MOET in ultrafine Merinos: An experimental evaluation

T. WULIJI, J. ASPINALL[1], J.T.J. LAND[2], G.H. SHACKELL, K.G. DODDS, R.N. ANDREWS AND J. ROGERS[2]

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand.

ABSTRACT

Results of an experimental MOET programme in elite ewes selected from within an ultrafine Merino flock are presented. Of the treated ewes, 60% provided transferable donors with a mean of 16.7 ovulations and 10.4 embryos recovered, 7.8 embryos transfered, 3.9 lambs born and 3.1 lambs weaned per donor ewe. The wool produced by hoggets from this elite embryo transfer group was 0.9μm ($P<0.01$) finer than that from their contemporaries in the ultrafine selection flock and 2.5 μm ($P<0.01$) finer than a random Merino control group. The improvement in fibre diameter was better than expected from the selection differentials, but not significantly so. The h^2 estimates for liveweight, clean fleece weight, fibre diameter and yield in three flocks of fine woolled Merinos were 0.50, 0.32, 0.39 and 0.58 respectively. The MOET programme was successfully applied to increase the number of progeny from elite ultrafine Merino ewes.

Keywords: ovulation; embryo transfer; ultrafine; fibre diameter; Merino.

INTRODUCTION

While there are considerable advantages to exploiting genetic superiority of individual Merino ewes with ultrafine fibre, natural reproductive constraints such as seasonality and low (70%) lambing rates limit the contribution to genetic gains. Most genetic progress is therefore made through ram selection. Although some advanced technologies such as Multiple Ovulation and Embryo Transfer (MOET), *in vitro* fertilization and cloning are available, their acceptance is often limited by cost and variable success rates. The present case study summarises an experimental MOET programme conducted on the ultrafine Merino research flock at the AgResearch Tara Hills High Country Research Station, Omarama. The programme was undertaken in three consecutive years, commencing in 1991. The aim was to establish an elite line of ultrafine Merino ewes, with a mean fibre diameter below 16.5 μm, for research and commercial development.

MATERIAL AND METHODS

Animal selection

Three to five rams and 15-20 Merino ewes from an ultrafine Merino flock, established at Tara Hills by screening commercial flocks (Wuliji *et al.*, 1990) were used in a MOET programme each year from 1991-1993. Ewes with progeny whose fibre diameter was consistently below 16.5 μm as hoggets were selected as donors. The sires were similarly chosen for the low fibre diameter of their progeny. Recipients (n=4-8 per donor) were mature Merino ewes which had previously successfully reared a lamb.

MOET procedure

Oestrus in the donor ewes was synchronised by treatment with an intravaginal progesterone releasing device (CIDR-G®; Carter Holt Harvey, Hamilton, NZ). CIDRs were inserted for 11-13 days and replaced with fresh CIDRs on day 8-10. In the first two years donor ewes were superovulated by i.m. injection of an equal dose of ovine FSH (Ovagen; ICP, Auckland, NZ) twice daily for four days (totalling 1.8 units) with a single injection of 300 iu Pregnant mareís Serum Gonadotrophin (PMSG) (Folligon; Intervet) given at the first injection and CIDRs removed at the time of the 7th injection. In 1993, donor ewes were treated with a series of 6 reducing doses (totalling 24mg) of FSH-P (Schering Corp, USA) injected i.m. at half day intervals during the last 3 days of CIDR treatment. A single i.m. injection of 200 iu PMSG (Pregnecol; Horizen Technology Limited) was administered at the same time as the first FSH-P injection. In all years recipients were synchronised for 11 -12 days with CIDRs, withdrawn so that oestrus occurred at the same time as in the donor ewes. Donors were joined in a single sire 'pen mating' system and embryos were collected on Day 7 following mating. Embryos were recovered by a surgical technique (Tervit and Havik, 1976) or a modification of the laparoscopic technique described by McKelvey *et al.* (1986). Embryos were examined under a light microscope to determine their suitability for transfer. One or two embryos were transferred to each recipient ewe by a minimal surgical technique. The tip of a uterine horn ipsilateral to *corpus luteum* was exteriorised and the embryo(s) introduced into the horn towards the utero-tubal junction. All donor ewes were injected with 125μg of prostaglandin (Estrumate; Coopers Tierarzneimittel, Germany) following embryo recovery and naturally mated.

Records and measurements

Ovulation rate (OR), number of embryos recovered (NER), number of embryos transfered (NET), number of lambs born (NLB) and number of lambs weaned (NLW) per donor ewe were recorded. Progeny were individually identified at birth and pedigree, birth date and rearing rank, birth weight, and weaning, autumn (ALW), spring (SLW), and

[1]Woodlands Research Station, Woodlands, New Zealand.
[2]Tara Hills High Country Research Station, Omarama, New Zealand.

summer liveweights and greasy fleece weights (GFW) were recorded. Midside fleece samples were measured for clean fleece weight (CFW), yield (%, at 16% moisture) and mean fibre diameter (FD) (LaserScan, NZWTA). Performances were compared between the elite embryo transfer group (ET), ultrafine 'closed' breeding flock (UF) and the control flock (C) described by Wuliji *et al.* (1990). The UF and C flock ewes were mated naturally within the selection program. All flocks were grazed and managed together except when ewes were drafted into their respective sire groups for lambing. In addition, ET progeny born in 1991 or 1992 and their C and UF contemporaries were monitored for adult SLW, CFW, Yield FD and lambing performance in two consecutive years.

Statistical analyses

The reproductive variates were analysed over years by one-way analysis of variance. The adult ewe performances were analysed by two-way analysis of variance including interactions, with age/year as one factor and flock group as the other. The production traits (liveweight, fleece weights and wool characteristics) from birth to one year of age were analysed by residual maximum likelihood (REML; Patterson and Thompson 1971) with a model that included year, group, sex, birth/rearing rank, dam age (2, 3 or 4 and older), and year by sex interaction as fixed effects, birth date as a covariate and sire as a random effect. Paternal half-sib heritability (h^2) estimates were obtained from the same set of analyses, which included progeny of 44 sires from either the three flock groups described above or those from an ultrafine 'open' breeding flock.

To investigate whether there was an effect of MOET *per se* (rather than through increased selection intensity) on FD, a genetic evaluation, incorporating MOET status as a factor, was performed. The genetic evaluation consisted of an animal model BLUP, performed with PEST (version 3.1; Groeneveld and Kovac, 1990) from the data described above, except that the control flock was omitted, and animals born from 1988 onwards were included. The analysis assumed a heritability of 0.5 for FD, and included the same fixed effects as in the REML model described above, except that the group effect was MOET vs natural, and birth date effects were allowed to vary across years. The BLUP analysis allows the effect of MOET, adjusted for the genetic contribution of the parents, to be calculated.

RESULTS AND DISCUSSION

Among ewes in the ET group the OR was highest ($P<0.05$) in 1991, NER was lowest ($P<0.05$) in 1992, but none of the other differences between years obtained significance (Table 1). The OR and NER were similar to data previously reported in sheep (Armstrong and Evans, 1983; Larson *et al.*, 1991), and the NET, NLB and NLW results were comparable to those reported by Shackell and Isaacs (1991), and Larson *et al.* (1991).

On average only 60% of treated donor ewes provided tranferrable embryos. This was in part due to a high failure rate of fertilisation in the donor ewes especially in 1991 when the fertilisation rate was 33% compared to 81% and 65% in 1992 and 1993 respectively. Fertilisation failure was usually associated with high ovulation rates, as also observed by Armstrong and Evans (1983). In 1991 several donors were recorded as having 'hyperstimulated' ovaries. Earlier Armstrong and Evans (1983) noted a similar condition, but found this phenomenon to be associated with PMSG-treated donors rather than those treated with FSH.

Mean liveweights of the three progeny hogget groups are given Table 2. Although some differences were statistically significant they were small in productive terms and therefore neither MOET nor selection for low FD had a major effect on live weight parameters.

Mean fleece weight, yield and fibre diameters in the three flocks are presented in Table 3. Both GFW and CFW were lower in ET hoggets ($P<0.05$) compared with UF and C

TABLE 1: Results of MOET and number of lambs born and weaned in three consecutive breeding seasons.

Year	Donors	Ovulation rate	Embryos recovered	Embryos transferred	Lambs born/donor	Lambs weaned/donor
Mean		16.7	10.4	7.8	3.9	3.1
1991	5	24.0^{b}	15.4^{b}	8.2	3.2	2.6
1992	13	15.2^{a}	8.5^{a}	6.9	3.1	2.6
1993	11	15.2^{a}	10.4^{ab}	8.5	5.3	3.9
S.E.D		2.9	2.4	2.71^{ns}	1.5^{ns}	1.5^{ns}
Total	29	485	302	225	113	90

^{ns}: non significant; ^{acb}: means with different superscripts differ at $P<0.05$.

TABLE 2: Mean (± SE) live weights (kg) of Control (C), Ultrafine (UF) and Elite embryo transfer (ET) progeny groups (pooled for birth years 1991,1992 and 1993).

Group	Number	Birth Weight	(S.E.)	Weaning Weight	(S.E.)	Autumn Weight	(S.E.)	Spring Weight	(S.E.)	Summer Weight	(S.E.)
C	516	3.6^{a}	0.05	23.0	0.29	32.8	0.40	38.0	0.54	54.5	0.76
UF	716	3.7^{b}	0.05	23.4	0.26	31.9	0.37	36.8	0.55	54.2	0.69
ET	93	3.8^{b}	0.10	23.5	0.51	32.1	0.63	37.2	0.94	54.2	1.50

^{ab}: means within columns with different superscripts differ at $P<0.05$.

animals. The FD of ET hoggets was significantly finer (P<0.01) than UF (0.9 μm) and C (2.5μm) animals respectively.

The effect of MOET (adjusted for the genetic contribution of the parents) was estimated as reducing FD by 0.21μm (SE 0.15). Although wool of the ET progeny was finer than expected, a significant result was not established. The analysis made a number of assumptions. One of these is that a single linear regression (within year) on birth date is appropriate for both the naturally bred and the earlier born MOET progeny. As there is no overlap in the birth dates of these two groups this assumption cannot be tested. Using the estimated regressions from the BLUP analysis indicates that the MOET progeny would be expected to be 0.45μm stronger than the others, due to birth date alone. If this is an over-estimate, then the estimated MOET effect should be larger. The birth date effect also suggests that the genetic gain from using MOET in the manner described here will not be fully realized (phenotypically) in hogget progeny. To clearly identify an effect of MOET it will be neccessary to simultanously mate MOET and natural breeding dams.

Another assumption in the BLUP analysis is the heritability of FD. If it is set to a lower value, this would result in a larger estimate of the MOET effect. Lowering the heritability to 0.4 still did not produce a significant effect (0.25 μm; SE 0.15). Alternatively, a much larger heritability would be necessary to eliminate the estimated MOET effect. Although other studies have found effects of reproductive technologies on productive traits such as birth weight (Wilson *et al.*, 1995), we have not been able to conclude that there are such effects on FD.

The heritability (h^2) estimates for lamb or hogget traits are shown in Table 4. The h^2 estimates of live weight, fleece weight and FD of these fine wool Merinos agree closely with values from previous studies in Merino and Merino-derived sheep (Mortimer and Atkins, 1989; Reid, 1987).

Mean performances of ET progeny at 2 and 3 years of age are compared with UF and C contemporaries in Table 5. The ET ewes were generally heavier but significantly so on only one occasion (P<0.05), compared with C animals. GFW, yield and CFW remained lower (P<0.05) for ET ewes born in 1991 compared to C or UF ewes, while there were no such difference in the 1992 born animals. The SLW, GFW, Yield%, and CFW of ET ewes was slightly lower in 1994 compared with 1993, while GFW and CFW (P<0.01) were lower in both C and UF ewes for 1994 compared to 1993.

TABLE 3: Mean (± SE) fleece weights, yield and fibre diameter comparison in Control (C), Ultrafine (UF) and Elite embryo transfer (ET) progeny groups (pooled for birth years 1991, 1992 and 1993), at hogget shearing.

Group	Greasy Fleece weight (kg)	(S.E.)	Yield (%)	(S.E.)	Clean Fleece Weight (kg)	(S.E.)	Fibre Diameter (μm)	(S.E.)
C	2.74^{b}	0.03	71.8	0.5	1.96^{b}	0.03	18.4^{c}	0.10
UF	2.72^{b}	0.03	72.1	0.5	1.96^{b}	0.03	16.8^{b}	0.09
ET	2.58^{a}	0.06	71.1	0.7	1.84^{a}	0.05	15.9^{a}	0.15

^{abc}: means within columns with different superscripts differ at P<0.05.

TABLE 4: Heritability (h^2) estimates of lamb and hogget live weights and of fleece weights, yield and fibre diameter in Merino hoggets (pooled birth years 1991, 1992 and 1993)

Parameter	Birth weight	Weaning Weight	Autumn Weight	Spring Weight	Summer Weight	Greasy Fleece Weight	Clean Fleece Weight	Yield	Fibre diameter
h^2	0.13	0.22	0.38	0.50	0.41	0.30	0.32	0.58	0.39
S.E.	0.06	0.07	0.10	0.13	0.15	0.09	0.09	0.13	0.10

TABLE 5: Mean performances of Control, Ultrafine and Elite embryo transfer (ET) progeny ewes in two consecutive prodnction years

Year of birth	Age	Group	Number	ALW (kg)	SLW (kg)	GFW (kg)	Yield (%)	CFW (kg)	FD (μm)	FD change (μm)	NLB	NLR
1991	2	Control	27	48.8^{a}	45.1	4.12^{b}	76.5^{b}	3.14^{b}	20.2^{c}	2.2^{c}	0.85	0.82
		Ultrafine	51	49.8^{ab}	45.1	4.03^{b}	76.7^{b}	3.09^{b}	17.4^{b}	0.8^{b}	0.88	0.78
		ET	8	54.4^{b}	48.0	3.35^{a}	71.6^{a}	2.40^{a}	15.9^{a}	-0.0^{a}	1.00	1.00
		Av.S.E.D		1.6	1.8^{ns}	0.18	1.4	0.15	0.3	0.3	0.16^{ns}	0.17^{ns}
1992	2	Control	28	47.6	43.5	3.76	76.0	2.83	19.6^{c}	0.8	0.89	0.82
		Ultrafine	50	48.3	43.5	3.77	76.8	2.88	17.3^{b}	0.6	0.94	0.82
		ET	11	50.2	43.9	3.54	76.8	2.72	16.6^{a}	0.3	0.91	0.82
		Av S E D		1.4^{ns}	1.6^{ns}	0.17^{ns}	1.4^{ns}	0.13^{ns}	0.3	0.2^{ns}	0.15^{ns}	0.16^{ns}
	3	Control	27	49.8	45.4	3.65^{b}	76.6^{b}	2.79^{b}	19.9^{c}	1.9^{b}	1.22	1.07
		Ultrafine	47	50.0	45.8	3.65^{b}	76.9^{b}	2.82^{b}	17.1^{b}	0.5^{a}	1.06	0.96
		ET	8	51.1	45.7	2.86^{a}	70.3^{a}	2.02^{a}	15.9^{a}	-0.0^{a}	1.25	1.25
		Av. S.E.D		1.6^{ns}	1.8^{ns}	0.19	1.3	0.13	0.3	0.3	0.16^{ns}	0.17

Note: SLW: Spring live weight; ALW: Autumn live weight; GFW: greasy fleece weight; CFW: clean fleece weight; FD: fibre diameter; FD change: increase in fibre diameter from hogget measurement; NLB: number of lamb born/ per ewe; NLR: number of lamb reared/per ewe; ^{ns}: non significant; ^{abc}: means with different superscrits differ at P<0.05.

FD was consistently lowest for the ET group and highest for the C group ($P<0.05$). The increase in FD with age was significantly less in ET ewes ($P<0.001$) than in the UF and C flocks. FD increased by 0.8 - 2.2 μm in C and 0.5 - 0.8 μm in UF from their hogget records (unadjusted for birth effects), whereas the increase in ET was 0.3 μm for those born in 1992 while the born 1991 ewes were unchanged. There were no significant differences between flocks in either NLB or number of lambs reared per ewe.

The advantages obtained from MOET are the combination of the increased selection intensity in the ultrafine flock, compared with the control, as well as the superiority of donor ewes and sires within the ultrafine flock.

MOET technology has made it possible to raise selection responses through increased selection pressure and shorter generation intervals. Smith (1986) predicted a substantial benefit for the sheep industry from MOET. He calculated that a high rate of embryo transfer (10 progeny per donor) could double the rate of genetic change compared to increase from natural reproduction. Moderate rates of embryo transfer (5 progeny per donor), would result in gains of 50 - 70%. The poor acceptance of MOET by sheep breeders is due to the variability of results achieved and high costs. While MOET considerably increases the reproductive capability of females, overall the selection gains are limited compared to those attainable from using elite males for artificial insemination. As in vitro culture (IVM/IVF) techniques develop (Tervit, 1989; Shorgan *et al.*, 1990; Thompson *et al.*, 1992; Pugh *et al.*, 1994) the ability to produce large numbers of uniform progeny from elite ultrafine individual ewes becomes an exciting prospect.

ACKNOWLEDGEMENTS

We appreciate the assistance of Ian Scott, Dave Anderson, Phil Neill, Allen Bradley, Kaye Isaacs, and other staff at Tara Hills for undertaking the MOET programmes and animal management.

REFERENCES

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

Groeneveld, E and Kovac, M. (1990). A generalized computing procedure for setting up and solving mixed linear models. *Journal of Dairy Science* **73:** 513-531.

Larson, B; Gustafsson, A.; Nasholm, A and Bjurstrom, L. (1991). A programme for oestrus synchronization and embryo transfer in sheep. *Reproduction in Domestic Animals* **26:** 301-308.

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

Mortimer, S.I and Atkens, K.D. (1989). Genetic evaluation of production traits between and within flocks of Merino sheep. I. Hogget fleece weights, body weight and wool quality. *Australian Journal of Agriculture Research* **40:** 433-443.

Patterson, H.D. and Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* **58:** 545-554.

Pugh, P.A.; Thompson, J.G.; Logan, K and Tervit, H.R. (1994). In vivo survival of transferred sheep embryos following puncture of the zona pellucida and in vitro culture. *Animal Reproduction Sciences* **35:** 81-89.

Reid, R. N. D. (1987). A study of repeatability and heritability in Polwarth sheep. Proceedings of a National Symposium: Merino Improvement Program in Australia (Editor: B.J. McGuirk). p.181- 183.

Shackell, G.H and Isaacs, K. L. (1991). A simplified MOET technique for Merino ewes carrying a double copy of the Booroola FecB gene. *Proceedings of New Zealand Society of Animal Production* **51:** 107-109.

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

Shorgan, B; Suolian, Z; Xiaoxian, X; Yiefei, P; Hongwu, C; Xihe, L; Qing lan, H and Qin. S. (1990). In vitro development of ovine oocytes matured and fertilized in vitro and lambing after embryo transfer. *Japanese Journal of Animal Reproduction* **36:** 225-229.

Tervit, H.R. (1989). Embryo transfer and sperm sexing. *In* Proceedings of 19th Seminar: Sheep & Beef Cattle Society of New Zealand Veterinary Association: 144-157.

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

Thompson, J.G.; Simpson, A.C.; Pugh, P.A.; McGowan, L.T.; James, R.W.; Berg, D.K.; Payne, S.R and Tervit, H.R. (1992). Effect of glucose level in culture medium on survival of in vitro cultured sheep embryos following transfer to recipient ewes. *Proceedings of New Zealand Society of Animal Production* **52:** 255 -256

Wilson, J.M.; Williams, J.D.; Bondioli, K.R.; Looney, C.R.; Westhusin, M.E and McCalla, D.F. (1995). Comparison of birth weight and growth characteristics of bovine calves produced by nuclear transfer (cloning), embryo transfer and natural mating. *Animal Reproduction Science* **38:** 73-83.

Wuliji, T.; Land, J.T.J.; Andrews, R.N and Dodds, K.G. (1990). Fleece weight and wool characteristics of Merino ewes screened into a superfine selection flock. *Proceedings of New Zealand Society of Animal Production* **50:** 301-303.