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MULTIPLICATION OF FIVE BREEDS OF "EXOTIC" SHEEP IN NEW ZEALAND USING THE TECHNIQUE OF EMBRYO TRANSPLANTATION

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

MARIA DATTENA

1989

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ABSTRACT

Sheep of five* breeds imported into New Zealand and held at a quarantine farm were increased in number through the use of embryo transplantation (ET) procedures. For this, donor ewes after superovulation treatment were inseminated with fresh semen of rams of their own breed and flushed to recover embryos 5 or 6 day after onset of oestrus. The embryos after classification and processing through ten washes of flushing media were transported to a secondary quarantine farm and transferred into synchronised recipient Coopworth and Romney ewes. Some of the surrogate ewes received intravaginal progesterone supplementation (CIDR) for 14 days after transfer. Details of lamb production were recorded. This thesis reports the results of some factors that can affect the succes of a commercial ET programme.

Among the donor ewes 87-89% of the animals were in oestrus after synchronisation and gonadotrophin treatment. The ewes not in heat often had low or absent superovulatory responses. Superovulation was induced with either FSH-P or Folltropin given as 7 or 8 intramuscular injections at 12 h interval begining 72 h before withdrawal of the progesterone treatment (CIDR). The gonadotrophin dose levels were modified according to the breed and also as the programme progressed. Most of the data were examined within breed.

The superovulatory response to either the type of gonadotrophin preparation or the dose levels did not differ significantly.

* Texel (from two sources, Danish (DT) and Finnish (FT) and considered as separate breeds), Finnish Landrace (Finn), Gotland Pelt (Got), Oxford Down (OXD), White Headed Marsh (WHM) breeds.

The variation in recovery rate of embryos was not affected by the type of gonadotrophin used, whether the animals treated were flushed on one or two occasions, whether flushing was done 5 or 6 days after oestrus, or according to the ovulatory response (classed as 1-8, 9-16 and >16 corpora lutea counted).

Overall 85% of the ova were fertilised and had developed into embryos. There were no significant differences in fertility between inseminations done in the morning or the afternoon, or when flushed once or twice, or when AI was followed by natural mating, or relative to the ovulatory response. Moreover there was no significant difference within the six breeds in the fertility rate.

The quality of the embryos, classified as "good" or "poor" on the basis of their appearance and stage of development consistent with the day of flushing (developmental age), was significantly affected in several of the breeds by the type of gonadotrophin, the dose levels, the ovulatory response and the age of the embryos when recovered.

The pregnancy rate after the transfer of two embryos was 59%, 72%, 60%, 53%, 53%, 46% for recipients carrying DT,FT,FINN,GOT,OXD,WHM embryos, respectively. The comparable values for embryo survival were 46%, 57%, 54%, 42%, 40%, 34%, respectively. In general among the factors studied involving breed of recipient, degree of synchronisation between donor and recipient, ovulation rate in the recipient, interval from flushing to transplantation and progesterone supplementation it was found that only the latter factor in the DT, FT and OXD breeds of embryos was significant.

The results from this multiplication programme after considering 409 treatments with gonadotrophin gave an average of 6.0 corpora lutea, 3.9 eggs recovered, 3.4 eggs fertilised and 1.5 lambs born per treatment. It is concluded that the low recovery rate and poor survival rate of the embryos are important factors to be overcome if a significant increase in the number of lambs born is to be expected from embryo transplantation. Work to overcome these problems is necessary, but attempts should also be made to increase the OR through modification of the gonadotrophin treatments. Support for this idea is suggested because in some animals with a high OR satisfactory numbers of eggs were fertilised and an increase of good quality embryos was recorded.

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CHAPTER I: INTRODUCTION

Genetic improvement in the sheep industry is desirable as a means to increase productivity and net economic returns. The importation of new breeds of sheep and the need to evaluate their performance either as purebred animals or in crosses in comparison with existing animals is a current objective of major importance. There has been much interest in determining the contribution that new genetic material could make to the New Zealand sheep industry. Breeds that could improve reproductive performance, liveweight growth and carcass characteristics have been of considerable interest and two recent importations of sheep to New Zealand have been made.

To multiply these animals as quickly as possible requires the use of several techniques in addition to natural breeding. It is required that the nucleus animals be increased rapidly and in sufficient numbers for proper comparison with the existing breeds of animals. If these exotic breeds have a real contribution to made then their numbers will also need to be increased for commercial use.

The importation of five breeds of ewes and rams (Texel sheep, sourced from Denmark (DT) or Finland (FT), Finnish Landrace (FINN), Gotland-Pelt (GOT), Oxford Down (OXD), and White Headed Marsh (WHM)), has meant that several methods of propagation need to be evaluated. It was decided to use embryo transfer procedures to increase the number of offspring. The work now to be presented is an evaluation of factors that can affect the success of a commercial ET programme. The data used are from the first year of a programme conducted by the LambXL company and known as the Exotic sheep project.

CHAPTER II: LITERATURE REVIEW

FACTORS AFFECTING SUCCESS OF AN ET PROGRAMME

An ET programme involves the collection of embryos from donor animals and their continued development in the recipient mother through to the birth of live offspring. Some of the factors of a technical nature that can be varied and influence the number of lambs are considered in this section.

2.1. SYNCHRONISATION OF OESTROUS ACTIVITY

Embryo Transfer programme can be facilitated by the use of methods to synchronise the breeding cycle of the donor animal with those of the recipient animals. The objective is to programme for a particular day or days a desirable number of superovulated donor ewes to be in oestrus and for these to be mated or inseminated at an appropiate stage of the cycle and subsequentely flushed for embryos; sufficient recipient ewes need to be available for transplantation of the embryos.

Control of the time of oestrus and ovulation can be achieved using two main methods: 1) the administration of high levels of Progesterone (P) or related compounds to suppress follicular growth until after cessation of the progestagen treatment, when pre-ovulatory release of LH will occur, 2) treatment with prostaglandins to cause luteolysis.

Progestagens such as MAP (medroxy-progesterone acetate) and FGA (fluorogestone-acetate) have been administered by injection, orally,

intravaginal pessary or subcutaneous implant. In New Zealand during the last few years, a new intravaginal device (CIDR) has been developed containing 9% or 12% of P (0.3g or 0.5g respectively). This method causes an earlier onset of oestrus than in animals treated with sponges containing an analogue of P; also the synchrony of onset of oestrus may be better (Welch et al., 1984, Rhodes & Nathanielsz, 1988). The use of CIDRs or sponges provide the most convenient method for control of oestrus in ewes.

The basic technique involves intravaginal treatment for 12-14 days. The interval from withdrawal of the device to oestrus depends on several factors including: 1) the dose level of progestagen (Robinson,1970), 2) method of preparation of the sponges (Robinson et al.,1968; Robinson,1970) 3) administration of superovulating hormones such as Pregnant Mare's Serum Gonadotrophin (PMSG) or Follicle Stimulating Hormone (FSH) (Colas et al.,1973; Gordon,1975; Botha et al.,1975; Wright et al.,1981). The technique can be considered satisfactory when 80 to 90% of the treated ewes come in heat within a period of 36-48 h after progesterone withdrawal(Lamond,1964).

As already mentioned, induction of luteolysis in ewes at a chosen time, can synchronize the oestrous cycles. Douglas and Ginter (1973) and Howk (1973) observed that doses of 10-15 mg of Prostaglandin (PGF) in a single intramuscular injection could induce regression of the corpus luteum (CL) in sheep. Trounson et al. (1976) have demonstrated that a dose of 100 ng of the analogue Cloprostenol was effective in inducing luteal regression in the sheep.

The CL of the ewe is responsive to PGF_2 between Day 4 and Day 14 of the oestrous cycle (Douglas & Ginther,1973). To ensure that all sheep in the flock are at an appropiate stage of the oestrous cycle to respond, the usual recommendation is to give two $PGF_{2\alpha}$ injections 9 to 14 days apart (Haresing,1980; Gordon,1983). Reports show that oestrus occurs about 40 h after $PGF_{2\alpha}$, normally lasting around 36 h, with ovulation taking place about 70 h from the time of $PGF_{2\alpha}$ injection (Acritopoulou & Haresing,1980).

2.2. SUPEROVULATORY RESPONSES

The use of a satisfactory method to cause superovulation in the donor ewes is a major factor in the success of an ET programme. Considerable variation exists in superovulation response and factors of importance include the breed of the ewe,age, stage of the breeding season, or anoestrous season as well as the method of hormonal treatment used. This review concerns the later factor.

2.2.1. Physiological Mechanisms Controlling Ovulation

The normal process of follicular growth in sheep, leading to the production of an ovum, requires an adequate exposure to the pituitary gonadotrophins, FSH and LH. At the time of luteal regression there is an increase in the frequency of LH pulses due to the decline of P (Legan & Karch,1979), which stimulates androgen synthesis by the theca cells of the developing follicle (Baird,1978). Antral follicles of 2-4 mm diameter, the granulosa cells of which have a very high aromatase

activity, can maintain a highly oestrogenic environment by converting androgens to oestradiol (Baird,1983; McNatty et al.,1985). At this point the rising level of oestradiol secreted by the dominant follicle into the ovarian vein suppresses the secretion of FSH. Inhibin produced by the dominant follicle might also contribute to this process (Miller et al.,1981). In this way those follicles which are at a slightly less advanced stage of development are deprived of FSH (Baird et al.,1983). The possibility that this fall in FSH is probably responsible for inhibiting the development of other antral follicles is supported by the fact that administration of exogenous FSH in the form of Human Menopausal Gonadotrophin (hMG) during the follicular phase stimulated the development of more than one preovulatory follicle (Edwards et al.,1972; McNatty,1985). However,despite the falling plasma FSH concentrations during the preovulatory period,sustained follicular development still requires some FSH. Further reduction in plasma FSH concentrations by administration of bovine follicular fluid (a rich source of inhibin) prevents follicles from attaining preovulatory maturity (Henderson et al.,1986).

There is also evidence that the sensitivity of sheep granulosa cells to FSH increases as follicle size increaes, (Henderson et al.,1985,1987). Only those follicles in which the granulosa cells have an increased sensitivity to FSH, may be able to continue their development despite low plasma FSH concentrations. Furthermore follicles less sensitive to FSH are likely to undergo atresia due to the lack of FSH (Henderson et al.,1988). On this basis in 1988 Henderson et al.,were able to demonstrate, that relatively small changes in plasma FSH concentration during the preovulatory period have

a marked effect on follicular development and subsequent ovulation rate. Partial or complete prevention of the normal preovulatory decline in plasma FSH concentrations were associated with a subsequent increase of follicles developing. In contrast to FSH,infusion of LH did not increase the mean ovulation rate (Henderson et al.,1988). During the preovulatory period,plasma LH concentrations are increased (Baird,1983). Therefore, it appears to be the availability of FSH, rather than LH, which is the major factor which limits the number of follicles maturing to ovulation.

It is still possible that the administration of LH together with FSH may allow more follicles to attain ovulatory maturity in synchrony than by giving FSH alone (Henderson et al.,1988). Chupin et al. (1986) obtained improvement of synchronisation of oestrus in ewes and increases of ovulation rate during the breeding season with the addition of LH at the end of the FSH treatment.

2.2.2. Mechanisms of Action of Exogenous Hormones

2.2.2.1. FSH-LH ratio

The ability to increase the ovulation rate in domestic animals by gonadotrophin treatment originated from experiments carried out more than sixty years ago (Parkes,1929). The most commonly used method to superovulate sheep has been treatment with PMSG (Hunter et al.,1955; Cumming,1965; Eastwood & McDonald,1975; Tervit et al.,1976; Gherardi & Martin 1978; Gherardi & Lindsay, 1980 and others). However, induction of superovulation in ewes with pituitary gonadotrophins of

equine or pig origin has been reported (Moore and Shelton 1962,1964a; Crosby et al.,1980; Armstrong and Evans,1983;1984; Torres & Cognie',1984; Torres et al.,1986; Chupin et al.,1986).

A common result reported from superovulatory treatments is a high variability in the ovulation rate in response to a standardized amount of injected hormone. This high variability has also been reported in cows by Hammond & Battacharya(1944). Reasons for this variability has been suggested by Monniaux (1983) and Moor et al.,(1984),and include factors involving the dynamics of follicular development during the oestrous cycle and differences in the relative abundance of FSH and LH activity in the various hormone preparations. In fact it is well known that commercial preparation of FSH can have high and variable LH content (Murphy et al.,1984; Chupin et al.,1984; Armstrong and Evans,1984, Lindsell et al.,1986).

The superovulatory response in cattle injected with a commercial FSH preparation is reduced dependant on the LH contamination (Donaldson & Ward 1985;1986). Ovulation rates in sheep and goats are inversely proportional to the amount of LH activity in the FSH preparations. Armstrong and Evans (1984) keeping constant the amount of FSH and reducing the amount of LH in three different groups of animals obtained ovulation rates of 11.2, 15.8 and 25.6 respectively.

Moor et al.,(1984) showed that an excess of LH in a gonadotrophin preparation hormone causes premature stimulation of the oocyte in superovulated ewes. Furthermore it has been shown that normal pre-ovulatory P ,LH and FSH concentrations are necessary for optimal

embryo production in superovulated cows (Donaldson,1985b;Callessen et al.,1986). Abnormal concentrations of P,LH and FSH are followed by abnormal follicular/oocyte maturation and reduced embryo production.

LH contamination of FSH preparations is one of the likely causes of abnormal pre-ovulatory oocyte. Donaldson and Ward (1986) confirmed that contamination of FSH with LH reduced fertilisation rates of cow ova produced by superovulation. LH appears specifically to block fertilisation. The mechanism may be through premature stimulation of the maturing oocyte (Moor et al.,1984) so that it becomes incapable of being fertilised. These suggestions are supported by an earlier study showing that fertilisation problems in superovulated cows cannot be overcome by multiple insemination with several doses of semen (Donaldson,1985a).

Moreover Donaldson and Ward (1986) suggested that the LH content in the FSH increased the variability of some of the responses. This was seen in the number and percentage of eggs fertilised and in transferable embryos. Thus, variability in the superovulatory response and embryo quality may be reduced by controlling the levels of LH in the superovulatory hormones. Similar conclusions were reported by Moor et al.,(1984)in sheep. It was postulated that the number of prematurely stimulated follicles will be substantially reduced by manipulating the FSH and LH levels during superovulation, considering that a reduction in the LH content of pituitary gonadotrophin preparation should decrease premature activation of oocytes during superovulation.

2.2.2.2. Time of injection and time of exposure to FSH

Henderson et al. (1988) showed that raising mean plasma FSH for 20 h starting at the time of Cloprostenol injection or 24 h after did not result in an increase in the mean ovulation rate. Continuous exposure to elevated plasma FSH concentration therefore seems necessary to bring these additional follicles to preovulatory maturity. In addition an increase in the frequency of double ovulations can be achieved by infusing FSH for a 24 h period in the 48 h before initiating luteolysis (McNatty et al.,1985). Presumably this treatment advances the maturation of a follicle, which would normally have undergone atresia, thereby allowing it to "survive" the preovulatory fall in plasma FSH concentrations and attain ovulatory maturity. In agreement with this hypothesis, repeated injections of FSH-P starting before the onset of luteolysis and continuing through out the follicular phase, have increased ovulation rate in sheep (Wright et al.,1981; Armstrong & Evans,1983;1984; Torres and Cognie',1984; Torres et al.,1987). However,if FSH-P infusion was given for only the first 24 h from the start of luteolysis, the treatment failed to increase the ovulation rate (Henderson,1988). Similarly, if the start of the infusion was delayed for longer than 12 h after the initiation of luteolysis no increase in ovulation rate occurred.

In ewes ovulating 2 eggs there is a higher plasma FSH concentrations from 48 h to 24 h and 24 h to 0 h before the onset of luteolysis than in ewes ovulating a single follicle (McNatty et al., 1985). Henderson (1988) showed that the mean ovulation rate of ewes infused for 48 h with 0.5mg FSH-P/h was similar to that obtained in

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Romney ewes given a similar total dosage as multiple injections over a 96 h period, starting 48 h before the removal of vaginal progestagen sponge. Thus, time of injection and duration of exposure to FSH seem to be very important factors in the ovulatory response of the animals. The objective is to treat animals in an attept to achieve a constant and reliable response with minimal variation to the superovulatory treatments.

2.2.2.3. Dose of gonadotrophin

In a commercial ET programme the decision about which dose to use is extremely important, not only in order to obtain a good superovulatory response, but also in order to avoid wasteful use of the material and added costs. Also the dose may have to be given in relation to the breed of sheep. Armstrong and Evans (1984) found no difference in superovulatory response between 32 and 16 mg of FSH-P (7.5 vs 7.6 CL) used during the non breeding season with purebred Suffolk ewes; but greater than 15 ovulations were obtained with 22.5 mg of FSH-P during the breeding season. In 1987 Torres et al., obtained a good ovulation rate (9,12,19.5 CL) with 16 mg FSH-P in Prealpes,Lacuane and Romanov x Prealpes ewes respectively. Wright et al. (1981) obtained an ovulation rate of 8.2. in 10 month old Colombia ewes with a dose of 24 mg. Torres and Cognie'(1984) injected Prealpes du Sud adult ewes with 12 mg of FSH-P and 8.1 corpora lutea per ewe were reported. Cognie' et al. (1986) injecting 16 mg FSH-P into Ile-de-France ewes during the breeding season obtained a mean of 18.6 corpora lutea. Likewise with cattle, Donaldson (1984) has evaluated various superovulatory treatments. There was a significant effect of

dose of FSH-P on embryo production; total embryos collected declined from 14.9 to 6.8 and the percentage of transferable embryos from 57% to 40% when the dose was increased from 28 mg to 60 mg.

In conclusion it appears that a "standard" dose of FSH will give unpredictable results. This is further complicated by variations in the activity of different products (Lindsell et al.,1986).

2.3. QUALITY OF EMBRYO AND CONTRIBUTING FACTORS

2.3.1. Drug: FSH and PMSG

A primary goal in the application of ET procedures is the development of a superovulatory regime which will yield a satisfactory number of good quality embryos. The use of superovulatory hormones alters the quality of the embryos (Gordon,1982; Moor et al.,1984; Moor et al.,1985;). Newcomb(1982) indicated that 55% of the embryos recovered on day 8 from superovulated donor cows showed developmental abnormalities. These abnormalities probably occur because of an unfavourable uterine environment induced by the grossly distorted level of circulating steroids (Booth et al.,1975). On the other hand, inappropiate interactions between exogenous gonadotrophins and partially differentiated thecal or granulosa cells could equally initiate lesions during oocyte maturation which may not be expressed until the morula or blastocyst stage (Moor et al.,1984). A clear example of delayed expression of an early abnormality in sheep has been reported by Moor and Trounson (1977) who found that oocytes matured in

the presence of inadequate levels of oestrogen underwent fertilisation and cleavage, but later showed aberrant blastulation patterns.

Moor et al.,(1984) have examined the hypothesis that egg quality is predetermined by the interaction between exogenous gonadotrophin and follicular cells. The experiment was carried out using ovine follicles obtained from untreated animals or after treatment with FSH or PMSG, and showed that 95% of the follicles from animals treated with FSH had a protein profile which characterized the inactivated germinal vesicle stage of development. These results suggest that FSH stimulates follicular development without prematurely activating the associated oocyte. The effect of PMSG was different. A superovulatory dose of 1250 i.u not only stimulated follicular development, but also resulted in the premature activation of over 35% of the oocytes. In addition oestrogen secretion per mg of follicular tissue within 24 h was about double in PMSG-treated ewes compared with FSH-treated ewes. When high doses of FSH were used problems similar to those with PMSG were found.

Murphy et al.,(1984) and Donaldson and Ward (1985) indicated that reduction in the LH content of the FSH-P product increased the yield of good quality embryos, presumably by controlling follicular and oocyte synchrony. Reduction of the quantity of FSH in the first one or two injections, in order to initiate multifollicular development without ovulation also may be helpful (Foote and Ellington,1988). However, Callessen et al. (1986) and Callessen et al. (1987) reported that treatment of cows with FSH or PMSG can cause premature ovulations presumably as a result of the LH component in the hormone preparation

and the presence of antral follicles at a different stage of development when superovulatory treatments are initiated (Foote and Ellington,1988).

2.3.2. Ovulation Rates

Failure of fertilisation and decreased rate of embryo survival were found in animals with high ovulation rates (>16 corpora lutea Armstrong & Evans, 1983). On the other hand Torres and Cognie (1984) and Torres et al., (1987) found a positive correlation between number of corpora lutea, and the number of morulae recovered. Yet,no decline in embryo recovery or fertilisation rates was observed in ewes responding with a greater than average number of ovulations under FSH treatment in the work of Wright et al., (1981). Tervit (1987) reported that goats superovulated with FSH produced more ovulations (15.1 vs 9.1),more embryos (13.0 vs 7.8), and more transferable embryos (11.0 vs 5.1) than those treated with PMSG. Although the use of different hormones could be the cause of the difference in the results, the highest ovulation rate was positively related to the quality of the embryo.

2.3.3. Day of Flush

Ovulation in the ewe occurs approximately 24 h after the onset of oestrus and first cleavage of fertilized eggs occurs about 24 h after ovulation. Thus, late on Day 1 some cleaved eggs will be observed, but the highest proportion of fertilized eggs will be in the one cell stage. Killeen, (1981) and Torres & Sevellec (1988) reported the

distribution of cell stages of embryos recovered at various times after oestrus as follows:

Day of recovery after onset of oestrus	Predominant Stage
2-3	2 cells
3-4	8 cells
4	32 cells (morula)
5	>64 cells (late morula)
6	late morula and blastocyst
7	unhatched and hatching blastocyst
10	hatched blastocyst
14	elongation of blastocyst

Although embryos collected up to day 14 are capable of normal development in recipients (Peterson et al.,1976), collection is generally attempted between days 3 and 6. After day 6 hatched blastocysts are difficult to distinguish among the cellular debris in the flushing media. Collection before day 3 is generally not attempted because it has long been recognised that 2 and 4 cell embryos are more sensitive to manipulation than later stages (Moore and Shelton,1964b). Moreover, Averill and Rowson (1958) showed that no two-cell and only 16% of 4-cell sheep embryos developed into viable lambs when transferred into the uterus.

According to Killeen (1981) when a few embryos are two to three

stages of development behind the majority of the embryos collected from the same animal, they should be considered retarded and their viability questionable. Embryos assessed as being behind their developmental age and hence retarded, result in lower pregnancy rates than occur in ewes with normally developed embryos (Eldsen et al.,1978). The reasons for embryos of several ages being found in the same flush have been suggested by Donaldson (1986) and include: 1) not all ova are ovulated simultaneously 2) not all ova are fertilised simultaneously and 3) even if two ova are fertilised at the same time, the rate of cleavage for each may differ slightly. Thus, after 5 to 7 days of development, these effects will produce embryos at different morphological stages of development. Lindner and Wright (1983) have assigned a code called the "developmental age" that corresponded to the estimated age of the embryo,regardless of the day after oestrus on which it was collected. Donaldson (1986) using this system found that the distribution of each embryonic stage of development varied with the day of collection. Embryos of a given stage exhibited a distinct maximum frequency dependant on the day of collection. For instance on day 6, 92% of embryos were early or late morulae and only 8% were blastocysts, but by day 7 and 8, 58% and 91% were blastocysts. In that study, "developmental age" was shown to affect pregnancy rate. Thus , the day of flush was related to stage of embryonic development and this in turn influenced the survival rate observed.

2.4. FACTORS INFLUENCING EMBRYO SURVIVAL RATE

Many factors can affect embryo survival and this is to be expected considering the numerous processes occuring during pregnancy.

2.4.1. Degree of Synchronisation

In the planning of embryo transfer programme precise synchronisation of oestrus between the donor and the recipient is required for a high embryo survival rate to be achieved. Moor and Shelton (1964b) and Rowson and Moor (1966) reported that acceptable survival rates can be obtained when there is an asynchrony of ±1 day, although the best results were obtained when the onset of oestrus in the donor and recipient was exactly synchronised (Hancock and Hovell,1961; Cumming, 1965). Seventy two hours difference between the onset of oestrus in the donor and the recipient appears to be incompatible or to result in very low pregnancy rates (Rowson and Moor,1966). Although the ovarian venous blood of the sheep shows little variation in progesterone concentration during the period extending from Day 7 to Day 15 after oestrus, the endometrium or its secretion must presumably be undergoing continuous and rapid changes and these effects must be appropriate for the age of the transferred embryo (Wilmut et al.,1985).

The most likely reason why the embryo dies is because the uterine environment may not be suitable for the 'out-of-phase' embryo (Rowson and Moor (1966). Another possibility could be that the 'out-of-phase' embryo is incapable of exerting a sufficient luteotrophic action on the recipient's corpus luteum, resulting in luteolysis and consequently the embryo is lost (Wilmut et al.,1985).

Donaldson (1985c) has pointed out the need for more detailed information on the interrelationship between embryo classification

(stage and grade), synchrony of oestrus between donors and recipients and the resultant pregnancy rates. In that work more developed embryos (late and hatched blastocysts) were transferred into recipients that were in oestrus before the donors (negative recipients,-12 or -24 h). Less developed embryos (early and late morula) were transplanted into recipient cows which were in heat after the donors (positive recipients, +12 or +24 h); and embryos at early blastocyst stage were transferred into exact oestrus-synchrony cows. The results did not show any significant advantages of this method. On the other hand, Lindner and Wright (1983) obtained a higher pregnancy rate (88%) with a ±1 day recipient-embryo syncrony compared to that (74%) based on ±1 day recipient-donor cycle synchrony. This result suggests that transfer of bovine embryos based on synchrony between day of recipient cycle and state of embryonic development provides higher pregnancy rate than transfer based on recipient-donor cycle synchrony. Both the work of Lindner and Wright (1983) and Donaldson (1985c) classified the embryo using a 'developmental code' or 'estimated age' based on the stage of development (i.e. morula, compact morula, early blastocyst) with each stage having an estimated age (5-6-7 Day) from the day of oestrus.

2.4.2. Number of Embryos Transferred

The survival rate of transplanted embryos varies according to the number of embryos present initially. Moore (1968) reported 75% survival when a single embryo was transplanted per recipient. Killeen (1980) found that when embryos were transferred singly or in pairs to the uterus on Day 3, 79% survived. The differences between the

transfer of one or two embryos are usually small and not statistically signficant (Armstrong and Evans,1983). The results obtained by Trounson(1983) with 75% of the ewes lambing when two embryos were transferred were similar to those obtained by Torres et al.,(1987), but Trounson (1983) obtained 92% pregnancy when three embryos were transplanted. Larsen (1971) found that an increase in the number of ova transferred resulted in a lowering of the ovum survival rate (66% vs 59%) when one or three ova were transferred although the difference was not statistically significant. The proportion of ova suffering pre-natal mortality increased as the number of ova transferred increased (Moore et al. 1960; Cumming & McDonald 1970; Moore, 1968). On the other hand Moore and Shelton (1962) reported a marginal improvment in conception rate by transferring two or three embryos rather than one in the same recipient.

The decision of whether to transfer one or two embryos in an ET programme will depend on the objectives of the programme and including factors like the value of the offspring, the cost of the recipients and other costs associated with the transplantation procedure. In general two embryos are usually transplanted in the same recipient ewe.

2.4.3. Quality of Embryo Transferred and Site of Transfer

Embryos classified as morphologically normal will include those which eventually develop and lead to normal pregnancies. Killeen and Moore (1971) indicate that small anucleated fragments do not affect the capacity of sheep embryos to continue their development following transfer. Retarded cleaved embryos, irregular cleavage and

fragmentation all have major effects on the developmental potential of animal embryos (Shea,1981).

In sheep, embryos collected up to 3.5 days after oestrus show better survival rates when transferred to the oviducts than to the uterus (Moore & Shelton,1964). Rowson and Moor (1966) obtained survival rates of 75% and 71% from morulae and blastocysts respectively when transplanted into the uterus. In contrast no two-cell and only 16% of four-cell embryos developed into viable lambs after transfers made into the uterus (Averill & Rowson,1958). Maximum efficiency can be achieved by collecting embryos of eight or more cells and transfering them into the uterus at a rate of two per animal (Moore and Shelton, 1962). Shea (1981) reported that a high pregnancy rate in cows was obtained when the embryos were between late morula and hatched blastocyst. Armstrong and Evans (1983) found that a high percentage of embryo survival was obtained when all embryos up to the 8-cell stage were transferred into the oviduct (40% for 9-16 cell; 28% for morula; 50% for blastocyst). The same authors made a comparison of site of transfer (oviduct vs. uterus) with embryos at more advanced stages then 8 cell and no significant differences in survival could be attributed to the site of transfer (40% vs. 47% respectively).

2.4.4. Number of Corpora Lutea and Exogenous Progesterone in the Recipient Ewes

Analyses of embryo survival in relation to the number of CL in the recipient ewes have failed to show any significant effect (Moore et al. 1960; Cumming & McDonald,1970). If the main factor in maintaining a pregnancy is the P profile, rather than the number of corpora lutea, this type of investigation is probably inadequate to estimate the P production. However, there are indications that Finnish Landrace ewes (Lawson and Rowson,1972; Bradford et al.,1974) and Border Leicester ewes (Moore,1968) tend to maintain larger litters more regularly than less fecund breeds with which they were compared. This may be due to a difference in luteal activity, ie. progesterone level between different breeds of recipient. This point has been clarified by Parr et al., (1982) when different doses of P (5,10,15, 20 or 25/mg) have been given to ewes which received embryos by transfer; at day 21 after oestrus the survival rates were 69%, 83%,79%,90% and 82% respectively.

Supplementation with P to maintain a high level of P at the start of pregnancy has been suggested as a method to increase embryo survival rate. Most studies on this possible relationship have been made with animals after natural mating or AI and only a few after ET. Peterson et al. (1984) showed that P therapy for 6 days (Day 10 to 16) after removal of a synchronizing device increased the lambing rate from 67% to 95% probably by a reduction in embryo mortality. Also mild superovulation with PMSG has a similar effect,possibly due to an increase in the amount of luteal tissue (Davis et al.,1985). McMillan et al.,(1987) found that P supplementation tended to improve pregnancy

rates in adult ewes. It also tended to improve the incidence of multiple foetuses in hoggets,but the effect was not apparent in mature ewes. Likewise, Kerton et al. (1986) noted that insertion of FGA sponges for 6 days starting at Day 8 after AI did not reduce embryonic mortality.

2.4.5. Time of Transplantation

In commercial embryo transfer programmes good organization is required to ensure that embryos are transferred at short intervals after recovery from the donors. Results from several studies demonstrate that an interval of up to two hours after collection can be tolerated by the embryo without compromising its viability (Moore and Shelton, 1962).

Successful short term storage of, sheep embryos in vitro have been reported by Averill (1956) and involved periods up about 1 - 2 h with the embryos maintained at 30°C. However, in the same work it was clearly shown that viability can be maintained for longer periods when temperatures were nearer 8°C. The use of a dialysis chamber to store the embryos assists in the retention of viability (Averill & Rowson, 1958). Using this system Moore and Shelton (1962) recorded a lambing rate of 67% when the maximum time elapsing between collection and transfer was 2 h. With the development of technology for frozen storage of embryos the period from flushing to freezing may also be varied. Thus, in cows (Pettit,1985) found that there was a significant decline in pregnancy rate when embryos were cultured for more than three hours after collection. Eggs cultured for more than five hours

resulted in a pregnancy rate 33% lower than embryos which were frozen within three hours. Accordingly Wright (1985) found that the overall pregnancy rates when embryos were frozen within four hours of collection were higher than when 12 h passed between collection and freezing.

2.5. TECHNICAL CONTRIBUTIONS TO THE SUCCESS OF ET

A successfull ET programme needs attention to several individual procedures, all of which can be limiting. These are as follow:

2.5.1. Insemination of Donor Ewes

Both natural mating and AI are utilized to provide fertilised eggs for ET. With AI both cervical and intra uterine insemination methods have been developed and the letter has real advantages if frozen semen is to be used in sheep (Killeen & Caffery,1982). Intrauterine AI can give high fertilisation rates above 90% (Killeen and Moore,1971; Trounson and Moore,1974) and use only small quantities of semen (Davis et al.,1984; McKelvey and Robinson,1986). This is an advantage especially if a large number of ewes need to be inseminated at the same time and with single sires. In addition doses as low as 2 to 20 million spermatozoa may be used (Walker et al.,1984) . However, in superovulated ewes the number of spermatozoa should be increased to at least 100 million motile sperm (Maxwell,1987).

2.5.2. Embryo Recovery Technique

Several surgical methods to recover embryos at laparotomy have been developed (Averill et al., 1956); Tervit & Havik,1976). The use of a laparoscopic technique for recovering embryos which eliminates the need for exteriorising the genital tract and almost totally prevents the formation of post-operative adhesions has recently been developed (McKelvey & Robinson, 1985). However, this technique has a relatively low (50%) recovery rate with respect to the laparotomy technique, comparable figures being 79%, Moore and Shelton,(1962); 73%, Cumming and McDonald (1967); 83%, Tervit and Havik (1976) and 76% Torres and Cognie' (1984). In a commercial programme the recovery rate obtained with the laparoscopic technique might be still too low, but on the other hand more flushings might be possible from the same animals (McKelvey et al.,1986).

2.5.3. Embryo Transplantation Technique

Lamond and Urquhart (1961), Killeen (1981), and Moore (1982) have reported a surgical technique by laparotomy for embryo transplantation. Recently a laparoscopic method for transplantation has been developed and a pregnancy rate of 33% reported (Schiew et al.,1984). For commercial ET the surgical technique might still be preferred as it is quite rapid to perform and probably gives a higher pregnancy rate.

CHAPTER III: MATERIALS AND METHODS

3.1. ANIMALS

Sheep of six breeds (300 ewes and 43 rams, 2-4 years old) comprising animals of the Danish Texel (DT) and Finnish Texel (FT) breeds (Texel sheep were from two sources of origin Denmark and Finland, and therefore considered as separate breeds) Finnish Landrace (FINN) Gotland Pelt (GOT), Oxford Down (OXD) and White Headed Marsh (WHM) breeds, were imported into New Zealand in February 1986. At the time of importation many of the ewes were pregnant and these animals subsequently lambed during the period from February to April 1986. Attempts at remating the animals naturally in late May failed and subsequent treatment with Progesterone and Pregnant Mare's Serum Gonadotrophin resulted in 85% of them lambing again within the same year.

These animals were used to produce embryos in a commercial embryo transplantation (ET) programme during April to June 1987. The recipient animals were either of the Coopworth or Romney breeds.

The donor ewes were kept on a primary quarantine farm at Awahuri, Manawatu and the recipient ewes on a secondary quarantine farm at Cheltenham, Manawatu. The two quarantine farms were 26 km apart. At both farms the sheep grazed ryegrass-clover pasture and were subjected to normal husbandry procedures so as to maintain satisfactory liveweights. Animals were treated for control of internal and external parasites.

All animals were identified individually with plastic and brass ear tags. After embryo transfer, the recipient ewes were again tagged with one of six different colours in order to identify the breed of the embryos transplanted.

Rams of all the six breeds were available for natural mating and/or artificial insemination (AI). These rams were grazed close to the yards until required. Vasectomised rams harnessed with a sire sine colour raddle were used for detection of oestrus among donor and recipient ewes. A ratio of 1:10 donor ewes to recipients was used.

3.2. SYNCHRONISATION OF OESTROUS CYCLES

Control of breeding in the donor ewes was attempted with intravaginal application of internal drug release devices (CIDR) containing 0.3g of progesterone (Plastic Moulding Co.,New Zealand) and left in place for 12 to 14 days. Ten days after CIDR insertion it was replaced with a new one in order to maintain a high circulating level of progesterone.

The recipient ewes were synchronised with vaginal sponges containing medroxyprogesterone acetate (Repromap MPA 60mg, Upjohn Veterinary Products) left in place for 12 to 14 days.

All the animals (donors or recipients) were put immediately with harnessed vasectomised rams after CIDR or sponge removal. They were examined twice daily (08.00 h, 20.00 h) for mating marks.

3.3. SUPEROVULATION TREATMENTS

Superovulation was attempted with two different gonadotrophin preparations: FSH-P (Schering Corporation, U.S.A) and Folltropin (Vetrapharm Inc.,London). Animals were treated with 7 or 8 intramuscular injections at 12 h interval in one of a series of treatments starting 72 h before CIDR removal. The treatments varied according to the breed and were chosen on the basis of "trial and error" as the work progressed. Table 3.1 shows details of the various treatments used.

Table 3.1. Distribution of drugs,doses, and number of injections per treatment (TRT) given to the various breeds of ewes.

TRT	Breed	Drug		Total Dose mg.	Dose of single injection. mg.	No. of injections
		FSH-P	Foll.			
1	FINN,GOT	FSH-P	Foll.	26	4,4,3,3,3,3,3,3	8
2	DT,FT,OXD,WHM	FSH-P	Foll.	30	4,4,4,4,4,4,3,3	8
3	DT,OXD,WHM	FSH-P	Foll.	36	7,6,5,5,5,4,4	7
4	FT	FSH-P	Foll.	34	5,5,4,4,4,4,4,4	8
5	DT,FT,OXD,WHM	FSH-P	Foll.	38	7,7,6,6,4,4,4	7
6	FINN,GOT	FSH-P	Foll.	30	6,6,4,4,4,4,2	7
7	FT,GOT	FSH-P	Foll.	32	5,5,4,4,4,4,3,3	8

3.4. DETECTION OF OESTRUS AND TIME OF INSEMINATION

The donor ewes marked in the morning were inseminated intra utero in the afternoon (14.00 h to 17.00 h). The donor ewes marked in the night were pen-mated immediately after checking for mating marks and inseminated intra utero the next morning (0.8.00 h to 12.00 h). Thus, donor ewes marked in the morning were inseminated at an average of 6-9 h from detection of heat. Donor ewes marked at night were inseminated at an average of 12-16 h from the time of recording the heat, but they were also naturally mated immediately after the observation of oestrus.

3.5. ARTIFICIAL INSEMINATION TECHNIQUE

Semen was collected by artificial vagina. It was held at 35°C in a water bath and diluted, 1:2, with a standard egg yolk diluent prepared by the New Zealand Dairy Board and was used within two hours after collection. The semen was examined, after collection and dilution, to determine the motility and quality.

3.5.1. Intrauterine Insemination

Insemination was carried out with animals under acetyl promazine tranquilliser (1 ml injected intramuscular) and local anaesthesia (1 ml of 2% xylocaine) while the ewes were restrained in a laparotomy cradle.

The technique was based on Killeen and Caffery (1982) with the following modifications: after visualisation of the uterus with the

aid of a laparoscope and probe, a trocar and cannula (internal diameter of 6mm) was inserted mid-ventrally between the telescope and the probe. A French AI pistolet containing a 0.25 ml straw filled with diluted fresh-semen was fitted with an aspic with a 5mm needle (IMV) and inserted through the cannula. A good fit of the aspic to the pistolet allowed close contact between the straw and the base of the needle. Thus loss of semen during insemination was avoided. One horn of the uterus was manipulated into position with the probe and the aspic needle stabbed through the uterine wall into the lumen (photo 1) The plunger of the pistolet was pressed half way down by an assistant to expel one half dose of semen (0.12 ml) with the remaining half dose injected into the other uterine horn. Antibiotic injections were given to all animals after insemination.

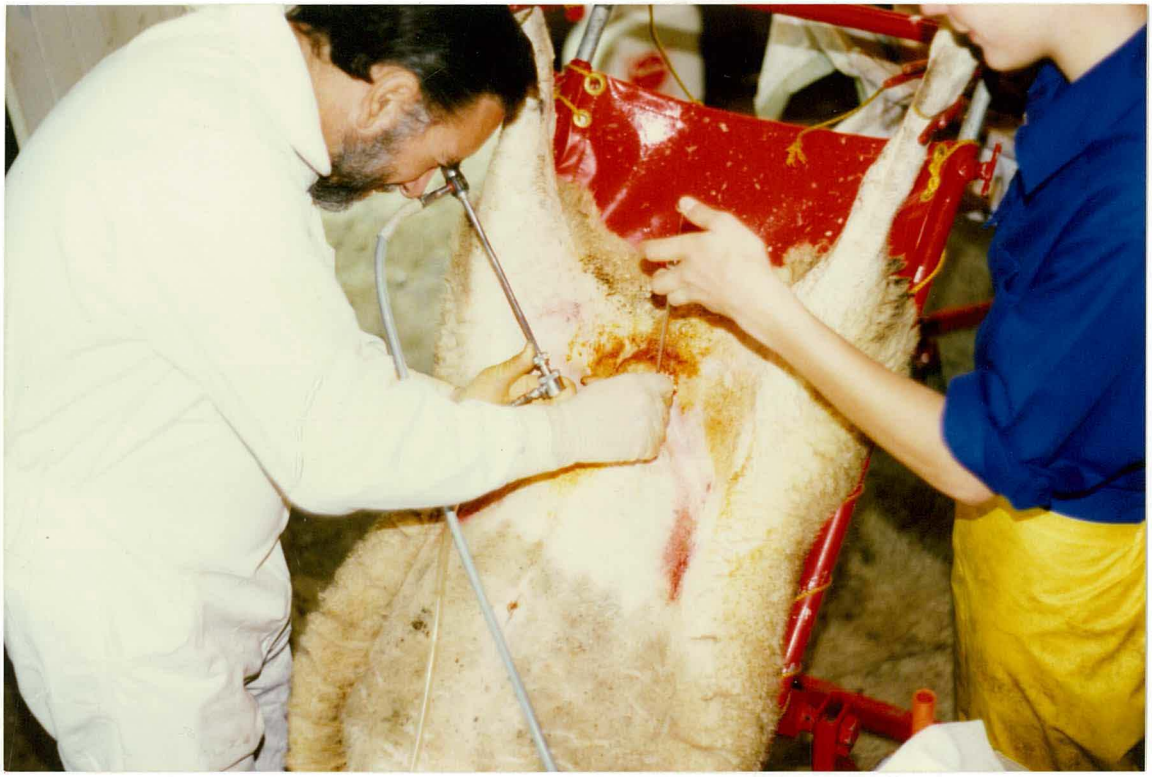

Photo 1. Intrauterine artificial insemination technique with the aid of a telescope (left side) and French pistolet (right side).

3.6. RECOVERY OF EMBRYOS

The embryos were recovered surgically between Day 5 and 6.5 after onset of the oestrus (Day 0). The donor ewes were starved for a minimum of 12 h before surgery. General anesthesia was initially induced by injecting 5% (w/v) of Thiamylal sodium (Bio-Tal) at a dose level of 20ml/50 kg bodyweight. It was maintained, during surgery, with Halothane and oxygen. A mid-ventral incision was made on the abdominal wall and the uterus and ovaries were exteriorised. The number of corpora lutea on both ovaries were recorded. For simplicity of data analysis and discussion the superovulatory response was grouped into 3 classes according to the number of CL counted at the time of flushing as follow:

Class 0-8 CL -low response;

Class 9-16 CL -medium response;

Class >16 CL -high response.

Each uterine horn was flushed according to the technique described by Tervit and Havik (1976). Each donor ewes was injected with prostaglandin after surgery in order to avoid possible pregnancy if not all embryos were flushed from the tract.

Embryo storage did not exceed 4 h at 35°C in the flushing medium (Phosphate Buffered Saline with 4% w/v Bovine Serum Albumin, Immuno Chemical Products Ltd. New Zealand). Antibiotic injections were given after flushing.

The search for embryos was carried out immediately after flushing using a stereoscopic microscope. They were transferred into a small

Petri dish containing fresh flushing media for classification.

3.6.1. Embryo classification

The embryos were examined and classified on the basis of "stage" (embryo development) and "grade" (embryo morphology) as described in table 3.2. In order to study the success rate of ET in relation to the quality of embryos transferred, they were then classified into either of two categories "good quality" and "poor quality" on the basis of "stage" and "grade" combined as described in Table 3.3.

Table 3.2 Classification of embryos according to the stage and grade

Stage	Description	Grade	Description
1	Unfertilised ova	1	Perfect, no irregularities
2	2-8 cells	2	One irregularity
3	16 cells	3	Two or more irregularities
4	Early morula	4	Many irregularities or
5	Morula		partly degenerated
6	Late morula	5	Degenerated
7	Blastocyst		
8	Expanded blastocyst		
9	Hatched blastocyst		

Table 3.3 Criteria for the classification of good and poor quality embryos

Good Quality		Poor Quality	
Grade 1	Stage 4	Grade 4	Stage 2
2	5	5	3
3	6		
	7		
	8		
	9		

After classification, the embryos were washed 10 times by shifting through 10 drops of fresh flushing media located in a Petri dish. Then the embryos were transferred with some media into a 4 ml autoanalyser cup. The cup was placed into a thermobottle and transported by car to the second quarantine farm. Before transfer a second evaluation of the embryos was carried out to confirm or alter the initial observation.

3.7. TRANSFER PROCEDURE

All the recipients were tranquillised with acepromazine maleate (ACP) at dose level of 0.10mg/kg bodyweight. Two embryos were surgically transferred into the upper third of each horn as described by Boundy et al.,(1985). The number of the corpora lutea were recorded. Antibiotic (i.m.) injection was given after the procedure. Some of the recipients received one or three embryos depending on quality and availability. Some recipients of each breed received a CIDR after the transplantation and these were left in situ for 14 days.

3.8. PREGNANCY TEST AND LAMBING

Approximately 60 days after surgery the ewes were tested for pregnancy using an ultrasaund technique.

The recipients were synchronised to lamb, 48 h before the expected parturition by injecting intramuscularly with 6 ml of Dexadreson (Intervet, Australia) containing 12 mg of Dexamethasone sodium phosphate. This therapy was used in order for lambing to occur under supervision in a shed and so that any cases of dystocia or other complications could be attended to. Full records of the identity of ewes and lambs were collected.

3.9. ANALYSIS OF DATA

The number of observations for each factor analysed differ according to the validity of the comparisons that could be made. Although 300 adult ewes were available for the programme several observations were excluded from certain analyses while in others they were included and the retreatment of some ewes resulted in more than 300 observations being considered in other analyses.

The variation of ovulation rate, fertilisation rate and recovery rate of the embryos was analysed using a Generalized Linear Model Computing package (SAS, 1985).

The general linear model used for analysis of ovulation rate after gonadotrophin treatment was:

$$Y_{ijk} = M + TRT_i + DRUG_j + TRT_i \text{ X } DRUG_j + E_{ijk}$$

where:

M= general mean

TRT_i= the fixed effect of the i^{th} TRT (i= 1,.....,7)

$DRUG_j$= the fixed effect of j^{th} Drug (j=1 or 2)

$(TRT \text{ X } DRUG)_{ij}$= effect of the interaction between the i^{th} TRT and the j^{th} drug.

E_{ijk}= error peculiar to each Y_{ijk}

The general linear model used for analysis of recovery rate was:

$$Y_{ijkw} = M + NFL_i + DFL_j + ORC_k + Bree_w + (NFL \times BREE)_{iw} + (NFL \times ORC)_{ik} + (DFL \times ORC)_{jk} + (DFL \times BREE)_{jw} + (ORC \times BREE)_{kw} + E_{ijkw}$$

where:

M= general mean

NFF_i= the fixed effect of the i^{th} Number of Flush (i= 1 or 2)

DFL_j= the fixed effect of the j^{th} Day of Flush (j= 5 or 6)

ORC_k= the fixed effect of the k^{th} Ovulatory Response classes (k= 1,..3)

$BREE_w$= the fixed effect of the w^{th} Breed (w= 1,.....,6)

$(NFL \times BREE)_{iw}$= effect of the interaction between the i^{th} Number of Flush and the w^{th} Breed.

$(NFL \times ORC)_{ik}$= effect of the interaction between the i^{th} Number of Flush and the k^{th} Ovulatory Response Classes

$(DFL \times ORC)_{jk}$= effect of the interaction between the j^{th} Day of Flush and the k^{th} Ovulatory Respone Classes

$(DFL \times BREE)_{jw}$= effect of the interaction between the j^{th} Day of Flush and the w^{th} Breed.

$(ORC \times BREE)_{kw}$= effect of the interaction between the k^{th} Ovulatory Response Classes and the w^{th} Breed

E_{ijkw}= error peculiar to each Y_{ijkw}

The general linear model used for analysis of Fertilisation Rate was:

$$Y_{ijkwx} = M + TAI_i + NFL_j + FER_k + ORC_w + BREE_x + (TAI \times FER)_{ik} + (TAI \times BREE)_{ix} + (NFL \times IT)_{jk} + (NFL \times BREE)_{jx} + (ORC \times BREE)_{wx} + E_{ijkwx}$$

where:

M= general mean

TAI_i= the fixed effect of i^{th} Time of Insemination (i= 1 or 2)

NFL_j= the fixed effect of j^{th} Number of Flush (j= 1 or 2)

IT_k= the fixed effect of k^{th} Insemination Technique (k= 1 or 2)

ORC_w= the fixed effect of w^{th} Ovulatory Response Classes (w= 1,...,3)

$BREE_x$= the fixed effect of x^{th} Breed (x= 1,.....,6)

$(TAI \times IT)_{ik}$= effect of the interaction between the i^{th} Time of Insemination and k^{th} Insemination Technique

$(TAI \times BREE)_{ix}$= effect of the interaction between the i^{th} Time of Insemination and the x^{th} Breed

$(NFL \times IT)_{jk}$= effect of the interaction between the j^{th} Number of Flush and the k^{th} Insemination Technique

$(NFL \times BREE)_{jx}$= effect of the interaction between the j^{th} Number of Flush and the x^{th} Breed

$(ORC \times BREE)_{wx}$= effect of the interaction between the w^{th} Ovulatory Response Classes and x^{th} Breed

E_{ijkwx}= error peculiar to each Y_{ijkwx}

Duncan's New Multiple Range Test was used when more than two means were involved in a significant F-test to compare differences among different levels of a factor.

The significance of the factors affecting the Onset of Oestrus, the Quality of the Embryos, the Pregnancy and the Survival rate were examined using Chi-square test by means of a contingency table analysis.

The following symbols were used to denote the levels of statistical significance.

Symbol	Level of significance
NS	0.10<P
+	P<0.10
*	P<0.05
**	P<0.01
***	P<0.001

CHAPTER IV: RESULTS

4.1. INCIDENCE AND DISTRIBUTION OF OESTRUS

4.1.1. Incidence of Oestrus

The incidence of oestrus after treatment with FSH-P and Folltropin for the 6 breeds is shown in Table 4.1. Analyses of these data did not find any significant effect of drug on the percentage of ewes showing oestrus following CIDR removal in any of the breeds studied.

Table 4.1. Effect of drug on the incidence of oestrus in six breeds.

Breed	FSH-P		Folltropin		Significance
	No.ewes	%	No.ewes	%	
DT	35	82.8	81	83.9	NS
FT	19	89.5	47	85.1	NS
FINN	24	100.0	49	100.0	NS
GOT	11	100.0	12	100.0	NS
OXD	53	79.5	62	87.1	NS
WHM	9	90.0	18	75.0	NS
Total	151	87.0	269	89.0	

Differences in the incidence of oestrus among the animals in the three O.R. classes were analysed within each breed and the results are shown in Table 4.2. There were significant differences among animals in different classes for DT,FT,and OXD breeds, with ewes in the medium and high classes having a higher incidence of oestrus than those in the low class. No significant difference was found in the remaining breeds.

Table 4.2 Effect of O.R. classes on the incidence of oestrous following CIDR removal and gonadotrophin treatment in the six breeds[1].

Breed	O.R. Classes						Sig.
	0-8		9-16		>16		
	No.ewes	%	No.ewes	%	No.ewes	%	
DT	70	73.0[a]	31	100.0[b]	15	100.0[b]	**
FT	42	78.0[a]	17	100.0[b]	7	100.0[b]	+
FINN	39	100.0[a]	10	100.0[a]	24	100.0[a]	NS
GOT	12	100.0[a]	7	100.0[a]	4	100.0[a]	NS
OXD	76	76.3[a]	25	96.0[b]	14	100.0[b]	*
WHM	9	73.0[a]	4	100.0[a]	3	100.0[a]	NS

1 Mean percentages with the same superscript within a breed are not significantly different (P>0.10).

4.1.2. Distribution of Onset of Oestrus

The effect of drug (FSH-P and Folltropin) on the number and percentage of ewes of the six breeds showing heat at 24, 36, 48 and 60 or more hours after CIDR removal are shown in Table 4.3. Significant ($P<0.10$) differences between drugs were found in DT and OXD breeds (Fig.1). No significant differences were found in the other breeds.

Table 4.4 shows the number and percentage of ewes in different O.R. classes showing oestrus at various times after CIDR removal. There were significant differences among these classes in the distribution of onset of heat in the DT and FINN ($P<0.01$) and in the WHM ($P<0.10$) ewes (Fig.2).

Table 4.3. Effect of FSH-P and Follotropin (Foll.) on the number and percentage(in bracket) of ewes coming into oestrus at different times after CIDR removal in the six breeds.

Drug	Time(h)	BREED					
		DT	FT	FINN	GOT	OXD	WHM
FSH-P	24	13(52)	5(42)	10(59)	5(56)	13(45)	1(20)
	36	11(44)	6(50)	7(41)	4(44)	15(52)	4(80)
	48	0(0)	0(0)	0(0)	0(0)	1(3)	0(0)
	≥60	1(4)	1(8)	0(0)	0(0)	0(0)	0(0)
Foll.	24	19(30)	6(17)	22(47)	4(40)	13(24)	3(18)
	36	29(45)	18(50)	15(32)	4(40)	30(56)	6(35)
	48	11(17)	9(25)	8(17)	1(10)	6(11)	4(24)
	≥60	5(8)	3(8)	2(4)	1(10)	5(9)	4(24)
Significance		+	NS	NS	NS	+	NS

Table 4.4. Effect of Ovulatory response classe (ORC) on the distribution of onset of oestrus after CIDR removal in Danish Texel (DT) Finnish Texel (FT), Finnish Lancrace (FINN), Gotland-Pelt (GOT), Oxford Down (OXD) and White Headed Marsh (WHM).

ORC		0-8				9-16				>16				Sig.
TIME(h)		24	36	48	≥60	24	36	48	≥60	24	36	48	≥60	
DT	No.	10	22	10	6	13	15	1	0	9	3	0	0	**
	(%)	(21)	(46)	(21)	(12)	(45)	(52)	(3)	(0)	(75)	(25)	(0)	(0)	
FT	No.	3	16	6	4	5	6	2	0	3	2	1	0	NS
	(%)	(10)	(55)	(21)	(14)	(38)	(46)	(15)	(0)	(50)	(33)	(17)	(0)	
FINN	No.	14	11	8	2	2	7	0	0	16	4	0	0	**
	(%)	(40)	(31)	(23)	(6)	(22)	(78)	(0)	(0)	(80)	(20)	(0)	(0)	
GOT	No.	2	6	0	1	5	1	1	0	2	1	0	0	NS
	(%)	(22)	(67)	(0)	(11)	(71)	(14)	(14)	(0)	(67)	(33)	(0)	(0)	
OXD	No.	12	25	6	5	9	13	1	0	5	7	0	0	NS
	(%)	(25)	(52)	(12)	(10)	(39)	(57)	(4)	(0)	(42)	(58)	(0)	(0)	
WHM	No.	0	6	4	4	2	2	0	0	2	2	0	0	+
	(%)	(0)	(43)	(29)	(29)	(50)	(50)	(0)	(0)	(50)	(50)	(0)	(0)	

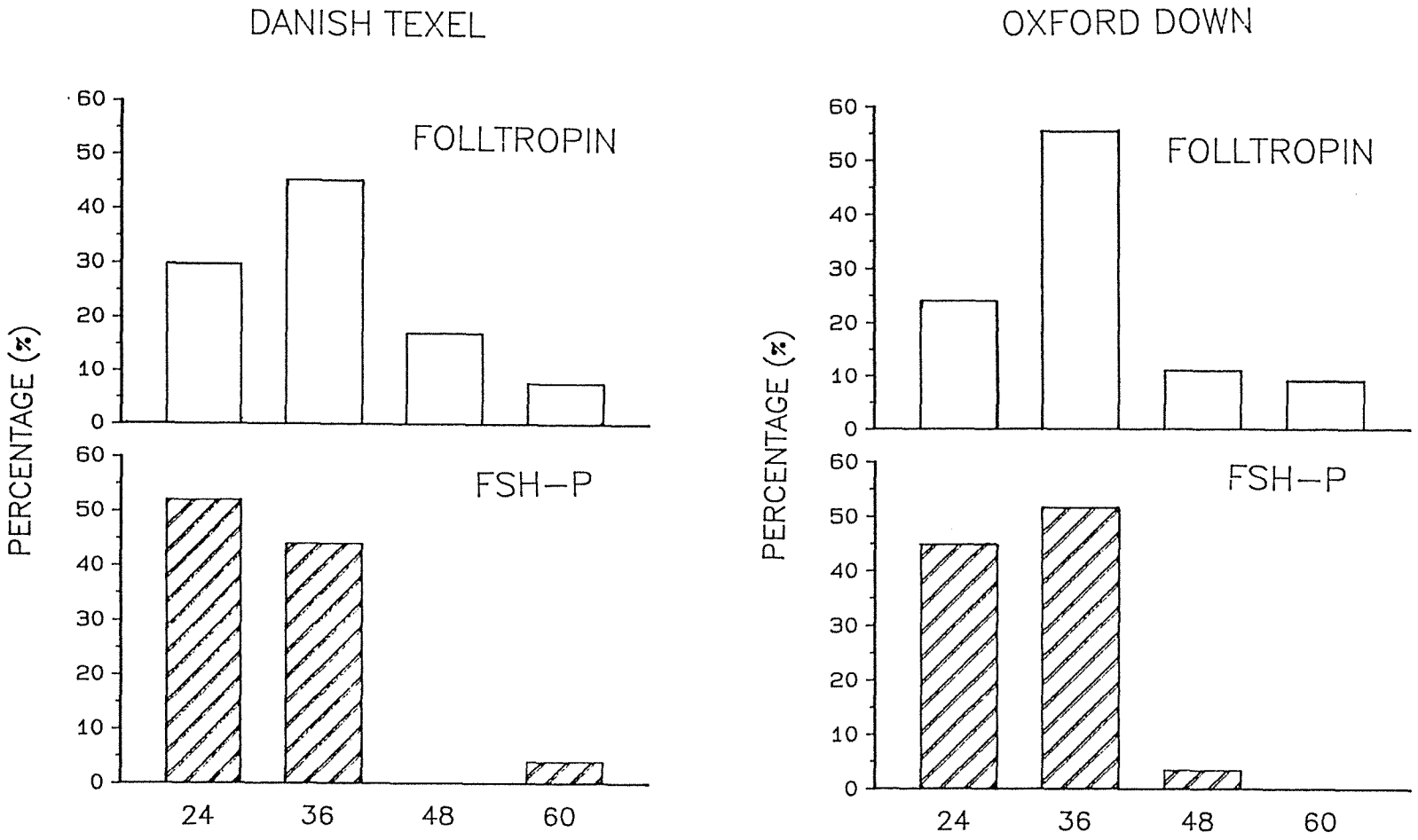

Fig. 1. Percentage of Danish Texel (left panel) and Oxford Down (right panel) ewes treated with either FOLLTROPIN (top) or FSH-P (bottom) showing oestrus at 24, 36, 48 and ≥60 hours following CIDR removal.

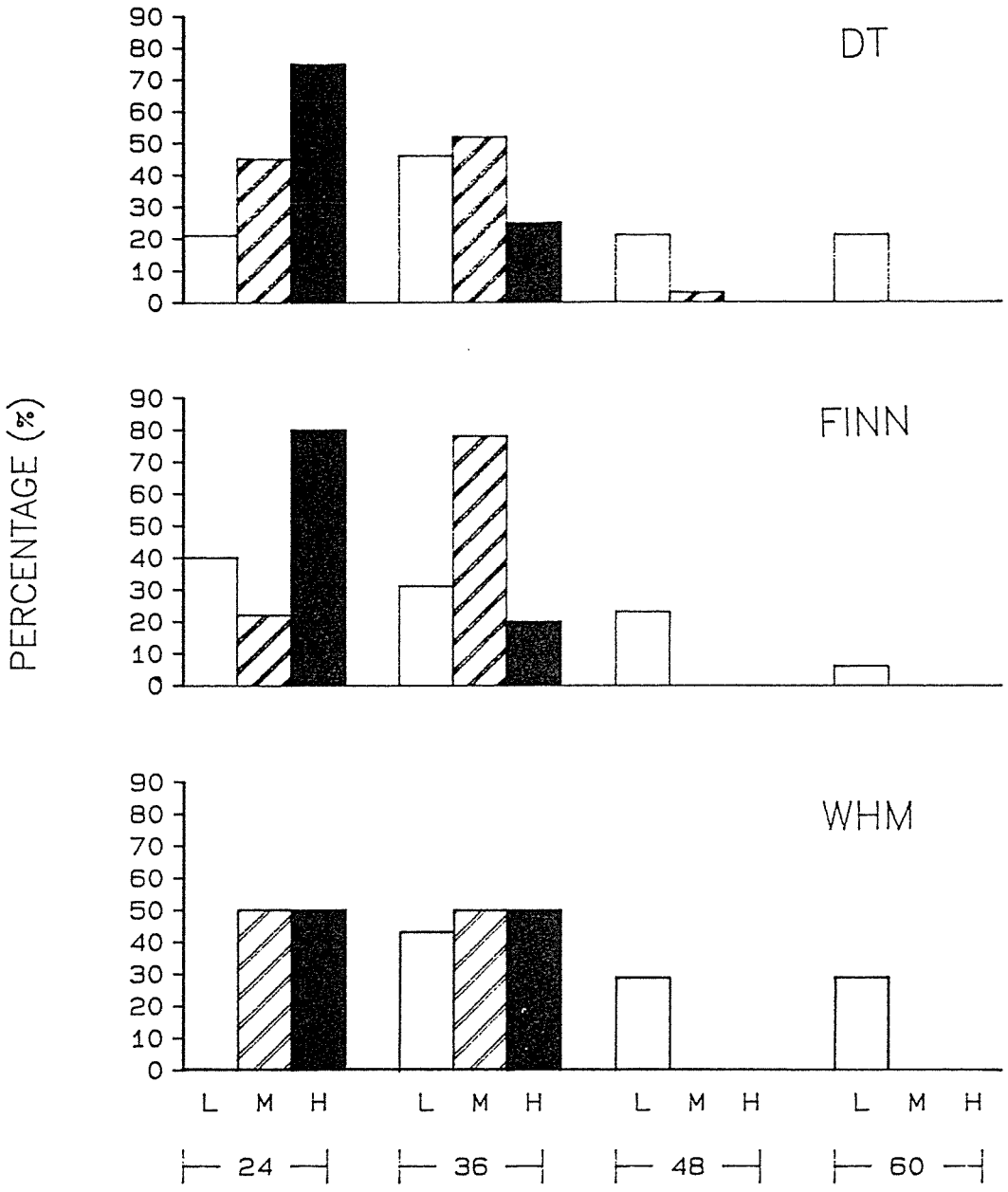

Fig. 2. Percentage of Danish Texel (DT), Finnish Landrace (FINN) and White Headed Marsh (WHM) ewes in the low (L, 0-8), medium (M, 9-16) and high (H, >16) ovulatory response classes showing oestrus at 24, 36, 48 and ≥60 hours following CIDR removal.

4.2. OVULATORY RESPONSE

Two hundred and four observations in six breeds were used to study the ovulatory responses following injections of either FSH-P or Folltropin at various doses considered. Table 4.5 summarises the numbers of ovulations recorded in each of the six breeds. Because of differences among breeds in the quantity of the gonadotrophins given analyses of the effects of drug and dose were made within breeds and the results are presented separately in Table 4.6 to 4.12 and Fig. 3. There was no significant effect of drug, dose or their interactions on the ovulatory response in any of the breeds studied.

Table 4.5. Mean(±se) ovulation rate (OR) after gonadotrophin treatment for the six breeds.

Breed	No.ewes	OR	SE
DT	63	9.6	(±0.08)
FT	28	9.9	(±1.20)
FINN	40	15.5	(±1.40)
GOT	22	10.2	(±1.71)
OXD	32	11.5	(±1.08)
WHM	19	6.3	(±1.43)
Total	204	10.9	

Table 4.6. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in Danish Texel ewes.

Drug	FSH-P			Folltropin			Significance		
Dose	30	36	38	30	36	38	Drug	Dose	DrugxDose
No ewes	17	9	4	2	19	12			
OR	7.9	10.0	13.0	3.0	11.2	9.4	NS	NS	NS
SE	(±1.03)	(±2.30)	(±4.18)	(±1.00)	(±2.01)	(±2.08)			

Table 4.7. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in Finnish Texel ewes.

Drug	FSH-P			Folltropin			Significance		
Dose	32	34	38	32	34	38	Drug	Dose	DrugxDose
No ewes	5	2	2	7	6	6			
OR	10.0	15.5	7.5	7.3	12.7	9.2	NS	NS	NS
SE	(±2.09)	(±5.50)	(±7.50)	(±1.22)	(±3.07)	(±3.26)			

Table 4.8. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in Finnish Landrace ewes.

Drug	FSH-P		Folltropin		Significance		
Dose	26	30	26	30	Drug	Dose	DrugxDose
No.ewes	22	2	14	2			
OR	17.3	15.5	12.6	17.5	NS	NS	NS
SE	(±2.17)	(±1.50)	(±1.93)	(±2.50)			

Table 4.9. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in Gotland-Pelt ewes.

Drug	FSH-P		Folltropin		Significance		
Dose	26	32	26	32	Drug	Dose	DrugxDose
No.ewes	8	3	7	4			
OR	10.2	8.7	8.6	14.2	NS	NS	NS
SE	(±3.53)	(±3.17)	(±2.19)	(±5.02)			

Table 4.10. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in Oxford Down ewes

Drug	FSH-P		Folltropin		Significance		
Dose	36	38	36	38	Drug	Dose	DrugxDose
No.ewes	7	5	9	11			
OR	11.3	14.0	11.5	10.4	NS	NS	NS
SE	(±2.67)	(±2.23)	(±2.22)	(±1.81)			

Table 4.11. Effect of drug and dose of gonadotrophin (mg) on the Mean(±se) ovulation rate (OR) in White Headed Marsh ewes.

Drug	FSH-P		Folltropin	
Dose	30	38	36	38
No ewes	8	1	6	4
OR	4.7	20	9.5	1.2
S.E	(±1.29)	-	(±2.81)	(±0.47)

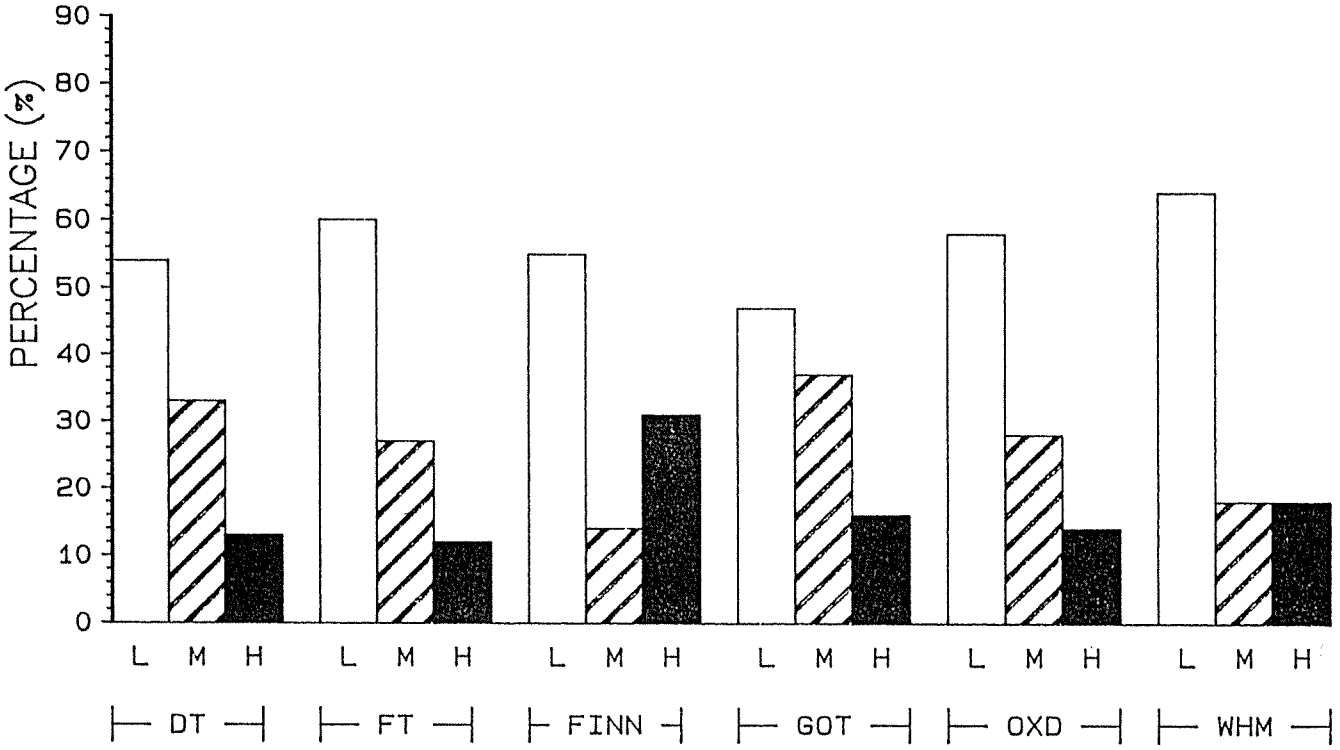

Fig. 3. Percentage of Danish Texel (DT), Finish Texel (FT), Finnish Landrace (FINN), Gotland-Pelt (GOT), Oxford Down (OXD) and White Headed Marsh (WHM) ewes in the low (L, 0-8), medium (M, 9-16) and high (H, >16) ovulatory response classes

4.3. RECOVERY RATE

A total of 221 ewes of six breeds were available to study embryo recovery rate (where recovery rate= number of egg recovered/number of corpora lutea). Table 4.12 is a summary of the number and percentage of eggs recovered (EGGR) from the various breeds of ewes. There was no significant difference among the breds in recovery rate. The data were further examined in relation to the drug used (FSH-P and Folltropin), number of flushes attempted (1 or 2),the day of flushing after oestrus dectetion (5 or 6) and ovulatory response classes (ORC) counted at surgery (Table 4.13). The analysis of these data did not show any significant difference for any of the factors considered

Table 4.12. Effect of breeds on mean±se embryo recovery rate

Breed	No.ewes	EGGR	Recovery rate
DT	58	387	63.0±0.03
FT	36	195	53.1±0.06
FINN	47	444	67.0±0.04
GOT	14	86	66.1±0.07
OXD	53	355	66.1±0.03
WHM	13	68	59.1±0.07
Total	221	1535	63.2

Table 4.13. Effect of drug (FSH-P, Folltropin),Number of Flushing (1 vs 2), Day of Flushes (day 5 vs 6) and Ovulatory Response Classes (1-8 vs 9-16 vs >16) on mean±se Embryo Recovery Rate[1]

Variables		No.of ewes	Recovery rate	Significance
Drug	FSH-P	66	63.0±0.03	NS
	Foll.	155	65.0±0.02	
No. of flushing	1	148	64.1±0.02	NS
	2	73	65.1±0.03	
Day of flushing	5	133	64.8±0.02	NS
	6	88	63.8±0.03	
ORC	1-8	101	63.8±0.03	
	9-16	69	63.6±0.03	NS
	>16	51	66.7±0.03	

[1] Due to a lack of breed effect, data were pooled across all six breeds.

4.4. FERTILISATION RATE

Results from 201 ewes of six breed were available to study fertilisation rate (where fertilisation rate = number of eggs fertilised/number of eggs recovered). Table 4.14 summarizes the number eggs recovered and fertilisation rate from the various breeds. The breeds did not differ significantly in their fertilisation rates. The data were further analysed in relation to the time of AI, numbers of flushes (1 vs 2), insemination technique either uterine AI or uterine AI plus natural mating (1 vs 2) and ovulatory response classes (ORC) (Table 4.15). There were no significant effect of any of the factors studied on fertilisation rate.

Table 4.14. Effect of breeds on mean ±se fertilisation rate.

Breed	No.ewes	EGGR	Fertilisation rate
DT	55	387	84.5±0.04
FT	28	195	91.4±0.05
FINN	41	422	78.7±0.06
GOT	14	86	71.4±0.13
OXD	52	355	93.3±0.04
WHM	11	64	80.1±0.12
Total	201	1509	85.4

Table 4.15. Effect of time of AI (am. vs pm.), Number of Flushing (1 vs 2), Insemination Technique (1 or 2) and Ovulatory Response Classes (1-8 vs 9-16 vs >16) on mean(±se) Embryo Fertilisation Rate[1]

Variables		No.ewes	Fertilisation rate	Significance
Time of AI	am	139	86.1 (±0.02)	NS
	pm	62	83.8 (±0.04)	
No of Flushing	1	135	86.3 (±0.02)	NS
	2	66	83.5 (±0.04)	
Ins. Tech.	1	137	83.6 (±0.03)	NS
	2	64	89.1 (±0.03)	
ORC	1-8	89	80.6 (±0.04)	NS
	9-16	63	87.6 (±0.03)	
	>16	49	91.3 (±0.03)	

[1] Due to lack of breed effect, data were pooled across all six breeds.

4.5 QUALITY OF EMBRYOS

The effect of drug (FSH-P Folltropin), dose(depending on breed), ovulatory response classes (1-8, 9-16, >16) and age of the embryos on the percentage of good quality embryos recovered are shown in table 4.16 to 4.22 for each of the six beeds.

a) Danish Texel- Table 4.16

All the factors significantly affected ($P<0.001$) the quality of embryos. Folltropin gave more good quality embryos than FSH-P (84.5 vs 73.6). The percentage of good quality embryos incresed with the dose of drug administered although the difference between the two lower doses was not significant. The ovulatory response classes also affected the percentage of good embryos and ewes in the "high" class had a higher percentage of good embryos than those in "medium" or "low" classes which did not differ significantly. The percentage of good quality embryos was lower if flushing was carried out on day 5 compared to other days. But there were no significant difference among flushings carried out on days 5.5, 6 or 6.5.

b) Finish Texel -Table 4.17

Drug, dose and OR class did not affect significantly the percentage of good quality embryos. Age had a similar effect on embryo quality in this breed as in Danish Texel.

c) Finnish Landrace -Table 4.18

Drug, dose and age of embryos did not affect significantly the percentage of good quality embryos. Ovulatory response class significantly ($P<0.001$) affected the quality of embryos. Ewes in the "medium" and "high" classes had higher percentages of good quality embryos than animals in the low class, with no significant difference between ewes in the "medium" and "high" classes.

d) Gotland Pelt -Table 4.19

Folltropin produced a significantly ($P<0.01$) higher percentage of good quality embryos than FSH-P (90.5 vs 78.6). In this breed,ewes treated with 30 mg of either FSH-P or Folltropin had a lower ($P<0.10$) percentage of good quality embryos than those treated with other doses, but the number of embryos in this class was too small for reaching any definite conclusion. The ovulatory response classes also affected the percentage of good embryos and ewes in the "low" class had a significantly ($P < 0.05$) lower percentage of good embryos than those in "medium" or "high" classes which did not differ significantly. The percentage of good quality embryos was lower if flushing was carried out on day 5 compared to other days. But there were no significant difference among flushing carried out on days 5.5, 6 or 6.5.

e) Oxford Down -Table 4.20

Folltropin produced a significantly ($P < 0.01$) higher percentage of good quality embryos than FSH-P (81.3 vs 71.8). The lowest dose level of

drug resulted in a significantly ($P<0.01$) lower percentage of good quality embryos compared the two higher classes which did not differ significantly. Animals in the low OR class had a significantly ($P<0.05$) lower percentage of good quality embryos than ewes in the other two classes which did not differ significantly. The age of embryos did not significantly affect the percentage of good embryos.

f) White Headed Marsh -Table 4.21

No significant difference was foun for any of the factors studied.

Table 4.16 Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in DT ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	140	73.6[a]	***
	Foll.	251	84.5[b]	
Dose	30mg	83	67.5[a]	***
	36mg	149	76.5[a]	
	38mg	159	91.2[b]	
ORC	1-8	54	75.9[a]	***
	9-16	177	70.6[a]	
	>16	160	93.1[b]	
Age	5.0	26	53.9[a]	***
	5.5	129	79.8[b]	
	6.0	181	81.8[b]	
	6.5	55	90.9[b]	
Total		391	80.5	

[1] Mean percentages within each factors carrying the same superscript are not significantly different ($P>0.10$).

Table 4.17. Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in FT ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	73	91.8[a]	NS
	Foll.	114	88.6[a]	
Dose	30mg	34	88.2[a]	NS
	32mg	43	83.7[a]	
	34mg	41	97.6[a]	
	38mg	69	89.9[a]	
ORC	1-8	56	87.5[a]	NS
	9-16	60	90.0[a]	
	>16	71	91.5[a]	
Age	5.0	18	66.7[a]	*
	5.5	40	100 [b]	
	6.0	43	88.4[b]	
	6.5	86	90.7[b]	
Total		187	89.8	

Table 4.18.Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in FINN ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	214	93.9[a]	NS
	Foll.	151	93.4[a]	
Dose	26mg	324	93.5[a]	
	28mg	15	100 [a]	NS
	30mg	26	92.3[a]	
ORC	1-8	33	78.8[a]	
	9-16	66	97.0[b]	***
	>16	266	94.7[b]	
Age	5.0	21	95.2[a]	NS
	5.5	95	96.8[a]	
	6.0	180	91.7[a]	
	6.5	69	94.2[a]	
Total		365	93.7	

Mean percentages within each factors carrying the same same superscript are not significantly different (P>0.10).

Table 4.19.Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in GOT ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	84	78.6[a]	**
	Foll.	95	90.5[b]	
Dose	26mg	104	84.3[a]	+
	28mg	4	100[a]	
	30mg	10	50.0[b]	
	32mg	65	90.5[a]	
ORC	1-8	33	72.7[a]	*
	9-16	57	82.5[b]	
	>16	89	91.0[b]	
Age	5.0	9	55.6[a]	*
	5.5	26	92.3[b]	
	6.0	107	84.1[b]	
	6.5	37	89.2[b]	
Total		179	84.9	

Table 4.20.Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in OXD ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	177	71.8[a]	**
	Foll.	225	81.3[b]	
Dose	30mg	87	65.5[a]	**
	36mg	154	81.8[b]	
	38mg	161	78.9[b]	
ORC	1-8	109	69.7[a]	*
	9-16	164	79.9[b]	
	>16	129	79.8[b]	
Age	5.0	23	78.3[a]	NS
	5.5	196	71.4[a]	
	6.0	106	84.0[a]	
	6.5	77	81.8[a]	
Total		402	77.1	

[1] Mean percentages within each factors carrying the same same superscript are not significantly different (P>0.10).

Table 4.21.Effect of Drug , Dose, Ovulatory Response Classes (ORC) and Age of the Embryos on mean percentage of Good Quality Embryos in WHM ewes[1]

Variables		Total No.Embryo	Good Quality Embryo	Significance
Drug	FSH-P	30	93.3[a]	NS
	Foll.	36	88.9[a]	
Dose	30mg	12	100[a]	NS
	36mg	19	84.2[a]	
	38mg	35	91.4[a]	
ORC	1-8	23	87.0[a]	NS
	9-16	6	100[a]	
	>16	37	91.9[a]	
Age	5.0	2	100[a]	NS
	5.5	37	91.9[a]	
	6.0	11	100[a]	
	6.5	16	81.2[a]	
Total		66	90.9	

[1] Mean percentages within each factors carrying the same same superscript are not significantly different (P>0.10).

4.6. PREGNANCY RATE AND SURVIVAL OF THE EMBRYOS

Data on pregnancy following ET was obtained from 169,71,146, 64,135 and 22 recipients receiving 2 embryos from each of the six breeds respectively. The recipient ewes were of either Coopworth or Romney breed. The effect of breed of recipient (Coopworth or Romney), degree of synchrony (Sunch.) between donor and recipient (-24 h, 0 h or +12 h; where -24 h=recipients detected on heat 24 h earlier than the donor; +12 h=recipient detected on heat 12 h later than donor; 0 h= recipient detected on heat at the same time as the donor),the interval from recovery of embryo to transplantation (Time) (1=100 minutes;2=101-150 minutes;

3=151-240), the number of corpora lutea (CL) recorded in the recipient ewes (1 or 2) and the effect of progesterone supplementation given as a CIDR (CIDR + or -) after transfer were analysed within each breed. Results on the pregnancy rate and survival rate are shown in Table 4.22 and 4.23 respectively. Table 4.24 shows the overall pregnancy rate and survival rate for each of the six breed.

4.6.1 Factors affecting Pregnancy rate

a) Breed of recipient:

The breed of recipient did not affected significantly the pregnancy rate of most of the breed, however Coopworth ewes transplanted with FINN embryos had a significantly ($P < 0.01$) higher pregnancy rate than Romney ewes (69.0 vs 46.8).

b) Degree of synchronisation

The pregnancy rate was not significantly affected by the degree of synchronisation between donors and recipients in most of the breeds exept for GOT embryos where recipients that were in oestrus 12 later than their corresponding donor had higher ($P < 0.01$) pregnancy rate than recipients in perfect synchrony with their donors (synch.0) (62.2 vs 40.7). Not data were available for recipients that were in oestrous 24 h later than donors.

b) Time of transplantation from flush

The time of transplantation from flush did not have any signficant effect on the pregnancy rate of ewes carring FT, FINN, GOT and OXD embryos. However, significant difference ($P<0.05$), were found in ewes receiving DT embryos where pregnancy rates were higher if transfer was carried out within 100 minutes from the flush than that carried out later. The ewes carrying WHM embryos were exclude from the analysi because of the small number of observations.

d) Number of CL in the recipient ewes

The pregnancy rate of the ewes was not significantly affected by the numbers of CL, however the recipient carring WHM had a lower pregnancy rate when two CL were in the surrogate mother.

e) Progesterone supplementation (CIDR)

The ewes receiving DT, FT and OXD embryos had significantly higher pregnancy rates when progesterone supplementation, as CIDR, was given compared with the corresponding recipients not suppleimented with progesterone. A similar trend existed for GOT and WHM, but the differences did not reach significance.

4.6.2 Factors affecting Survival rate

a) Breed of recipient

Breed of recipients did not affect significantly the survival rate of embryos of most breeds, however the FINN and the OXD embryos had significantly higher survival rate when they were transferred into Coopworth compared to Romney ewes.

b) Degree of synchronisation

The survival rate was not significantly affected by the degree of synchronisation in most of the breeds of embryos. However the GOT embryos transplanted into recipients that were on heat 12 h later than their corresponding donor had higher ($P < 0.10$) survival rate than recipients in perfect synchrony with the donors (45.9 vs 37.0) .

c) Time of transplantation from flushing

The time of transplantation from flush did not have any significant effect on embryo survival rate in any breeds.

d) Number of CL in the recipients

Embryos from WHM donors had a signignificantly ($P<0.05$) higher survival rate when transfered to recipients with one CL than those with two CL. However, survival rates of embryos from all other breeds were not significantly affected by the number of the CL in the recipient.

e) Progesterone supplementation (CIDR)

The survival rates of DT, FT and OXD embryos were significantly higher in recipients supplemented with progesterone than in those not supplemented with progesterone. No significant effect of progesterone supplementation was found fro embryos of the other breeds.

Tabel 4.22 Effect of Recipient breed (C or R),Synchrony (-24,0,+12),Time of trans.(1 or 2 or 3) Number of CL (1 or 2), Progesterone Supplementation (CIDR; + or -) on pregnancy rate(PR) of the six breeds.

Breed		Recipient Breed		Synchrony			CL		CIDR		Time		
		C	R	-24	0	+12	1	2	+	-	1	2	3
DT	No[a]	114	55	12	104	53	51	118	67	102	17	59	93
	PR	57	62	58	54	68	59	59	73	41	82	59	54
	Sig.	NS			NS		NS		**			*	
FT	No[a]	39	32	4	36	31	25	45	35	36	0	20	51
	PR	74	69	100	64	77	64	78	86	58	0	70	72
	Sig.	NS			NS		NS		+			NS	
FINN	No[a]	84	62	8	66	72	52	94	33	113	7	32	107
	PR	69	47	75	52	65	54	63	58	67	57	66	58
	Sig.	**			NS		NS		NS			NS	
GOT	No[a]	42	22	0	27	37	18	46	34	30	6	29	29
	PR	55	50	0	41	62	56	52	56	50	50	48	59
	Sig.	NS			+		NS		NS			NS	
OXD	No[a]	82	53	0	40	95	47	88	36	99	15	55	65
	PR	54	51	0	58	51	51	53	72	45	53	45	59
	Sig.	NS			NS		NS		**			NS	
WHM	No[a]	22	0	0	4	18	8	14	12	10	0	3	19
	PR	46	0	0	25	50	75	29	50	40	0	0	53
	Sig.	-			NS		*		NS		Not Analysed		

[a] No= Number of ewes.

Table 4.23 Effect of Recipient breed (C or R),Synchrony (-24,0,+12),Time of trans.(1 or 2 or 3) Number of CL (1 or 2), Progesterone Supplementation (CIDR; + or -) on embryo Survival rate (SR) of the six breeds.

Breed		Recipient Breed		Synchrony			CL		CIDR		Time		
		C	R	-24	0	+12	1	2	+	-	1	2	3
DT	No[a]	228	110	24	208	106	102	236	134	204	34	118	186
	SR	47	45	38	44	52	46	46	59	38	65	48	39
	Sig.	NS			NS		NS		**		NS		
FT	No[a]	78	64	8	72	62	50	90	70	72	0	40	102
	SR	60	53	75	47	66	56	59	67	42	0	60	56
	Sig.	NS			NS		NS		+		NS		
FINN	No[a]	168	124	16	132	144	144	188	226	66	14	64	214
	SR	65	38	69	45	60	46	58	52	59	57	66	58
	Sig.	**			NS		NS		NS		NS		
GOT	No[a]	84	44	0	54	74	36	92	68	60	12	58	58
	SR	42	43	0	37	46	44	41	44	40	42	40	45
	Sig.	NS			+		NS		NS		NS		
OXD	No[a]	164	106	0	80	190	94	176	72	198	30	110	130
	SR	44	34	0	44	39	37	41	54	35	47	34	44
	Sig.	*			NS		NS		**		NS		
WHM	No[a]	44	0	0	8	36	16	28	20	24	0	6	38
	SR	36	0	0	12	36	50	25	30	38	0	0	53
	Sig.	-			NS		*		NS		Not Analysed		

[a] No= number of embryos.

Table 4.24 Pregnancy rate and Survival rate of embryos from the six breeds after embryo transfer.

Donor Breed	No. recipients	Pregnancy rate	No. Embryos	Survival rate
DT	169	58.6	338	46.1
FT	71	71.8	142	57.0
FINN	146	59.5	292	53.7
GOT	64	53.1	128	42.2
OXD	135	52.5	270	40.0
WHM	22	46.6	44	34.0

4.7 LAMB PRODUCTION

Table 4.25 shows the total number and the mean of corpora lutea, eggs recovered (EGGR), eggs fertilised (EGGF) and lambs born from the observations considered. These results were obtained with variable dose levels of gonadotrophin between and within breeds. These data also include most of the animals treated and involve repeated treatment of some ewes.

Table 4.25 Total and Means of corpora lutea (CL),eggs recovered (EGGR),eggs fertilised (EGGF) and numbers of lambs born (LB).

Breed	No. ewes Treat.	CL		EGGR		EGGF		LB	
		Total	Mean	Total	Mean	Total	Mean	Total	Mean
DT	117	666	5.7	431	3.7	363	3.1	156	1.3
FT	58	384	6.6	218	3.8	214	3.7	117	2.0
FINN	71	587	8.2	405	5.7	356	5.0	181	2.5
GOT	32	139	4.3	103	3.2	92	2.9	36	1.1
OXD	101	552	5.5	376	3.7	322	3.2	122	1.2
WHM	30	134	4.5	78	2.6	50	1.7	15	0.5
Total	409	2462	6.0	1611	3.9	1397	3.4	627	1.5

CHAPTER V: DISCUSSION AND CONCLUSION

5.1. INCIDENCE AND DISTRIBUTION OF OESTRUS

Overall the breeds studied, the use of FSH-P and Folltropin resulted in 87% and 89% respectively of the ewes showing heat. In both the FINN and the GOT breeds all ewes came into heat. Other workers such as Torres and Cognie (1984) who used commercial FSH-P also observed a high oestrous response. These results are comparable with those where single injection of PMSG was used. According to the studies of Lamond (1964),Larsen (1971), Clark (1973) and Rangel (1986) treatment with PMSG can be considered satisfactory when 80% to 90% of the treated ewes came into heat. Thus, it can be assumed that the use of either FSH-P or Folltropin is as satisfactory as PMSG. Most of the ewes which did not show heat were grouped in the class of low ovulatory response (Table 4.2).

The distribution of the onset of heat suggested a difference among the two drugs used. It is not clear as to the reason for FSH-P causing an earlier occurrence of oestrus,but the relative proportions of LH and FSH might account for the difference.

Changes in ovulatory secretion of oestradiol have been shown as a consequence of LH infusion (McCracken et al.,1969) and after each LH pulse during the natural follicular phase (Baird,1978). If the repeated LH pulses induce bursts of oestrogen secretion, and these summate to produce a rise in plasma oestrogen concentration, these increased oestrogen levels will have two effects: 1) induce oestrous

behaviour and 2) reduce FSH secretion, since oestrogens are a component of the negative feedback on FSH in sheep (Goodman et al.,1981). Thus, if a commercial FSH-P product contains a higher level of LH than Folltropin, it, could induce, an earlier secretion of oestrogen and so cause earlier oestrous behaviour than in animals treated with Folltropin. The writer is unaware of comparative LH profile reported after FSH-P and Folltropin treatment.

In three of the breeds (DT,FINN,WHM) there was evidence of earlier oestrus being associated with the higher OR (Fig.2). Similar results have been reported by Torres and Cognie' (1984) and moreover Torres et al.,(1987) indicated that a higher OR corresponded with an oestrus time interval from sponge removal of only 24 h to 36 h. They also noted that when the interval between sponge removal and onset of oestrus was more than 48 h the ovulation rate was lower (5 vs 2). On the basis of these results it could be strongly suggested that animals with follicular activity, resulting in high ovulation rates, reach the levels of oestrogen that will bring them into oestrus earlier than in animals with low ovulation rate.

5.2.OVARIAN ACTIVITY

Because variable doses of FSH-P and Folltropin both within and between breeds were used for induction of superovulation, it was necessary to exclude many observations from the comparison of ovulation rate. When this was done it was clear that the two preparations gave a similar response. This result was in agreement with that of Tervit

(1989) using the same two preparations in Coopworth ewes. In all cases there was also no significant variation due to the dose levels used.

These results might be surprising considering that commercial preparation of gonadotrophin such as FSH-P differ in levels of LH hormone depending on procedure of manufacture (Chupin et al. 1984; Armstrong & Evans, 1984; Lindsell et al.,1986). FSH-P has a variable level of LH while Folltropin has a constant by weight (5%) quantity of LH (Tervit,1989). Control of FSH/LH ratio at 0.8 has led to improved superovulation response (Torres et al.,1977). Work with cattle has also shown that different FSH/LH ratios elicit differences in degree of superovulation (Chupin et al.,1984; Murphy et al.,1984).

The superovulatory response to commercial gonadotrophin will also be modified by the breed being treated. Thus, with cattle Donaldson (personal communication with Chupin,1986) has reported that Brahaman cows treated with FSH-W (woman,low content of LH) had a better response than those of European breeds. This is in agreement with the need for higher doses of LH observed in Charolais cows, compared with Fresian animals (Chupin et al.,1985). In the present work with six breeds of sheep it was impossible to compare the response to a standard dose of gonadotrophin, but it is suggested that different breeds might need different preparation for optimum results.

In contrast to the above (Cahill & Fry,1986) in their studies found that the ovarian responses varied greatly and showed no relationship to either FSH content, LH content, or LH:FSH ratio. The authors suggested that factors present in the follicular fluid can have

a direct effect on the ovary and therefore modulate the gonadotrophin effects, leading to the possibility that local factors may play an important role in determining the ovulation rate. It is probable that the "state" of the ovary at the time of stimulation, in terms of the content of follicular fluid or the number of large follicles and the sensitivity to the local factors influence the eventual ovulation rate. Several studies have shown that when there are many large follicles present at the time of gonadotrophin treatment the ovulation rate is low. The lack of significant variation between doses in the present study could be due to the dose level being already too high (26 to 38mg) in relation to an expected curvilinear response. Moreover, perhaps the difference among doses was not big enough to detect significant variation. In this regard Tervit (1989) found a significant linear increase in ovulation rate as level of FSH-P or Folltropin was increased over the range of 0, 8, 16, 24 mg. However, because the maximum dose was 24 mg, it is not possible to confirm the dose level above which no further significant increase occurred.

On the other hand Armstrong and Evans (1984) with a slightly bigger range of doses (18 to 27 mg),but overall lower then the one used in the present study, did not find any difference in the ovulation rate. An ovulation rate similar to the one obtained in this study has been reported by injecting ewes with 12 mg of FSH-P (Torres & Cognie,1984), 16 mg (Torres et al., 1987), 20 to 24 mg (Armstrong & Evans,1983), and 24 mg (Wright et al.,1981) involving several breeds. This could suggest that a reduction in the levels of drug used across the breeds might still induce superovulatory responses similar to those found in the present work. This is further supported by the fact that

there is an increase in variability of the response when FSH level is increased (Tervit,1989). Table 4.5 shows the OR among the breeds, but valid comparisons could not be made. However, FINN ewes had the highest OR and they are known to be a more prolific breed than the others. Whether a genotype x dose of gonadotrophin interaction might be present could not be tested.

5.3.RECOVERY RATE

The successful implemeantation of an ET programme will be aided by a good embryo recovery rate. None of the factors studied (drug, number of flushes, day of flush after oestrus, classes of ovulatory response significantly affected the recovery rate (Table 4.12 and 4.13). The results of 63% embryo recovery was somewhat low when compared with other work: 83% ,Tervit and Havik,1976; 73%, Cumming and McDonald,1967; 79%, Moore and Shelton, 1962; 80%, Trouson and Moore, 1974; 74%, Torres and Cognie',1984 and 80%, Tervit,1989.

Repeated surgery on the donors that might have coused the development of adhesions on the reproductive tract did not significantly affect the recovery rate in this work. This is in contrast to Torres and Sevellec (1987) who noted that with repeated surgery a significant reduction in the recovery rate occurred after the first flush (88% vs 52%). Because of this other techniques that could reduce the incidence of adhesions and consequently avoid reduction in recovery rate has been attempted (Mckelvey et al.,1986).

Likewise the day of flush after oestrus detection did not affect the percentage of embryos found (65% vs 64%). These results were expected, because it is known that normally at days 4 and later all the embryos are in the uterus (Killeen,1981; Torres & Sevellec,1988).

The recovery rate in relation to the class of ovulatory response showed that there was no statistically significant difference. Armstrong and Evans (1983) found that the number of embryos recovered increased progressively with increased ovulation rate when goats were treated with FSH-P and also Wright et al.,(1981) using FSH-P noted that embryo recovery did not decline in ewes responding with a greater than average number of ovulations. Previous studies with ewes (Betterige & Moore,1977) indicated that embryo recovery rate decreased as the number of ovulations increased. However, in that study PMSG treatment was used and perhaps the "hyperstimulation" of the ovary often caused by PMSG, can be considered as a likely cause of the decreased recovery rate.

5.4.FERTILISATION RATE

The time when AI was conducted was examined as a possible factor affecting fertilisation rate. In general, the onset of oestrus in superovulated ewes starts 24-36 h after removal of the progestagen treatment (Moore,1982). A similar pattern was observed in most of the animals in this study. Moreover, Whyman et al. (1979) reported a median time for the first ovulation of 23.6 h after the onset of oestrus in PMSG superovulated ewes. It is on the basis of these two

observations that the decision for the insemination time was suggested to be at 44-48 h after sponge withdrawal (Killeen et al., 1982; Armstrong and Evans, 1984; Evans et al.,1986; Hunton et al.,1986; Maxwell & Evans,1987;) In this study the insemination was carried out between 6 and 16 h after onset of oestrus (30 to 52 h from CIDR removal). According to these results animals inseminated at 6 to 9 h after the onset of oestrus did not show any decline in fertilisation nor did animals inseminated even at 16 h after the onset of oestrus. These results are comparable to other studies such as Ryan et al. (1984) where insemination at 24 h after sponge removal resulted in a fertilisation rate of 80%. A fertilisation rate of >90% had been obtained at 12 h from onset of oestrus (Wright et al., 1981). Insemination 24 h after detection of oestrus gave 94% fertilisation (Torres and Cognie,1984).

There was also no significant difference between first and second flushes in the fertilisation rate (86% vs. 84%; Table 4.15). Although it could be expected that adhesions might occur as result of surgery, in the present study a second surgery was still not enough to cause serious reduction in fertilisation rate.

Analysis of the two fertilisation techniques used (ie. intrauterine or in addition natural mating) did not show a significant difference (84% vs.89%; Table 4.15). On the other hand Tervit (1989) did not find any difference between hand mated and artificially intrauterine inseminated animals, provided the rams had good quality semen and good libido. Considering the general reduction in embryo recovery when intra-uterine artificial insemination is applied

(Robinson at al.,1989; Tervit,1989), hand mating should be taken into consideration, but because single sire mating may be required then this technique can be a limitation.

There was no significant effect of ovulatory response classes on the fertilisation rate, although a gradual increase seemed to follow when ovulation rate increased (table 4.15). These results are comparable with other studies where the presence of unfertilised eggs could not be attributed to excessive superovulation since these eggs were found more frequently in ewes with fewer corpora lutea (Torres and Cognie',1984). In addition in the work of Wright et al.,(1981) no decline in fertilisation rate was observed in ewes showing the highest ovulation rate.

5.5.QUALITY OF THE EMBRYOS

The use of Folltropin gave a higher percentage of good quality embryos in three breeds (DT,GOT,OXD). In the other breeds there was no effect of the type of gonadotrophin used. The better results with Folltropin have also been reported in other studies eg.Holstein cattle (Page et al.,1989) and McMillan (unpublished) with goats found a higher rate of embryo survival when Folltropin and FSH-P were compared (69% vs 51%). Reasons for the improvement in the quality of the embryos are unknown, but events that occur leading to fertilisation must be involved. Donaldson et al.,1984 have shown that LH imbalance may result in disturbance of normal oocyte and follicle maturation and consequentely in poor ovum quality and reduced fertilisation rate.

Reference has been made earlier to the different ratios of FSH/LH in the gonadotrophin preparation used and the effect that this might have on oocyte development and formation of the embryo. Better control of the level of LH might therefore yield a higher incidence of good quality embryos. However, with different breeds there might be different requirements for LH or FSH to yield high quality embryos. Considering the data from all the breeds it was evident that there was no consistent relationship between quality of the embryos and dose of gonadotrophin. This is in contrast to work of Donaldson (1984) with cattle in which a reduction from 5.9 to 2.7 of transferable embryos occurred when FSH-P was increased from 28 mg to 60 mg.

In the present study improved embryo quality frequently occurred with animals which had the highest superovulation response. It is difficult to suggest a reason for this observation, but it is in agreement with those of Torres & Cognie, 1984, Cognie et al., 1986, and Page et al., 1989.

Comparison of embryos recovered at day 5, 5.5, 6 and 6.5 after oestrus shows that after day 5 there was an increase of percentages of good quality embryos in almost all the breeds. This finding is in agreement with that of Donaldson (1986) where the frequency of transferable embryos were found to increase with the later date of flush.

5.6 PREGNANCY RATE AND SURVIVAL RATE

Transplantation of embryos into either the Coopworth or Romney recipient ewes did not affect the incidence of pregnancy and the survival rate. This finding is similar to results of Larsen (1971) and Hanrahan (1979) who showed that the genotype of the recipient did not affect survival rate of the embryos. However, in the case of the embryos from FINN ewes the pregnancy ($P<0.01$) and survival rates ($P<0.001$) were significantly affected by the breed of the recipient when embryos were transplanted into Coopworth rather than Romney ewes (pregnancy rate 69 vs 47; survival rate 65 vs 38). The survival rate of the embryos from OXD ewes was also significantly ($P<0.05$) affected by the breed of the recipient ewes.

Lawson and Rowson (1972) and Bradford et al.,(1974) came to the conclusion that Finnish Landrace ewes tended to maintain larger litters more regularly than did less fecund breeds. On this basis it might be assumed that embryos coming from FINN and OXD ewes also might need a prolific recipient breed more than embryos from less prolific breeds. If this is the situation this might indicate an effect because of higher progesterone production (associated with more corpora lutea) or the uterine enviroment is more suitable in the prolific breed.

Variation in the number of CL in the recipients (1 or 2) did not significantly affect pregnancy and survival rates in any of the six breeds. This result is in agreement with other studies (Moore et al.,1960; Cumming & McDonald,1970).

However, progesterone supplementation has sometimes been reported as helpful in improving embryo survival. In the present study higher pregnancy and survival rates were found in CIDR-treated animals carrying DT,FT and OXD embryos (Table 4.22 and 4.23). Not all the results of studies with progsterone supplementation confirm this (Peterson et al.,1984; Davis et al.,1985; McMillan et al.,1987; Walsh, 1989). However it should be emphasized that in the present work the supplementation was after embryo transfer and perhaps exogenous progesterone is more effective because of the more stresfull condition of the embryo and the surrogate mother.

The succes of ET was affected by the interval between collection and transfer for DT and WHM embryos (Table 4.22 and 4.23). As most of the embryos were reinserted into the uterus of the recipient within four hours from collection it is apparent that the embryos were tolerant to this relatively short period outside of the reproductive tract. Thus, the present study confirms the results of earlier work of Averill,(1956) and Moore & Shelton,(1962). The significant effect of transplantation time on pregnancy rate in DT and WHM indicate that the storage time should be kept to a minimum.

5.7 LAMBS PRODUCED

The 409 treatments administered resulted in an average of 1.5 lambs born per donor. Other workers have reported similar results, ie. 1.6 lambs Armstrong & Evans (1983); 2.0, Armstrong & Evans (1984); 1.6, Walker et al. (1986); 1.7, Torres et al. (1987). The succes of

an ET programme will depend upon many factors, but the ovulation rate in conjunction with a high rate of recovery of fertilised eggs is very important. The literature shows that several types of gonadotrophins have been used and variable ovulatory responses obtained. However, if a 60% survival rate is assumed, it has been calculated that 2.1 lambs could be produced per treatment (Tervit,1989). In the present programme several animals were treated on more than one occasion. It could be expected that with repeated embryo collections more embryos would be produced in total, but at the second and later flushes a reduction in the recovery rate and fertilisation rate is quite likely. This was not the case with the present work; instead a low recovery rate occurred throughout all the programme and the survival rate was lower than the 60% calculated by Tervit (1989). However, the general conclusion is that this ET programme was moderately succesfull expecially considering the large number of animals involved and that some of the personel were being trained as the programme progressed.

It is appropiate to compare the number of lambs produced in this ET programme (1.5 lambs/treatment) with that possible if other multiplication procedures had been employed. Accelerated lambing systems have been reported to be successful with selected breeds (Gordon,1983). For example Foster et al. (1977) has reported 1.8 lambs weaned/ewe/year and Thimonier and Cognie,(1977) 1.8 and 2.2 lambs/ewe/year using methods of hormonal treatment, indoor lambing and early weaning. Other methods which are much cheaper to apply include mild superovulation in the normal breeding season (26% increase in lambing rate, Robinson,1980) and steroid immunisation (25% increase in lambing rate, Smith et al.,1985). The advantage that ET has over these

methods is that in addition to the lambs born to the surrogate mothers the donor itself can also produce one or more lambs in that same season. In this project this option was not chosen and the animals were prepared for a further series of embryo recoveries early in the next season.

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APPENDIX

2.8. BREEDS DESCRIPTION

2.8.1. Texel

This breed has its origin in the island of Texel in the Netherlands and it is known around the world especially in lamb-producing countries. The breed is hardy and is frequently kept outdoors even in the harsh Northern European winter.

Many studies have been carried out to evaluate the performance of this breed. Latin and Owen (1980) compared the performance and carcass composition of Texel- and Suffolk-sired lambs from Finnish Landrace x Dorset Horn ewes. It was found that Texels had significantly heavier carcasses (17.2 vs 16.4 Kg) and significantly higher dressing percentages (50.7 vs 49.2) than Suffolks. Texel-cross carcasses had 3% more lean meat, but 4% less total fat. This could indicate the suitability of Texel-sired lambs for intensive lamb production. However, trials by Wolf et al.,(1980), although confirming the data of Latin and Owen (1980), showed that the Texel cross lambs grew more slowly than cross lambs of other breeds such as the Oxford Down and Suffolk.

There is little published information on the reproductive potential of Texel ewes.Hanrahan (1979) reported a mean of ovulation rate of 1.77 which would be expected to yield a litter size of 1.56.

Comparative aspects of the fecundity (No lambs born/No ewes mated) of Texel and Texel-cross ewes are reported by Hanrahan,1977. Results from this study suggest that Texel pure breed increases fecundity relative to Galway pure breed. It was indicated that the Texel should be 0.1 to 0.2 better than the Galway for ovulation rate or litter size.

Performance of superovulated Texel ewes has been tested by Hanrahan and Quirke (1977). In this trial the superovulatory hormone was PMSG (1000 i.u. to 1200 i.u.). The mean number of corpora lutea was 7.6 , the number of eggs-recovered per ewe 4.5 and the fertilisation rate 84%.

2.8.2. Finnish Landrace

The Finnish Landrace is a member of the Scandinavian short-tailed family of sheep. There is some evidence of relationship to another higly fertile breed, the Russian Romanov. Originally there where two types of sheep in Finland: one was a small inbred animal weighing only 20 to 40 Kg at maturity; the other was much bigger, with mature ewes weighing between 40 to 50 Kg (Eastwood et al.,1978).

In recent years, the Finnish Landrace breed has been succesfully used in improving the reproductive performance of many sheep breeds (Land and McClelland,1971; Terril, 1974; Timon,1975). Finnish Landrace x Dorset Horn ewes are now well known for their early puberty, high fertility and fecundity (Robinson and Orskov,1975). They are, therefore,suitable for breeding out of season and frequent lambing (Land and McClelland,1971), i.e for the very intensive systems of lamb production.

Latin and Owen (1979) reported that the fertility and prolificacy of Finnish Landrace x Dorset Horn ewes were 95% and 190% respectvely, and were not affected by sire breed. It can be assumed that this high prolificacy is attributable to Finnish Landrace. Moreover Hanrahan (1973) reported a litter size of 2.5 lambs for purebreed Finnish Landrace ewes.

The performance of superovulated Finnish Landrace ewes has been evaluated by Quirke and Hanrhan (1973). PMSG (1000 i.u. to 1500 i.u.) was used as superovulatory hormone and the mean number of corpora lutea was 7.3 and 10.5 respectively for each of the doses used.

2.8.3. Gotland-Pelt

The most common breed in Sweden is the Swedish Pelt sheep, to which about 60% of the total number of ewes in Sweden belong. Swedish Pelt sheep originate from the island of Gotland in the Baltic and the breed belongs to the North European short-tailed group of sheep. Nowadays the Pelt-sheep are all over Sweden and to some extent also Denmark and Finland. There are also small populations of this breed in Great Britain, France and Holland. Adult rams weight 75-90 Kg and adult ewes 55-70 Kg. The lambs are slaughtered when they are 5-6 months old. The average carcass weight is about 15-16 Kg.

The lamb skins are prepared and sold at auctions. The skins are used for fur jackets which are sold both in Sweden and abroad under the trademark "Viking Lamb". Nowadays all Peltsheep are grey with a black head and black legs.

The breeding season is resticted to the autumn and most of the lambs are born in April. The ewe lambs are mated in their first autumn and about 95% of them lamb when they are one year old (Brasch,1982). The Swedish Peltsheep have high fertility. The average litter size at birth is 1.86 and for one-year old ewes 1.22 (Brasch,1982).

2.8.4. Oxford Down

This is the largest of the Down breeds. The Oxford Down has Cotswold blood. The large, prolific Cotswold were crossed with Hampshires,and the progeny interbred to produce the Oxford Down, which look like big Hampshires.

By repute this breed is the largest (mature animals 98 Kg) and fastest growing of all British breeds. More O'Ferral and Timon (1977) showed that Oxford Down sire impart rapid early growth rate to their progeny and as a result could be racommended as sires for early lamb production. However, this breed compared with Suffolk or Hampshires seems to have heavier bones, longer than average legs and bigger carcasses, factors indicating poorer carcass conformation.

Oxford Down are prolific and good milkers, and their fecundity is high, matching that of Border Leicester(Eastwood, 1978). However More O'Ferral and Timon (1977) found in a comparison with other breeds that Oxford Down had 4% higher perinatal mortality than Suffolk-cross lambs.

2.8.5. White Headed Marsh

This breed originated in the area of the North Sea marshes of West Germany. The breed originated from crossings in the mid-19th century between the North German Marsh sheep, the local milk sheep and various imported British longwool breeds, including the Cotswold. The average weight of an adult male is around 109 Kg and of a female 84 Kg. Lambs will reach weights of 63 Kg at six months of age. The fleece weight of the adult ewes is 5.4 Kg and 7.5 Kg in adult rams. This breed can be used through crossbreeding to increase the size and fecundity of breeds of small size and low reproductive performance. Moreover this breed performs exceedingly well in very wet conditions.

The White Headed Marsh is more fecund than Romney sheep with a lamb drop of around 180% (Performance data from Sheep 88).