SIRE AND GENOTYPE EFFECTS ON SURVIVAL OF TRANSFERRED OVINE EMBRYOS

G N HINCH, G H SHACKELL, C J THWAITES and J M THOMPSON

Dept Animal Science, University of New England, Armidale, NSW 2350.
AgResearch Invermay Agricultural Centre, PB 50034 Mosgiel, New Zealand.

SUMMARY
Two flocks of ewes were used as sources of embryos for embryo transfer programs: a Merino flock comprising 3 lines differentially selected on weaning weight and a Coopworth flock consisting of three lines differentially selected for backfat thickness. Effects of embryo genotype, year of transfer, sire and number of embryos transferred on embryo survival were examined. No significant line or sire effects were found for either flock with a mean survival of 43.2% for directly transferred Merino embryos and 21.9% for frozen Coopworth embryos. Although not significant there was a trend for improved survival with increased number of embryos transferred per recipient.

Keywords: embryo survival, embryo transfer, sheep, sire, genotype.

INTRODUCTION
There have been several reports published over the last 30 years describing embryo transfer programs in sheep. A number of these reports have evaluated the factors associated with the variation observed in embryo survival (eg Moore et al. 1960), and the possibility that genetic factors may contribute to variation in embryo survival has frequently been suggested.

Chromosomal abnormalities have been identified as one source of ‘genetic’ loss although this appears to be of minor importance in the ewe (Long and Williams 1980). While most between and within breed variation appears to be associated with differences in dam ovulation rate (Bolet 1986), it has been suggested that the ram may also contribute to embryo mortality through variation in sperm quality (Courot and Colas, 1986) and possibly via breed or strain differences such as those reported by Burfening et al. (1977) for high and low prolificacy rams, and by Maxwell et al. (1992) for different Merino strains.

This paper reports the results of two embryo transfer programs using sires selected for different production characteristics in 3 lines of either Merino or Coopworth sheep. The effects of both selection line and sire on embryo survival after surgical transfer were examined.

MATERIALS AND METHODS

Flock 1 - Weaning weight selected lines
Multiparous ewes from three lines of Medium Peppin Merinos were used in the first study. The flock consisted of: a random bred line (R), a line selected for increased weaning weight (W+), and a line, selected for decreased weaning weight (W-). The details of the selection procedures applied are outlined by Davis (1988).

The experiment ran for three years on the Kirby Research Station, Armidale, with mating/embryo transfer being conducted in the autumn (March) of 1984-86. Oestrous was synchronised in both donor and recipient ewes using progestagen impregnated sponges (Repromap, Upjohn), and PMSG (800 or 1000 iu, Folligon - Intervet) was injected into donor ewes 12 hours prior to pessary withdrawal. Donors were placed with fertile rams immediately after sponge withdrawal while recipients were joined with vasectomised rams (5%). All rams were fitted with mating harnesses and the marked ewes were drafted from the flock morning and afternoon so that the stage of oestrus of donor and recipient ewes would be synchronised within 12 hours. In the first two years recipients were multiparous Merino ewes while in the final year multiparous Merino x Border Leicester ewes were used. The same nine Merino sires were used each year, three rams from each line. Semen quality of rams was assessed as ‘good’ prior to joining each year. In all years the donors and recipients grazed improved pastures prior to and after surgery.

Embryos were collected from the uterus at day four post-mating using the technique described by Tervit and Havik (1976). The embryos were then classified (non-fertilized, fertilized, and developmental stage) and maintained in an incubator at 35°C for 15 to 30 minutes before being transferred to recipient ewes. Only embryos between the eight cell and early morula stages were transferred. Surgical transfer was conducted on recipient ewes under general anaesthesia. The tip of the uterine horn was exposed and the embryos placed as
close as possible to the utero-tubal junction by making a small puncture wound in the uterus and using a pipette to deliver the embryos. All recipients were observed to have at least one functional corpus luteum (CL) and in situations where only one embryo was transferred it was placed in the horn ipsilateral to the CL. Twin placement was bilateral. A total of 345 embryos were transferred over the three years of the program and 198 recipients were used (Table 1).

Table 1. The number of recipients used in each year and for each flock

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Flock1</td>
<td>1984</td>
</tr>
<tr>
<td></td>
<td>1985</td>
</tr>
<tr>
<td></td>
<td>1986</td>
</tr>
<tr>
<td>Flock2</td>
<td>1989</td>
</tr>
<tr>
<td></td>
<td>1990</td>
</tr>
</tbody>
</table>

Flock 2 - Backfat selected lines

The Coopworth embryos were collected at Invermay Agricultural Centre, New Zealand, from three lines of Coopworth ewes that had been selected for either decreased or increased backfat thickness or were randomly selected. The details of the selection procedures used were reported by Fennessy et al. (1982). There were 34 Coopworth sires represented in the embryo transfer program and nine of these were represented by 10 or more embryos transferred. The recipients were multiparous Border Leicester x Merino ewes, located on Kirby Research Station Armidale, and were synchronised with CIDRs (EAZI Breed) for a 10 day period. Three hundred i.u. of PMSG (Folligon, Intervet) was injected 24 hours prior to CIDR withdrawal and ewes were drafted from the flock, morning and evening when marked by vasectomised rams (5%). In both years embryos were transferred to the recipients on day 7 post-oestrus. The embryos, shipped to Australia after eight months storage, were thawed and then one or two embryos were transferred to each recipient so that pedigree information could be obtained. As for Flock 1 all recipient ewes were in ‘good’ condition and were maintained on improved pastures prior to and after surgery.

A modified laparotomy procedure was used for transfer. The uterine horn of recipient ewes was first located by laparoscopy and then the tip of the uterine horn was exposed through a 1 to 2 cm incision using Babcock forceps. All recipient ewes were sedated using 0.1 to 0.2 ml of Xylazine and a local anaesthetic (Lignocaine, Astra) was injected at the site of the incisions. A total of 347 embryos were transferred over the two years of the program and 213 recipients were used (Table 1).

The mean number of embryos transferred per recipient was 1.75 and 1.70 for Flocks 1 and 2 respectively and the number of embryos surviving was determined from recipient lambing records.

Statistical Analyses

Data on the recipient, embryo genotype, year of transfer, sire and number of embryos transferred were recorded for each embryo and used as fixed effects in a generalised linear model (logit link function) to determine their effects on embryo survival. Sires were nested within lines and were analysed accordingly.

RESULTS

Embryo survival

For Flock 1, sires within lines had no significant effect on embryo survival (P=0.26) with a range in survival of 22 to 59%. There were no significant differences between lines in embryo survival and the number of embryos transferred per recipient also did not significantly alter embryo survival. However there was a trend for more than two embryos per recipient to reduce the probability of survival (P=0.17). A significant year effect was noted (Table 2, P<0.05) and survival levels declined over the three years of collection for Flock 1, particularly for W+ animals (Figure 1).
Embryo survival in Flock 2 ranged from 0 and 67% between sires but the effect was not significant. When sires with less than 10 embryos were excluded the range in survival was 10.7 to 41.7%. Within line, sire effects were not significant, but survival in the lean-line was consistently lower than for the other two lines (Table 2), particularly in 1989. As for Flock 1, the number of embryos transferred in Flock 2 did not have a significant effect on survival, although the increase in survival of 6.8% between 1 and 2 embryos transferred was greater than the 3.1% observed for Flock 1 (Table 2). Year differences in survival were not significant (Table 2) although survival tended to be higher in 1990.

**Table 2. The effects of line, year and number of embryos transferred on the survival of embryos**

<table>
<thead>
<tr>
<th>Line</th>
<th>% survival</th>
<th>Year</th>
<th>% survival</th>
<th>No. embryos transferred</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Flock1</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W+</td>
<td>38.8</td>
<td>1984</td>
<td>54.2</td>
<td>1</td>
<td>43.1</td>
</tr>
<tr>
<td>W-</td>
<td>51.2</td>
<td>1985</td>
<td>41.1</td>
<td>2</td>
<td>46.2</td>
</tr>
<tr>
<td>Random</td>
<td>39.8</td>
<td>1986</td>
<td>31.1</td>
<td>3</td>
<td>28.6</td>
</tr>
<tr>
<td><em>Flock2</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>16.7</td>
<td>1989</td>
<td>19.6</td>
<td>1</td>
<td>18.3</td>
</tr>
<tr>
<td>Fat</td>
<td>27.1</td>
<td>1990</td>
<td>27.8</td>
<td>2</td>
<td>25.1</td>
</tr>
<tr>
<td>Random</td>
<td>25.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Although there was a large range in embryo survival between sires these differences were not statistically different for either flock. Differences between lines were also not significant, although the lean line in Flock 2 produced embryos with the lowest survival. Line/strain differences of the same magnitude have been reported for ‘natural’ embryo loss (Burfeind *et al.* 1977, Maxwell *et al.* 1992) and support the contention that at least part of the variation in embryo survival may be related to genetic factors. The relatively low survival level observed for the W+ embryos is in accord with the report of Bradford *et al.* (1986) of greater prenatal losses in sheep lines selected for increased weaning weight compared with controls. The fact that line differences were not significant in this experiment may be associated with the screening of embryos (advanced to, or beyond the eight cell stage) and the standardization of maternal environment. However the latter is unlikely to be important as there appears to be little genetic variability in maternal traits for embryo survival.

The non-significant increase in survival of embryos when two rather than one were transferred is in agreement with Moore *et al.* (1960), who reported that transfers of less than two did not increase the likelihood of pregnancy.
The overall survival level for embryos in the two experiments was within the range reported by Bolet (1986) in his review of embryo loss (20 to 70%), although for embryos transferred directly (Flock 1) survival levels were slightly lower (43.2%) than the 58% reported by Kelly et al. (1983) using a similar protocol. The survival levels of the frozen-thawed embryos from Flock 2 were at the low end of reported values (Szell and Windsor 1994).

The consistent decline in embryo survival over the years of experiment 1 is difficult to explain given the relatively fixed treatment regime and age structure of the recipient ewes. It is possible that repeated use of superovulatory regimes may have a negative impact on embryo development/quality although this does not appear to have been reported previously.

ACKNOWLEDGEMENTS

Our thanks to all those who assisted with the embryo collection and transfer programs, in particular to Mr B Johnston for his skilled assistance with animal anaesthesia. Both of these projects were funded by the MRC.

REFERENCES