Journal of Reproduction and Fertility* 46:151-154.

Wilmut, I. (1987). Embryo Transfer. In: Fayez, I., Marai, I. And Owen, J.B. (eds.). *New Techniques in Sheep Production*, Butterworths, pp. 79-89.

Wilmut, I. and Sales, D.I. (1981). Effect of an asynchronous environment on embryonic development in sheep. *Journal of Reproduction and Fertility* 61:179-184.

Wilmut, I., Sales, D.I. and 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.

Wolf, B.T. and Mylne, M.J.A. (1994). Influence of age of donor ewe on MOET in Texel sheep. *Proceedings of the British Society of Animal Production (Winter Meeting)*, Paper No. 86.

Wray, N.R. and Goddard, M.E. (1994). MOET breeding schemes for wool sheep. 1. Design alternatives. *Journal of Animal Production* 59:71-86.

Wright, R.W., Bondiolo, K., Grammer, J., Kunzan, F. and Menino, A.Jnr. (1981). FSH or FSH plus LH super-ovulation in ewes following medroxy-progesterone acetate pessaries. *Journal of Animal Science* 52:115-118.

Chapter Seven

Appendix I

Table 7.2.1 Individual recovery rate for each breed

Breed	No. ewes	Recovery rate (%)	± SE
Texel	92	72.2	3.7
Awassi	75	80.5	4.8

Table 7.2.2 Percentage of ova recovered relative to surgeon for the Texel data set

Surgeon code	No. ewes	Recovery rate (%)	± SE	p-value
1	41	71.8	5.7	
2	42	72.1	5.3	0.99
3	3	69.4	14.7	

Table 7.2.3 Effect of ovulatory response on recovery rate in the Texel data set

Number of ovulations	No. ewes	Recovery rate (%)	±SE	p-value
1-6	29	68.9	6.2	
7-10	40	78.0	8.0	0.68
11+	23	67.3	5.5	

Table 7.2.4 Effect of previous flushing on recovery rate in ewes in the Awassi data set

Previously flushed	No.ewes	Recovery rate (%)	±SE	p-value
Yes	26	92.8	8.9	
No	22	88.2	5.6	0.71

Table 7.3.1 Effect of the sire on the fertilisation rate for the Texel data set

Sire code	No. ewes	No.embryos recovered	Fertilisation rate (%)	p-value
1	28	192	99.5	
2	34	220	94.1	0.64
3	26	180	90.0	

Table 7.3.2 Effect of the sire on the fertilisation rate in the Awassi data set

Sire code	No. ewes	No.embryos recovered	Fertilisation rate (%)	p-value
1	21	125	77.0	
2	7	43	72.1	
3	18	85	62.4	0.30
4	23	146	89.7	
5	3	16	62.5	
6	2	8	87.5	

Table 7.3.3 Effect of ovulatory response on fertilisation rate for the Texel data set

Number of ovulations	No. ewes	No.embryos recovered	Fertilisation rate (%)	p-value
1-6	27	105	90.5	
7-10	38	269	96.3	0.87
11+	23	218	94.5	

Table 7.3.4 Effect of ovulatory response on fertilisation rate for the Awassi data set

Number of ovulations	No. ewes	No.embryos recovered	Fertilisation rate (%)	p-value
1-6	31	131	69.5	
7-10	33	198	76.8	0.22
11+	10	99	89.9	

Table 7.3.5 Effect of previous flushing on fertilisation rate in the Awassi data set

Previous flushing	No.ewes	No.embryos recovered	Fertilisation rate (%)	p-value
Yes	24	164	81.1	
No	51	264	75.4	0.51

Table 7.4.1 Effect of ovulatory response on embryo quality in the Texel data set

Number of ovulations	No.ewes	No.fertile embryos	Good quality embryos (%)	p-value
1-6	27	95	97.9	
7-10	38	259	96.5	0.23
11+	23	206	82.5	

Table 7.4.2 Effect of ovulatory response on embryo quality in the Awassi data set

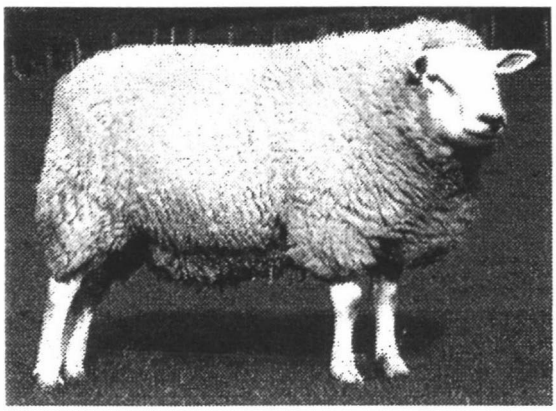
Number of ovulations	No.ewes	No.fertile embryos	Good quality embryos (%)	p-value
1-6	31	91	83.5	
7-10	33	152	80.9	0.82
11+	10	89	90.0	

Appendix II: Breed descriptions

Texel

The Texel is a sheep breed originating from the Dutch island of Texel. The breed is well established in Europe, but is relatively new to New Zealand, having been released from quarantine in 1990.

Figure IV. Texel ram.



In October 1983 a proposal was approved to import several new sheep breeds from Denmark and Finland, countries identified as meeting New Zealand's strict animal health regulations. Sheepac, a joint venture between the Ministry of Agriculture and 43 sheep breeders, imported the Texel with several other exotic breeds.

In November 1984, 136 embryos from 46 Texel ewes were imported from Denmark. From these embryos, 35 Texel lambs were born by August 1985. A second larger importation of Texels, along with other breeds, was made in February 1986 by Animal Enterprises Ltd and MAF who imported sheep and embryos from Scandinavia for quarantine.

The Texel is an open faced breed with a lamb drop usually in the 150-170% range. The breed is hardy and is frequently kept outdoors, often in winter environments harsher than those experienced in New Zealand (Allison *et al.*, 1989). The Texel has good muscle conformation and produces exceptionally lean meat. Furthermore it has a superior carcass formation (shorter and blockier) than other breeds available in New Zealand.

Allison *et al* (1989) reported on several trials in the United Kingdom and Ireland that evaluated the performance of the Texel. Compared to the Suffolk, another popular meat

producing breed, the purebred Texel was found to grow 8-9% slower, but Texel ram lambs have almost 8% less total carcass fat and at least 7.5% more carcass lean. Texel crosses have also been shown to be up 30% leaner than Suffolk crosses.

Other benefits of the Texel are the ability to produce heavier carcasses without becoming too fat, natural worm resistance, less dags and flies, a placid nature, good grazing, the ability to settle quickly after weaning, strong survival instincts of new born lambs, hardiness and adaptability and the heavy stamping of first cross lambs with Texel characteristics.

The disadvantages of the Texel include reduced wool production (15-20% less than Border Leicester crosses) however, the wool is white with high bulk. Slow weight gain and the corresponding failure to catch early markets, particularly in areas with low levels of summer feed. There are also claims that the Texels are marginally more prone to lambing problems and more susceptible to dystocia problems.

The superovulatory response of Texel ewes to exogenous gonadotrophins has been documented by several workers. Crosby *et al.*, (1981) observed an average ovulatory response of 12.6CL's resulting in 7.3 transferable embryos per donor ewe. Boundy *et al.*, (1985) reported 2.2 and 6 CL's with 1.8 and 4.2 transferable embryos in two trials. Dattena recovered 4.5 embryos per donor ewe.

Awassi

The Awassi is a fat-tailed sheep that is widespread throughout much of the Middle East. It is the dominant breed in Iraq, the most important sheep in Syria and the only indigenous breed of sheep in Lebanon, Jordan and Israel. It is primarily a meat breed, although an Israeli strain has been developed for milk production.

Figure V. Awassi ram.

The breed is calm around people and has the advantage of natural hardiness and grazing ability. The breed is well suited to a grazing production system as well as a confinement operation. The Awassi has a brown face and legs with the fleece varying in colour from brown to white. Individuals can also be found with black, white, grey or spotted faces. The males are horned and the females are usually polled. The fleece is mostly carpet type with a varying degree of hair. Dairy ewes are capable of producing over 300 litres of milk during a 210 day lactation period.

Embryos were imported to New Zealand from Israel in 1991 to improve milk yields of milking sheep and provide a terminal sire for live sheep exports to the Middle East, where consumers prefer this type of lean fat-tailed sheep (Knight *et al.*, 1994). The breed was developed in semi-arid climates and the strain imported to New Zealand was selected for milk production under zero grazing conditions. Therefore there is only limited data on its performance in a temperate climate.

Kassem *et al.*, (1989) has studied the reproductive aspects of the Awassi in Syria over a 7 year period and found the number of lambs produced per ewe lambing ranged

between 1.11-1.17, however the percentage of ewes lambing per ewe mated was between 62.1% and 93.1%.

New Zealand studies have compared the performance of Awassi-cross and Texel-cross ram lambs. Pre- and post-weaning growth rates of the Awassi crosses were lower than the Texel crosses as was the muscle to bone ratio (Holloway *et al.*, 1994). Awassi crossbreds also have lower fleece weights and poorer wool quality than Texel crossbreds (Knight *et al.*, 1994).

Information on the superovulatory response of Awassi ewes to exogenous gonadotrophins is very limited. In this study the mean ovulation rate was 7.08, with 4.48 embryos recovered per ewe and 2.10 lambs born per ewe flushed.