Viability of frozen sheep embryos and semen imported from Europe

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ABSTRACT

In 1984 selected Oxford Down (O) and Texel (T) sheep in Denmark and T and Finnish Landrace (F) sheep in Finland were purchased or leased and then assembled for treatment. The ewes were synchronised and superovulated before being subjected to surgical embryo recovery. Recovered embryos with at least 8 cells and an intact zona pellucida were then deep frozen. The mean ovulation rate, number of eggs recovered and embryos frozen from the O and T in Denmark were 5.4, 4.4, 1.5 and 7.1, 5.0 and 3.0, respectively. Results for T and F in Finland were 7.5, 4.9, 4.1 and 10.0, 7.0 and 5.8, respectively. A total of 548 embryos was imported into New Zealand. Two-hundred and twenty embryos were thawed and transferred to Coopworth recipients in March and 46 in May 1985. The percentages of embryos surviving to term for the various breeds in March and May were: Denmark O, 43 and 20; Denmark T, 59 and 7; Finland T, 59 and 55; Finland F, 64 and 50, rcsepctively. Thirty-two Coopworths were inseminated with thawed F sperm deposited via endoscopy and 24 (75%) lambed producing 28 lambs. The importation of both embryos and semen was very successful.

Keywords Sheep; Finnish Landrace; Oxford Downs; Texel; importation; frozen embryos; semen

INTRODUCTION

The New Zealand sheep industry has traditionally been largely based on one breed, the Romney. This breed has high fleece weights but only mediocre fertility, milk production and lamb growth rate. Numerous overseas breeds are known to be far superior to the Romney and local breeds for fertility or growth rate and since it can be argued that the genetic base of the local sheep industry should be broadened, 2 attempts have been made by the Ministry of Agriculture and Fisheries to import exotic sheep breeds. The first occurred in 1972 when Finnish Landrace (F), East Friesian, German Whiteheaded Mutton and Oxford Down (O) sheep were imported from Britain. Unfortunately scrapie was diagnosed in some animals and so all the imported animals and their descendants were slaughtered. The second importation occurred in 1984 and involved introducing frozen embryos collected from O and Texel (T) animals in Denmark and T and F animals in Finland. Frozen F semen was also introduced. This paper describes the procedures used and the results from this recent importation.

MATERIALS AND METHODS

Selection of Ewes and Rams

Denmark

Both the O and T breeds when established in New Zealand are most likely to be utilised as terminal sire breeds and so ewe growth rate and lean growth rate should have been important selection criteria. However, since no objective information was available on ewes little selection pressure could be put on these characters. Factors considered were reproductive performance, pedigree, size and structural faults. A central performance test for ram lambs has been operating in Denmark

since 1979. Thus O rams selected were above average for growth and loin eye area and below average for fat thickness. In the case of the T rams more emphasis was put on growth rate.

The Danish ewes (46 O; 47 T) and rams (14 O; 11 T) were leased by the New Zealand government.

Finland

Two different samples of F sheep were obtained. One was from industry flocks which were members of the national performance recording scheme. Ewes selected (29) were structurally sound animals with good fleece characters and as high a breeding value for reproductive performance as could be obtained. Rams selected (9) were genetically superior animals for reproductive performance based on the performance of dams, maternal granddams and paternal granddams. The second sample was from the flock maintained at the Kuuma Agricultural Research Station. Here ewes (18) with superior genetic merit for growth and conformation but average reproductive performance were selected. Rams (8) were chosen from Kuuma stock and also from central performance tested ram lambs.

The T ewes and rams were selected from 1 large flock (200 to 250 ewes). The ewes (23) were selected for reproductive performance and freedom from structural faults and the rams (5) for size, structural soundness and fleece characteristics.

With the exception of the Kuuma F's, all the sheep were purchased by the New Zealand government.

Animal Housing

After being tested negative for maedi visna, chlamydia and brucella ovis, animals in Denmark were assembled at a barn approximately 30 km from Copenhagen. Animals in Finland were assembled at a barn approximately 7 km from Jokioinen. In both countries they were housed in the barns until the end of the treatments. The Danish treatments began in late August 1984 and finished in late October. The Finnish treatments began in late September and finished in late November.

Ewe Hormone Treatment

Controlled internal drug release devices (CIDRs; Alex Harvey Industries, New Zealand) were inserted into all donor ewes for 15 or 16 days. Ewes were checked daily for CIDR loss and, except where device loss occurred within 1½ d of planned removal, new devices were inserted. The synchronised oestrus after device removal was detected by harnessed vasectomised rams and ewes were then superovulated by an intramuscular injection of pregnant mares' serum gonadotrophin (PMSG; Intervet, Holland) administered 12 d after oestrus. Ewes which failed to show oestrus after CIDR removal were treated with 125 μ g Estrumate (ICI, England) and superovulated 12 d after the induced oestrus. The dose of PMSG administered was based on superovulation results from the same breeds treated in New Zealand (Tervit *et al.*, 1976) and Europe (Hanrahan and Quirke, 1982). The ewes in each country were divided into 3 groups and the groups were treated over 3 consecutive weeks. Some ewes in the first group were subjected to endoscopy about 3 d after the PMSG-stimulated oestrus to determine ovulation rate. The results obtained were used to adjust the dose of PMSG administered to subsequent animals. About 3 d after surgery all ewes were injected with 500 μ g Estrumate to abort any unrecovered embryos. The subsequent oestrus was detected with vasectomised rams.

Donor Entire Mating

All rams were electroejaculated and their semen samples examined before they were used to mate their allotted superovulated ewes.

Denmark

The Danish matings in the first 2 weeks involved placing each donor with her harnessed entire ram immediately after PMSG injection. The ram and ewes (maximum 1:2) were then run together with matings being observed at least twice daily (0800 and 1600 h) until the ewe(s) ceased to mate. Egg fertilisation using this mating system was poor and in the third week ewes were placed with vasectomised rams after PMSG injection. Oestrus was observed twice daily and the ewes were placed with their entire rams at the oestrus observation subsequent to the one at which they were first observed in oestrus. The rams were observed to actually penetrate their ewes.

Finland

The donors were placed with vasectomised rams after PMSG and oestrus was recorded 3 times daily (0800, 1500 and 2100 h). At the oestrus observation subsequent to the one at which she was first observed in oestrus each donor was placed in a mating pen with her allotted ram and observed to mate twice. She was then removed from the ram and reintroduced at each subsequent oestrus recording until she would not mate.

Surgery

Surgery was conducted 5½ to 7 d after onset of the entire mating oestrus. At surgery the ovarian response was recorded and the eggs recovered from the uterus using a uterine flush technique (Tervit and Havik, 1976). The flushing medium was warm (37 °C) enriched Dulbecco's phosphate-buffered saline (PBS; Tervit *et al.*, 1972).

Embryo Manipulation and Freezing

Embryos were located in the flushing medium and then carefully examined to determine the state of the zona pellucida. Embryos with cracked zonas were rejected as were embryos of less than 8 cells. The remaining

embryos were washed 10 times in small volumes of PBS with the glassware being changed between each wash. They were then frozen using ethylene glycol as cryoprotectant. The method used has been published (Tervit and Goold, 1984) and basically involved a 3-step addition of cryoprotectant (0.5, 1.0, 1.5 M), sealing in ampoules and freezing in a Planer freezer (Planer Products, UK). The programme used was -1 °C/min from ambient to -7 °C at which temperature the ampoules were seeded, -0.3 °C/min to -35 °C, -0.1 °C/min to -38 °C and then plunging into liquid nitrogen.

After appropriate disease and customs clearance the frozen embryos were flown to New Zealand.

Embryo Thawing and Transfer

Embryos were thawed on Somes Island Quarantine Station during March and May 1985 and transferred to Coopworth ewes which had had their oestrus synchronised with medroxyprogesterone acetate (MAP, Upjohn, 70 mg) intravaginal sponges inserted for 14 or 15 d. The surgical transfers occurred approximately 6 d after the synchronised oestrus and each ewe usually received 2 embryos, one transferred into each uterine horn.

The thawing method has been published (Tervit and Goold, 1984) and basically involved thawing in a 37 °C water bath, recovering the embryos and removing the cryoprotectant in one step by placing them into PBS containing 0.5 M sucrose, and finally transferring the embryos into PBS.

Recipient ewes were run with harnessed vasectomised rams after transfer and return oestrus dates recorded. They were examined for pregnancy by real-time ultrasound between 49 and 91 d after surgery and non-pregnant animals slaughtered. Pregnant animals lambed on the island and full lambing data were recorded.

Importation and Insemination of Semen

Semen from 4 F rams was frozen in a Tris-based diluent in 0.25 ml straws in Finland. Each straw contained 200 x 10^6 sperm. The straws were transported by air to Somes Island where some straws were thawed (37 °C for 12 seconds) and used to inseminate 32 Coopworth ewes in May 1985.

The ewes were synchronised with CIDRs inserted for 14 d and at the oestrus subsequent to the synchronised one were inseminated using an endoscopic technique (Tervit *et al.*, 1984). Prior to insemination the ewes were fasted and run with harnessed vasectomised rams and were examined twice daily (0800 and 1600 h) for mating marks. Ewes marked between successive 0800 h recordings (i.e. over a 24 h period) were inseminated at 0900 on the morning they were found marked. In preparation for insemination each ewe was tranquilsed, loaded onto an endoscopy cradle, her belly prepared and her abdomen inflated with CO_2. The reproductive tract was viewed through an endoscope and 0.05 ml semen containing 40 x 10^6 sperm was injected through a glass pipette into the lumen of each uterine horn.

The ewes were run with vasectomised rams and return oestrus dates recorded. They were examined for pregnancy about 49 d after insemination and pregnant ewes were followed through to lambing.

Treatment of Data

The animals in the study were treated to maximise the number of embryos frozen. This meant that modifications were frequently made to treatments and therefore that the data are difficult to analyse. Thus the results presented in this paper have been subjected to minimal statistical analyses and are presented as a summary of results obtained.

RESULTS AND DISCUSSION

The loss rate of CIDRs from ewes in Denmark was very low (O, 2/46; T, 1/47) and was also low for Ts in Finland (1/23). However, 18 of 47 F ewes (38%) lost their devices. Fourteen of these animals had replacement devices inserted (one animal had 3 replacement devices inserted, one 2 devices and 12 one device). The 4 ewes which did not receive replacement devices lost CIDR's within 0.5 to 1.5 d of planned removal time. It is presumed that the high loss rate in the F ewes was largely due to their advanced age and resultant large vaginas.

The oestrous response of the ewes to CIDR treatment is shown in Table 1. The data are only from donors which had their devices removed at 1600 h on their removal day. This deletes some animals from the data as their devices were removed at 0800 h. The synchronised Danish O and T ewes showed oestrus at a similar time after device removal (39.8 and 42.7 h after removal, respectively). The T ewes treated in Finland showed oestrus far earlier than their Danish counterparts (26.2 h) and the F ewes showed the earliest oestrus of all (20.5 h). Higher fecundity breeds often show oestrus earlier after synchronisation than lower fecundity breeds (Eastwood and McDonald, 1975) and so the F result was not unexpected. The difference in onset of Ts in the 2 countries was unexpected and could be due to seasonal or environmental effects. Synchrony of oestrus was very satisfactory in the ewes treated in Finland and was satisfactory in Denmark.

The Danish donor mean ovulation and embryo responses for the various treatment weeks are shown in Table 2 and the Finnish responses in Table 3. The doses of PMSG used for each breed were chosen to produce, on average, 7 to 8 ovulations per donor. The O donors did not ovulate particularly well and this was largely

TABLE 1 Oestrous response of ewes treated with CIDRs.

	Breed	No. treated	No. showing oestrus by various intervals (h) after CIDR removal						No. not synchronised
			0-15	16-24	25-39	40-48	49-63	64-72	
Denmark	O	30	—	2	12	6	5	1	4
	T	33	—	1	9	12	2	3	6
Finland	T	23	3	6	11	—	1	—	2
	F	42	10	18	10	1	—	—	3

TABLE 2 Denmark donor ovulation and embryo results.

Breed	Week No.	No. ewes (a)	No. ewes (b)	Mean dose PMSG (i.u.)	Ovulations	Mean no. Eggs	Mean no. Embryos	Embryos frozen	Total embryos frozen
O	1	14	14	1600	7.9	6.6	2.4	1.7	24
	2	16	13	1600	4.8	4.0	1.4	1.3	17
	3	16	16	1600	3.8	2.8	1.4	1.4	23
		46	43		5.4	4.4	1.7	1.5	64
T	1	11	11	1550	8.8	5.9	2.3	1.6	18
	2	17	16	1875	7.9	5.3	4.3	3.2	51
	3	19	19	1400	5.4	4.2	3.6	3.5	67
		47	46		7.1	5.0	3.5	3.0	136

a Superovulated
b Operated on

TABLE 3 Finland donor ovulation and embyro results.

Breed	Week	No. ewes (a)	No. ewes (b)	PMSG dose (i.u.)	Ovulations	Mean no. Eggs	Mean no. Embryos	Embryos frozen	Total embryos frozen
T	1	8	8	1500	8.1	4.0	2.9	2.9	23
	2	8	8	1500	6.9	6.5	5.9	5.6	45
	3	7	7	1500	7.6	4.0	4.0	3.9	27
		23	23		7.5	4.9	4.3	4.1	95
F	1	15	15	1200	6.3	4.6	4.6	4.4	66
	2	15	15	1500	9.3	6.9	5.1	4.8	72
	3	17	14	1500	14.6	9.7	8.4	8.2	115
		47	44		10.0	7.0	6.0	5.8	253

a Superovulated
b Operated on

due to a fall in ovulation rate over succeeding weeks using the same dose of PMSG. Reasons for this are unknown. It was anticipated from overseas reports (Hanrahan and Quirke, 1982) that T ewes would be difficult to superovulate and so in Denmark 2 different PMSG batches were used. The doses given were adjusted after the first weeks treatment on the basis of endoscopy of a few donors prior to surgery and on their final ovulation rate recorded at surgery. The adjustments did not always produce the desired effect (Table 2). Overall though, the Danish T ovulation rate was close to that desired. Both breeds in Finland superovulated well (Table 3) with the Fs as expected (Hanrahan and Quirke, 1982) giving high ovulation rates from relatively low doses of PMSG. The egg recovery rate was very satisfactory for the Os (81%) but was lower than expected for the F and T donors. This could perhaps be due to the large size of the T and particularly the F uteri which meant that the flushing medium had to perfuse a large tract volume.

Very real difficulties were experienced with the entire mating of O donors. This was largely due to poor ram performance. In general they had poor libido and only 9 of 14 available rams successfully mated. Ram age may have contributed to this problem since 3 of 6 ram lambs did not mate compared to 2 of 8 older rams. Fertilisation rates were very poor in the ewes which mated (40% of recovered eggs fertilised; Table 2). Reasons for this are unclear. One explanation during the first 2 weeks could have been that the rams, since they were running continuously with the ewes, were exhausting their libido and/or sperm reserves early in oestrus and therefore depositing few sperm about the time of ovulation. The results of the altered mating system in the last week suggests that this may partly explain the problem since for the first time more fertilised than unfertilised eggs were recovered (51% eggs fertilised *v* 35% and 37% for surgery weeks 3, 2 and 1, respectively). The Danish T rams performed well with all rams mating successfully. Even so, egg fertilisation rate (71%; Table 2) was lower than desired. This was largely due to poor fertilisation rates in some donors with high ovulation rates. The mating system used in Finland was very successful with high egg fertilisation rates being achieved in both breeds (T, 88%; F, 85%; Table 3). As a corrollary, the good result was achieved at the expense of considerable time and effort. Low fertilisation rates are often recorded when ewes are superovulated (e.g. Tervit *et al.*, 1976) and the results from the present trial must be considered satisfactory.

The donor hormone regime was designed to give an average of 4 embryos frozen per donor treated. This was achieved for both breeds in Finland (Table 3) but for neither breed in Denmark (Table 2). However, not all the embryos frozen in each country were at the desired stage for freezing (i.e. tight morula to expanded blastocyst) or of good quality. For Denmark the numbers of poor quality and/or incorrect cleavage stage embryos frozen were: O, 15 (23% of embryos frozen); T, 22 (16%) and for Finland ewes were: T, 18 (19%); and F 33 (13%). It is anticipated that these embryos will give reduced survival post-thaw.

The actual freezing technique gave no problems and a total of 548 embryos was imported into New Zealand. A total of 221 embryos were thawed and 220 transferred to recipients in March 1985 and 47 thawed and 46 transferred in May 1985. The 2 untransferred embryos were not recovered from the ampoules after thawing. The survival of the embryos to birth is shown in Table 4. The results from the March thaw were very satisfactory. The overall embryo survival was similar to that recorded by us during development of the technique (Tervit and Goold, 1984) and substantially better than reported by others (e.g. Willadsen *et al.*, 1977). It was anticipated that the May thaw results would be poorer than in March since the transfers were into recipients which, because they failed to conceive to the March transfers, may have been of lower fertility. Also, in the case of the Os, poorer quality embryos were transferred. The poor survival of T embryos from Denmark was very surprising. The embryos were of good quality before and after freezing and the recipients were of the same quality and stage of the oestrous cycle as those used for the other breeds. Overall though, the results of this first year's thaw were very satisfactory. The remaining embryos will be thawed in 1986 and, because more poor quality and/or incorrect cleavage stage embryos will be transferred, it is anticipated that embryo survival will be lower than recorded to date.

Twenty-four of the 32 Coopworth ewes (75%) subjected to the endoscopic insemination lambed producing 28 Finn-cross lambs. This result is most satisfactory and again demonstrates the effectiveness of this technique for enabling high lambing rates to be achieved from low numbers of frozen-thawed sperm. The technique makes the use of frozen semen feasible since the results are far superior to those achieved by us when thawed semen is deposited via the vagina into the cervix (Tervit *et al.*, 1984).

Provided the animals which result from this importation of embryos and semen do not develop any undesirable diseases, the importation of the T and F genotypes must be considered very successful. Fewer O embryos than desired were imported and hence a narrower range of genotypes than planned is available for multiplication and release to the industry. Overall though, the importation demonstrates that satisfactory results can be achieved when the relatively new techniques of embryo freezing and intrauterine insemination are used to import new sheep genotypes.

TABLE 4 *Survival of thawed embryos.*

Thaw month	Source	Breed	No. embryos Frozen	No. & (%) lambs born Transferred	from embryos frozen
March	Denmark	O	40	40	17 (43)
		T	64	64	38 (59)
	Finland	T	37	36	22 (59)
		F	80	80	51 (64)
			221	220	128 (58)
May	Denmark	O	10	10	2 (20)
		T	16	15	1 (7)
	Finland	T	11	11	6 (55)
		F	10	10	5 (50)
			47	4o	14 (30)

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