A STUDY OF THE RESULTS OF AN EMBRYO TRANSFER PROGRAMME CONDUCTED DURING TWO SEASONAL PERIODS USING FIVE IMPORTED BREEDS OF SHEEP

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Agriculture Science
in Animal Science
at Massey University

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1991

ABSTRACT

The overall objective of this trial was to compare embryo transfer results from two seasonal periods (out-of-season vs in-season) in order to determine the effect of the season on the different parameters of reproductive performance such as; the incidence of oestrus, ovarian response to exogenous gonadotrophins, fertilisation rates and the number of lambs born per donor ewe programmed and flushed. Five breeds of imported sheep (Danish Texel {DT}, Finnish Texel {FT}, Gotland Pelt {GOT}, Oxford Down {OXD}, and the White Headed Marsh {WHM}) consisting of two age groups (14-16 month-old and 26-28 month-old) in a commercial embryo transfer programme (LambXL, Manawatu) were used.

A total of 553 ewes out-of-season and 234 ewes in-season were studied. Two data sets were selected from these seasonal groups;

(1) The random data set.

(2) The repeat data set, which consisted of the same donor ewes in each seasonal group.

Oestrous synchronisation was attempted with a double CIDR-G regime and a super-ovulatory treatment consisting of an initial PMSG injection (200-300IU) and a series of six descending doses of FSH-P (total dose 24-36mg). The ewes were inseminated *intra-uterine* with fresh diluted semen from a ram of the same breed on the basis of oestrous detection. Embryo recovery was attempted on day 6.5-7 after oestrous detection using a laparoscope-aided uterine flush technique. Two embryos were transferred to each synchronised recipient ewe within two hours of recovery.

The incidence of oestrus for the out-of-season and in-season groups was 93.3% and 100%, for the random data set compared to 93.9% and 100% for the repeat data set, respectively. The ovulatory response to the super-ovulatory treatments

was significantly affected by the interaction of the breed and age of the donor in the random data set, but the repeat data set ovulation rate was not significantly affected by any of the variables recorded in this study. This interaction was attributable to the GOT breed having a higher ovulation rate in the older age group relative to the younger age group which was the reverse trend exhibited by the remaining breeds. However, there was an overall tendency for the out-of-season ovulation rate to be higher than that in-season, 7.64CL vs 6.60CL for the random data set and a difference (out-of-season - in-season) of +2.86CL was recorded for the repeat data set. The embryo recovery rates were 53.4% out-of-season and 53.5% in-season for the random data set and a difference of -0.7 percentage points was recorded for the repeat data set. The fertilisation rate was not significantly affected by the season with 75.5% out-of-season and 65.7% in-season from the random data set and a difference of -4.5 percentage points was recorded for the repeat data set. The yield of good quality transferable embryos was significantly affected by the season with 78.2% out-of-season and 83.7% in-season from the random data set but the repeat data set was not significantly affected by the season with a difference of -11.0 percentage points. The embryo survival rate to birth was not significantly different for the two seasonal periods with 66.3% out-of-season and 52.4% in-season of the embryos surviving to birth for the random data set and a difference of +9.7 percentage points was recorded for the repeat data set. This resulted in an average of 1.66 lambs born per donor ewe programmed out-of-season, which was not significantly different from 1.00 lambs born in-season for the random data set compared to a difference of +0.11 lambs born per donor ewe programmed in the repeat data set.

This work clearly demonstrates the inter-dependence of several factors affecting the number of lambs born per donor ewe in an embryo transfer programme. However it is concluded that out-of-season embryo transfer is as effective as that conducted in-season, under these embryo transfer conditions.

ACKNOWLEDGEMENTS

I wish to thank my supervisor Dr M F McDonald.

My sincere gratitude goes to Dr H William Vivanco for his patience and efforts throughout the entire duration of this study. He taught me most of the technical skills required in embryo transfer procedures as well as the theoretical knowledge which enabled me to conduct the appropriate analysis and compile this thesis.

Thanks also must be given to the technical staff at LambXL for their direct assistance with the experimental work. The financial support given to me by LambXL is also gratefully acknowledged.

The assistance and helpful suggestions of Raymondo Rangel-Santos are acknowledged.

I am indebted to Dr Hugo Varela and Dr D J Garrick for statistical advice and extreme patience, especially in the latter case.

The helpful suggestions and support of the Animal Science Department are gratefully acknowledged.

Finally very special thanks to my family, in particular my fiance, Catherine, without whose support and encouragement this thesis would not have been possible.

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CHAPTER ONE

INTRODUCTION

Embryo transfer is currently used to introduce new genetic material and to multiply genotypes and, to a lesser extent, to increase the rate of genetic gain by reducing the average age of the breeding females and hence the generation interval (Smith, 1986). Current embryo transfer technology results in relatively modest embryo harvests and hence low numbers of lambs born per donor ewe subjected to embryo transfer (average 2.1 lambs born per mature donor ewe; Tervit, 1989).

To effectively utilise the available embryo transfer technology, the number of lambs born per donor ewe per year needs to be increased. This can be achieved in a number of ways. The first area of improvement is to increase the reliability of response to super-ovulatory treatments (McMillan, 1991). Both embryo recovery and fertilisation rates have the potential to be in the range of 90-95%, however such results are infrequently reported. Hence the techniques involved in these two areas require further refinement. The yield of good quality transferable embryos is adequate at this stage of our knowledge and should continue to improve as appropriate super-ovulatory treatments are developed to allow higher ovulation rates and more favourable uterine environments to successfully develop the ova. The average embryo survival rate is approximately 60% (Tervit, 1989); hence this is an area where considerable potential for improvement remains.

Embryo transfer is traditionally conducted during the breeding season only, however a few recent reports indicate that embryo transfer can be successfully conducted during the non-breeding season (Torres et al., 1987; Lopez-Sebastian et al., 1990). The addition of effective out-of-season embryo transfer to an in-

season embryo transfer programme has the potential to increase the number of lambs born per donor per year.

When embryo transfer is used in a genetic improvement programme, the rate of genetic gain is directly affected by the number of progeny born per ewe (among other variables). Smith (1986) showed that the theoretical rate of genetic gain increased by 50-70% for five lambs born per donor per year, but with ten lambs born per donor per year the genetic gain increased by 89-102%. This latter number of lambs born per year may be achieved with embryo transfer conducted during both seasonal periods.

Within the commercial embryo transfer programme at LambXL, Manawatu, it was possible to arrange a comparison of donor ewes subjected to multiple ovulation and embryo transfer (MOET) during both the out-of-season and in-season periods. Thus the overall objective of this trial was to compare results of MOET conducted during the non-breeding season and the breeding season in order to determine whether out-of-season embryo transfer is as effective as that conducted in-season.

CHAPTER TWO

CHAPTER TWO

LITERATURE REVIEW

The success of embryo transfer procedures is determined by six variables;
(1) The incidence of oestrus, that is the percentage of ewes detected in oestrus within a given period of time (usually 48 hours) after progesterone source removal.
(2) The number of ovulations, represented by the number of corpora lutea (CL) present on the ovaries at the time of embryo recovery.
(3) The embryo recovery rate, that is the number of eggs recovered expressed as a percentage of the corpora lutea present on the ovaries.
(4) The fertilisation rate, that is the number of fertilised eggs recovered expressed as a percentage of the total eggs recovered.
(5) The yield of good quality transferable embryos, that is the number of embryos at the appropriate stage of development which are structurally and morphologically normal for the time after oestrous detection at which embryo recovery is performed, expressed as a percentage of the total eggs fertilised.
(6) The percentage of transferred embryos surviving to birth which is the number of lambs born expressed as a percentage of the number of embryos transferred.

The following review of literature attempts to identify some of the factors which influence these variables and hence the level of success of an embryo transfer programme.

2.1 Oestrous synchronisation

Oestrous synchronisation is necessary to allow super-ovulatory treatments to be administered at the correct stage of the oestrous cycle of the donor ewe and to ensure close oestrous synchrony between the donor and recipient ewes, as well as to ensure effective utilisation of the available labour and technical resources. The techniques of oestrous synchronisation have been reviewed by several workers (Robinson 1959; Lamond, 1964; Moore, 1982).

Prostaglandin (PGF2∝) treatment is an effective oestrous synchronisation method (Trounson et al., 1976) but is not widely used with super-ovulatory gonadotrophins (Moore, 1982). Two PGF2∝ treatments (9-14 days apart) are necessary to ensure all of the ewes are at the same stage of the oestrous cycle because the corpora lutea (CL) are responsive to the luteolytic effect of PGF2∝ only from day 4-14 of the oestrous cycle (Gordon, 1983). Most workers have detected oestrus approximately 40 hours after PGF2∝ treatment (Trounson et al., 1976, Acritopoulou and Haresign, 1980; Gordon 1983).

Progesterone (and progestagen) treatments have been used extensively in conjunction with super-ovulatory gonadotrophin treatments in ewes (Moore, 1982). Progesterone was initially used in 1948 by Dutt and Cassida to manipulate the oestrous cycle of the dog. In the early work with sheep, the hormone was dissolved in oil and administered over 8-10 days as a series of daily injections (Averill, 1958). The development of the intra-vaginal sponge (Robinson and Lamond, 1966) removed the need for this labour-intensive series of injections.

The current methods of progesterone supplementation in the ewe are;
(1) The sponge, which uses progestagens, that is either medroxyprogesterone acetate (MAP) or flurogestone acetate (FGA; Gordon, 1971).
(2) The CIDR (controlled internal drug release device) which uses progesterone as its source (30 or 50mg; 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 the added advantages of being easy to insert and a higher vaginal retention rate than the sponge (Welch et al., 1984).

An oestrous synchronisation programme is considered effective if 80-90% of the ewes programmed are detected in oestrus within 48 hours of progesterone source removal (Lamond, 1964). The CIDR and sponge may be used to synchronise oestrus under natural mating conditions. A reduction in fertility following oestrous synchronisation with vaginal sponges was reported by Robinson and Smith (1967) and was thought to be due to impaired sperm transport and survival following natural mating. More recent work has shown normal levels of fertility following oestrous synchronisation using CIDR's and cervical insemination of fresh semen (Harvey et al., 1984). McMillan (1986) found that 74% of Romney ewes synchronised with a CIDR had mated within three days of CIDR withdrawal compared to 79% following sponge (70mg MAP) withdrawal.

Ewes synchronised with a CIDR exhibit oestrus earlier than ewes synchronised with a sponge (Clarke et al., 1984; Maxwell and Hewitt, 1986). However, there was no significant difference in the fertilisation rate for the two devices under natural ovulation rates (Maxwell and Hewitt, 1986). There is some loss of synchrony of the pre-ovulatory surge of luteinising hormone {LH} (and possibly ovulation) with the onset of oestrus (Parr et al., 1982). By injecting LH to simulate the pre-ovulatory surge and using higher sperm numbers at artificial insemination, this effect may be reduced (Parr et al., 1982).

There have been several unsuccessful attempts at increasing the efficiency of oestrous synchronisation with the addition of gonadotrophins (PMSG) at the end of the progesterone treatment (Foord, 1966; Clarke, 1973). However, there is an increase in the efficiency of oestrous synchronisation when this treatment (PMSG at progesterone withdrawal) is applied out-of-season (Robinson and Smith 1967).

There are few reports comparing the effectiveness of oestrous synchronisation procedures during both the out-of-season and in-season periods. Out-of-season

oestrous synchronisation procedures (in addition to the super-ovulatory follicle stimulating hormone {FSH} treatments) are very effective. Armstrong and Evans (1984a) used sponges containing various types of progestagens for 12-14 days in conjunction with a super-ovulatory treatment of FSH-P to induce an acceptable out-of-season incidence of oestrus of 78.3%. Lopez-Sebastian et al. (1990) recorded all of the ewes programmed detected in oestrus following FGA (30mg) sponges inserted for 13 days (plus super-ovulatory doses of FSH-P) during both the out-of-season and in-season periods.

2.2 Number of ovulations

The number of ovulations dictates the overall potential number of embryos, and hence the number of lambs born, from an embryo transfer programme. The availability of reliable methods to super-ovulate sheep appears to have the greatest influence on the overall effectiveness of a MOET programme (Armstrong, 1991). The variation in response to super-ovulatory treatment is large, particularly the difference in response between animals (McMillan, 1991). There are a number of different sources of super-ovulatory drugs currently commercially available, usually with different FSH and LH activities, which contributes to the variability of response (Tervit, 1989; McMillan, 1991).

It is pertinent to outline the physiology of the oestrous cycle and how the exogenous gonadotrophin treatments affect the ovulatory responses.

2.2.1 Physiology of the oestrous cycle and ovulation

The ovary of an adult ewe contains between 12000 and 86000 primordial follicles and between 100 and 400 growing follicles, of which 10 to 40 are visible on the surface of the ovary (Cahill et al., 1979; McNatty et al., 1982). In the absence of exogenous gonadotrophin treatments, usually only one follicle ovulates at the end of each oestrous cycle. This is the result of a complex interaction of the ovarian follicles and the hypothalamo-pituitary axis, as well as some intra-ovarian regulation.

The differentiation of the ovulatory follicle is a two step process in which the large antral follicles are brought forth from the total available follicular pool, when exposed to sufficient gonadotrophin stimulation (Di Zerga and Hodgen, 1981). The first step is follicular recruitment. From these recruited follicles, a single follicle (in monotocous species) continues maturation, that is the follicle is selected and becomes dominant. By removing part of the recruited follicle population (by unilateral ovariectomy or follicle cautery) during the early follicular phase, there is no resultant increase in the interval between luteolysis and

ovulation in the ewe, which indicates that at the time of recruitment the follicle which will eventually ovulate has not as yet been selected (Findlay and Cumming, 1977; Tsonis et al., 1982). The stages of follicular development and the factors affecting this development are further outlined below.

Follicular recruitment occurs around 48 hours before the pre-ovulatory LH peak. This has been demonstrated by the following experimental findings;
(1) If a recently ruptured follicle is cauterised at the end of oestrus, a new follicle is present within 48 hours, and able to generate another pre-ovulatory LH peak (Smeaton and Robertson, 1971).
(2) If a mature CL is induced to regress (by prostaglandin treatment) another LH peak will occur within 48-55 hours (Acritopoulou et al., 1977).
(3) LH (or human chorionic gonadotrophin; hCG) administered during seasonal anoestrus will result in an LH peak 30-50 hours after treatment (McNatty et al., 1981).

Prostaglandin F2∝ (PGF2∝) is the luteolytic factor which induces regression of the CL (Scaramuzzi and Baird, 1976). Sufficient PGF2∝ reaches the ovary by day 13 to cause a decline in progesterone secretion, which in turn induces a marked increase in the frequency of LH pulses (Karsch et al., 1978). In contrast, there is no major change in the follicle stimulating hormone {FSH} secretion which coincides with luteolysis (Cahill et al., 1981).

In ewes where follicular development is blocked (by depression of gonadotrophin secretion) exogenous FSH is able to induce recruitment (Picton et al., 1990). The induction of ovulation during the anoestrous period requires FSH (Karsch et al., 1984). Basal levels of LH are also involved in recruitment (McNeilly et al., 1990) however no modulatory effects of LH pulses on recruitment have been found (Driancourt, 1991). Recruitment can occur during the early follicular phase and the early luteal phase, whilst the patterns of LH pulses vary markedly for these two phases (Driancourt et al., 1989). The presence of large follicles (> 4mm diameter) in ewes which were actively immunised against LH (hence lacking LH pulses) also illustrates that LH pulses are unlikely to play a role in follicular recruitment (Roberts and Reeves, 1989; Driancourt, 1991). Hence FSH is the key

hormone for follicular recruitment in the ewe (Driancourt, 1991).

The minimum size (diameter) of the recruited follicle is 2mm (McNatty et al., 1982; Driancourt and Cahill, 1984). Follicles less than 2mm are less sensitive to gonadotrophins as shown by the observation that follicular growth progresses to 2mm in hypophysectomised ewes (Dufour et al., 1980). The number of recruited follicles (> 2mm) is dependent on the FSH levels during the 24 hours preceding luteolysis (Driancourt et al., 1985). The recruited follicle begins to secrete more oestrogen. Despite the decreasing amplitude of LH pulses at this stage (due to oestrogen feedback; Goodman et al., 1981) more oestradiol is secreted in response to each LH pulse than at earlier stages of the oestrous cycle (Baird, 1978). These increased levels of oestrogen induce oestrous behaviour and reduce FSH secretion via negative feedbacks (Goodman et al., 1981).

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 of selection are still unclear, but in most cases (70%) the largest healthy follicle is selected (Driancourt et al., 1985). There are currently two hypotheses on the mechanisms of selection (as suggested by Driancourt et al., 1985);

(1) There is a given stage of follicular maturity, at which the morphological and functional adaptations which have occurred, prevent the follicle from being affected by the decreasing FSH levels which urge other follicles on to atresia.

(2) A more preferred suggestion is that selection is an active process, where one mature follicle actually inhibits the growth of other follicles.

Once a dominant follicle is selected, its growth rate decreases while atresia progresses in the non-selected follicles and recruitment is blocked (Driancourt and Cahill, 1984). FSH levels decrease due to the increased feedback of both oestrogens and inhibin but in spite of this the dominant follicle remains healthy (as indicated above). This may be due to the high intra-follicular FSH levels, or the high blood flow or (the most probable explanation) a high oestradiol content within the dominant follicle (Driancourt and Cahill, 1984). Dieleman et al. (1983)

has shown that the FSH concentrations in the pre-ovulatory (bovine) follicle are highly variable. Bruce and Moor (1976) found no difference in the blood flow to large follicles at the beginning of oestrus (no dominant follicle) as compared to late oestrus (in the presence of a dominant follicle). Hence these two reports indicate that the first two suggestions (of high intra-follicular FSH levels and high blood flow) should be rejected. The role of intra-follicular oestradiol in maintaining the dominant follicle is supported by the fact that oestrogens sensitise ovarian granulosa cells to gonadotrophins, possibly by triggering the differentiation of gonadotrophin receptors (Richards, 1979).

The pre-ovulatory surges of LH and FSH are provoked by a rise in oestrogen secretion in the pre-ovulatory follicle (Baird et al., 1981). Passive immunization of ewes against oestradiol prevents the LH surge (Scaramuzzi, 1975). Increased frequency of GnRH discharges induce high oestradiol levels which result in a rapid elevation of the LH levels (Reeves et al., 1971). The pre-ovulatory surge of LH initiates a sequence of changes in the steroidogenesis of the dominant follicle, and in its structure, as it prepares to expel the oocyte.

The gonadotrophin preovulatory surge induces an immediate and temporary rise in steroid levels due to an increased secretion of progesterone. Later, oestradiol and prostaglandin F2 secretion is also augmented. Hence the gonadotrophin surge induces ovulation by a cascade of biochemical changes.

The LH surge (at oestrus) induces ovulation and the subsequent formation of the corpus luteum (Karsch, 1980). 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 pre-ovulatory oestradiol rise which subsequently induces an LH surge, resulting in ovulation (McNatty et al., 1981).

The cumulus cells of the growing follicles actively secrete glycoproteins which form a viscous mass enclosing the oocyte and its corona. The follicular volume rapidly increases in the few hours preceding ovulation without any increment of follicular fluid pressure (Rondell, 1970). The wall of the follicular apex becomes

exceedingly thin and bulges on the surface of the ovary. At ovulation this bulge ruptures at the apex, releasing some of the follicular fluid and the viscous glycoprotein mass embedding the oocyte. This viscous mass spreads at the ovarian surface to facilitate the "pick up" of the oocytes by the fimbria. The oviduct curls over the ovary to facilitate egg "pick up" by the mucosal folds 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).

Starting on day three (after oestrus) the CL secretes increasing quantities of progesterone until day seven, when the level of secretion plateaus. LH is secreted in pulses during this period and each pulse induces an increase in oestradiol and androgen concentrations (Scaramuzzi and Baird, 1976). Irregular FSH pulses also occur during this period. Contrary to earlier reports that follicular growth occurs in waves of 4-6 days duration (Mattner and Braden, 1972) it is now thought that follicular growth during the luteal phase occurs at random (Driancourt et al., 1985). The follicles grow until they reach 4-6mm diameter and then regress in the absence of the appropriate hormonal conditions to induce ovulation.

2.2.2 The seasonality of reproduction

The sheep has seasonal periods of reproductive activity and the timing of these periods is such that lambs are conceived at a time of the year which ensures lambing occurs when the environmental conditions are most favourable for the survival of the newborn lamb (Hafez, 1952). The ewe is a short day breeder, hence the ewe becomes sexually active in the late summer to early autumn (Karsch et al., 1984). Unless the ewe becomes pregnant, repeated 16-17 day oestrous cycles persist until late winter when the anoestrous season begins. This anoestrous season is of varying length depending on breed.

Two types of experiments have demonstrated that photoperiod is the primary synchronising agent for the annual reproductive cycle of the ewe:
(1) Photoperiodic reversal; Transferring ewes across the equator results in a six

month change in photoperiod. After a period of adjustment, the annual reproductive cycles of ewes shifted across the equator are found to have altered in accordance with the new locale (Thwaites, 1965).
(2) Artificial photoperiods; Artificially alternating photoperiods between long and short days every 90 days caused the oestrous cycles to terminate when the shift was from short to long days, however the oestrous cycles were reinstated after a lag of about 50 days when the ewes were shifted back into short days, (Karsch et al., 1984).
The results of both of these types of experiments lend strong support to the view that daylength is the primary synchronising agent for the annual reproductive cycle of the ewe (Karsch et al., 1984).

One central mechanism which processes environmental signals is the "LH pulse generator" (Karsch, 1980). This neuroendocrine mechanism gives rise to the pulsatile mode of gonadotrophin secretion and serves to mediate the reproductive response to daylength. The LH pulse generator produces bursts of GnRH release from the hypothalamus which are coupled with, and presumably the cause of, the pulses of LH discharged from the anterior lobe of the pituitary. The nature of the LH pulse pattern changes dramatically throughout the oestrous and seasonal reproductive cycles.

The LH pulse generator is controlled by steroids (progesterone and oestradiol) in addition to the external environmental olfactory and photic cues. The immediate induction of hourly LH pulses in anoestrous ewes following exposure to pheromones from an unfamiliar ram illustrates the activation of the LH pulse generator by olfactory cues (Poindron et al., 1980). Regulation of the pulse generator by daylength is evident throughout the course of the annual reproductive cycle. In ovariectomised ewes the LH pulses are relatively infrequent but large during the long days of summer. They gradually shift to high-frequency, low-amplitude pulses during the short days of winter and then revert to the low-frequency pattern the next summer (Goodman et al., 1982). Since this effect of photoperiod is evident in the absence of gonadal steroids (the ewes were ovariectomised) it is termed the "direct photoperiodic drive" on the LH pulse

generator (Karsch et al., 1984).

In ovariectomised ewes administered with oestradiol (to maintain a constant circulatory level) the LH pulses were obliterated during anoestrus (Goodman et al., 1982). However during the breeding season (with these same ewes) a high frequency of LH pulses persisted, which indicates that oestradiol had lost its ability to slow the LH pulse generator. Thus the inhibitory photoperiods of the anoestrous period enable oestradiol to act within the brain to depress LH pulse frequency, but this action is totally absent during the inductive photoperiods of the breeding season (Goodman et al., 1982). This change in the ability of oestradiol to suppress the LH pulse generator constitutes the basis of the seasonal shift in the negative feedback action of this steroid on gonadotrophin secretion (Legan et al., 1977). This is supported by a number of experimental findings as reported by Karsch (1980);

(1) The LH pulse generating system is compromised during anoestrus because the LH pulse frequency is low.

(2) The ovary is not limiting during anoestrus because;

(a) It can respond normally to LH.

(b) It can generate a preovulatory oestradiol rise in response to a sustained tonic LH rise.

(c) Ovulation and CL formation can occur following an artificially induced LH surge.

(3) The LH surge system is not limiting during anoestrus because a preovulatory LH surge can be induced by a rise in oestradiol which mimics that of the normal follicular phase.

(4) The capacity of the pituitary to respond to high-frequency pulses of GnRH is not limiting during anoestrus.

(5) The spontaneous transition into the breeding season induces an increase in frequency of the LH pulses.

From the above findings, it appears that there is sufficient evidence to suggest that the photoperiod regulates the timing of the annual reproductive cycle by directing the activity of the LH pulse generator.

A number of reports suggest that retinal photoreceptors are required for the timing of the annual reproductive cycle in the ewe (Legan and Karsch, 1983; Karsch et al., 1984). In the absence of these receptors, the ewe may receive photoperiodic information indirectly via the ram, possibly by means of olfactory cues. When deprived of both the photoreceptor and the ram, the ewe remains seasonally reproductive, although the seasonal transitions may occur at a slightly different time from one year to the next (Karsch et al., 1984).

A monosynaptic tract, which links the retina to the suprachiasmatic nuclei of the hypothalamus, has been identified in a number of species including the sheep (Eichler and Moore, 1974). It has been suggested by O'Callaghan et al. (1987) that the role of the suprachiasmatic nuclei in biological timekeeping and seasonal breeding, which has been described in rodents (Karsch et al., 1984), can be extended to sheep.

The pineal gland is essential to mediate the effect of photoperiod on ovarian cyclicity in the ewe. This function is likely to be affected through changes in potency of oestradiol in the feedback inhibition of tonic LH secretion (Karsch et al., 1984). Hence the pineal gland responds to both inductive and inhibitory daylengths, suggesting that it actively participates in the measurement of daylength (Karsch et al., 1984).

Melatonin secretion (from the pineal gland) is rhythmic and occurs only at night, that is secretion is effectively suppressed by light (Goodman et al., 1981). Hence the photoperiod shapes the melatonin rhythm in the ewe, so that its secretory pattern changes during the course of the year (Rollag et al., 1978). Melatonin has all the properties of a "timekeeping" hormone;
(1) Its secretion is sensitive to light and changes under different photoperiods.
(2) It originates from a structure known to mediate the photoperiod response.
(3) It has the characteristics of a circadian rhythm (Karsch et al., 1984).

Hence the pineal gland drives reproductive induction in short days through its nocturnal secretion of melatonin, because the nocturnal rise of melatonin

mediates the suppressive effect of long days, as well as the inductive effect of short days. Thus the circadian rhythm of melatonin secretion determines the reproductive responses to photoperiod. Of the four parameters used to describe the melatonin secretion pattern (amplitude, duration, phase relative to the 24 hour light/dark transitions, or the specific shape of the nocturnal elevation curve) it appears that the critical feature in the ewe is the duration of melatonin secretion (Karsch et al., 1984).

The frequency of the LH pulse generator is known to be susceptible to regulation by the pineal gland, both in the presence and absence of steroids (Bittman et al., 1983). With prolonged exposure to a short-day pattern of melatonin, an increase in frequency of LH pulses in the presence of oestradiol was found by Bittman et al. (1983). It was suggested by Karsch et al. (1984) that the primary actions of melatonin in this system are directed toward the neural elements of the LH pulse generator.

To summarise the seasonality of reproduction;
Light cues activate retinal photoreceptors and are transmitted via a monosynaptic tract to the suprachiasmatic nuclei of the hypothalamus. After interacting with the circadian system, the photic information is relayed to the pineal gland which transforms the neural message into a hormonal signal in the form of a circadian rhythm of melatonin secretion. The pattern of this melatonin signal, which is interpreted as inductive or suppressive, sets the frequency of the LH pulse generator and determines the capacity of this neural oscillator to respond to the negative feedback action of oestradiol. The resultant changes in the episodic pattern of gonadotrophin secretion in turn dictate whether or not oestrous cycles can occur.

2.2.3 Manipulation of the number of ovulations using exogenous gonadotrophins

Historically the most common super-ovulatory regime for sheep is a single dose of PMSG (Gheradi and Lindsay, 1980; Armstrong, 1991; Driancourt, 1991). A major problem with this treatment is the variable response from a constant dose rate. However, the induction of super-ovulation in ewes with pituitary gonadotrophins is now relatively common (Armstrong and Evans, 1983, 1984a). Again, these regimes have highly variable responses. Reasons for this include factors involving the dynamics of follicular development during the oestrous cycle and the differences in FSH and LH concentrations in the hormonal preparations (Driancourt et al., 1985; McMillan, 1991).

Henderson et al. (1988) showed that continuous exposure to elevated plasma FSH concentration is necessary to bring additional follicles to pre-ovulatory maturity. McNatty et al. (1985) also reported that an increase in the frequency of double ovulations was achieved by the infusion of FSH over a 24 hour period, during the 48 hours before initiating luteolysis. Presumably this treatment advances the maturation of a follicle which would normally have undergone atresia, thereby allowing it to survive the pre-ovulatory fall in plasma FSH concentrations and attain ovulatory maturity. This was also illustrated by Ryan et al. (1984) who used PMSG at 48 hours before sponge removal to increase the size of the recruited follicular pool, whilst still utilising an FSH regime. Ryan et al. (1984) showed an increase in ovulation rate of 8.3CL vs 12.7CL for 0 vs 800IU PMSG, with 12mg FSH in both treatments. Wright et al. (1981) also showed an increase in ovulation rate using repeated FSH-P injections, starting before the onset of luteolysis and continuing through out the follicular phase. This work was confirmed by others (Armstrong and Evans, 1983, 1984a; Torres and Cognie, 1984). Henderson et al. (1988) found that if FSH-P infusion was given for only the first 24 hours from the start of luteolysis, the treatment failed to increase the ovulation rate. Also if the start of infusion was delayed for longer than 12 hours after the initiation of luteolysis, no increase in ovulation rate occurred.

Super-ovulation has been induced in sheep by the administration of PMSG

(Moore and Rowson, 1960) and pituitary extracts, for example HAP (horse anterior pituitary extract; Moore and Shelton, 1964a) and FSH (Wright et al., 1981), but responses between animals have been highly variable (McMillan, 1991). Increasing the super-ovulatory dose of PMSG can increase the number of persistent large follicles, resulting in a decrease in the ovulation rate (Shelton and Moore, 1967). This has been attributed to the prolonged half-life of PMSG, which may cause an abnormal endocrine status which is thought to be detrimental to the fertilisation, transport, and survival of the embryos (Du Mesnil Du Buisson et al., 1977). Ryan et al. (1984) recorded a rise in the number of persistent follicles as the PMSG dose increased. This is in agreement with other workers (Boland and Gordon, 1978). The high oestrogen output of these follicles is thought to increase the rate of transport of ova through the oviducts and decrease recovery rates (Quirke and Hanrahan, 1975). This may explain the lower recovery rates obtained in ewes receiving 1600IU PMSG doses in the work of Ryan et al. (1984)(22% vs 63% recovery for PMSG doses of 1600 and 800IU, respectively).

The number of persistent follicles remains relatively constant as the dose of HAP administered to ewes increases (Shelton and Moore, 1967). This is the main benefit of pituitary extracts, compared to PMSG, in the production of large numbers of fertilised sheep embryos and is thought to be a consequence of the short half life of exogenous pituitary gonadotrophins in the ewe (Akbar et al., 1974).

A variable LH concentration in FSH preparations is one of the most likely causes of abnormal pre-ovulatory oocytes (Moor et al., 1984). 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). Donaldson (1985a) supported this theory by showing that fertilisation problems in super-ovulated cows could not be overcome by multiple inseminations of several doses of semen.

Armstrong and Evans (1984a) suggested that the super-ovulatory response of the

ewe is inversely proportional to the amount of LH activity in the exogenous FSH preparations. This was illustrated by maintaining a constant amount of FSH and reducing the amount of LH. They recorded ovulation rates of 11.2CL, 15.8CL and 25.6CL from LH:FSH ratios of 0.205, 0.042 and 0.008, respectively (Armstrong and Evans, 1984a). Moor et al. (1984) showed that an excess of LH in a gonadotrophin hormone treatment causes premature stimulation of the oocyte in super-ovulated ewes. Furthermore, it has been shown that normal pre-ovulatory progesterone, LH and FSH concentrations are necessary for optimal embryo production in super-ovulated cows (Donaldson, 1985b). Abnormal concentrations of progesterone, LH and FSH are followed by abnormal follicular/oocyte maturation and a reduced embryo production (Donaldson, 1985b).

Walker et al. (1989) suggested that the addition of GnRH to a super-ovulatory gonadotrophin hormonal regime is necessary to synchronise the pre-ovulatory LH surge (as well as ensuring that the LH surge does in fact occur). The exogenous FSH has a negative feedback effect on endogenous GnRH production (via increased oestradiol and inhibin concentrations) which decreases endogenous LH (and FSH) production. Hence the pre-ovulatory LH surge may not occur, unless assisted as suggested above (Walker et al., 1989). The same authors also suggested that the viability of spermatozoa in the reproductive tract is adversely affected by the exogenous progesterone-gonadotrophin treatments.

Ryan et al. (1984) showed that the ovulation rate of ewes increased with increased FSH-P dose, independent of PMSG dose (no PMSG and no FSH-P, 2.0CL; 18mg FSH-P and no PMSG, 13.2CL; 1600IU PMSG and no FSH-P, 15.0CL; 1600IU PMSG and 18mg FSH-P, 19.7CL).

Torres et al. (1987) were able to illustrate a breed effect on super-ovulatory response to a constant 16mg of FSH-P; 9CL in Preaples, 12CL in Lacuane, 19.5CL in Romanov x Preaples ewes. Torres and Cognie (1984), using Preaples du Sud ewes, achieved 8.1CL per ewe with 12mg FSH-P. Using Ile-de-France ewes in the breeding season, Cognie et al. (1986) obtained an ovulation rate of 18.6CL with 16mg FSH-P.

Rhind and McNeilly (1986) illustrated the effect of body weight on ovulation rate using Scottish Blackface ewes, the high body condition group having a higher ovulation rate than the lower body condition group (2.86CL vs 1.84CL, respectively). The number of small follicles present in each group did not differ with body condition. During the luteal and follicular phase there was no difference in the LH profile, but FSH and prolactin were increased in the high body condition group. However, there was no effect of FSH concentration on ovulation rate. All subsequent luteal function (as measured by progesterone concentration) was normal and did not illustrate any influence of body condition. Rhind and McNeilly (1986) concluded that body condition affected the size of the large follicle (> 4mm diameter) population through changes in FSH secretion, and possibly pulsatile LH secretion and prolactin secretion, during the luteal phases of the cycle and that the number of follicles that were potentially ovulatory was probably determined during the luteal phase of the cycle.

2.2.4 Out-of-season hormonal regimes

To the author's knowledge there are few reports quoting super-ovulatory treatments out-of-season, especially reports quoting the super-ovulatory response recorded out-of-season relative to that in-season. Robinson and Smith (1967) used PMSG at progesterone withdrawal to induce oestrus activity during the out-of-season period but the ewes were not super-ovulated. McLeod et al. (1982) noted that the use of a series of small doses of GnRH was one method of breaking anoestrus, but that super-ovulation was not induced with this treatment. More recent work with out-of-season super-ovulation tends to use exogenous sources of FSH (Armstrong and Evans, 1984a; Torres et al., 1987; Lopez-Sebastian et al., 1990).

The use of a series of small doses of GnRH (every two hours for 48 hours) induces the episodic LH secretion pattern found during the follicular phase, which in turn induces follicular maturation (McLeod et al., 1982). The pre-ovulatory LH surge, after multiple GnRH injections, is a result of the positive feedback from the increasing concentration of oestradiol, not as a direct result of the GnRH

injections (McLeod et al., 1982).

The dose of GnRH (25-250ng) varies the episodic pattern of LH secretion although, with high dose rates, the pituitary becomes refractory, especially after the first 12 hours (McLeod et al., 1983). The interval from the start of GnRH treatment to oestrus is usually 35-37 hours and is not affected by the GnRH dose rate. McLeod et al. (1983) suggested that the above responses are due to an increase in the mean LH concentrations, rather than the episodic pattern of LH secretion per se, which is responsible for promoting follicular maturation and oestrogen secretion.

A single injection of 150-300μg GnRH into anoestrous ewes causes an immediate pre-ovulatory type LH surge and ovulation, however luteal function is absent due to inadequate follicle development prior to induction of ovulation. Normal luteal function occurs when the animal is pre-treated with progesterone for 10-14 days. GnRH treatments are currently not a common method of breaking anoestrus, predominantly because of the requirement for frequent injections (every two hours) and the relative success achieved with FSH treatments out-of-season (Armstrong and Evans, 1984a).

Armstrong and Evans (1984a) used FSH-P to successfully induce super-ovulation during the out-of-season period. The ovulation rates recorded following administration of 16-32mg FSH-P (8 injections over 4 days) were 9.8CL out-of-season compared to 13.4CL in-season (Armstrong and Evans, 1984a). There was no effect of dose rate (16 vs 32mg FSH-P) on the degree of super-ovulation recorded out-of-season (7.6 vs 7.5CL, respectively; Armstrong and Evans, 1984a). Torres et al. (1987) used 12-16mg FSH-P (4 injections over two days) and induced a lower super-ovulatory response out-of-season compared to in-season (8.4CL vs 11.2CL, respectively). Lopez-Sebastian et al. (1990) used 16mg FSH-P (two injections per day over three days, with the last two injections containing an additional 100μg LH) and achieved an ovulation rate of 7.1CL out-of-season compared to 7.3CL in-season. From the above reports, it is evident that super-ovulation can be induced out-of-season using FSH-P, although response is often less than that achieved during the breeding season.

2.3 Recovery rate

Embryo transfer procedures rely heavily on effective embryo recovery techniques. The early work (with oviduct flushes) tended to give higher recovery rates but resulted in more adhesions of the reproductive tract (Tervit and Havik, 1976). Recent developments have focused on the need for techniques of repetitive embryo recoveries (on the same donor ewe) with a minimum of trauma and damage (to the reproductive tract) whilst still achieving adequate embryo yields (McKelvey et al., 1986).

The embryo recovery rate is predominantly affected by the following factors, which are discussed in more detail in the following sections:

(1) The embryo development rate; the correct section of the reproductive tract needs to be flushed in order to recover the embryos of a specific stage of development, hence an understanding of the development of the embryos is essential.

(2) Day of flush; this may be altered to maximise the recovery rate of the desired stage of embryo development.

(3) Number of ovulations; the available literature on this point is contradictory, hence no clear trend is evident.

(4) Embryo recovery technique; developments in this area have mainly been in the area of reducing the damage to the reproductive tract of the donor ewe during the embryo recovery process, whilst maintaining the embryo recovery rate.

(5) Previous number of flushes; most reports agree that the embryo recovery rate declines as the number of previous flushes increases, predominantly due to the build up of post operative adhesions on the reproductive tract.

(6) Out-of-season embryo recovery; there are few reports discussing the effect of the season on the recovery rate, however the few that have find no difference in the embryo recovery rates. There is a lack of reports which discuss the effect of the seasonal period on the embryo recovery rate.

2.3.1 Embryo development rate

In the ewe, ovulation follows oestrus by approximately 24 hours and the first cleavage of the fertilised egg occurs about 24 hours after fertilisation (Killeen, 1981).

Embryos collected up to day 14 (after oestrus, day 0) are capable of normal development in the recipient ewe (Peterson et al., 1976). However, most embryos are flushed between days 3 and 6. After day 6-6.5, the hatched blastocysts are difficult to distinguish among the cellular debris in the flushing media. Moore and Shelton (1964b) illustrated that 2- and 4-cell embryos are more sensitive to manipulation than later stages of embryo development, hence flushing is generally not attempted before day 3. Averill and Rowson (1958) showed that no 2-cell and only 16% of 4-cell sheep embryos developed into viable lambs when transferred into the uterus. The survival rates of these younger embryos are relatively low as indicated by Armstrong and Evans (1983); 5- to 8-cell, day 3 embryos, 28% vs blastocyst stage, day 7 embryos, 50.5% survival. If embryos recovered from a single donor exhibit varied stages of development, they may be considered retarded and of poor viability (Killeen, 1981). Eldsen et al. (1978) showed that embryos assessed as being behind their developmental age, and hence retarded, resulted in lower pregnancy rates than those for ewes with normally developed embryos. Donaldson (1986) suggested several reasons to explain this variation in embryo development;

(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 be different.

Linder and Wright (1983) have assigned a code called the "developmental age" that corresponds to the estimated age of the embryo in reference to the day after oestrus on which it was collected. This is similar to the distribution of cell-stages as presented in Table 2.1 (source; Torres and Sevellec, 1987). The distribution of each embryonic stage of development varies with the day of collection, hence

embryos of a given stage exhibited a distinct maximum frequency dependent on the day of collection (Donaldson, 1986). For example; on day 6, 92% of the embryos were early or late morulae and only 8% were blastocysts but, by day 7 and 8, 58% and 91% of the embryos were blastocysts, respectively (Table 2.1). In this study, "developmental age" was shown to affect the pregnancy rate (Donaldson, 1986).

Table 2.1 Embryo development rate

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

2.3.2 Day of flush

Morula and blastocyst stage embryos are present in the uterine horns at days 5-8 and result in the highest survival rates of any of the embryo stages transferred (Shea, 1981).

The recovery rate is not affected by the day of flush, unless the flush is at the extremes of the developmental scale, with oviductal flushes on day 3 and day 5 recording acceptable recovery rates (which were not significantly different) of 82% and 73%, respectively (Tervit and Havik, 1976).

In summary, the day of flush has little effect on the recovery rate, assuming the day of flush is between days 3 and 7.

2.3.3 Number of ovulations

Betteridge and Moore (1977) showed that the embryo recovery rate decreased as the number of ovulations increased, however this work used PMSG as the super-ovulatory drug and hence "hyper-stimulation" of the ovary (typical of PMSG-treated donors; Armstrong and Evans, 1983) may have caused this decline in recovery rate. This trend was also confirmed by Hanrahan and Quirke (1982) who noted that the recovery rate declined in Galway ewes when the ovulation rate exceeded five. A decline in recovery rate was noted in donor ewes with more than 16 ovulations in a trial by Armstrong and Evans (1983). However this trend was reversed for goats in the same trial. In contrast to these reports, Wright et al. (1981) found no decline in embryo recovery and fertilisation rate in ewes with greater than the average ovulation rate in that trial. Torres et al. (1987) identified a positive correlation between the number of corpora lutea and the number of morulae recovered.

2.3.4 Embryo recovery technique

Most of the early embryo transfer work utilised the oviductal flushing technique outlined by Hunter et al. (1955). Tervit and Havik (1976) showed that this technique had a similar recovery rate to uterine flushes; 78% vs 83%, respectively (for day 5 flushes). However, the degree of post-operative adhesion formation associated with the oviductal flush technique was much greater than with the uterine flush technique. This particularly involved adhesions between the fimbria and ovary, which reduces the recovery rate from successive flushes and the chance of natural conception.

The oviductal flush technique was developed into the uterine flush technique in an attempt to further minimise the occurrence of post-operative adhesions (Tervit and Havik, 1976). As indicated above, these workers showed that the recovery rates from this technique were similar to those from the oviductal flush, and the degree of post-operative adhesion formation was markedly decreased in donors subjected to the uterine flush technique (16% vs 56% of the donors flushed

formed post-operative adhesions for the uterine and oviductal flushes, respectively).

McKelvey et al. (1986) have developed a laparoscope-aided uterine flush technique which does not exteriorise the reproductive tract. This technique was developed in order to further reduce the incidence of post-operative adhesions, when compared to uterine flushes. The recovery rate with this "non-surgical" technique is relatively low compared to recovery rates obtained using uterine flushes (50%; McKelvey et al., 1986 vs 76%; Torres and Cognie, 1984). In a commercial embryo transfer programme, this decline in the embryo recovery rate associated with the "non-surgical" technique must be considered against the possibility of more flushings on the same donor ewe, thus potentially yielding more embryos per donor ewe per unit of time (McKelvey et al., 1986)

2.3.5 Number of previous flushes

A decline in recovery rate may be expected, as a donor ewe is subjected to more embryo flushings, due to the potential build up of post-operative adhesions. A reduction in the recovery rate of 10-20% is expected for subsequent attempts at embryo collection as indicated by Hanrahan and Quirke (1982). Torres and Sevellec (1987) found repeated surgeries significantly decreased the subsequent recovery rates (88% vs 52% vs 24%).

2.3.6 Out-of-season embryo recovery

Assuming the recovery technique is the same as that used during the breeding season there is no reason to expect a different recovery rate out-of-season (apart from the points already identified, such as increased flushings leading to a decline in the recovery rate). There was no significant difference in the number of fertilised embryos recovered from Suffolk ewes flushed during the out-of-season and in-season periods in the work conducted by Armstrong and Evans (1984a). Torres et al. (1987) recorded similar embryo recovery rates during both the out-of-season and in-season periods (84.5% vs 83.0%, respectively). Lopez-

Sebastian et al. (1990) also recorded no effect of the season on the recovery rates (79% out-of-season and 77% in-season). These three reports confirm that the out-of-season embryo recovery rate can be expected to be similar to that recorded during the in-season period.

2.4. Fertilisation rate

Natural mating has historically been very effective, however economic pressures, the need to use semen of selected sires and the desire to achieve ever higher genetic gains has lead to the advent of a number of different artificial insemination (AI) techniques. The increasing usage of embryo transfer has placed pressure on the libido and semen producing abilities of rams. Hence AI has found increasing popularity under these conditions, in particular to try to reduce the incidence of fertilisation failure which was common under the early embryo transfer regimes using natural mating and relatively unrefined gonadotrophin.

The failure of fertilisation is considered to be one of the main factors limiting the success of embryo transfer (Betteridge and Moore, 1977; Armstrong and Evans, 1983). This has been attributed mainly to a decline in sperm transport within the female reproductive tract, particularly within the cervix (Trounson and Moore, 1974; Armstrong and Evans, 1984b). Walker et al. (1989) suggested that fertilisation failure can be "eliminated" through judicious use of gonadotrophin releasing hormone (GnRH) and properly timed intra-uterine insemination.

To the author's knowledge there are no reports which discuss the effect of season on the fertilisation rates following embryo transfer or which compare the fertilisation rates from the out-of-season period with those recorded in-season. However, Armstrong and Evans (1984a) showed that the percentage of ewes yielding fertilised ova was similar for the two seasonal periods (61% vs 77.4%, respectively).

Factors affecting the fertilisation rate are:

2.4.1 Artificial insemination technique

There are three AI techniques currently used, however natural mating is used in some small scale embryo transfer programmes, where single sire matings do not cause problems with semen availability (Tervit, 1989):

(1) Vaginal; This is rarely used in embryo transfer work but is common in large-scale breeding programmes, such as the one utilised by the Australian Merino Society (Clarke et al., 1984). Fertilisation rates vary between 18% for frozen/thawed semen (Maxwell and Hewitt, 1986) 74% for fresh semen (Clarke et al., 1984).

(2) Anterior cervical; Again this technique is rarely used in embryo transfer work, but is used by commercial breeders. Fertilisation rates range from 10.1% (Armstrong and Evans, 1984b) to 59% (Vivanco and Alarcon, 1987) for frozen/thawed semen and from 65% (Clarke et al., 1984) to 82% (Armstrong and Evans, 1984b) for fresh semen.

(3) Intra-uterine; This is the most common AI technique used in embryo transfer work, especially if frozen/thawed semen is used (Clarke et al., 1984). The major advantage of intra-uterine AI is that it uses the lowest dose of semen, whilst still yielding the highest fertilisation rates of the three techniques mentioned here (Clarke et al., 1984). Tervit (1989) suggested that the most useful application of intra-uterine AI is for the insemination of ewes which have not been detected in oestrus (that is, "fixed time" inseminations) and which would have otherwise not been mated at all and hence their progeny lost to the embryo transfer programme. However, intra-uterine AI is the most expensive and demanding of technical skill of the three techniques mentioned here (Clarke et al., 1984). Clarke et al. (1984) recorded 83% fertilisation with intra-uterine AI using fresh semen, which was similar to the report by Dattena (1989) with a fertilisation rate of 85%. Maxwell et al. (1984), using frozen/thawed semen, clearly illustrated the difference in fertilisation rate for the three techniques mentioned here; vaginal 17%, cervical 30%, intra-uterine 60%.

2.4.2 AI time relative to oestrus and progesterone removal

Oestrus usually precedes ovulation by approximately 24 hours in the ewe (Torres et al., 1987). Oestrus follows progesterone source removal by 6-40 (mean 24) hours, which represents the variability in oestrous detection (Moor et al., 1984). Ova will remain viable for 12-24 hours after ovulation, whereas sperm will retain its fertilising ability for 24-48 hours after ejaculation (in the reproductive tract). The

sperm must undergo a capacitation period of 2-6 hours before fertilisation can occur (Mattner, 1963).

Most workers suggest the optimum time for intra-uterine AI is between 44 and 60 hours after sponge removal (Armstrong and Evans, 1984b; Maxwell et al., 1984). Moore (1982) is of the opinion that insemination should be carried out around the time of onset of oestrus because insemination near the time of ovulation can depress the recovery rate of the embryos (disturbance of the fimbrial collection mechanism is more likely if insemination is closer to ovulation). Wright et al. (1981) obtained greater than 90% fertilisation using a 12 hour interval between the onset of oestrus and intra-uterine insemination. Ryan et al. (1984) recorded a fertilisation rate of 80% following insemination 24 hours after sponge removal. Using the same interval, Torres and Cognie (1984) achieved 94% fertilisation.

Oestradiol secretion has been shown to be influenced by LH pulses during the follicular phase (Baird, 1978). If the repeated LH pulses induce bursts of oestrogen secretion, which summates to a rise in plasma oestrogen concentration, there are two results;
(1) Induction of oestrous behaviour.
(2) A decline in FSH secretion, because oestrogens are a component of the negative feedback of FSH in sheep (Goodman et al., 1981). Hence if a commercial FSH product contains a higher level of LH than another product, it could induce a relatively earlier oestrogen secretion and so cause earlier oestrous behaviour than that observed using the lower LH concentration product (Goodman et al., 1981).

Walker et al. (1986) noted that GnRH administration synchronised the timing of ovulation and reduced the interval between the first and last ovulation. Walker et al. (1989) concluded their work by stating that appropriate timing of both GnRH treatment and intra-uterine AI will "eliminate" fertilisation failure in embryo transfer programmes. These workers recorded a fertilisation rate of 72% following administration of GnRH at 24 hours after progesterone source removal (and before oestrous detection) compared to 52% in the absence of the exogenous

GnRH treatment.

It has been shown by Torres et al. (1987) and Dattena (1989) that oestrus occurs earlier in ewes with higher than average ovulation rates. In the work of Torres et al. (1987) this corresponded to a period of only 24-36 hours between sponge removal and oestrus. On the basis of these results Torres et al. (1987) suggested that animals with high ovulation rates reach the levels of oestrogen that will bring them into oestrus earlier than in ewes with low ovulation rates. Moore (1982) recorded no difference in the fertilisation rates for the ewes detected in oestrus at different times after progesterone source removal, including ewes which did not show oestrus but were inseminated.

2.4.3 Semen processing

Semen collection methods and semen evaluation techniques have been reviewed elsewhere (Salamon, 1976; Evans, 1987).

Intra-uterine AI gives high fertilisation rates, using small quantities of semen (McKelvey et al., 1985). Doses as low as 2-20 million spermatozoa may be used (Walker et al., 1984). However in super-ovulated ewes, the number of spermatozoa should be increased to at least 100 million motile sperm (Maxwell and Hewitt, 1986).

Semen should be used fresh and kept at 30°C while awaiting insemination, usually within 2-4 hours of collection for maximum fertilisation rates (Evans, 1987).

2.5 The yield of good quality transferable embryos

To the author's knowledge there are few reports available which discuss the factors affecting the yield of good quality transferable embryos, hence a review of the documented factors affecting this variable is restricted.

Lopez-Sebastian et al. (1990) recorded no significant difference in the yield of good quality transferable embryos out-of-season compared to in-season (58% vs 59%, respectively). To the author's knowledge this is the only report available which illustrates the effect of the season on the yield of good quality transferable embryos.

Dattena (1989) recorded a higher percentage of good quality transferable embryos using Folltropin compared to FSH-P, for three of the six breeds used in that trial. The reasons for the apparent increase in embryo quality with Folltropin relative to FSH-P are not known, but Dattena (1989) suggested that the events that occur prior to fertilisation could be involved. Donaldson (1984) has shown that LH imbalance may result in the disturbance of normal oocyte and follicle maturation and consequently in poor ovum quality and reduced fertilisation rates. With the different breeds used in the work of Dattena (1989) there may be different requirements for LH or FSH to yield high quality embryos.

Dattena (1989) was able to show an increase in embryo quality with increased super-ovulatory response. This is in agreement with other workers (Cognie et al., 1986; Page et al., 1989).

2.6 Embryo survival rate

The embryo survival rates are affected by several factors, including those that influence the naturally developing embryo in addition to the effects that the embryo transfer procedure may have on the embryo survival rate.

Factors affecting the embryo survival rate are:

2.6.1 Embryo quality and stage of development

Shea (1981) noted a common problem with work that attempts to rate embryo quality with survival rates, that is the reports are not uniformly successful. Shea (1981) also noted that groups of embryos with evidence of degeneration resulted in lower pregnancy rates when compared to embryos with a normal morphology. However, some of those donor ewes yielded poorly rated embryos which resulted in high pregnancy rates and some donors yielded superior rated embryos which failed to produce a pregnancy. This illustrates that the establishment of a pregnancy is not solely determined by the quality or condition of the embryo (Shea, 1981). The same author also noted that debris on the zona pellucida did not affect the pregnancy rate, unless the debris was the result of an infection. The failure of some blastomeres to divide also did not affect pregnancy rates. Shea (1981) reported that a high pregnancy rate (in cows) was obtained when the transferred embryos were between the late morula and the early blastocyst stages.

Linder and Wright (1983) achieved a higher pregnancy rate with a ±1 day recipient oestrous/embryo development synchrony than transfer based on ±1 day recipient/donor oestrous synchrony (88% vs 74%, respectively). This result suggests that transfer of (bovine) embryos based on synchrony between the day of recipient oestrous cycle and the stage of embryonic development will provide higher pregnancy rates than transfer based on recipient/donor oestrous synchrony.

Armstrong and Evans (1983) recorded the survival rates for various stages of

embryo development (following transfer to the oviduct): 2- to 4-cell, 0%; 5- to 8-cell, 28.0%; 9- to 16-cell, 39.8%; morula, 28.6%; and blastocyst stage embryos, 50.0%.

2.6.2 Number of embryos transferred per recipient

In prolific ewes gestating their own embryos (natural pregnancy) it appears that embryo mortality increases as the number of ovulations increases. Meyer et al. (1983), using Booroola cross ewes, found that the survival rates were; 85% for ewes with 2 ovulations; 80% for 3 ovulations; and 70% for 4 ovulations. This trend was confirmed by Cumming et al. (1975) who showed survival rates to day 26 of 58-91% for single ovulations compared to 53-84% for twin ovulations. Armstrong and Evans (1983) also confirmed this trend with survival rates of 35.1% for one embryo vs 31.8% for two embryos.

The difference in survival rates for one vs two embryos transferred is usually small and not significant (Armstrong and Evans, 1983). Larsen (1971) found that an increase in the number of embryos transferred resulted in a lowering of the survival rate for one vs three embryos (66% vs 50%), although the difference was not significant. Trounson (1983) reported a 75% survival for two embryos transferred, which was similar to the 76% obtained by Torres et al. (1987). However, Trounson (1983) achieved a 92% survival rate when three embryos were transplanted per ewe.

The effect of embryo migration on the survival rate is also best illustrated with ewes gestating their own embryos. Embryo migration occurs in 84% of unilateral twin ovulators and this migration has been shown to increase the embryo mortality rate by 4-12% above that of bilateral ovulations (Knight, 1982). However, the cause of the increased embryo mortality is unclear; it could be due to the embryo being lost during the migration or to the absence of a CL on the ovary ipsilateral to the other uterine horn. Thus embryos which migrate to a uterine horn with no adjacent CL have decreased survival rates, but this is more than offset because the non-migrating embryo's chances of survival are

enhanced by the consequent removal of uterine crowding effects (Knight, 1982).

The proportion of embryos suffering pre-natal mortality increased as the number of embryos transferred increased in the work of Cumming and McDonald (1970). This is in contrast to Moore and Shelton (1964b) who reported a marginal improvement in conception rate by transferring two or three embryos rather than one, to the each recipient. The same workers concluded that maximum efficiency can be achieved by collecting embryos of 8 or more cells and transferring them at a rate of 2 embryos per recipient.

2.6.3 Recipient synchrony treatment

Recipient ewes for in-season embryo transfer work usually only require progesterone synchronisation treatment to achieve adequate oestrous synchrony with the donor ewes. There have been several unsuccessful attempts at increasing the efficiency of this oestrous synchronisation treatment with the additional use of gonadotrophins (PMSG) at the end of the progesterone treatment (Foord, 1966; Clarke, 1973). However, there is an increase in the efficiency of oestrous synchronisation only when this treatment (PMSG at progesterone source removal) is applied out-of-season (Robinson and Smith, 1967).

Oestrous synchronisation may also be achieved with a single dose (150-300μg) of GnRH following progesterone pre-treatment for 10-14 days (McLeod et al., 1982). The progesterone treatment has no effect on the pituitary response to the GnRH treatment. However, if the GnRH treatment is used without progesterone pre-treatment, luteal function is absent due to inadequate follicle development (McLeod et al., 1982).

2.6.4 Recipient synchrony

Accurate synchrony between the oestrus of donor and recipient ewes is necessary to achieve high embryo survival rates (Wilmut et al., 1985). Acceptable survival rates can be achieved when there is an asynchrony of ±1

day, although best results are obtained when the oestrous cycles of the donor and recipient ewes are exactly synchronised (Cumming, 1965; Rowson and Moor, 1966). Rowson and Moor (1966) showed that a lag of 72 hours between the onset of oestrus in the donor and recipient ewes appears incompatible and results in low embryo survival rates. In an attempt to explain this variation in embryo survival rates, Wilmut et al. (1985) found little variation in the progesterone concentration in the ovarian venous blood, during the period 7-15 days after oestrus. However, it was also suggested that the uterine endometrium (and its secretions) undergo continuous and rapid changes, and that these effects must be appropriate for the age of the transferred embryo.

The most obvious 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 sufficient luteotrophic action on the recipient's corpus luteum, resulting in luteolysis of the corpus luteum and consequently the death of the embryo (Wilmut et al., 1985).

2.6.5 Site of transfer

To achieve optimum survival rates, the site of transfer would be expected to be the same as the site of collection, that is the embryo should be returned to the same section of the tract, at the same stage of development, as that from which it has been recovered.

Moore and Shelton (1964b) showed that embryos collected up to day 3.5 after oestrus have higher survival rates when transferred to the oviduct rather than to the uterus. Rowson and Moore (1966) recorded survival rates of 75% and 71% from morulae and blastocysts stage embryos, respectively, when transferred into the uterus. In contrast to this, no 2-cell and only 16% of 4-cell embryos developed into viable lambs following transfer to the uterus (Averill and Rowson, 1958). Armstrong and Evans (1983) compared the oviduct vs the uterus as the site of transfer for embryos at more advanced stages than 8-cell, and there was

no significant difference in survival rates (40% vs 47%, respectively).

2.6.6 Interval between collection and transfer

Most workers agree that the interval between collection and transfer can be up to three hours without detrimentally affecting the embryo survival rate (Averill, 1956; Moore and Shelton, 1964b; Thatcher et al., 1985). All of the above workers also agree that a minimum of time outside the reproductive tract gives the best chance of achieving good embryo survival rates.

Tervit (1967) showed that a 40 minute delay between recovery and transfer had no effect on the embryo survival rates, but culture (in pure PBS at 35-36°C) for 24 hours gave an embryo survival rate of 6%, whereas for culture times of 48 and 72 hours the embryo survival rates were zero. Averill (1956) cultured embryos in PBS at 5-8°C for 24, 48 and 72 hours with survival rates of 47%, 25% and 22%, respectively. Tervit (1967) showed that the incidence of abnormal ova decreased with the first 24 hours of culture, but dramatically increased for longer culture times. Dattena (1989) found that an increase in the interval between embryo collection and transfer significantly decreased the embryo survival rates in only one of the six breeds in that trial (using bovine serum albumin {4%} added to the PBS flushing media).

2.6.7 Number of corpora lutea present in the recipient

The effect of the number of CL present in the recipient ewe on the survival rate of the embryos is negligible (Moore and Rowson, 1960; Cumming and McDonald, 1970). Moore and Rowson (1960) suggested that more CL would elevate the progesterone profile. Finnish Landrace and Border Leicester ewes tend to maintain larger litters more regularly than less fecund breeds (Bradford et al., 1974; Moore, 1968, respectively) which may be due to a difference in luteal activity, and hence different progesterone levels between the breeds of recipient ewe.

2.6.8 The effect of supplementary progesterone

Embryo survival rates maybe elevated by progesterone supplementation at the start of pregnancy. The majority of studies on this possible relationship have been conducted with animals following natural mating or AI and only a few have been conducted following embryo transfer. However, Dattena (1989) found significantly higher embryo survival rates when recipient Romney ewes received progesterone (CIDR) following transfer of embryos from two of the six donor breeds used in that trial. Interestingly these two donor (and embryo) breeds were the least fecund of the six breeds used in that trial.

Wilmut et al. (1985) made three conclusions about progesterone supplementation work;

(1) The timing of uterine development and secretions is controlled by changes in the time of increase in progesterone concentrations to levels typical of the luteal phase.

(2) Pregnancy will only occur if certain minimal levels of progesterone are present during the luteal phase.

(3) A lower, but equally important, level of peripheral progesterone is required from the time of oestrus to the time of increase to the luteal levels.

Wilmut and Sales (1981) showed that embryos transferred into advanced asynchronous recipients failed to implant because their development was "fatally modified". Hence they failed to inhibit the luteolysis of the resident CL. The same workers showed that a synchronised embryo prevents luteolysis and establishes pregnancy, but is unable to protect an asynchronous embryo (Wilmut and Sales, 1981). Lawson et al. (1983) showed that progesterone treatment (25mg/day) on day 0-3 after oestrus, significantly decreased the duration of the oestrous cycle, that is the exogenous progesterone advanced the uterine development rate. This was indicated by the survival of day ten embryos in a day six uterus, given this progesterone treatment.

The mechanisms by which the embryo prevents luteolysis in the ewe are not

known. Two hypotheses are (as suggested by Findlay, 1984);

(1) An effect of the embryo on the synthesis and release of PGF2∝ by the uterine endometrium.

(2) An action at the ovarian level to prevent luteolysis.

Maternal recognition of pregnancy must have occurred before day 12-13, otherwise uterine-secreted PGF2∝ will cause the CL to regress, hence terminating the luteal phase and allowing the follicular phase to begin (Rowson and Moor, 1966; Findlay, 1984). Peak progesterone production in the pregnant ewe occurs on day 12-13 (Bindon et al., 1971). Wilmut et al. (1985) showed that the embryo survival rate was affected by the season and that the effect of season (on the survival rate) was mediated entirely by progesterone.

Bindon et al. (1971) showed that the daily progesterone requirement (to day 20) is in the range 4-16mg/day. This allows correct embryo/uterine development to occur, followed by implantation (on day 26-35). Wilmut et al. (1985) have narrowed this "critical" range of progesterone supplementation to 4-10mg/day. Parr et al. (1982) illustrated the effect of the dose of exogenous progesterone on the embryo survival rate (to day 21); ewes given progesterone doses of 5, 10, 15, 20, or 25mg/day, had embryo survival rates of 69%, 83%, 79%, 90%, and 82%, respectively.

A lower level of progesterone must be present until the time of the rise to levels typical of the luteal phase (day 4-5) or very few pregnancies will occur (Wilmut et al., 1985). Progesterone therapy for six days (day 10-16) after removal of the synchronising progesterone increased the lambing rate from 67% to 95%, probably by reducing the incidence of pre-natal embryo mortality (Peterson et al., 1984). The CL persists throughout pregnancy, however bilateral ovariectomy during the latter half of pregnancy does not cause abortion, because the placenta produces adequate quantities of progesterone at that stage of gestation (Kelly et al., 1974).

2.6.9 Recipient breed

Most workers agree that the breed of recipient ewe has little effect on the embryo survival rates (Larsen, 1971; Hanrahan, 1979). Dattena (1989) found no significant difference in the embryo survival rates for five of the six embryo breeds used in that trial, when transferred to either Coopworth or Romney recipient ewes. However, a recipient breed effect for the Finnish Landrace embryos was found, with survival rates of 65% and 38% for the Coopworth and Romney recipient breeds, respectively (Dattena, 1989). Bradford et al. (1974) concluded that Finnish Landrace ewes tend to maintain larger than average litters, hence it may be assumed that Finnish Landrace embryos also may need a prolific recipient breed, more so than embryos from less prolific breeds.

2.6.10 Recipient nutrition

The level of feeding has a well defined effect on embryo mortality, as indicated by Cumming et al. (1975) using the lambing rates of naturally mated ewes; ewes fed 25% maintenance (lambing rate; 112%), 100% maintenance (119%), 200% maintenance (104%). These nutritional regimes were implemented from day 2-16 after oestrus.

The level of nutrition directly affects the plasma level of progesterone. High levels of nutrition cause the progesterone concentration to drop, whereas low levels of nutrition result in elevated levels of progesterone (Edey, 1970). Tervit (1989) showed that the decreased progesterone level under high feeding levels is a result of an increase in the metabolic clearance rate of this hormone. Hence progesterone deficiency under poor nutrition may be ruled out as a cause of increased embryo mortality. It has been shown by Rhind et al. (1983) that there is a basal level of progesterone which will maintain gestation, and that this level varies with breed.

Kelly et al. (1974) suggested that, following embryo transfer, recipient ewes should not be subjected to any nutritional stress particularly within the period up to day 28-35, that is pre-implantation.

CHAPTER THREE

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental animals

Five imported breeds of sheep (Danish Texel {DT}, Finnish Texel {FT}, Gotland Pelt {GOT}, Oxford Down {OXD}, and White Headed Marsh {WHM}) from the LambXL commercial embryo transfer programme in the Manawatu were used in this trial, during November and December, 1989 (out-of-season) and March and April, 1990 (in-season). All animals were individually identified with brass and plastic ear tags.

3.1.1 Donor ewes

A group of 553 ewes, consisting of two age groups (14-16 months and 26-28 months old, at the beginning of the out-of-season work) across the five breeds were used in this trial (Table 3.1). These animals represented all of the 14-16 month-old ewes and a random sample of the 26-28 month-old ewes available as donors for embryo transfer at LambXL during the 1989 breeding season. The 26-28 month-old ewes had been subjected to a maximum of four embryo flushes prior to the beginning of this trial whereas the 14-16 month-old ewes had been flushed only once. The last flushing was five months prior to the out-of-season trial.

Table 3.1 The number and age of the donor ewes

Breed Age	Out-of-season Nov and Dec, 1989 14-16 mth	26-28 mth	In-season March and April, 1990 14-16 mth	26-28 mth
DT	127	10	10	0
FT	115	9	12	21
GOT	70	10	67	7
OXD	144	9	5	59
WHM	49	10	36	17
Totals	505	48	130	104
All	553		234	

The donor ewes were grazed on ryegrass-white clover pasture. Nutritional "flushing" on ad-libitum pasture began six weeks before the initiation of the super-ovulatory treatments for the respective out-of-season and in-season groups. The average, minimum and target donor ewe body weights (target weights were based on historical growth records for each breed, as obtained by LambXL) at the beginning of this trial (November, 1989) are shown in Table 3.2.

Table 3.2 Body weights of the 14-16 month-old donor ewes (kg)

Breed	No. ewes	Mean	Minimum	Target
DT	127	54.1	38.0	40.0
FT	115	50.2	42.5	40.0
GOT	70	35.4	27.6	35.0
OXD	144	58.3	42.3	45.0
WHM	49	47.3	36.0	40.0

3.1.2 Rams

A total of 35 rams of the same breed and age groups as the donor ewes (Table 3.3) were available. A semen morphology test was conducted on all rams prior to the trial; those with greater than 20% abnormal sperm were not used.

The rams were exposed to oestrous ewes twice a week from the conclusion of the previous embryo transfer programme (that is five months prior to this trial). Also the rams were collected, using an artificial vagina, for semen quality assessment every week over the preceding five months. This "training period" was to ensure the rams remained sexually active during their normal out-of-season period. The rams were housed from 4pm to 8am each day and their diets supplemented with maize starting eight weeks prior to this trial. The same rams were used both out-of-season and in-season.

Table 3.3 The numbers and ages of the rams used

Breed	No. 14-16 months	No. 26-28 months
DT	6	4
FT	5	3
GOT	3	4
OXD	4	3
WHM	2	1

3.1.3 Recipient ewes

The recipient ewes were 3-4 year old multiparous Romneys. Prior to nutritional flushing on ad-libitum pasture six weeks before surgery, the average body weight was 57kg with a minimum of 25.5kg. Recipient ewes that did not reach 50kg body weight prior to this trial were not used.

3.1.4 Teaser rams

A total of 55 vasectomized Romney rams were available as "teasers" for oestrous detection, using Sire sine harnesses and crayons. The teaser rams were used at a rate of 10% over the number of ewes, for both donors and recipients. These rams were also exposed to oestrous ewes twice a week for the five months preceding this trial.

3.2 Experimental plan

In November and December 1989, 553 ewes were programmed for embryo transfer (the out-of-season group). In March and April 1990, the whole LambXL population of donor ewes were programmed for embryo transfer (3760 donors programmed) and from these 234 ewes were randomly selected within each breed and age group for use as the in-season group (Table 3.1). These ewes were assigned to the same hormonal and embryo transfer regimes as that used in the out-of-season trial in order to estimate the effect of the season on the efficiency of embryo transfer (Table 3.4).

Table 3.4 The numbers and ages of all of the donor ewes programmed within regime and age groups

Breed	Age	Regime	Out-of-season	In-season
DT	14-16	3	10	0
		4	117	10
	26-28	4	10	0
FT	14-16	3	111	12
		4	4	0
	26-28	4	9	21
GOT	14-16	1	11	0
		2	59	67
	26-28	2	10	7
OXD	14-16	3	86	5
		7	58	0
	26-28	4	9	59
WHM	14-16	4	9	0
		8	31	0
		9	9	0
		10	0	36
	26-28	5	10	0
		6	0	17

Note; see section 3.4 for description of hormonal regimes.

There were a number of ewes which were programmed for embryo transfer during both seasons (Table 3.5). Some of the ewes were programmed twice out-of-season and there were also some ewes which were programmed twice out-of-season and once in-season (Table 3.5).

Table 3.5 The number of times the donor ewes were programmed

Breed	Once only	Twice OOS	Once in both seasons	Twice OOS and once INS
DT	110	8	9	1
FT	104	12	13	1
GOT	35	1	45	9
OXD	177	8	12	0
WHM	20	1	33	8
Totals	446	30	112	19

Note; OOS = out-of-season. INS = in-season.

From these two seasonal groups two data sets were selected;

(1) Only ewes which were programmed in one seasonal period (the random group). If a ewe was programmed in both seasonal groups only the in-season record was used in this comparison. Due to the variability in the number of previous programmings these donor ewes had been subjected to, the out-of-season programming was assumed to have no effect on the ewes in-season response (Appendix 1, Tables 7.1.1 and 7.1.2). This data set attempted to minimise any individual animal effects by randomising them for both seasonal groups.

(2) Only ewes which were programmed in both seasonal periods (the repeat group). If a ewe was programmed twice out-of-season, the second (chronologically) programming record was used in this comparison. Where possible the ewe was subjected to the same hormonal regime during both seasonal periods (Appendix 2, Tables 7.2.1 and 7.2.2). This data set attempted to minimise any individual animal effects by recording the response of the same animal in both seasons in order to analyse the difference in response between the two seasons for each donor ewe.

3.3 Synchronisation of oestrus

Progesterone impregnated (9%) controlled internal drug release devices (CIDR-G; Carter-Holt-Harvey, NZ) were used for 11-13 days, to attempt oestrous synchrony in the donor ewes. The CIDR-G was replaced after 8-10 days (Thompson and Smith, 1988).

The recipient ewes were synchronised in oestrus with a single CIDR-G for 11-13 days, plus 500IU (out-of-season) or 200IU (in-season) PMSG (pregnant mare serum gonadotrophin; Pregnecol, Heriot Developments Ltd, Australia) at CIDR-G removal.

3.4 Super-ovulatory hormonal regimes

The super-ovulatory hormonal regime was a series of six descending doses of follicle stimulating hormone (FSH-P; Schering Corp, USA) with an initial PMSG (Pregnecol; as above) injection (Table 3.6). The FSH-P injections were given at 8am and 4pm, over the 3 days preceding CIDR-G removal, with the PMSG administered at the same time as the first FSH-P injection.

On the basis of previous work with these breeds (Dattena, 1989; H R Tervit, personal communication; H W Vivanco, personal communication) the PMSG dose was either 200 or 300IU and the FSH-P dose varied between 24 and 36mg (Table 3.6) depending on the breed and age of the ewe (Table 3.4). Each age group within a breed received the same hormonal regime for the two seasonal periods, where possible (Table 3.4).

The FSH:LH ratios in the hormonal preparations used were determined by MAF (Wallaceville) using NIHovine FSH-S17 as the FSH standard and NIHovine LH-S24 as the LH standard. Out-of-season the FSH:LH ratio was 9:1 for the FSH-P (batch no. 586H89) and 4:1 for the PMSG (batch no. 452930). In-season the FSH:LH ratio was 10:1 for the FSH-P (batch no.578B89) and 4:1 for the PMSG (batch no. 452930, that is the same as that used out-of-season).

Table 3.6 Super-ovulatory hormonal regimes

Regime code	Total FSH-P (mg)	Daily FSH-P (mg)	Total PMSG (IU)
1	24	6,6,4,4,2,2	200
2	26	7,7,4,4,2,2	200
3	28	8,8,4,4,2,2	200
4	32	8,8,5,5,3,3	200
5	34	8,8,6,6,3,3	200
6	36	9,9,6,6,3,3	300
7	28	8,8,4,4,2,2	200 +G
8	36	9,9,6,6,3,3	200
9	30	8,8,4,4,3,3	200
10	34	8,8,6,6,3,3	300

Note;
+G = 250mg GnRH (gonadotrophin releasing hormone; Fertagyl, Intervet, Australia) administered at oestrous detection.

3.5 Oestrous detection

Teaser rams, with Sire sine harnesses and crayons, were run with the donor ewes from four hours after CIDR-G removal. Oestrous ewes were drafted out at 8am, 12 noon and 6pm daily, following CIDR-G removal at 4pm the previous day. If a ewe was not tupped after 40 hours from CIDR-G removal it was either;
(1) Given 250mg GnRH and artificially inseminated six hours later (inseminated "fixed time").
(2) Not artificially inseminated but reprogrammed.

Recipient ewes had the CIDR-G removed at either 8am or 4pm (half of each group respectively), 36 hours (out-of-season) or 24 hours (in-season) before the corresponding donor group, to ensure the recipients' oestrus coincided with that of the donors (Torres et al., 1987). Teaser rams were run with these ewes

following CIDR-G withdrawal and oestrous ewes were drafted at 8am and 4pm daily.

3.6 Timing of insemination

The donor ewes were inseminated *intra-uterine* on the basis of oestrous detection, according to Table 3.7.

Table 3.7 Timing of insemination

Time of day marked ewes drafted off	Distribution of possible onset of oestrus	Time of AI	Hours since possible onset of oestrus
8 am	4pm - 8am	2 pm	6 - 22
12 noon	8am - 12noon	5 pm	5 - 9
6 pm	12noon - 6pm	7 am	13 - 19

3.7 Artificial insemination technique

The semen was collected via an artificial vagina. The motility of the semen was assessed by microscopic evaluation of the mass movement of the semen and graded from one to five (Salamon, 1976). The concentration of the semen was subjectively assessed on the gross density and colour of the ejaculate and scored one to five (Salamon, 1976). Only ejaculates with good motility (graded four or five) and good concentration (graded four or five) were used. The semen was then diluted at a ratio of 1:2 (semen:diluent) with a standard egg yolk diluent and kept at 30°C for a maximum of two hours prior to insemination. Each individual straw was re-checked for semen quality and motility prior to insemination.

The donor ewes were starved from the time they were detected in oestrus, to the time of artificial insemination (Table 3.7). The ewes were tranquillised with 1ml Acepromazine Maleate (ACP, Ethical Agents Ltd, NZ) and a local anaesthetic (5ml Lopaine; 2% Lignocaine, Ethical Agents Ltd, NZ) was injected in the area

where each trocar and cannula were to be placed. The ewes were restrained and inclined in a laparotomy cradle for the insemination.

The technique used was based on that outlined by Killeen and Caffery (1982) with modifications as indicated by Dattena (1989). Total sperm dose was 200-400 million per straw. Antibiotic injections were given to all ewes after insemination.

3.8 Embryo recovery technique

Embryo recovery was attempted on day 6.5-7 after oestrous detection (day 0). The donor ewes were starved for 24 hours prior to surgery. General anaesthesia was initially induced by Thamylal Sodium (Bio-Tal 5% weight:volume) at a dose of 20ml per 50kg body weight and maintained during surgery with halothane and oxygen.

The laparoscope-aided uterine flush was based on the technique outlined by McKelvey et al. (1986). The flushing media was complete enriched PBS (phosphate buffered saline; glucose 1gm and 36mg sodium pyruvate per litre and 10% heat inactivated sterilised steroid free sheep serum; Immuno-Chemical Products Ltd, NZ) at 37°C, which was allowed to cool to a constant 20°C after embryo flushing.

The embryos were removed from the flushing media, into a small petri dish containing fresh media, for classification (section 3.9). For the in-season group, the embryos were then placed into a 4ml autoanalyser cup with some media, and transported to another location 5km distant. (Note; Dattena {1989} also transported embryos over the same distance using this technique; no decline in embryo survival was observed as long as the embryos were implanted within three hours of recovery.) The out-of-season transfers were at the same site as the donor ewes.

All donor ewes received an antibiotic injection after surgery and an injection of a prostaglandin analogue (1ml PGF2α, Estrumate; ICI, England) two days after surgery.

3.9 Embryo classification

The eggs were examined and classified into five "quality" classes (Q1-Q5) on the basis of the evidence of fertilisation and of the appropriate stage of development for the embryo's age (Shea, 1981) and the presence of irregularities or degenerate areas (Table 3.8).

Table 3.8 Embryo classifications

Classification	Description
Q 1	Embryos of the appropriate stage of development and with no signs of irregularities or degenerate areas.
Q 2	Embryos of the appropriate stage of development but showing some irregularities and/or degenerate areas.
Q 3	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.
Q 4	Empty zona pellucida.
Q 5	Unfertilised ova.

3.10 Transfer to recipient

The embryos were re-appraised prior to transfer to confirm earlier quality determinations. Normally, two Q1 embryos were transferred per recipient, however Q1 and Q2 embryos were paired if possible, and Q3 embryos were transferred to make up triplets.

The time of oestrus was preferably in synchrony for the recipient and donor ewes, however up to 12 hours difference between oestrous detection times was acceptable. All embryos were implanted within two hours of recovery.

The recipient ewes were tranquillised with 1 ml Acepromazine Maleate (ACP). The transfer technique was based on that described by Boundy et al. (1985) with the following modification;

The number of corpora lutea was determined via laparoscopy, with the ipsilateral uterine horn exteriorised via forceps through a 3cm mid ventral incision, hence the ovaries remained inside the abdominal cavity. A CIDR-G was inserted and an antibiotic injection given after the transfer was completed (Dattena, 1989).

3.11 Management of the recipient ewe after transfer

The CIDR-G implanted after transfer was replaced on day 20 (after recipient oestrus, which was approximately day 13 after transfer; Dattena, 1989) to be removed when the ewe was real-time ultrasound scanned to estimate the embryo survival rate, that is day 50-55.

The recipients grazed pasture ad-libitum for two weeks after transfer. The ewes were then shifted to a near maintenance (1.25 kg DM/hd/d offered) level of feeding until the last third of gestation. The recipients were then divided into carriers of single and twin lambs, on the basis of scanning results, those with twins received 1.75 kg DM/hd/d, while the single carriers received 1.5 kg DM/hd/d, until parturition.

3.12 Pregnancy testing and lambing

All recipients were subjected to real-time ultrasound scanning on day 50-55, to determine the number of foetuses present and hence estimate the embryo survival rate (Lindahl, 1968).

Recipient lambing was synchronised with an intra-muscular injection of 6ml Dexadreson (Intervet, Australia) 48 hours before the expected time of parturition (Dattena, 1989). This treatment allowed supervised lambing (indoors) hence allowing easy access to ewes with parturition problems and the collection of accurate lambing data.

3.13 Analysis of the data

The random data set was analysed to compare the response of the variables which affect the efficiency of embryo transfer (see below). The repeat data set was analysed to compare the difference in response between the two seasonal periods for the variables which affect the efficiency of embryo transfer. For the repeat data set the average value of each of the variables recorded for both seasons is presented in conjunction with the average of the difference between the two seasons (for each ewe; section 3.2) . The two data sets were analysed using the same models unless otherwise stated.

The data were analysed utilising the following "general model" with a number of different factors added to the "general model" depending on the variable being analysed (either the incidence of oestrus, time of oestrous detection, ovulation rate, recovery rate, fertilisation rate, yield of good quality transferable embryos {%}).

The following generalised linear model was used as the "general model" in the analysis;

$$Y_{ijkl} = \mu + A_i + B_j + C_k(A_i) + D_l + AB_{ij} + AD_{il} + BC_{jk} + BD_{jl} + CD_{kl} + E_{ijkl}$$

where:

Y_{ijkl} = the variable being analysed (the incidence of oestrus, time of oestrous detection, ovulation rate, recovery rate, fertilisation rate, yield of good quality transferable embryos {%} for each season, donor breed, donor age, and hormonal regime combination).

μ = the overall mean of the variable being analysed.

A_i = the donor breed (DT, FT, GOT, OXD, or WHM).

B_j = the donor age (14-16 or 26-28 months).

$C_k(A_i)$ = the hormonal regime used (1-10, see Table 3.6) nested within donor breed.

D_l = the season (out-of-season or in-season).

AB_{ij} = interaction between donor breed and donor age.

AD_{il} = interaction between donor breed and the season.

BC_{jk} = interaction between donor age and the hormonal regime used.

BD_{jl} = interaction between donor age and the season.

CD_{kl} = interaction between the hormonal regime used and the season.

E_{ijkl} = the error unique to each Y_{ijkl}, where the E_{ijkl}'s were assumed to be independent and normally distributed with common variance.

Note; Only two-way interactions were included in the model, because three- and four-way interactions were not relevant to the overall objective of this trial.

3.13.1 Incidence and time of oestrous detection

The incidence of oestrus was defined as the percentage of programmed ewes detected in oestrus within 40 hours of CIDR-G removal. The time of oestrous detection was defined as the percentage of programmed ewes detected in oestrus up to 16 hours after CIDR-G removal {category 1}, from 16-26 hours after CIDR-G removal {category 2}, 26-40 hours after CIDR-G removal {category 3}, not detected in oestrus {category 4}.

The incidence and time of oestrous detection for the random data set were analysed with the Chi-square test of independence (SAS, 1988) using the "general model" (without interactions). The donor breed, donor age and hormonal regime (nested within breed) were each separately tested against the incidence and time of oestrous detection to test for independence. The analysis of the repeat data set used the same models as the random data set, however an additional variable was added to the models; the individual animal effect (donor ewe identification number, see below). This was necessary because the same animals were used in both of the season groups.

To analyse the incidence of oestrus in the repeat data set, the following factor was added to the "general model";

G_y = the individual animal identification tag (repeat data set only).

To analyse the time of oestrous detection in the repeat data set, the following factors were added to the "general model";

G_y = the individual animal identification tag (repeat data set only).
H_z = ovulation rate class (ORC; where ORC 1 = 0-3CL {includes ewes which were not flushed but which had ovulated}, ORC 2 = 4-7CL, ORC 3 = 8-12CL, ORC 4 >12CL). The range of each ORC was chosen based on the practical categories used by LambXL, where donor ewes with three CL or less were considered as not super-ovulated, four to seven CL is an average super-ovulatory

response, eight to twelve is an above average super-ovulatory response and more than twelve CL is a very high super-ovulatory response).

3.13.2 Ovulation rate

The ovulation rate was defined as the total number of corpora lutea present on both of the ovaries, at the time of embryo recovery (day 6.5-7 after oestrus). It was assumed that the samples (season, donor breed, donor age, hormonal regime, etc) drawn from the raw ovulation rate data (raw data mean = 7.49 ± 0.16 SE, range 0-22CL) were of sufficient size to assume that each sample approximated a normal distribution, hence allowing ANOVA procedures to be conducted. Bartlett's test for homogeneity of variance was undertaken for all season, donor breed, donor age, and hormonal regime combinations for the ovulation rate data. The tests were non-significant.

The ovulation rate data were analysed using ANOVA procedures (SAS, 1988) with the "general model" and following additional factors. Non-significant interactions were then dropped from the complete model and the reduced model fitted. If the main effects or any of the interactions were significant, all possible probability values for the hypothesis of equal least square means were requested. Means with the same subscript were not significantly different.

E_x = the presence of unruptured large {potentially ovulatory} follicles (present or absent).
F_y = the corpora lutea quality (good or poor; as subjectively determined by the donor surgeon at the time of embryo recovery).
G_z = the time of oestrous detection after CIDR-G removal (16, 26, 40 hours or not detected in oestrus but the ewes were inseminated "fixed time").

Note; interactions between the E_x, F_y and G_z with the other four main effects (A_i, B_j, C_k, D_l) were not relevant to the overall objective of this trial and hence were not included in the model.

3.13.3 Recovery and fertilisation rates, and the yield of good quality transferable embryos

The recovery rate was defined as the total number of embryos recovered ÷ the number of corpora lutea, as a percentage, for each ewe. (Note; only ewes with an ovulation rate of three or greater were flushed because it was deemed uneconomical to subject a ewe to the trauma and potential damage of an embryo flush to recover a maximum of two embryos).
The fertilisation rate was defined as the total number of fertilised ova recovered ÷ the total number of ova recovered, as a percentage.
The percentage of good quality transferable embryos recovered from each ewe, was defined as the number of good quality transferable embryos recovered (number of embryos classified as Q1 and Q2) ÷ the total number of fertilised embryos recovered, as a percentage.

It was assumed that the data for the embryo recovery and fertilisation rates, and the yield of good quality transferable embryos were each approximately normally distributed. The following generalised linear models were analysed using ANOVA procedures utilising the SAS package (1988). Non-significant interactions were then dropped from the complete models and the reduced models fitted. If the main effects or any of the interactions were significant, all possible probability values for the hypothesis of equal least square means were requested. Means with the same subscript were not significantly different.

To analyse the recovery rate data, the following factors were added to the "general model";

E_u = the presence of unruptured large {potentially ovulatory} follicles (present or absent).
F_v = the corpora lutea quality (good or poor; as subjectively determined by the donor surgeon at the time of embryo recovery).
G_w = ovulation rate class (ORC; where ORC 1 = 0-3CL {only includes ewes with 3CL because ewes with less than 3CL were not flushed}, ORC 2 = 4-7CL, ORC 3 = 8-12CL, ORC 4 > 12CL).

H_x = the time of oestrous detection after CIDR-G removal (16, 26, 40 hours or not detected in oestrus but the ewes were inseminated "fixed time").
M_y = donor surgeon (five donor surgeons were used; 1-5).
N_z = the flush technique (laparoscope-aided uterine or surgical uterine flushes).

Note; interactions between the E_u, F_v, G_w, H_x, M_y and N_z with the other four main effects in the "general model" (A_i, B_j, C_k, D_l) were not relevant to the overall objective of this trial and hence were not included in the model.

To analyse the fertilisation rate data, the following factors were added to the "general model";

E_t = the presence of unruptured large {potentially ovulatory} follicles (present or absent).
F_u = the corpora lutea quality (good or poor; as subjectively determined by the donor surgeon at the time of embryo recovery).
G_v = ovulation rate class (ORC; where ORC 1 = 0-3CL {only includes ewes with 3CL because ewes with less than 3CL were not flushed}, ORC 2 = 4-7CL, ORC 3 = 8-12CL, ORC 4 > 12CL).
H_w = the time of oestrous detection after CIDR-G removal (16, 26, 40 hours or not detected in oestrus but the ewes were inseminated "fixed time").
M_x = the ram used (used 35 rams, see Table 3.3).
N_y = the inseminator used (used three inseminators; 1-3).
P_z = the time of insemination relative to oestrous detection (three times were used, see Table 3.7).

Note; interactions between the E_t, F_u, G_v, H_w, M_x, N_y and P_z with the other four main effects in the "general model" (A_i, B_j, C_k, D_l) were not relevant to the overall objective of this trial and hence were not included in the model.

To analyse the yield of good quality transferable embryos, the following factors were added to the "general model";

E_w = the presence of unruptured large {potentially ovulatory} follicles (present or absent).
F_x = the corpora lutea quality (good or poor; as subjectively determined by the donor surgeon at the time of embryo recovery).
G_y = ovulation rate class (ORC; where ORC 1 = 0-3CL {only includes ewes with 3CL because ewes with less than 3CL were not flushed}, ORC 2 = 4-7CL, ORC 3 = 8-12CL, ORC 4 > 12CL).
H_z = the time of oestrous detection after CIDR-G removal (16, 26, 40 hours or not detected in oestrus but the ewes were inseminated "fixed time").

Note; interactions between the E_w, F_x, G_y, and H_z with the other four main effects in the "general model" (A_i, B_j, C_k, D_l) were not relevant to the overall objective of this trial and hence were not included in the model.

3.13.4 Embryo survival to scanning or birth, and the number of lambs born per donor ewe programmed and flushed

The embryo survival was defined as the percentage of embryos transferred present as either foetuses (at day 50-55, that is survival to scanning) or as lambs (that is survival to birth). The embryo survival to scanning and to birth were estimated using the number of good quality (Q1 and Q2) embryos transferred to each recipient only. Recipient ewes which were implanted with Q3 embryos were not used in this analysis. This is because the embryo survival rates may have been elevated as a result of transferring the Q3 embryos but not including them in the estimation of the embryo survival rates. Also it was not possible to estimate the survival rates of the Q3 embryos per se because they were not transferred to recipients by themselves (note; Q4 is zona pellucida's and Q5 is un-fertilised ova).

The embryo survival rate to scanning and to birth were both assumed to be approximately normally distributed. The number of lambs born per donor ewe programmed and flushed were assumed to be approximately normally distributed.

To analyse the embryo survival to scanning and to birth and the number of lambs born per donor ewe programmed and flushed ANOVA procedures (SAS, 1988) using the following generalised linear model were conducted. Non-significant interactions were then dropped from the complete models and the reduced models fitted. If the main effects or any of the interactions were significant, all possible probability values for the hypothesis of equal least square means were requested. Means with the same subscript were not significantly different.

$$Y_{ijklvwxyz} = \mu + A_i + B_j + C_k + D_l + E_v + F_w + G_x + H_y + M_z + E_{ijklvwxyz}$$

where:

$Y_{ijklvwxyz}$ = the embryo survival rate to scanning or birth and the number of lambs born per donor ewe programmed and flushed for each combination of the following variables.

μ = the overall mean embryo survival rate to scanning or birth, or the number of

lambs born per donor ewe programmed and flushed.

A_i = the season (out-of-season or in-season).

B_j = the donor breed (DT, FT, GOT, OXD, or WHM).

C_k = the donor age (14-16 or 26-28 months).

D_l = the donor/recipient oestrous synchrony (either synchrony or ±12 hours).

E_v = the embryo quality (Q1 or Q2).

F_w = the recipient oestrous synchrony technique (200 or 500IU PMSG at CIDR-G removal).

G_x = recipient surgeon (used five recipient surgeons; 1-5).

H_y = number of corpora lutea present in the recipient ewe.

M_z = the delay between embryo recovery and transfer (0-30, 31-60, 61-90, 91-120 minutes; maximum delay was two hours).

$E_{ijklvwxyz}$ = the error unique to each $Y_{ijklvwxyz}$, where the $E_{ijklvwxyz}$'s were assumed to be independent and normally distributed with common variance.

Note; interactions were excluded from this model because they were not relevant to the overall objective of this trial.

The following symbols for significance levels are used;

NS	$P > 0.05$	
*	$0.05 < P > 0.01$	(significant)
**	$0.01 < P > 0.001$	(highly significant)
***	$P < 0.001$	(highly significant).

CHAPTER FOUR

CHAPTER FOUR

RESULTS

The results from the analysis of the incidence of oestrus, the time of oestrous detection, the ovulation rate, the recovery rate, the fertilisation rate, the yield of good quality transferable embryos, the embryo survival to scanning and birth, and the number of lambs born per donor ewe programmed and flushed are presented in the following sections. For each of the above variables the results from the analysis of the random data set are presented first followed by the results from the repeat data set.

4.1 Incidence and time of oestrous detection

4.1.1 The incidence of oestrus

The Chi-square tests showed that incidence of oestrus was independent of the season, for both data sets. The incidence of oestrus was not significantly affected by the donor breed, donor age, or the hormonal regime used.

The overall incidence of oestrus for the random data set is presented in Table 4.1.1 and the incidence of oestrus for the repeat data set is presented in Table 4.1.2.

The incidence of oestrus for the five breeds for each seasonal period is presented in Appendix 1, Table 7.1.3 for the random data set and in Appendix 2, Table 7.2.3 for the repeat data set.

Table 4.1.1 The effect of season on the incidence of oestrus for the random data set

Out-of-season		In-season	
n	%	n	%
373	93.3	234	100.0

Note;

n = the number of ewes programmed.

% = the percentage of ewes detected in oestrus within 40 hours of CIDR-G removal.

Table 4.1.2 The effect of season on the incidence of oestrus for the repeat data set

Out-of-season		In-season	
n	%	n	%
131	93.9	131	100.0

4.1.2 The time of oestrous detection

The Chi-square tests showed that the time of oestrous detection was independent of the season, for both data sets. The time of oestrous detection was not significantly affected by the donor breed, donor age, or the hormonal regime used.

The time of oestrous detection for the random data set is presented in Table 4.1.3 and in Table 4.1.4 for the repeat data set.

The time of oestrous detection for the five breeds for each seasonal period is presented in Appendix 1, Table 7.1.4. for the random data set and in Appendix 2, Table 7.2.4 for the repeat data set.

Table 4.1.3 The effect of season on the time of oestrous detection for the random data set

Season	n	16h (%)	26h (%)	40h (%)	NT (%)
Out	373	26.5	59.8	7.0	6.7
In	234	26.5	67.1	6.4	0

Note;
h = interval in hours from CIDR-G removal to oestrous detection.
(%) = the percentage of ewes programmed detected in oestrus.
NT = ewe not tupped after 40 hours from CIDR-G removal.

Table 4.1.4 The effect of season on the time of oestrous detection for the repeat data set

Season	n	16h (%)	26h (%)	40h (%)	NT (%)
Out	131	35.9	52.7	5.3	6.1
In	131	22.1	71.8	6.1	0

Although the effect of the ovulation rate class (ORC) on the time of oestrous detection was not significant, it was of interest to determine whether the time of oestrous detection was affected by the ORC. This analysis is presented in Appendix 1, Table 7.1.5 for the random data and Appendix 2, Table 7.2.5 for the repeat data.

4.2 Ovulation rate

Only donor ewes which were detected in oestrus or were not detected in oestrus but were inseminated "fixed time" were used in this analysis. (Note; some of the ewes in the in-season group could not be inseminated {due to excessive adhesions} hence these ewes {7} were unavailable for the ovulation rate data.) A total of 371 ewes out-of-season and 227 ewes in-season, were available to study the effect of season on the ovulatory response to the super-ovulatory regimes from the random data set. From the repeat data set a total of 130 ewes out-of-season and 126 ewes in-season were available for the ovulation rate analysis.

The only significant estimable interaction in the random data set was that between the donor breed and donor age, which is presented in Table 4.2.2. The ovulation rate data for the rest of the donor breed and age combination are presented in Appendix 1, Table 7.1.7. The ovulation rate for each breed and seasonal period is presented in Appendix 1, Table 7.1.6. The overall ovulation rate for the two seasonal periods is presented in Table 4.2.1. The ovulation rate for each donor breed, age, hormonal regime and seasonal group is presented in Appendix 1, Table 7.1.8.

For the repeat data set the ovulation rate was not significantly affected by any of the interactions. There was no significant effect of the season, donor breed, donor age, hormonal regime, the time of oestrous detection after CIDR-G removal, the presence of unruptured large {potentially ovulatory} follicles or the corpora lutea quality on the ovulation rate. The overall ovulation rate for the two seasonal periods is presented in Table 4.2.3 and the ovulation rate for each breed within season is presented in Appendix 2, Table 7.2.6. The ovulation rate for each donor breed, age, hormonal regime and seasonal group is presented in Appendix 2, Table 7.2.7.

Table 4.2.1 Overall ovulation rate for the two seasonal periods for the random data set

Season	No. ewes	Ovulation rate (mean)	±SE
Out	371	7.64	0.22
In	227	6.60	0.26

Table 4.2.2 Ovulation rate for the donor breed by donor age interaction for the random data set

Breed	Age (mths)	No. ewes	Ovulation rate (mean)	±SE	Sig.
DT	14-16	118	7.47	0.35	a
	26-28	10	4.60	1.22	
FT	14-16	102	8.25	0.46	a
	26-28	22	5.32	0.80	
GOT	14-16	74	7.91	0.53	b
	26-28	14	10.00	1.74	

Table 4.2.3 Ovulation rate within season and donor breed for the repeat data set

Season	No. ewes	Ovulation rate	±SE	n	Diff.	±SE
Out	130	8.62	0.47	124	+2.86	0.50
In	126	6.45	0.35			

Note;

n = the number of ewes which generated ovulation rate data in both seasons, hence allowing the difference in ovulation rate between the two seasons to be calculated.

Diff. = the mean difference in the out-of-season ovulation rate minus the in-season ovulation rate for each ewe.

4.3 Recovery rate

Only ewes with three or more corpora lutea were flushed and hence available for the recovery rate analysis. From the random data set 322 (out-of-season) and 176 (in-season) ewes were available to estimate the recovery rate, whereas from the repeat data set 108 (out-of-season) and 91 (in-season) ewes were available.

From the analysis of the random data set there were no significant interactions. The recovery rate was significantly affected by the time of oestrous detection after CIDR-G removal and the donor surgeon. There was no significant effect of the season, donor breed, donor age, hormonal regime, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality, the ORC and the flushing technique used on the recovery rate.

For the random data set, the overall recovery rates for the two seasonal periods is presented in Table 4.3.1. The recovery rate data, for the five breeds within season group, are summarised in Appendix 1, Table 7.1.9. The recovery rates for the four oestrous categories are presented in Table 4.3.2. The recovery rates for each donor surgeon used is presented in Table 4.3.3.

The analysis of the repeat data set revealed no significant interactions. The donor surgeon had a significant effect on the recovery rate. There was no significant effect of the season, donor breed, donor age, hormonal regime, the time of oestrous detection after CIDR-G removal, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality, the ORC and the flushing technique used on the recovery rate.

The recovery rates for the two seasonal periods is presented in Table 4.3.4 with the breed by season recovery rates presented in Appendix 2, Table 7.2.8. The effect of the donor surgeon on the recovery rate is presented in Table 4.3.5.

Table 4.3.1 Recovery rate for the two seasonal periods for the random data set

Season	No. ewes	Recovery rate (%)	±SE
Out	322	53.4	1.7
In	176	53.5	2.6

Table 4.3.2 The effect of the time of oestrous detection on the recovery rate for the random data set

Interval from CIDR-G removal to oestrus (hours)	No. ewes	Recovery rates (%)	±SE	Sig.
16	146	58.4	2.4	a
26	305	53.5	1.8	a
40	32	46.4	5.5	b
Not tupped	15	26.8	7.4	b

Note;

Most of the ewes not tupped at 40 hours post CIDR-G out, were inseminated "fixed time", hence these ewes were flushed.

Table 4.3.3 The effect of donor surgeon on the recovery rate for the random data set

Surgeon code	No.	Rec. rate	±SE	Sig.
1	235	50.5	2.1	ac
2	124	51.2	2.5	ac
3	103	65.4	2.9	b
4	12	31.4	9.0	c
5	24	52.2	8.0	ab

Table 4.3.4 Recovery rate for the two seasonal periods for the repeat data set

Season	No. ewes	Recovery rate (%)	±SE	n	Diff.	±SE
Out	108	56.4	3.7	79	-0.7	4.5
In	91	57.3	3.5			

Note;

n = the number of ewes which generated recovery rate data in both seasons.

Diff. = the mean difference in the recovery rate out-of-season minus the in-season recovery rate for each ewe.

Table 4.3.5 The effect of donor surgeon on the recovery rate for the repeat data set

Surgeon code	No.	Rec. rate	±SE	Sig.
1	96	54.4	3.4	a
2	47	56.1	4.5	a
3	45	64.4	4.8	a
4	4	15.5	8.1	b
5	7	68.8	11.4	a

4.4 Fertilisation rate

Only ewes from which ova were recovered were used for the fertilisation rate analysis. Out-of-season 302 ewes were available to estimate the fertilisation rate, whereas in-season 155 ewes were used from the random data set. From the repeat data set 98 ewes were available out-of-season for the analysis of the fertilisation rate compared to 82 ewes in-season.

The analysis of the random data found no significant interactions. The fertilisation rate was significantly affected by the time of oestrous detection after CIDR-G removal. There was no significant effect of the season, donor breed, donor age, hormonal regime, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality, the ORC, the ram used, the inseminator or the time of insemination relative to oestrous detection on the fertilisation rate.

The overall fertilisation rate for the two seasonal periods is presented in Table 4.4.1, with the breed by season fertilisation rates presented in Appendix 1, Table 7.1.10. The effect of the time of oestrous detection after CIDR-G removal on the fertilisation rate is illustrated in Table 4.4.2.

From the repeat data there were no significant interactions found. There was no significant effect of the season, donor breed, donor age, hormonal regime, the time of oestrous detection after CIDR-G removal, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality, the ORC, the ram used, the inseminator or the time of insemination relative to oestrous detection on the fertilisation rate.

The overall fertilisation rate for both seasons is presented in Table 4.4.3, with the breed by season fertilisation rates presented in Appendix 2, Table 7.2.9.

Table 4.4.1 Fertilisation rate within season and breed for the random data set

Season	No. ewes	Fertilisation rate (%)	±SE
Out	302	75.5	2.1
In	155	65.7	3.3

Table 4.4.2 The effect of interval from CIDR-G removal to oestrous detection on the fertilisation rate for the random data set

Interval from CIDR-G removal to oestrus (hours)	No. ewes	Fertilisation rate (%)	±SE	Sig.
16	140	70.1	3.2	a
26	278	76.0	2.2	a
40	29	49.2	8.6	b
Not tupped	10	60.0	16.3	ab

Table 4.4.3 Fertilisation rate for the two seasonal periods for the repeat data set

Season	No. ewes	Fertilisation rate (%)	±SE	n	Diff.	±SE
Out	98	63.7	3.9	66	-4.5	6.0
In	82	63.2	4.5			

Note;

n = the number of ewes which generated fertilisation rate data in both seasons.

Diff. = the mean difference in fertilisation rate for the two seasons for each ewe.

4.5 Embryo quality

Only ewes from which fertilised ova were recovered were used for the analysis of the embryo quality. The yield of good quality transferable embryos was estimated with 262 ewes out-of-season and 124 ewes in-season from the random data set. From the repeat data set out-of-season 82 ewes were available and 65 ewes were used in-season.

There were no significant interactions for the random data set. The yield of good quality transferable embryos was significantly affected by the season, donor breed and the time of oestrous detection after CIDR-G removal. There were no significant effects of the donor age, hormonal regime, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality and the ORC on the yield of good quality transferable embryos.

The overall yield of good quality transferable embryos for the two seasonal periods is presented in Table 4.5.1, with the breed by season data presented in Appendix 1, Table 7.1.11. The different yields of good quality transferable embryos for the five donor ewe breeds is presented in Table 4.5.2. The effect of the time of oestrous detection on the yield of good quality transferable embryos is illustrated in Table 4.5.3.

There were no significant interactions for the repeat data set. The yield of good quality transferable embryos was significantly affected by the season. There were no significant effects of the donor breed, donor age, hormonal regime, the time of oestrous detection after CIDR-G removal, the presence of unruptured large {potentially ovulatory} follicles, the corpora lutea quality and the ORC on the yield of good quality transferable embryos.

The overall yield of good quality transferable embryos for the two seasonal periods is presented in Table 4.5.4, with the breed by season yields presented in Appendix 2, Table 7.2.10.

Table 4.5.1 The yield of good quality transferable embryos for each seasonal period for the random data set

Season	No. ewes	Good quality embryos (%)	±SE	Sig.
Out	262	78.2	4.0	*
In	124	83.7	4.5	

Table 4.5.2 The yield of good quality transferable embryos for each donor breed for the random data set

Breed	No. ewes	Good quality embryos (%)	±SE	Sig.
DT	88	79.7	3.8	a
FT	80	81.9	3.7	a
GOT	51	72.5	5.1	b
OXD	135	88.7	4.0	a
WHM	32	81.2	5.7	a

Table 4.5.3 The effect of interval from CIDR-G removal to oestrous detection on the yield of good quality transferable embryos for the random data set

Interval from CIDR-G removal to oestrus (hours)	No. ewes	Good quality embryos (%)	±SE	Sig.
16	119	85.0	2.7	a
26	243	84.1	2.7	a
40	18	65.5	9.2	b
Not tupped	6	16.7	16.7	c

Table 4.5.4 The yield of good quality transferable embryos the two seasonal periods for the repeat data set

Season	No. ewes	% good quality embryos	±SE	n	Diff.	±SE	Sig.
Out	82	68.8	4.4	49	-11.0	8.2	*
In	65	82.1	4.1				

Note;

n = the number of ewes which generated embryo quality data in both seasons.

Diff. = the mean difference in the yield of good quality transferable embryos between the two seasons for each ewe.

4.6 Survival of the embryos to day 50-55 after transfer

The embryo survival rate to scanning for the random data set was estimated with 932 embryos out-of-season and 439 embryos in-season, whereas the repeat data set used 278 embryos out-of-season and 242 embryos in-season. Only embryos classified as Q1 or Q2 were used for this analysis.

For the random data set there were no significant effects of the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer (0-30, 31-60, 61-90, 91-120 minutes, maximum two hours) on the embryo survival rate to scanning.

The overall embryo survival rate to scanning for the two seasonal periods is presented Table 4.6.1, with the survival data for the five breeds in each season presented in Appendix 1, Table 7.1.12.

Analysis of the repeat data set revealed no significant effects of the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer on the embryo survival rate to scanning.

The overall embryo survival rate to scanning for the two seasonal periods is presented Table 4.6.2, with the survival data for the five breeds in each season presented in Appendix 2, Table 7.2.11.

Table 4.6.1 Embryo survival rate to scanning for the random data set

Season	No. embryos	% survival	±SE
Out	932	70.8	1.3
In	439	65.1	1.9

Note;

No. embryos = the number of good quality (Q1 or Q2) embryos transferred. The number of recipient ewes involved was not presented in the interest of clarity, see section 3.10.

Table 4.6.2 Embryo survival rate to scanning for the two seasons for the repeat data set

Season	No. E's	Survival rate (%)	±SE	n	Diff.	±SE
Out	278	63.6	1.2	155	+14.7	9.6
In	242	64.8	1.6			

Note;

No. E's = the number of good quality (Q1 or Q2) embryos transferred.

n = the number of embryos generating embryo survival rate data for both seasons.

Diff. = the mean difference in embryo survival rate for the two seasons for each ewe.

4.7 Embryo survival to birth

The embryo survival rate to birth was estimated with 932 embryos out-of-season and 439 embryos in-season for the random data set compared to 278 embryos out-of-season and 242 in-season for the repeat data set. Only embryos classified as Q1 or Q2 were used for this analysis.

For the random data set, there were no significant effects of the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer (0-30, 31-60, 61-90, 91-120 minutes, maximum two hours) on the embryo survival to birth.

The embryo survival rate to birth for the random data set is summarised for the two seasonal periods in Table 4.7.1, with the breed by season embryo survival rates to birth presented in Appendix 1, Table 7.1.13.

For the repeat data set, the embryo survival rate to birth was not significantly affected by the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer on the embryo survival to birth.

The embryo survival rate to birth for the repeat data set is summarised for the two seasonal periods in Table 4.7.2, with the breed by season embryo survival rates to birth presented in Appendix 2, Table 7.2.12.

Table 4.7.1 Embryo survival rate to birth for the random data set

Season	No. embryos	% survival	±SE
Out	932	66.3	2.3
In	439	52.4	1.2

Note;

No. embryos = the number of good quality (Q1 or Q2) embryos transferred. The number of recipient ewes involved was not presented in the interest of clarity, see section 3.10.

Table 4.7.2 Embryo survival rate to birth for each season for the repeat data set

Season	No. E's	Survival rate (%)	±SE	n	Diff.	±SE
Out	278	67.3	2.1	155	+9.7	8.9
In	242	57.4	1.6			

Note;

No. E's = the number of good quality (Q1 or Q2) embryos transferred.

n = the number of embryos generating embryo survival rate data for both seasons.

Diff. = the mean difference in embryo survival rate for the two seasons for each ewe.

4.8 Lambs born

The number of lambs born per donor ewe programmed and flushed was estimated with 618 lambs out-of-season and 234 lambs in-season for the random data set whereas the repeat data set used 187 lambs out-of-season and 139 lambs in-season.

For the random data set, there were no significant effects of the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer on the number of lambs born per donor ewe programmed or flushed for the random data.

The total number of lambs born per donor programmed and flushed for the random data set is presented in Table 4.8.1, with the breed by season data presented in Appendix 1, Table 7.1.14.

The number of lambs born per donor ewe programmed or flushed from the repeat data set was not significantly affected by the season, foetus breed, donor age, donor/recipient oestrous synchrony, embryo quality (Q1 or Q2), recipient oestrous synchrony technique (500 or 200IU PMSG at CIDR-G removal), recipient surgeon, number of corpora lutea present in the recipient ewe, or the delay between recovery and transfer.

The total number of lambs born per donor programmed and flushed for the repeat data set is presented in Table 4.8.2, with the breed by season data presented in Appendix 2, Table 7.2.13.

Table 4.8.1 Number of lambs born for the random data set

Season	No. donor ewes	Total No. embryos transferred	Total No. lambs born	No. lambs born per donor programmed	No. lambs born per donor flushed
Out	262	932	618	1.66	1.92
In	124	439	234	1.00	1.33

Note;

No. donor ewes = the number of donor ewes from which Q1 or Q2 embryos were recovered.

Table 4.8.2 Number of lambs born for the repeat data set

Season	No. donor ewes	Total No. embryos transferred	Total No. lambs born	Diff.	Total No. lambs born per donor programmed	Diff.	Total No. lambs born per donor flushed	Diff.
Out	82	278	187	+15	1.43	+0.11	1.73	-0.02
In	65	242	139		0.99		1.53	

Note;

Diff. = the mean difference in embryo survival across the seasons for each donor ewe.

4.9 Summary of the overall MOET programme

A summary of the number of embryos and lambs resulting from this trial for the two seasons, is presented in Table 4.9.1 for the random data and in Tables 4.9.2 and 4.9.3 for the repeat data set. The summary of the number of embryos and lambs resulting from this trial for all of the breed and season combinations, is presented in Appendix 1, Table 7.1.15 for the random data and in Appendix 2, Tables 7.2.14 and 7.2.15 for the repeat data set. The overall responses for the out-of-season and in-season periods for the random and repeat data sets, respectively were:

(1) Incidence of oestrus:

random data; 93.3% out-of-season (373 ewes programmed) vs 100% in-season (234 ewes programmed).

repeat data; 93.9% out-of-season (131 ewes programmed) vs 100% in-season(131 ewes programmed).

(2) Ovulation rate:

random data; 7.64CL vs 6.60CL, respectively.

repeat data; 8.62CL vs 6.45Cl, difference +2.86Cl, respectively.

(3) Recovery rate:

random data; 53.4% (4.69 eggs) vs 53.5% (4.55 eggs), respectively from the donors which were actually flushed.

repeat data; 56.4% (5.85 eggs) vs 57.3% (5.12 eggs), difference -0.7%

(4) Fertilisation rate:

random data; 75.5% (3.77 embryos) vs 65.7% (3.39 embryos), respectively for the donors from which ova were recovered.

repeat data; 63.7% (4.11 embryos) vs 63.2% (3.59 embryos), difference -4.5%.

(5) Yield of good quality embryos:

random data; 78.2% (3.56 embryos) vs 83.7% (3.39 embryos), for the donors from which fertilised ova were recovered.

repeat data; 68.8% (3.37 embryos) vs 82.1% (3.72 embryos), difference -11.0%.

(6) Embryo survival to birth:

random data; 66.3% vs 52.4%.

repeat data; 67.3% vs 57.4%, difference +9.7%.

(7) Total number of lambs born:

random data; 618 lambs from 373 donors programmed (1.66 lambs born per donor ewe programmed) vs 234 lambs from 234 donors programmed (1.00 lambs born per donor ewe programmed).

repeat data; 187 lambs from 131 donors programmed (1.43 lambs born per donor ewe programmed) vs 139 lambs from 131 donors programmed (0.99 lambs born per donor ewe programmed), difference +0.11 lambs.

Table 4.9.1 Summary of the results of the overall MOET programme for the random data set

Season	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
		n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
Out	373	371	7.6	322	4.69	302	3.77	262	3.56	618	1.66
In	234	227	6.6	176	4.55	155	3.39	155	3.39	234	1.00

Note;

No. of ewes = the number of donor ewes programmed, n_1 = the number of ewes which were examined for ovulatory activity, n_2 = the number of ewes which were subjected to an embryo flush, n_3 = the number of ewes from which ova were recovered, n_4 = the number of ewes from

which fertilised ova were recovered, n_5 = the number of lambs born, μ_5 = the average number of lambs born per donor ewe programmed. The mean number of ova recovered and fertilised, and the number of good quality transferable embryos is based on the number of donor ewes indicated in each column (n_1-n_4).

Table 4.9.2 Summary of the results of the overall MOET programme for the repeat data set

Season	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
		n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
Out	131	130	8.6	108	5.85	98	4.11	82	3.37	187	1.43
In	131	126	6.5	91	5.12	82	3.59	65	3.72	139	0.99

Table 4.9.3 Summary of the difference in results of the overall MOET programme for the repeat data set

	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
		n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
Total	131	124	+2.9	79	-0.7	66	-4.5	49	-11.0	155	+0.11

CHAPTER FIVE

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

The overall objective of this trial was to compare embryo transfer results from two seasonal periods (out-of-season vs in-season) in order to determine the effect of the season on the different parameters of reproductive performance such as; the incidence of oestrus, ovarian response to exogenous gonadotrophins, fertilisation rates and overall the number of lambs born per donor ewe programmed and flushed. The conditions of this trial (which was conducted within the frame work of a commercial embryo transfer organisation) were not duplicated in any of the reports in the literature which were presumably conducted under more controlled research conditions, hence comparisons with the results reported in the literature should be taken with caution.

5.1 Incidence and the time of oestrous detection

5.1.1 The incidence of oestrus

The incidence of oestrus for the random data set was not significantly affected by the seasonal period with 93.3% of the ewes programmed during the out-of-season period detected in oestrus within 40 hours of CIDR-G removal compared to 100% for the in-season group (Table 4.1.1). The incidence of oestrus from the repeat data was also not significantly affected by the season, with 93.9% of the ewes programmed detected in oestrus out-of-season compared to 100% in-season (Table 4.1.2).

Moore (1982) suggested that the majority of animals should show oestrus 24 to 36 hours after removal of the progesterone pessaries. Thus the incidence of oestrus achieved during the two seasonal periods in both data sets is of an acceptable level and indicates that the oestrous synchronisation procedures used were successful.

Gordon (1971) reported an incidence of oestrus of 88% in Suffolk cross ewes during the non-breeding season, using progestagen and PMSG (250-750IU). Moore (1982) noted that progesterone-gonadotrophin treatment is effective for the induction of oestrus and super-ovulation in the ewe during the non-breeding season. Armstrong and Evans (1984a) recorded an incidence of oestrus of 78.3% out-of-season in Suffolk ewes (using 22.5mg FSH-P and progestagen sponges). Lopez-Sebastian et al. (1990) recorded all of the Manchega ewes programmed in oestrus between 24 and 32 hours after removal of the flurogestone acetate (FGA, 30mg) sponge in addition to a super-ovulatory treatment of FSH-P (16mg) during the non-breeding season. The out-of-season incidence of oestrus achieved in the current work (for both data sets) is comparable to that of similar out-of-season oestrous synchronisation work, as indicated above.

There are a number of reports illustrating that progesterone-containing CIDR will effectively synchronise oestrus in ewes and hoggets (which were not super-

ovulated) (Harvey et al., 1984; Maxwell and Barnes, 1986; McMillan, 1986) but there are few reports on the use of the CIDR with super-ovulatory doses of gonadotrophin in sheep. However Boland et al. (1983) showed that the incidence of oestrus was similar following a super-ovulatory dose of HAP (135mg, over three days) and either a CIDR or a 30mg flurogestone acetate sponge or 500mg progesterone sponge. Thompson and Smith (1988) confirmed that the CIDR can be used to successfully synchronise oestrus in donor ewes super-ovulated with FSH-P or Folltropin. Dattena (1989) recorded an incidence of oestrus of 88% following CIDR treatment and super-ovulation with FSH-P or Folltropin during the breeding season with the five breeds used here plus ewes of the Finnish Landrace breed.

From the above discussion it may be concluded that the oestrous synchronisation procedures employed effectively induced oestrous activity in both data sets.

There was no significant effect of any of the factors recorded in this study on the incidence of oestrus in either data set.

The incidence of oestrus was not significantly affected by the donor ewe breed (Appendix 1, Table 7.1.3 and Appendix 2, Table 7.2.3). Tervit et al. (1986) noted that the DT breed had a lower incidence of oestrus relative to the FT breed (82% vs 91%, respectively during the in-season period, following CIDR treatment and 1200-1875IU PMSG). These workers also recorded 87% of the OXD ewes programmed showing oestrus. These figures are comparable to the current work, but this work cannot confirm the difference between the DT and FT breeds.

Some ewes which did not show oestrus within 40 hours from CIDR-G removal were found to have ovulated and hence yielded embryos. This finding is in agreement with other workers (Cumming, 1965; Larsen, 1971; Clarke, 1973).

5.1.2 The time of oestrous detection

The time of oestrous detection was not significantly affected by the seasonal period (random data, Table 4.1.3; repeat data, Table 4.1.4). To the author's knowledge there are no reports which discuss the time of oestrous detection after CIDR-G removal in super-ovulated donor ewes during the out-of-season period.

The time of oestrous detection in either data set was not significantly affected by any of the factors recorded in this study.

The time of oestrous detection was not significantly affected by the donor ewe breed (Appendix 1, Table 7.1.4 and Appendix 2, Table 7.2.4). Tervit et al. (1986) noted that (during the breeding season) the FT breed showed an earlier mean oestrous detection time (after CIDR treatment and 1200-1875IU PMSG) relative to the DT breed (26.2 and 42.7 hours after CIDR removal, respectively) and that the OXD ewes also had a similar average oestrous detection time to the DT breed (39.8 hours).

The time of oestrous detection was not significantly affected by the ovulation rate class (random data, Appendix 1, Table 7.1.5.; repeat data, Appendix 2, Table 7.2.5). The out-of-season and in-season groups do not clearly illustrate an earlier oestrous detection time for the ewes in the higher ovulation rate classes as may have been expected from the literature. Torres and Cognie (1984) noted that ewes which were detected in oestrus relatively late had a lower average ovarian response and a lower percentage of good quality embryos. Thompson and Smith (1988) detected oestrus significantly earlier in ewes with higher ovulation rates. These workers suggested that the onset of oestrus is dependent upon the number of oestrogenic follicles present at CIDR removal (which may reflect the circulating level of oestrogen). Dattena (1989) recorded an earlier onset of oestrus after CIDR removal (in addition to 26-38mg FSH-P or Folltropin) for the DT and WHM breeds, which was associated with the higher ovulation rate classes.

5.1.3 Summary of the factors affecting the incidence and time of oestrous detection

There was no significant effect of the seasonal period (or any of the other factors studied) on the incidence or time of oestrous detection in the random data set which was confirmed by the repeat data set. The oestrous synchronisation procedures effectively induced oestrus activity during both seasonal periods. The incidence and time of oestrous detection were similar for the five breeds studied. This work does not clearly illustrate the tendency for ewes with higher ovulation rates to be detected in oestrus relatively early, as found by other workers (Torres and Cognie, 1984; Thompson and Smith, 1988).

5.2 Ovulation rate

The ovulation rate was significantly affected by the interaction between the donor breed and the donor age in the random data (Table 4.2.2) whereas the repeat data ovulation rate was not significantly affected by the season or any of the other factors recorded in this study.

5.2.1 The interaction between the donor ewe breed and age

The ovulation rate for the random data set was significantly affected by the interaction of the donor ewe breed and age, which was found to be attributable to the interaction of the Texel (DT and FT) and GOT breeds with the two age groups (Table 4.2.2). From this interaction it was evident that the ovulation rates of the two age groups of ewes for the two Texel (DT and FT) breeds were not significantly different. The younger ewes had higher ovulation rates than the older ewes (DT 14-16 months old, 7.47CL vs 26-28 months old 4.60CL; FT 14-16 months old 8.25CL vs 26-28 months old 5.32CL). This compares to the GOT breed for which a significantly lower ovulation rate was recorded for the younger donor ewes (7.91CL) compared to the older age group (10.00CL). The remaining donor ewe breeds also illustrated the same trend as that found in the Texel breeds, that is the younger donor ewes having higher ovulation rates than the older ewes (Appendix 1, Table 7.1.7).

From the above discussion it is evident that the two age groups of GOT ewes responded differently to the super-ovulatory treatments than the two age groups in each of the other four breeds used in this trial.

Torres et al. (1987) recorded a higher (not significantly) ovulation rate in six year old donors compared to three year old donor ewes (12CL vs 8.8CL, respectively for donor ewes of the Prealpes, Lacaune and Romanov breeds) following a super-ovulatory treatment of 16mg FSH-P. There is a lack of literature available on the effect of donor ewe age on the response to super-ovulatory gonadotrophin treatment, hence work using natural ovulation rates is quoted here. The finding

of a similar (or higher) ovulation rate for the younger relative to the older age group of Texel ewes is contrary to that reported in the literature for natural ovulation rates. Most workers show that the ovulation rate of immature (but post-puberal ewes) is lower than mature ewes and that there is no difference between age groups of mature ewes (Bindon et al., 1980; Montgomery et al., 1983). Lindsay et al. (1975) recorded a positive correlation (0.42) between ewe age and the ovulation rate. Wheeler and Land (1977) recorded ovulation rates for three breeds aged 18 or 30 months: Finnish Landrace, 2.5 vs 3.4CL; Merino, 1.0 vs 1.1CL; and Blackface, 1.2 vs 1.4CL; respectively.

Averill and Rowson (1958) noted a consistently higher ovulation rate from mature ewes (although not significant) when compared to younger ewes given PMSG, which is similar to the trend observed for the GOT breed in this study but contrary to that observed with the other four breeds.

Due to the repeated embryo transfer programmings in this trial it may be more relevant to discuss the effect of donor ewe age in relation to the number of previous embryo transfer programmings rather than the actual age of the donor ewe (in months). Hanrahan and Quirke (1982) noted a high repeatability for the number of CL from repeated super-ovulatory treatments, which was confirmed by Armstrong and Evans (1984a) and Torres and Sevellec (1987).

The ovulation rate recorded by the two age groups of Texel ewes compares favourably with that recorded by Hanrahan and Quirke (1982) also using Texel ewes (not specified whether of DT or FT origin), namely an ovulation rate of 6.6CL following the administration of PMSG (1500-2000IU). Tervit et al. (1986) recorded ovulation rates of 7.1CL for the DT breed and 7.5CL for the FT breed, but did not analyse this difference because the transfers were conducted under different conditions for each breed. Dattena (1989) noted similar ovulation rates for the DT, FT and GOT breeds (9.6CL, 9.9CL and 10.2CL, respectively).

5.2.2 The effect of the season on the ovulation rate

The overall mean ovulation rates (combining all breeds, ages and hormonal regimes) for the two seasonal periods were 7.64CL out-of-season vs 6.60CL in-season for the random data set (Table 4.2.1; note, for the random data set the significance of this effect was not estimable due to the donor breed by age interaction) and a difference of +2.86CL was recorded for the repeat data set (8.62CL out-of-season vs 6.45CL in-season, Table 4.2.3). The out-of-season ovulation rate tended to be higher (although not significantly) than the in-season ovulation rate for both data sets. The comparison of ovulation rates across the two seasonal periods for each of the donor breed-age-hormonal regime combinations is presented in Appendix 2, Table 7.2.7 for the repeat data set. From this table it is evident that there is a tendency for the out-of-season ovulation rate to be higher than that recorded in-season (six out of seven of the comparisons show a higher ovulation rate out-of-season; Appendix 2, Table 7.2.7).

This is contrary to the response recorded by Armstrong and Evans (1984a) with a significantly lower ovulation rate out-of-season of 9.8CL compared to 13.4CL in-season, using Suffolk ewes with a hormonal regime of 22.5mg FSH-P during both seasonal periods. Torres et al. (1987) also recorded a significantly lower ovulation rate out-of-season (8.4CL) compared to that recorded in-season (11.2CL) following a super-ovulatory treatment of 16mg FSH-P to Prealpes, Lacaune and Romanov ewes. However Lopez-Sebastian et al. (1990) found no significant difference between the seasons in Manchega ewes treated with 16mg FSH-P (7.1CL and 7.3CL, respectively).

From the work of Armstrong and Evans (1984a) and Torres et al. (1987) it was expected that the out-of-season ovulation rate would be less than that recorded in-season (note; the report by Lopez-Sebastian et al., 1990, was published after this trial had begun). Possible suggestions as to why this was not recorded are outlined below.

(1) There may have been some form of refractory response to the super-ovulatory treatments (the last treatment was five months prior to the out-of-season work and the in-season work was three months after that conducted out-of-season). This is especially relevant to the ovulation rate recorded in-season which was below expectations (based on the out-of-season ovulation rates) which may have been partially attributable to a refractory response. However a number of reports have shown no decline in the ovulation rates recorded from repeated super-ovulatory treatments (Hanrahan and Quirke, 1982; Armstrong and Evans, 1984a; Torres and Sevellec, 1987) hence this possible explanation is rejected.

(2) In order to determine the season effect, the same hormonal regimes had to be applied to both seasonal groups. The hormonal regimes used were considered to be optimum for the respective breeds when used out-of-season (H R Tervit, personal communication). The results from this trial suggest that the hormonal regimes used during the out-of-season period were not necessarily the optimum hormonal regimes to use during the in-season period for some of these breed-age combinations.

(3) Another possible explanation is related to the conditions under which this trial was conducted. Out-of-season, 553 ewes were programmed for embryo transfer over a period of two months (20-30 ewes per day) whereas in-season 3760 donor ewes were programmed over 3-4 months (50-60 ewes per day) of which the 234 ewes in the in-season group were a part. Thus the two seasonal groups may have been subjected to different levels of stress. For instance, the time the donor ewes spent in the yards awaiting the super-ovulatory hormonal injections was much greater for the in-season group, and as such these ewes were deprived of pasture and subjected to the stress of crowding for longer periods than the out-of-season group. Thus the "commercial nature" of this trial could have depressed the responses recorded in-season.

(4) The in-season group may have exhibited a relatively lower ovulation rate as a result of being programmed during the early part of the breeding season, as compared to the middle of the breeding season when the ovulation rate is

expected to be higher (Wheeler and Land, 1977). If the in-season group had been programmed at a later stage in the breeding season, the relatively low in-season ovulatory response recorded in some of the breed-age combinations may not have been observed.

(5) Oestrous cycles are absent out-of-season, and although follicular activity continues these follicles are not recruited and hence undergo atresia (Driancourt et al., 1985). Wheeler and Land (1977) recorded a higher follicular turnover during the non-breeding season (using the Finnish Landrace, Merino and Scottish Blackface breeds). Exogenous FSH administered during the non-breeding season (in the absence of an active oestrous cycle) does not have to compete with the in-vivo hormonal milieu associated with whatever stage of the oestrous cycle is present in the ewe at the time of administration. The out-of-season administration of exogenous gonadotrophins may result in a more efficient endocrine control of the oestrous cycle (and hence ovulation rate) than that achieved during the breeding season.

To summarise the effect of the season on the ovulation rate;
there was no significant difference between the ovulation rates recorded in both seasonal periods for the two data sets, however the out-of-season ovulation rate tended to be higher than the in-season ovulation rate. The out-of-season ovulation rate was expected to be lower than that recorded in-season (based on Armstrong and Evans, 1984a and Torres et al., 1987). The fact that the out-of-season ovulation rate recorded here was not significantly different from that recorded in-season suggests that super-ovulation can be effectively induced in these breeds during the out-of-season period.

5.2.3 The effect of the hormonal regime on the ovulation rate

There was no significant effect of the hormonal regime on the ovulation rate in the repeat data set (note; for the random data set the significance of this effect was not estimable due to the donor breed by age interaction).

The previous studies on the appropriate hormonal regime to use for each of these breed-age and season combinations (Dattena, 1989 {in-season only}; H R Tervit, personal communication; H W Vivanco, personal communication) helped to ensure the absence of a significant effect of the hormonal regime on the ovulation rate.

It may be suggested that an increase in the dose of FSH-P (or similar super-ovulatory gonadotrophin) will result in an increase in the ovulation rate up to a maximum level where the ovulation rate will begin to plateau or decline, presumably due to some form of refractory response. Tervit (1989) attempted to illustrate this using Coopworth ewes, but due to the relatively small dose rates used was only able to record a significant linear increase in the ovulation rate as the dose of FSH-P increased (0mg, 1.1CL; 8mg, 3.1CL; 16mg, 8.6CL; 24mg, 10.2CL). Armstrong and Evans (1984a) illustrated a maximum ovulatory response in Suffolk ewes out-of-season following the administration of 16mg FSH-P (7.6CL) compared to 32mg FSH-P (7.5CL) perhaps indicating a refractory response to the additional FSH-P. Tervit (1989) recorded an increase in the variability of the ovulatory response as the FSH-P dose increased. Tervit (1989) also found no significant difference in the ovulatory response from different commercial FSH preparations with different FSH:LH ratios (FSH-P, variable ratio; Folltropin, 5% LH by weight; Ovagen, 0.2% LH by weight). Torres and Cognie (1984) however suggested that the FSH:LH ratio should be constant at 4:1 for improved super-ovulation of the ewe. The current work used a FSH:LH ratio of 9:1 and 10:1, for the out-of-season and in-season groups respectively. Hence any variation in the ovulation rate is not likely to be due to the minimal difference in the FSH:LH ratios between the two seasonal periods.

5.2.4 Summary of the factors affecting the ovulation rate

There was no significant effect of the season on the ovulation rate. The ovulation rate for the random data was significantly affected by the interaction of the donor ewe breed and age which was found to be due to the two age groups of the GOT breed responding with the reverse trend to the remaining breeds. The ovulation rate was not significantly affected by the hormonal regimes used.

5.3 Recovery rate

5.3.1 The effect of the season on the recovery rate

The recovery rate in both data sets was not significantly affected by the seasonal period (53.4% out-of-season vs 53.5% in-season, for the random data, Table 4.3.1; a difference of -0.7 percentage points was recorded for the out-of-season recovery rate relative to in-season for the repeat data set {56.4% out-of-season vs 57.3% in-season} Table 4.3.4). Torres et al. (1987) recorded similar embryo recovery rates out-of-season to those recorded in-season (84.5% vs 83.0%, respectively). Lopez-Sebastian et al. (1990) also recorded no difference in recovery rate between the two seasons (81% out-of-season vs 77% in-season). There is a lack of reports quoting recovery rates out-of-season relative to in-season, especially utilising flushing techniques similar to the laparoscope-aided technique used in this trial. From the current work and in the absence of contradictory reports, it may be concluded that the embryo recovery rate was not significantly affected by the seasonal period.

The recovery rates found in the present work were lower than those observed by many other workers (who used surgical uterine flush techniques). Trounson and Moore (1974) obtained an 80% recovery rate (in-season), compared to Tervit and Havik (1976) with 83%, and 74% by Torres and Cognie (1984). However the recovery rates in the present work compare favourably with other (in-season) work using the same laparoscope-aided technique as that used here (50%; McKelvey et al., 1986).

5.3.2 The effect of the donor surgeon used on the recovery rate

There was a significant effect of the donor surgeon used on the recovery rate for both data sets (Tables 4.3.3 and 4.3.5). This was unexpected as the five donor surgeons used all yielded reasonable recovery rates considering the varied operational conditions under which each of them worked. Note, donor surgeon codes 1-3 used both embryo flushing techniques (laparoscope-aided uterine flush

and the surgical uterine flush) whereas donor surgeon codes 4 and 5 used only the surgical uterine flush technique. The effect of the flush technique used on the recovery rate was not significant.

The difference in the recovery rates for the five donor surgeons used (Tables 4.3.3 and 4.3.5) could be due to differences in surgeon skill. However, as noted earlier, donor surgeon codes 4 and 5 used a surgical uterine flush technique only. This was used for donor ewes with adhesions (as a result of previous embryo flushes) which prevented the use of the laparoscope-aided uterine flush technique. Hence the recovery rates for donor surgeon code 4 (and possibly 5, although the recovery rate for this donor surgeon was not significantly different from that of the other donor surgeons in either data set) could have been depressed by the presence of adhesions on the reproductive tract (McKelvey et al., 1986).

5.3.3 The effect of the time of oestrous detection on the recovery rate

The time of oestrous detection relative to CIDR-G removal had a significant effect on the recovery rate in the random data (Table 4.3.2) but not for the repeat data. This suggests that the effect of the time of oestrous detection on the recovery rate was confounded with the individual animal variation, because when this individual animal variation was minimised (in the repeat data set) there was no significant effect of the time of oestrous detection.

There was a significant decline in the recovery rate as the interval from CIDR-G removal to oestrous detection increased in the random data (Table 4.3.2). However the recovery rate for the ewes detected in oestrus 40 hours after CIDR-G removal was not significantly different from the recovery rate of the ewes which were not detected in oestrus (46.4% vs 26.8%, respectively). McKelvey et al. (1985) recorded a higher embryo recovery rate for ewes with a later average oestrous detection time (74% vs 85% for oestrous detection 27 and 37 hours after progesterone source removal, respectively) which is the opposite trend to the one recorded in the present work. However Torres et al. (1987) recorded a

similar trend to the one found in the current work; that is a recovery rate of 71.5% for ewes detected in oestrus 24 hours after sponge removal compared to 63% and 48% for ewes detected in oestrus 36 and 48 hours after sponge removal, respectively.

The ewes which were detected in oestrus relatively early may be expected to have higher levels of oestrogen (as a result of higher numbers of oestrogenic follicles) than ewes detected in oestrus later (Thompson and Smith, 1988; as discussed in section 5.1.2) which may help to reduce the spread of ovulation time. This may ensure that all the ova are collected by the fimbria and begin the developmental passage down the oviduct and hence are available for collection. This may partially explain the higher recovery rate for ewes detected in oestrus relatively early which was found in this work.

5.3.4 The recovery rate and the non-significant factors

The recovery rate was not significantly affected by the donor breed in either data set (Appendix 1, Table 7.1.9 and Appendix 2, Table 7.2.8). This was expected because the five breeds were subjected to the same embryo recovery techniques. By definition the recovery rate is affected by the ovulation rate, however from Appendix 1, Table 7.1.15 and Appendix 2, Tables 7.2.14 and 7.2.15 it can be seen that the number of ova recovered from each donor breed was similar. Tervit et al. (1986) found a lower recovery rate for the Texel breeds compared to the OXD breed (70% vs 81%, respectively) using a surgical uterine flush. It was suggested by those workers that the relatively large size of the Texel uterus, and hence a large tract volume for the flushing media to perfuse, may have been partially responsible for this decline in recovery rate. The current work cannot dismiss or confirm this suggestion. Dattena (1989) recorded no significant difference between the recovery rates for the five breeds used in this trial.

There was no significant effect of age of donor ewe on the recovery rates. Repeated attempts at embryo flushing increase the chance of post-operative adhesion formation which results in a decline in the recovery and fertility rates

(McKelvey et al., 1986). In this trial the older donor ewe age group (26-28 months old) had been subjected to a maximum of four previous flushes and therefore a lower recovery rate from this age group relative to the younger donor ewe age group (14-16 months old, with one previous flush) might have been expected (Torres and Sevellec, 1987). The 14-16 months old ewes tended to have higher recovery rates than the 26-28 months old ewes (random data, 55.1% ±1.5, 14-16 months old vs 46.1% ±3.6, 26-28 months old, respectively; repeat data, 58.3% ±2.4 vs 47.5% ±6.9, respectively). Torres and Sevellec (1987) showed that repeated embryo flushes significantly reduced the recovery rate after the first flush (88% vs 52% vs 24%). Hanrahan and Quirke (1982) recorded a reduction in the recovery rate of 10-20% for the second flushing of a donor ewe. Other workers have noted significant declines in the recovery rate for subsequent embryo flushes (as indicated above). Hence the absence of a significant effect of the donor ewe age (or more appropriately the number of previous flushes) on the recovery rate was unexpected.

The hormonal regime used had no significant effect on the recovery rates. This finding was expected, however an increase in the recovery rate was expected following the addition of GnRH to the hormonal regime (Walker et al., 1989). The comparison between regime 3 and 7 with OXD ewes out-of-season, illustrates the effect of GnRH (250mg) administration at the time of oestrous detection on the recovery rate. The recovery rate for no GnRH treatment was 66.7% ±33.3 compared to 55.0% ±3.7 when GnRH was administered for the random data set (note; this comparison was not available in the repeat data set). The lower recovery rate following GnRH treatment may be due to the relatively late GnRH administration (at oestrous detection). Walker et al. (1989) showed that relatively small doses of GnRH (25μg) administered at 24 or 36 hours after progesterone source removal (that is before the average time of oestrous detection in this report) had a beneficial effect on the recovery rates (47% and 44%, respectively) compared to 38% for no GnRH treatment. Another possible reason to explain the depressed recovery rate following GnRH administration recorded in the present work is that the dosage used was relatively large (250mg) compared to that {25μg} used by Walker et al. (1989). This could have affected the timing of ovulation and the number of ova collected by the fimbria and hence potentially

alter the number of ova available for recovery from the reproductive tract. Walker et al. (1986) noted that GnRH administration synchronised the timing of ovulation and reduced the interval between the first and last ovulation.

The quality of the corpora lutea and the presence of un-ruptured {potentially ovulatory} follicles had no significant effect on the recovery rates. One may have expected a lower recovery rate in the presence of un-ruptured {potentially ovulatory} follicles because of the high oestrogen output associated with these follicles, which increases the rate of ova transport through the oviducts and decreases recovery rates (Quirke and Hanrahan, 1975; Du Mesnil Du Buisson et al., 1977).

There was no significant effect of the ovulation rate class (ORC) on the recovery rates. From the available literature, it appears uncertain as to what effect the ovulation rate class has on the recovery rate. Betteridge and Moore (1977) showed that the embryo recovery rate decreases as the number of ovulations increases, but this work used PMSG as the super-ovulatory drug and hence "hyper-stimulation" of the ovary (typical of PMSG-treated donors; Armstrong and Evans, 1983) may have caused this decline in recovery rate. Hanrahan and Quirke (1982) showed a decline in recovery rate when the number of corpora lutea exceeded five, using Galway ewes. Wright et al. (1981) found that the recovery rate increases as the ovulation rate increases (in ewes treated with FSH-P) which was confirmed by Armstrong and Evans (1983) using goats treated with FSH-P. Dattena (1989) found no significant difference in recovery rate between the ORC's, but this may have been due to the large range in number of CL within each of the three ORC's used in that work.

There was no significant effect of the flush technique used on the recovery rates (laparoscope-aided, 54.1% ±1.5 vs surgical, 48.0% ±4.9 for the random data compared to 57.3% ±2.4 for the laparoscope-aided vs 53.3% ±7.3 for the surgical technique in the repeat data). It may be suggested that the relatively low (50.9%) recovery rates using the surgical uterine flush technique in this trial, may be due to adhesions on the reproductive tract of the donor ewes (as discussed in section 5.3.2). Thus if the two flush techniques were compared on donor ewes with the

same degree of adhesions, it might be expected that the surgical flushing would yield a higher recovery rate as can be seen from the reports quoting recovery rates from uterine flushing techniques (83%, Tervit and Havik, 1976; 74% Torres and Cognie, 1984).

5.3.5 Summary of the factors affecting the recovery rate

There was no significant effect of the seasonal period on the recovery rate.

The recovery rate was significantly affected by the time of oestrous detection after CIDR-G removal in the random data set only. Early oestrous detection after CIDR-G removal significantly increases the recovery rates; this finding is contrary to that of McKelvey et al. (1985).

The recovery rate was significantly affected by the donor surgeon used in both data sets. The difference in donor surgeon skill may partially explain the different recovery rates. The presence of adhesions (as a result of previous embryo flushes) may have depressed the recovery rate for (at least) two of the five donor surgeon codes.

5.4 Fertilisation rate

5.4.1 The effect of the season on the fertilisation rate

The fertilisation rate was not significantly affected by the seasonal period (75.5% out-of-season vs 65.7% in-season for the random data, Table 4.4.1; compared to a difference of -4.5 percentage points for the out-of-season fertilisation rate relative to in-season {63.7% vs 63.2%, respectively}, Table 4.4.3). The results of Armstrong and Evans (1984a) and Lopez-Sebastian et al. (1990) are in agreement with this finding, as both of these reports showed no effect of the season on the recovery of fertilised embryos. Hence it may be concluded that the fertilisation rate is not significantly affected by the seasonal period.

The fertilisation rate for the two seasonal periods was lower than that recorded by other workers using intra-uterine insemination of fresh diluted semen (83%, Clarke et al., 1984; and 85.4%, Dattena, 1989) but is comparable to the fertilisation rate of 64% using frozen/thawed semen (Maxwell and Hewitt, 1986). This was unexpected because the conditions of the present trial were very similar to those described by Dattena (1989).

5.4.2 The effect of the time of oestrous detection on the fertilisation rate

There was a significant effect of the time of oestrous detection relative to CIDR-G removal on the fertilisation rate for the random data set only (Table 4.4.2). This finding was not confirmed in the repeat data set. The ewes which were detected in oestrus relatively early had significantly higher fertilisation rates than those detected in oestrus later (16 and 26 hours after CIDR-G removal, 70.1% and 76.0% vs 40 hours, 49.2%). The ewes which were not detected in oestrus but were still "fixed time inseminated" had a similar fertilisation rate (60.0%) to the ewes which were detected in oestrus (at any time up to 40 hours after CIDR-G removal). Moore (1982) found no difference in fertilisation rates for ewes in oestrus at different times after progesterone source removal, which also included ewes which were "fixed time" inseminated. Donor ewes which were detected in

oestrus relatively late may be expected to have lower levels of oestrogen (as a result of a lower number of oestrogenic follicles; Thompson and Smith, 1988) and hence the spread of ovulation time may be increased. This may reduce the time some of the ova have to develop in the oviduct, prior to insemination (and hence potential fertilisation) which may reduce the fertilisation rate.

5.4.3 The fertilisation rate and the non-significant factors

There was no significant effect of the donor breed on the fertilisation rates (Appendix 1, Table 7.1.10 and Appendix 2, Table 7.2.9). No difference in fertilisation rate between donor ewe breed was expected, as shown by Dattena (1989). However Tervit et al. (1986) showed that the OXD breed had a lower fertilisation rate than the Texel breed (40% vs 71%, respectively; under a hand mating regime) which was thought to be due to poor ram performance rather than the breed of donor ewe.

The effect of the donor ewe age on the fertilisation rate was not significant for both data sets. It was thought that any difference in fertilisation rate between the age groups would be more a reflection of the number of previous flushes each age group had been subjected to rather than an effect of donor age per se. The flushing technique used in this trial (laparoscope-aided uterine flush) could be potentially damaging to the site of sperm storage within the oviduct (isthmic-uterine junction) because the flushing aspic (through which the flushing media passes into the uterine horn) is inserted cranial to this junction. Thus every time the uterus is flushed this junction is subjected to the full flow of the flushing media and all the potential damage associated with that, in addition to the damage made by inserting the aspic needle into the oviduct.

Hanrahan and Quirke (1982) recorded an average drop of 8% in the fertilisation rate for each subsequent embryo flush. McKelvey et al. (1986) suggested that the fertilisation rate may be decreased in ewes which have adhesions as a result of previous embryo recoveries. Torres and Sevellec (1987) recorded a substantial drop in fertilisation rate for the third flush, following natural mating of super-

ovulated 15 month-old Prealpes ewes (86.6% vs 93.5% vs 6.7%, respectively). In the above reports, the flushing technique used was similar to the surgical uterine flush outlined by Tervit and Havik (1976) with the flushing aspic introduced 2-3cm cranial to the utero-tubal junction, which suggests that the isthmic-uterine junction is subjected to the potential damage of each uterine flush.

The fertilisation rate was not significantly affected by the hormonal regime used. It was expected that the hormonal regime should not significantly affect the fertilisation rate, that is except for the addition of GnRH to the hormonal regime, which was expected to elevate the fertilisation rate. Walker et al. (1989) recorded fertilisation rates of 51.5% with no GnRH vs 71.7% with 25μg GnRH. The comparison between regimes 3 and 7 (within the OXD breed out-of-season) estimates the effect of GnRH (250mg) on the fertilisation rate with a constant 28mg of FSH-P. GnRH treatment did not cause an increase in the fertilisation rate (no GnRH, 80.5% ±4.1 vs GnRH, 75.3% ±3.4, random data set only, this comparison was not available in the repeat data set). The absence of a beneficial effect of the GnRH treatment on the fertilisation rate may be due to the relatively large dose of GnRH used or the relatively late timing of the administration, as discussed in section 5.3.4 (for the recovery rate).

There was no significant effect of the ram used on the fertilisation rate for either data set. All of the rams under-went a semen morphology test prior to the trial and each ejaculate was graded for semen quality (concentration and motility) after collection. Only rams with less than 20% abnormal sperms and good quality ejaculates were used. Both of these procedures attempted to ensure that the presence of abnormal sperms and differences in semen quality did not occur. It appears that the implementation of the above procedures successfully ensured that no difference in fertilisation rate occurred between the rams used.

There was no significant effect of the corpora lutea quality or the presence of unruptured {potentially ovulatory} follicles on the fertilisation rates. This is in agreement with other workers (Quirke and Hanrahan, 1975; Du Mesnil Du Buisson et al., 1977) although both of these reports noted that the increase in oestrogen output associated with the presence of unruptured follicles could

potentially decrease the fertilisation rates.

There was no significant effect of the ovulation rate class on the fertilisation rates. The fertilisation rate was not expected to vary for the different ORC's used. Wright et al. (1981), Torres and Cognie (1984), and Dattena (1989) also observed no decline in the fertilisation rate as the ovulation rate increased. Hanrahan and Quirke (1982) recorded a decline in the fertilisation rate in Finnish Landrace ewes when the ovulation rate exceeded fifteen.

There was no significant effect of the time of insemination after oestrous detection on the fertilisation rates (see Table 3.7 for insemination times). This finding was expected as the range of insemination times relative to oestrous detection quoted in the literature was similar to this trial (Wright et al., 1981, 12 hour delay between oestrous detection and insemination, 94%; Torres and Cognie, 1984, 24 hours delay, 94%; McKelvey et al., 1985, 20 hours delay, 95.2% fertilisation rate).

5.4.4 Summary of the factors affecting the fertilisation rate

There was no significant effect of the season on the fertilisation rate, which was confirmed by other reports (Armstrong and Evans, 1984a; Lopez-Sebastian et al., 1990).

There was a significant effect of the time of oestrous detection after CIDR-G removal in the random data set only. The ewes which were detected in oestrus relatively late had a significantly lower fertilisation rate.

The five donor ewe breeds had similar fertilisation rates.

5.5 Embryo quality

5.5.1 The effect of the season on the yield of good quality transferable embryos

The yield of good quality transferable embryos was significantly affected by the seasonal period for both data sets (78.2% out-of-season vs 83.7% in-season for the random data, Table 4.5.1; a difference of -11.0 percentage points was recorded out-of-season relative to in-season for the yield of good quality transferable embryos from the repeat data set {68.8% out-of-season vs 82.1% in-season} Table 4.5.4). Torres et al. (1987) recorded a significantly lower yield of good quality transferable embryos out-of-season compared to that in-season (40.6% vs 74.5%, respectively) which was confirmed by the current work. Lopez-Sebastian et al. (1990) found that the season did not significantly affect the yield of good quality transferable embryos (56.4% out-of-season vs 59.5% in-season) which is contrary to the finding of the current study.

From the discussion on the effect of the hormonal regime on the ovulation rate for the two seasonal periods (section 5.2.3) it was evident that the hormonal regimes used induced adequate super-ovulatory responses during both seasonal periods. However the lower yield of good quality transferable embryos out-of-season (in both data sets) may suggest that the hormonal regimes applied to the donor ewes during the out-of-season period did not create a suitable endocrine environment in which to adequately develop the ova. The out-of-season hormonal regimes in particular require further refinement to induce a more suitable endocrine environment, which is necessary to produce an acceptable percentage of good quality embryos (section 5.5.4 for further discussion on hormonal regimes).

5.5.2 The effect of the donor breed on the yield of good quality transferable embryos

The donor breed had a significant effect on the random data sets' yield of good quality transferable embryos (Table 4.5.2) although this was not confirmed by the repeat data. The GOT breed had a significantly lower yield of good quality transferable embryos than the other breeds. Dattena (1989) recorded a similar percentage of good quality embryos for all of the six breeds used in that trial (Finnish Landrace plus the five breeds used in this trial). Tervit et al. (1986) recorded 77% of the embryos recovered as good quality from the OXD ewes compared to Texel ewes with 84%.

The significantly lower yield of good quality transferable embryos for the GOT breed may indicate an LH imbalance (Donaldson, 1984) between the LH supplied by the super-ovulatory treatments and the LH required by that breed of donor ewe. Donaldson (1984) showed that an LH imbalance may result in a disturbance of the normal oocyte and follicle maturation process, which results in poor ovum quality and a reduced fertilisation rate.

5.5.3 The effect of the time of oestrous detection after CIDR-G removal on the yield of good quality transferable embryos

The random data recorded a significantly lower yield of good quality transferable embryos as the time from CIDR-G removal to oestrous detection increased (Table 4.5.3) which was not found in the repeat data set. The ewes detected in oestrus at 16 and 26 hours after CIDR-G removal yielded similar percentages of good quality transferable embryos (85.5% vs 84.1%, respectively) but the later oestrous detection times had significantly reduced yields of good quality transferable embryos (65.5% for 40 hours and 16.7% for non-tupped ewes). This finding may be due to the decreased levels of circulating oestrogen in those ewes detected in oestrus relatively late, as a result of fewer oestrogenic follicles (Thompson and Smith, 1988) which may spread the time of ovulation (first to last ovulation). This increase in the duration of the ovulation process would result in

varying degrees of ova development due to the varied time the ova had spent in the reproductive tract (from ovulation to recovery) in which to develop.

5.5.4 The yield of good quality transferable embryos and the non-significant factors

There was no significant effect of the hormonal regime used on the yield of good quality transferable embryos. Donaldson (1984) showed that the yield of good quality transferable embryos declined with increasing dose rates of FSH-P in cattle (57% for a low dose of FSH-P vs 47% for a high dose of FSH-P). There was no significant effect of GnRH (250mg) administration on the yield of good quality transferable embryos from the OXD ewes out-of-season in the random data set (no GnRH, regime 3; 87.8% ±3.6 vs GnRH, regime 7; 83.2% ±5.3). Walker et al. (1989) also did not find a significant effect of GnRH administration on the yield of good quality transferable embryos (85.5% vs 83.6%, respectively).

The yield of good quality transferable embryos was not significantly affected by any of the remaining factors recorded in this study.

5.5.5 Summary of the factors affecting the yield of good quality transferable embryos

From this section it may be concluded that the yield of good quality transferable embryos was significantly lower out-of-season than in-season in both data sets, which is contrary to the finding of Lopez-Sebastian et al. (1990). This finding may be partially attributable to the less than optimum endocrine environment created by the hormonal regimes used out-of-season, suggesting that further refinement of these hormonal regimes is required. The donor breed significantly affected the yield of good quality transferable embryos in the random data set, with the GOT breed having significantly lower yields than the other four breeds. The ewes detected in oestrus relatively late yielded significantly lower percentages of good quality transferable embryos than ewes detected in oestrus earlier in the random data set.

5.6 Embryo survival to scanning and birth

5.6.1 The effect of the season on embryo survival to scanning and birth

The embryo survival rate to scanning was not significantly affected by the seasonal period for both data sets (70.8% out-of-season vs 65.1% in-season for the random data set, Table 4.6.1; a difference of +14.7 percentage points was generated by the difference in the embryo survival rates to scanning by the repeat data set {out-of-season 63.6% vs 64.8% in-season} Table 4.6.2). The embryo survival to birth was not significantly affected by the season in either data set (66.3% out-of-season vs 52.4% in-season for the random data, Table 4.7.1; a difference of +9.7 percentage points was generated by the difference in embryo survival to birth by the two seasonal groups of the repeat data set {67.3% vs 57.4%} Table 4.7.2).

Armstrong and Evans (1984a) confirmed that the season did not affect the embryo survival rate to birth (35% out-of-season and 40% in-season). Torres et al. (1987) recorded no difference in the survival rate to birth of embryos transferred during the two seasonal periods (82.7% out-of-season vs 76.3% in-season). There is a lack of reports comparing embryo survival rates from two seasonal periods. However the embryo survival rates (to scanning and to birth) for both seasonal periods (from both data sets) are comparable to other (in-season) reports (63%, Hanrahan and Quirke, 1982; 69%, Moore, 1982).

5.6.2 The embryo survival to scanning and birth and the non-significant factors

The embryo survival rates to scanning and birth were not significantly affected by the embryo breed in either data set (Appendix 1, Table 7.1.12 and 7.1.13; Appendix 2, Table 7.2.11 and 7.2.12). Hanrahan and Quirke (1982) also noted that the genotype of the embryo (Finnish Landrace, Galway, or Texel) did not affect the survival rates.

There was no significant effect of the donor ewe age on the embryo survival rates to scanning or birth. Most reports indicate lower embryo survival rates for younger donor ewes (Du Mesnil Du Buisson et al., 1977, 6% survival to birth for embryos from pre-puberal donor ewes vs 39% from adult donor ewes; Quirke and Hanrahan, 1977, 33.3% survival birth for embryos from ewe lambs vs 72.9% for embryos from adult donor ewes). McMillan (1981) recorded a lower survival rate for embryos recovered from ewe lamb donors when transferred to hogget (48%) compared to mature (75%) recipient ewes, whereas embryos recovered from mature donor ewes showed no difference in survival rate for the two recipient ewe age groups. This strongly suggests some form of impairment in the development of the hogget ova. However this was not observed in this trial, presumably because of the relatively small difference in age between the two groups (14-16 months vs 26-28 months old).

The two recipient oestrous synchrony methods (CIDR-G plus 500IU PMSG out-of-season or 200IU PMSG in-season) did not significantly affect the embryo survival rates. The different levels of PMSG and the time of CIDR-G removal for the two seasonal groups (the out-of-season recipients' CIDR-G were removed 12 hours before the corresponding in-season recipients due to the longer interval from CIDR-G removal to oestrous detection observed out-of-season compared to that in the in-season group) were considered necessary to ensure close oestrous synchrony between the donor and recipient ewes in the respective seasons.

The embryo survival rates (to scanning and birth) were not significantly affected by the degree of oestrous synchrony between the donor and recipient ewes (either oestrous synchrony or ±12 hours asynchrony between the time of oestrous detection for the donor and recipient ewes). This is probably due to the relatively close oestrous synchrony achieved in this work. Most workers agree that the embryo survival rate is depressed as the degree of oestrous asynchrony increases (Moore, 1982; Hanrahan and Quirke, 1982; Tervit, 1989). Acceptable embryo survival rates can be obtained when there is an asynchrony of ± one day (Cumming, 1965; Rowson and Moor, 1966) but 72 hours asynchrony appears fatal for the embryo (Rowson and Moor, 1966).

The delay between embryo recovery and transfer to the recipient did not significantly affect the embryo survival rates, predominantly because of the relatively short period of time elapsing (all embryos were implanted within two hours of recovery). It is generally agreed that the interval between embryo collection and transfer can be up to three hours without detrimentally affecting the embryo survival rate and that a minimum of time outside of the reproductive tract is desirable (Averill, 1956; Moore and Shelton, 1964b; Thatcher et al., 1985).

The number of corpora lutea present in the recipient ewe did not significantly affect the embryo survival rates. All of the recipient ewes in this trial received progesterone supplementation, in the form of a CIDR-G after transfer (section 3.11). This exogenous source of progesterone may have masked any effect that the number of CL in the recipient ewe had on the embryo survival rates. Most workers have found no effect of the number of CL in the recipient ewe on the survival rate of transferred embryos (Moore and Rowson, 1960; Cumming and McDonald, 1970).

5.6.3 Summary of the factors affecting the embryo survival to scanning and birth

The embryo survival rates (to scanning and birth) were not significantly affected by the seasonal period and are comparable to other reports (Moore, 1982; Armstrong and Evans, 1984a). The embryo survival rates for the five donor breeds were similar. The age of the donor ewe did not significantly affect the embryo survival rates, contrary to other reports (McMillan, 1981). Due to the close donor/recipient oestrous synchrony (±12 hours) there was no significant effect of the degree of oestrous synchrony on the embryo survival rates. The relatively short interval between embryo recovery and transfer (2 hours) ensured there was no significant effect of this variable on the embryo survival rates. The number of corpora lutea present in the recipient ewe did not significantly affect the embryo survival rates, possibly because of the progesterone supplementation after transfer.

5.7 Number of lambs born

The number of lambs born per donor ewe programmed was not significantly affected by the seasonal period for either data set (1.66 out-of-season vs 1.00 in-season, for the random data set, Table 4.8.1; a difference of +0.11 lambs born per donor ewe programmed was recorded for the repeat data {1.43 out-of-season vs 0.99 lambs born in-season} Table 4.8.2). The number of lambs born per donor ewe actually flushed was also not significantly affected by the season in either data set (1.92 out-of-season vs 1.33 in-season for the random data set, Table 4.8.1; a difference of -0.02 lambs born per donor ewe flushed was recorded in the repeat data set {1.73 out-of-season vs 1.53 lambs in-season} Table 4.8.2).

The number of lambs born per donor ewe programmed or flushed was not significantly affected by any of the factors recorded in this study.

The number of lambs born as a result of implementing an embryo transfer programme is a function of the six variables discussed in the previous sections (the incidence and time of oestrous detection, ovulation rate, recovery rate, fertilisation rate, the yield of good quality transferable embryos and the survival of those embryos to birth). Each of these variables is dependent on at least one of the other variables (with the exception of the incidence and time of oestrous detection). In the present work the overall out-of-season ovulation rate tended to be (not significantly) higher than that recorded in-season (for both data sets) thus it may be expected that there will be a carry-over effect resulting in a higher number of transferable embryos recovered in the out-of-season group. However there was a significantly lower yield of good quality transferable embryos in the out-of-season group (for both data sets) relative to the in-season group. It is suggested that the difference (although not significant) in the overall number of lambs born for the two seasonal periods is a result of the factors influencing the variables which directly affect the embryo yield (for example, the ovulation rate and the yield of good quality transferable embryos) rather than the lambing rate per se. These factors have been discussed in the preceding sections. Torres et al. (1987) recorded a lower ovulation rate and yield of good quality transferable

embryos out-of-season relative to in-season, however the overall number of lambs born during the two seasonal periods was not significantly different.

The number of lambs born per donor ewe programmed is comparable to those of other embryo transfer studies such as: 2.7 lambs, Hanrahan and Quirke (1982); 1.6, Armstrong and Evans (1983); 2.0, Armstrong and Evans (1984a); 1.6, Walker et al. (1986); 1.5, Dattena (1989); 2.1, Tervit (1989). However, the in-season numbers of lambs born per donor programmed (1.00 random data and 0.99 repeat data) are relatively low compared with the reports cited above, although these results were not significantly different from the number of lambs born out-of-season (1.66 random data and 1.43 repeat data).

The number of lambs born per donor ewe programmed appears to be relatively low when compared to the number of lambs born from less expensive breeding options, such as the use of a mild dose of PMSG and synchronised natural mating (1.76 lambs born per ewe per year; Robinson, 1980). However, embryo transfer has the advantage of being able to be repeated several times throughout the year. For example, out-of-season embryo transfer could be conducted twice and in-season embryo transfer four times during the space of one calendar year, as has been done at LambXL. Using the results obtained from this trial, an estimate of the annual lamb production generated by implementing the techniques outlined above is 7.32 (for the random data set only) lambs born per donor ewe programmed per year (2x1.66 lambs born = 3.32 lambs born out-of-season per donor ewe programmed plus 4x1.00 lambs born = 4.00 lambs born in-season per donor ewe programmed).

5.8 Overall conclusions

The present work clearly demonstrates the inter-dependence of several factors affecting the number of lambs born per donor ewe in an embryo transfer programme. The importance of any one of these factors cannot be under-estimated because of the consequential effects that each factor may have on the overall effectiveness of the whole programme. This was clearly illustrated by the relatively low in-season ovulation rate which appeared to depress the number of lambs born during that period. However the only variable to be significantly affected by the season was the yield of good quality transferable embryos, with the out-of-season yield significantly lower than that recorded in-season, hence reversing the (non-significant) trend observed with the ovulation rate. The overall number of lambs born for the two seasonal periods was not significantly different, hence this important measure of the success of embryo transfer procedures illustrated that there was no effect of the seasonal period.

The overall conclusion from this trial is that out-of-season embryo transfer is as effective as in-season embryo transfer. The use of out-of-season embryo transfer in addition to that conducted during the in-season period, can increase the number of lambs born per donor ewe per year and hence decrease the fixed costs (associated with the donor ewes, buildings, equipment and staff) per lamb born. It is suggested that if embryo transfer (in-season) is being considered or is already used, the addition of out-of-season embryo transfer to the overall programme can only increase the speed and efficiency with which the desired objectives are achieved.

CHAPTER SIX

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CHAPTER SEVEN

CHAPTER SEVEN

APPENDICES

APPENDIX ONE - RANDOM DATA

Table 7.1.1 The number of donor ewes in the random group

Breed	Age (mths)	Out-of-season	In-season
DT	14-16	108	10
	26-28	10	0
FT	14-16	92	12
	26-28	5	21
GOT	14-16	9	67
	26-28	7	7
OXD	14-16	131	5
	26-28	2	59
WHM	14-16	7	36
	26-28	2	17
Total	14-16	347	130
	26-28	26	104

Table 7.1.2. The number of donor ewes in the random group for each breed, age and regime combination

Breed	Age	Regime	Out-of-season	In-season
DT	14-16	3	3	0
		4	105	10
	26-28	4	10	0
FT	14-16	3	89	12
		4	3	0
	26-28	4	5	21
GOT	14-16	2	9	67
	26-28	2	7	7
OXD	14-16	3	73	5
		7	58	0
	26-28	4	2	59
WHM	14-16	8	6	0
		9	1	0
		10	0	36
	26-28	5	2	0
		6	0	17

Table 7.1.3 The effect of season on the incidence of oestrus for each breed

Breed	Out-of-season		In-season	
	n	%	n	%
DT	118	93.2	10	100.0
FT	97	93.8	33	100.0
GOT	16	100.0	74	100.0
OXD	133	91.7	64	100.0
WHM	9	100.0	53	100.0
Total	373	93.3	234	100.0

Table 7.1.4 The effect of season on the time of oestrous detection for each breed

Breed	Season	n	16h	26h	40h	NT
DT	Out	118	14.4	66.1	12.7	6.8
	In	10	50.0	50.0	0	0
FT	Out	97	35.1	53.6	5.2	6.2
	In	33	21.2	75.8	3.0	0
GOT	Out	16	37.5	62.5	0	0
	In	74	35.1	60.8	4.1	0
OXD	Out	133	29.3	57.9	4.5	8.3
	In	64	25.0	64.1	10.9	0
WHM	Out	9	33.3	66.7	0	0
	In	53	15.1	77.4	7.6	0
Total	Out	373	26.5	59.8	7.0	6.7
	In	234	26.5	67.1	6.4	0

Note;

Figures are percentages of donor ewes programmed detected in oestrus.

h = interval in hours from CIDR-G removal to oestrous detection.

NT = ewe not tupped after 40 hours from CIDR-G removal.

Table 7.1.5 The effect of season and ovulation rate class on the time of oestrous detection

Breed	ORC	n	16h	26h	40h	NT
OUT-S	1	74	18.9	55.4	9.5	16.2
	2	119	23.5	60.5	7.6	8.4
	3	138	29.0	63.0	6.5	1.5
	4	42	40.5	54.9	2.4	2.4
IN-S	1	66	12.1	80.3	7.6	0
	2	73	26.0	72.6	1.4	0
	3	78	32.0	57.7	10.3	0
	4	17	58.8	35.3	5.9	0

Note;

Figures are percentages of donor ewes programmed detected in oestrus.

See section 3.13.1 for definition of the ORC's.

OUT-S = out-of-season.

IN-S = in-season.

Table 7.1.6 Ovulation rate within season and donor breed

Breed	Season	No. ewes	Ovulation rate (mean)	±SE
DT	Out	118	7.20	0.36
	In	10	7.80	1.37
FT	Out	96	7.97	0.50
	In	28	6.89	0.73
GOT	Out	15	12.27	1.53
	In	73	7.41	0.51
OXD	Out	133	7.29	0.30
	In	64	6.36	0.40
WHM	Out	9	7.22	1.12
	In	52	5.39	0.52
Total	Out	371	7.64	0.22
	In	227	6.60	0.26

Table 7.1.7 Ovulation rate within donor breed and donor age

Breed	Age (mths)	No. ewes	Ovulation rate (mean)	±SE
DT	14-16	118	7.47	0.35
	26-28	10	4.60	1.22
FT	14-16	102	8.25	0.46
	26-28	22	5.32	0.80
GOT	14-16	74	7.91	0.53
	26-28	14	10.00	0.53
OXD	14-16	136	7.23	0.30
	26-28	61	6.46	0.40
WHM	14-16	43	5.70	0.54
	26-28	18	5.56	0.99
Total	14-16	473	7.48	0.19
	26-28	125	6.38	0.37

Table 7.1.8 Ovulation rate for each breed, age, hormonal regime and season combination

Breed	Age	Regime	Out-of-season			In-season		
			n	Ovulation rate	±SE	n	Ovulation rate	±SE
DT	14-16	3	3	5.3	0.88	0		
		4	105	7.5	0.38	10	7.8	1.37
	26-28	4	10	4.6	1.22	0		
FT	14-16	3	89	8.2	0.50	10	7.9	1.46
		4	3	12.0	1.00	0		
	26-28	4	4	0.75	0.48	18	6.33	0.79
GOT	14-16	2	8	11.0	2.13	66	7.53	0.53
	26-28	2	7	13.7	2.23	7	6.29	1.98
OXD	14-16	3	73	6.9	0.39	5	4.8	1.59
		7	58	7.8	0.48	0		
	26-28	4	2	5.5	0.50	59	6.42	0.36
WHM	14-16	8	6	9.0	0.73	0		
		9	1	7.0	.	0		
		10	0			36	5.11	0.59
	26-28	5	2	2.0	0	0		
		6	0			16	6.0	1.16

Table 7.1.9 Recovery rate within season and breed

Breed	Season	No. ewes	Recovery rate (%)	±SE
DT	Out	103	50.7	3.0
	In	8	49.3	12.5
FT	Out	77	47.3	3.4
	In	23	48.3	8.4
GOT	Out	13	50.6	7.0
	In	58	60.1	4.0
OXD	Out	122	60.5	2.6
	In	53	47.3	5.1
WHM	Out	7	41.4	11.7
	In	34	56.6	5.5
Total	Out	322	53.4	1.7
	In	176	53.5	2.6

Table 7.1.10 Fertilisation rate within season and breed

Breed	Season	No. ewes	Fertilisation rate (%)	±SE
DT	Out	95	72.3	3.8
	In	7	57.5	13.7
FT	Out	71	83.3	3.8
	In	17	74.8	9.0
GOT	Out	13	42.6	12.3
	In	56	60.2	5.5
OXD	Out	117	77.0	3.4
	In	44	59.4	6.5
WHM	Out	6	75.8	15.8
	In	31	81.3	6.3
Total	Out	302	75.5	2.1
	In	155	65.7	3.3

Table 7.1.11 The yield of good quality transferable embryos within season and breed

Breed	Season	No. ewes	Good quality embryos (%)	±SE
DT	Out	82	78.2	4.0
	In	6	100	0
FT	Out	64	80.8	4.2
	In	16	86.8	13.0
GOT	Out	8	68.8	13.0
	In	43	73.1	5.7
OXD	Out	103	85.9	3.0
	In	32	97.8	13.9
WHM	Out	5	95.6	4.4
	In	27	78.5	6.6
Total	Out	262	81.9	2.0
	In	124	83.7	4.5

Table 7.1.12 Embryo survival rate to scanning

Breed	Season	No. embryos	% Surv.	±SE
DT	Out	244	70.6	2.6
	In	23	70.2	8.9
FT	Out	244	68.2	2.8
	In	62	60.2	8.1
GOT	Out	29	52.3	2.6
	In	144	69.3	4.1
OXD	Out	389	68.9	2.1
	In	113	61.5	4.7
WHM	Out	21	67.8	5.8
	In	102	61.7	5.2
Total	Out	932	70.8	1.3
	In	439	65.1	1.9

Note;

No. embryos = the number of good quality (Q1 or Q2) embryos transferred. The number of recipient ewes involved was not presented in the interest of clarity, see section 3.10.

Table 7.1.13 Embryo survival rate to birth for each season and breed

Breed	Season	No. embryos	% Surv.	±SE
DT	Out	244	72.5	2.8
	In	23	52.2	9.7
FT	Out	244	53.3	3.8
	In	62	35.5	7.6
GOT	Out	29	65.5	4.7
	In	144	72.0	2.1
OXD	Out	389	72.0	2.1
	In	113	46.0	6.1
WHM	Out	21	57.1	5.3
	In	102	52.9	5.5
Total	Out	932	66.3	2.3
	In	439	52.4	1.2

Table 7.1.14 Number of lambs born

Breed	Seas.	No. donor ewes	Total No. embryos transferred	Total No. lambs born	No. lambs born per donor programmed	No. lambs born per donor flushed
DT	Out	82	244	177	0.99	1.72
	In	6	23	12	1.20	1.50
FT	Out	64	244	130	1.34	1.69
	In	16	62	22	0.67	0.96
GOT	Out	8	29	19	1.19	1.46
	In	43	144	94	1.27	1.62
OXD	Out	103	389	280	2.11	2.30
	In	32	113	52	0.81	0.98
WHM	Out	5	21	12	1.33	1.71
	In	27	102	54	1.02	1.59
Total	Out	262	932	618	1.66	1.92
	In	124	439	234	1.00	1.33

Note;

No. donor ewes = the number of donor ewes from which Q1 or Q2 embryos were recovered.

Table 7.1.15 Summary of the results of the overall MOET programme for the random data

Breed	Seas	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
			n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
DT	Out	118	118	7.2	103	4.18	95	3.28	82	2.80	177	0.99
	In	10	10	7.8	8	4.81	7	3.16	6	3.69	12	1.20
FT	Out	97	96	8.0	77	4.70	71	4.25	64	3.81	130	1.34
	In	33	28	6.9	23	4.09	17	4.18	16	3.88	22	0.67
GOT	Out	16	15	12.3	13	7.23	13	3.15	8	3.63	19	1.19
	In	74	73	7.4	58	5.61	56	3.52	43	3.33	94	1.27
OXD	Out	133	133	7.3	122	4.81	117	3.86	103	3.77	280	2.11
	In	64	64	6.4	53	3.64	44	2.61	32	3.53	52	0.81
WHM	Out	9	9	7.2	7	3.85	6	3.50	5	4.02	12	1.33
	In	53	52	5.4	34	4.67	31	4.19	27	3.75	54	1.02
Total	Out	373	371	7.6	322	4.69	302	3.77	262	3.56	618	1.66
	In	234	227	6.6	176	4.55	155	3.39	124	3.39	234	1.00

Note;

No. of ewes = the number of donor ewes programmed, n_1 = the number of ewes which were examined for ovulatory activity, n_2 = the number of ewes which were subjected to an embryo flush, n_3 = the number of ewes from which ova were recovered, n_4 = the number of ewes from which fertilised ova were recovered, n_5 = the number of lambs born, μ_5 = the average number of lambs born per donor ewe programmed. The mean number of ova recovered and fertilised, and the number of good quality transferable embryos is based on the number of donor ewes indicated in each column (n_1-n_4).

APPENDIX TWO - REPEAT DATA

Table 7.2.1 The number of donor ewes in the repeat group

Breed	Age (mths)	Out-of-season	In-season
DT	14-16	10	10
	26-28	0	0
FT	14-16	10	10
	26-28	4	4
GOT	14-16	51	51
	26-28	3	3
OXD	14-16	5	5
	26-28	7	7
WHM	14-16	33	33
	26-28	8	8
Total	14-16	109	109
	26-28	22	22

Table 7.2.2 The number of donor ewes in the repeat group for each breed, age and regime combination

Breed	Age	Regime	Out-of-season	In-season
DT	14-16	4	10	10
FT	14-16	3	10	10
	26-28	4	4	4
GOT	14-16	2	51	51
	26-28	2	3	3
OXD	14-16	3	5	5
	26-28	4	7	7
WHM	14-16	8	25	0
		9	8	0
		10	0	33
	26-28	5	8	0
		6	0	8

Table 7.2.3 The effect of season on the incidence of oestrus

Breed	Out-of-season		In-season	
	n	%	n	%
DT	10	90.0	10	100.0
FT	14	92.9	14	100.0
GOT	54	90.7	54	100.0
OXD	12	100.0	12	100.0
WHM	41	97.6	41	100.0
Total	131	93.9	131	100.0

Table 7.2.4 The effect of season on the time of oestrous detection

Breed	Season	n	16h	26h	40h	NT
DT	Out	10	10.0	50.0	30.0	10.0
	In	10	50.0	50.0	0	0
FT	Out	14	0	85.7	7.1	7.1
	In	14	14.3	78.6	7.1	0
GOT	Out	54	59.3	29.6	1.9	9.3
	In	54	31.5	63.0	5.6	0
OXD	Out	12	8.3	91.7	0	0
	In	12	8.3	91.7	0	0
WHM	Out	41	31.7	61.0	4.9	2.4
	In	41	9.8	80.5	9.8	0
Total	Out	131	35.9	52.7	5.3	6.1
	In	131	22.1	71.8	6.1	0

Note;

Figures are percentages of donor ewes programmed detected in oestrus.

h = interval in hours from CIDR-G removal to oestrous detection.

NT = ewe not tupped after 40 hours from CIDR-G removal.

Table 7.2.5 The effect of season and ovulation rate class on the time of oestrous detection

Breed	ORC	n	16h	26h	40h	NT
OUT-S	1	24	29.1	45.8	4.25	20.8
	2	35	25.7	65.7	5.7	2.9
	3	47	40.4	51.1	6.4	2.1
	4	25	48.0	44.0	4.0	4.0
IN-S	1	43	7.0	86.1	7.0	0
	2	32	25.0	77.0	0	0
	3	47	23.4	66.0	10.6	0
	4	9	77.8	22.2	0	0

Note;

Figures are percentages of donor ewes programmed detected in oestrus.

See section 3.13 for definition of the ORC's.

OUT-S = out-of-season.

IN-S = in-season.

Table 7.2.6 Ovulation rate within season and donor breed

Breed	Season	No. ewes	Ovul. rate	±SE	n	Diff.	±SE
DT	Out	10	8.10	1.44	10	+0.30	1.07
	In	10	7.80	1.37			
FT	Out	14	9.36	1.51	11	+2.73	2.27
	In	11	7.18	1.27			
GOT	Out	53	9.00	0.82	52	+3.29	0.85
	In	53	7.19	0.56			
OXD	Out	12	8.08	1.47	11	+1.67	1.36
	In	12	6.42	1.00			
WHM	Out	41	8.17	0.74	40	+3.33	0.76
	In	40	4.95	0.54			
Total	Out	130	8.62	0.47	124	+2.86	0.50
	In	126	6.45	0.35			

Note;

Ovul. rate = ovulation rate.

n = the number of ewes available to calculate the difference in the ovulation rate between the two seasons.

Diff. = the mean difference in the out-of-season ovulation rate minus the in-season ovulation rate for each ewe.

Table 7.2.7 Ovulation rate for each breed, age, hormonal regime and season combination

Breed	Age	Regime	Out-of-season			In-season					
			n	Ovul. rate	±SE	n	Ovul. rate	±SE	n	Diff.	±SE
DT	14-16	4	10	8.10	1.44	10	7.80	1.37	10	+0.30	1.07
FT	14-16	3	10	11.40	1.55	8	7.13	1.44	8	-3.33	2.33
	26-28	4	4	4.25	2.17	3	7.33	3.18	3	+5.00	2.61
GOT	14-16	2	50	8.98	0.83	50	7.36	0.58	49	+5.00	3.51
	26-28	2	3	9.33	4.81	3	4.33	1.86	3	+3.18	0.89
OXD	14-16	3	5	5.20	1.11	5	4.80	1.59	4	+2.57	2.23
	26-28	4	7	10.14	2.12	7	7.57	1.17	7	+0.40	0.98
WHM	14-16	8	25	9.60	0.96	0			0	NA	
		9	8	7.88	1.06	0			0	NA	
		10	0			33	5.33	0.62	0	NA	
	26-28	5	8	4.0	1.40	0			0	NA	
		6	0			7	3.14	0.74	0	NA	

Note;

Ovul. rate = ovulation rate.

Diff. = the mean difference in the out-of-season ovulation rate minus the in-season ovulation rate for each ewe.

Table 7.2.8 Recovery rate within season and breed

Breed	Season	No. ewes	Recovery rate (%)	±SE	n	Diff.	±SE
DT	Out	10	32.9	11.9	8	-20.6	9.5
	In	8	49.3	12.5			
FT	Out	13	52.3	9.0	9	-9.7	17.0
	In	9	58.4	13.2			
GOT	Out	40	62.9	4.4	30	+3.9	7.4
	In	40	61.0	5.0			
OXD	Out	10	64.2	10.9	7	+6.2	12.4
	In	9	47.4	12.3			
WHM	Out	35	55.0	5.4	25	+1.5	8.1
	In	25	57.0	6.9			
Total	Out	108	56.4	3.7	79	-0.7	4.5
	In	91	57.3	3.5			

Note;

n = the number of ewes for which recovery rate data were available for both seasons.

Diff. = the mean recovery rate out-of-season minus the recovery rate in-season for each ewe, hence only ewes from which recovery rate data were available for both seasons contributed to this comparison.

Table 7.2.9 Fertilisation rate within season and breed

Breed	Season	No. ewes	Fert. rate (%)	±SE	n	Diff.	±SE
DT	Out	8	60.4	16.0	6	-1.5	23.8
	In	7	57.5	13.7			
FT	Out	11	54.7	13.4	7	+1.1	10.3
	In	7	65.5	13.7			
GOT	Out	37	53.4	6.4	25	-3.9	10.1
	In	38	54.2	6.7			
OXD	Out	10	75.0	13.4	6	-28.3	28.3
	In	8	52.5	18.1			
WHM	Out	32	75.8	5.8	22	-1.4	9.7
	In	22	83.6	6.7			
Total	Out	98	63.7	3.9	66	-4.5	6.0
	In	82	63.2	4.5			

Note;

Fert. rate = the fertilisation rate.

n = the number of ewes generating fertilisation rate data in both seasons.

Diff. = the mean difference in the fertilisation rate between seasons for each ewe.

Table 7.2.10 The yield of good quality transferable embryos within breed and season

Breed	Season	No. ewes	Good qual.(%)	±SE	n	Diff.	±SE
DT	Out	6	41.7	20.1	5	-50.0	22.6
	In	6	100	0			
FT	Out	8	72.4	14.2	5	+10.8	33.4
	In	7	85.7	14.3			
GOT	Out	30	67.7	7.1	16	-14.0	11.5
	In	27	79.6	6.4			
OXD	Out	8	91.4	5.7	3	-13.3	13.3
	In	5	100	0			
WHM	Out	30	68.4	7.8	20	-3.9	14.8
	In	20	74.2	8.4			
Total	Out	82	68.8	4.4	49	-11.0	8.2
	In	65	82.1	4.1			

Note;

Good qual. = the yield of good quality transferable embryos.

n = the number of ewes generating embryo quality data for both seasons.

Diff. = the mean difference in the % of good quality transferable embryos out-of-season minus the % in-season for each ewe.

Table 7.2.11 Embryo survival rate to scanning within season and breed

Breed	Season	No. E's	Survival rate (%)	±SE	No.	Diff.	±SE
DT	Out	7	66.5	2.4	5	+28.0	25.6
	In	23	70.2	8.9			
FT	Out	27	71.3	1.6	19	+12.5	22.8
	In	27	63.8	6.5			
GOT	Out	108	58.9	2.4	58	+4.3	8.6
	In	102	65.7	3.6			
OXD	Out	42	68.5	1.8	12	+3.4	12.9
	In	20	62.3	4.5			
WHM	Out	94	64.5	5.2	61	+26.5	18.5
	In	70	62.8	4.8			
Total	Out	278	63.6	1.2	155	+14.7	9.6
	In	242	64.8	1.6			

Note;

No. E's = the number of good quality transferable embryos transferred.

n = the number of embryos recovered from the same donor ewe in both seasons.

Diff. = the mean difference in embryo survival across the seasons for each donor ewe.

Table 7.2.12 Embryo survival rate to birth within season and breed

Breed	Season	No. E's	Survival rate (%)	±SE	n	Diff.	±SE
DT	Out	7	71.4	2.6	5	+20.0	28.7
	In	23	52.2	9.7			
FT	Out	27	63.0	3.4	19	+21.1	25.2
	In	27	40.7	6.7			
GOT	Out	108	66.7	1.8	58	+1.7	12.9
	In	102	64.7	2.9			
OXD	Out	42	69.0	4.3	12	+16.7	14.6
	In	20	55.0	4.5			
WHM	Out	94	68.1	2.7	61	+13.1	16.7
	In	70	55.7	6.2			
Total	Out	278	67.3	2.1	155	+9.7	8.9
	In	242	57.4	1.6			

Note;

No. E's = the number of good quality transferable embryos transferred.

n = the number of embryos recovered from the same donor ewe in both seasons

Diff. = the mean difference in embryo survival across the seasons for each donc ewe.

Table 7.2.13 Number of lambs born

Breed	Seas	No. donor ewes	Total No. embryos trans.	Total No. lambs born	Diff.	Total No. lambs born per donor programmed	Diff.	Total No. lambs born per donor flushed	Diff.
DT	Out	6	7	5	+1	0.50	+0.10	0.50	+0.03
	In	6	23	12		1.20		1.50	
FT	Out	8	27	17	+4	1.21	+0.29	1.31	+0.03
	In	7	27	11		0.79		1.22	
GOT	Out	30	108	72	+1	1.33	+0.02	1.80	+0.03
	In	27	102	66		1.22		1.65	
OXD	Out	8	42	29	+2	2.42	+0.17	2.90	+0.12
	In	5	20	11		0.92		1.22	
WHM	Out	30	94	64	+8	1.56	+0.20	1.83	-0.16
	In	20	70	39		0.95		1.56	
Total	Out	82	278	187	+15	1.43	+0.11	1.73	-0.02
	In	65	242	139		0.99		1.53	

Note;

No. donor ewes = the number of donor ewes from which Q1 or Q2 embryos were recovered.

Total No. embryos trans. = total number of embryos transferred.

Diff. = the mean difference in embryo survival across the seasons for each donor ewe.

Table 7.2.14 Summary of the results of the overall MOET programme

Breed	Seas	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
			n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
DT	Out	10	10	8.1	10	2.66	8	2.01	6	1.12	5	0.50
	In	10	10	7.8	8	4.81	7	3.16	6	3.69	12	1.20
FT	Out	14	14	9.4	13	5.27	11	3.41	8	3.39	17	1.21
	In	14	11	9.0	9	5.22	7	4.43	7	3.86	11	0.79
GOT	Out	54	53	9.0	40	7.50	37	4.33	30	3.60	72	1.33
	In	54	53	7.2	40	5.85	38	3.34	27	3.78	66	1.22
OXD	Out	12	12	8.1	10	6.22	10	4.67	8	5.25	29	2.42
	In	12	12	6.4	9	4.11	8	2.50	5	4.00	11	0.92
WHM	Out	41	41	8.2	35	5.26	32	4.36	30	3.15	64	1.56
	In	41	40	5.0	25	4.52	22	3.27	20	3.50	39	0.95
Total	Out	131	130	8.6	108	5.85	98	4.11	82	3.37	187	1.43
	In	131	126	6.5	91	5.12	82	3.59	65	3.72	139	0.99

Note; No. of ewes = the number of donor ewes programmed, n_1 = the number of ewes which were examined for ovulatory activity, n_2 = the number of ewes which were subjected to an embryo flush, n_3 = the number of ewes from which ova were recovered, n_4 = the number of ewes from which fertilised ova were recovered, n_5 = the number of lambs born, μ_5 = the average number of lambs born per donor ewe programmed. The mean number of ova recovered and fertilised, and the number of good quality transferable embryos is based on the number of donor ewes indicated in each column (n_1-n_4).

Table 7.2.15 Summary of the difference in results of the overall MOET programme

Breed	Seas	No. of ewes	No. CL		No. ova recovered		No. ova fertilised		No. good quality embryos		No. lambs born	
			n_1	μ_1	n_2	μ_2	n_3	μ_3	n_4	μ_4	n_5	μ_5
DT	Out	10	10	+0.3	8	-20.6	6	-1.5	5	-50.0	5	-0.10
FT	Out	14	11	+2.7	9	-9.7	7	+1.1	5	-10.8	19	+0.29
GOT	Out	54	52	+3.3	30	+3.9	25	-3.9	16	-14.0	58	+0.02
OXD	Out	12	11	+1.7	7	+6.2	6	-28.3	3	-13.3	12	+0.17
WHM	Out	41	40	+3.3	25	+1.5	22	-1.4	20	-3.9	61	+0.2
Total	Out	131	124	+2.9	79	-0.7	66	-4.5	49	-11.0	155	+0.11