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## Out-of-season embryo transfer in five breeds of imported sheep

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### ABSTRACT

The success of a multiple-ovulation and embryo transfer programme involving five breeds of sheep (Danish and Finnish Texel, Oxford Down, Gotland Pelt, and the White Headed Marsh) was compared across two seasonal periods. Results for the incidence of oestrous, ovulation rate, embryo recovery rate, fertilisation rate, and for the survival rate of the embryos to scanning and lambing in recipient ewes, were compared. The ovulation rate ( $7.8 \pm 0.19$  out-of-season compared to  $6.6 \pm 0.26$  in-season), was the only variable to be significantly affected by the season ( $P < 0.05$ ). The number of lambs born per donor programmed was 1.58 out-of-season vs 0.98 in-season. The results from this trial suggest that embryo transfer can be successfully conducted out-of-season in these imported sheep breeds.

**Keywords** Sheep, breed effects, multiple-ovulation, embryo transfer, season effects.

### INTRODUCTION

Multiple-ovulation and embryo transfer (MOET) in sheep is most frequently conducted during the breeding season. However, there are a few reports suggesting that embryo transfer can be conducted successfully out-of-season (Armstrong and Evans, 1984; Lopez Sebastian *et al.*, 1990). To increase the return on the capital invested in a large scale MOET scheme, it would be advantageous to increase the number of lambs born per donor per year, by performing embryo transfer throughout the year.

This trial uses data from a large commercial MOET programme, involving the Danish and Finnish Texel (DT and FT), Gotland Pelt (GOT), Oxford Down (OXD), and White Headed Marsh (WHM) sheep breeds (all of which were recently imported into New Zealand), to describe the effect of the season of treatment on the success of an embryo transfer programme.

### MATERIALS AND METHODS

#### Donor Ewes and Treatments

A total of 556 donor ewes, consisting of two age groups

(14-16 and 26-28 months old) across the five breeds, were available for embryo transfer in November and December 1989, (that is, outside of the normal breeding season of these sheep breeds in New Zealand) for use as the out-of-season group. From this out-of-season group, 248 ewes were randomly selected (within breed and age group) for use within the normal breeding season (in-season group), which was programmed in March and April 1990. These ewes were subjected to the same hormonal and embryo transfer regimes, as that used out-of-season.

Oestrous in the donor ewes was synchronised with CIDR-G (Carter-Holt-Harvey, Hamilton, NZ) treatment for 11-13 days (replaced after 8-10 days; Thompson *et al.*, 1988). A series of six descending doses of FSH-P (Schering Corp, USA), with an initial PMSG (Pregnenol, Heriot Developments, Australia) injection, began 60 h before CIDR-G removal. On the basis of previous work with these breeds (Dattena, 1989; Tervit, unpublished data) the total FSH-P dose varied between 24 and 36 mg, and the PMSG dose was either 200 or 300 IU, depending on the breed and age of the ewe.

The ewes were inseminated *intra uterine* with fresh diluted semen, from a ram of the same breed, 5-13

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hafter detection of oestrous, using the technique outlined by Killeen and Caffery (1982), with a total sperm dose of 200-400 million per insemination.

Embryo recovery was attempted on day 6.5-7 after the onset of oestrous (day 0), in ewes with an ovulation rate of three or greater. The laparoscope aided uterine flush was based on the technique outlined by McKelvey *et al.* (1986). The flushing media used was complete enriched PBS (Immuno-Chemical Products Ltd, NZ) at 37°C, which was then allowed to cool to 20°C.

### Recipient Ewes and Treatment

Recipient ewes (3-4 year old multiparous Romney) were synchronised for transfer with a CIDR-G for 11-13 days. Recipients used out-of-season, also received 500 IU PMSG at CIDR-G removal.

Two embryos (determined to be of good quality) were transferred to each recipient, within 2 h of collection. The out-of-season transfers were at the same site as the donor ewes, whereas the in-season embryos were transported 5 km to another site, and transferred to recipients located there. The transfer technique used was based on that described by Boundy *et al.* (1985), however the number of *corpora lutea* was determined via laparoscopy, and the ipsilateral uterine horn exteriorised using forceps through a 3 cm mid ventral incision, hence the ovaries remained inside the abdominal cavity. A CIDR-G was inserted into each recipient ewe on the day of transfer, replaced on day 20 (where day 0 is the day of recipient oestrous), and removed at day 50-55, when the ewe was ultrasound scanned, to determine the number of foetuses and hence estimate the embryo survival rate. Lambing was indoors to enable close observation and accurate recording of pedigrees.

### Analysis

The incidence of oestrous was analysed by Hiloglinear analysis using the SPSSx package. The ovulation rate, recovery rate, fertilisation rate, yield of good quality transferable embryos, and the embryo survival rate to scanning and birth were all analysed using an analysis of variance approach (SAS).

## RESULTS

Overall the incidence of oestrous in the donor ewes (out-of-season vs in-season) was not significantly different; with 93.4% (556 ewes) vs 94.3% (248 ewes) of the ewes programmed showing oestrous, respectively.

Out-of-season, the ovulation rate was  $7.8 \pm 0.19$  (SE) (552 ewes), compared to  $6.6 \pm 0.26$  (227 ewes) in-season, ( $P < 0.05$ ). There were significant interactions between the season of transfer and the donor breed, age, and the hormonal regime used.

The embryo recovery rate was  $53.4\% \pm 1.4$  (476 ewes) vs  $53.5\% \pm 2.6$  (176 ewes) respectively (NS).

The fertilisation rate of the eggs recovered was  $72.7\% \pm 1.8$  (438 ewes) vs  $65.7\% \pm 3.3$  (155 ewes) respectively (NS). There was a significant ( $P < 0.01$ ) effect of donor age on the fertilisation rate; 14-16 month old ewes  $73.2\% \pm 1.6$  (503 ewes) vs 26-28 month old ewes  $57.8\% \pm 4.6$  (90 ewes).

The yield of good quality transferable embryos was not different out-of-season,  $78.5\% \pm 1.8$  (375 ewes) compared to in-season,  $79.5\% \pm 3.1$  (124 ewes).

Embryo survival to scanning was  $68.8\% \pm 1.4$  (732 recipient ewes) out-of-season compared to  $64.8\% \pm 2.8$  (190 recipient ewes) in-season (NS). Embryo survival to birth was similar out-of-season  $66.7\% \pm 2.9$  (819 recipient ewes) to that in-season  $61.2\% \pm 1.5$  (203 recipient ewes) (NS).

The number of lambs born per donor programmed was 1.58 vs 0.98, whereas the number of lambs born per donor flushed was 1.91 vs 1.38, respectively.

## DISCUSSION

On the basis of these results, there is no reason to expect a lower response out-of-season. There was no influence of the season of treatment on any of the variables analysed, except the ovulation rate, which was significantly higher out-of-season.

The higher ovulation rate out-of-season, may be due to a lower level of stress in the out-of-season group, compared to that of the in-season group. We assume this, based on the total population that was subjected to embryo transfer during the respective seasons (556 donors programmed out-of-season compared to approximately 5000 donors programmed during the season, of which the 248 donor ewes of the in-season

group, were a part). Hence, within this donor population of 5000 ewes, the in-season group was more likely to be subjected to a higher degree of stressful management, relative to the out-of-season group.

It is also possible that the higher ovulation rate out-of-season, could be associated with a more efficient exogenous endocrinological control, compared to that achieved during the season when the endogenous system is fully active. In order to determine the season effect the same hormonal regimes had to be used for both seasonal groups, thus the results suggest that the hormonal regimes for the out-of-season period are not necessarily optimum for in-season treatments.

The number of lambs born per donor programmed (1.58 out-of-season and 0.97 in-season) appears to be relatively low when compared to the number of lambs born from less expensive breeding options, such as the use of a mild dose of PMSG and synchronised natural mating (for example, 1.76 lambs born per year; Robinson, 1980). However, embryo transfer has the advantage of being able to be repeated several times throughout the year. For example, out-of-season embryo transfer is conducted twice and in-season embryo transfer up to four times within one calendar year, in the commercial MOET programme at LambXL (H.W. Vivanco, unpublished data). Using the results obtained from this trial it is suggested that a total of 7.04 lambs born per donor per year could be generated by implementing the techniques outlined above (3.16 lambs born out-of-season and 3.88 lambs born in-season).

Based on the results obtained in this trial, it is

concluded that embryo transfer can be successfully conducted out-of-season, in these sheep breeds.

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