INSEMINATION OF SHEEP WITH FRESH OR FROZEN SEMEN

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SUMMARY

The percentages of ewes lambing after insemination with fresh semen at a synchronized or normal oestrus (Trial 1) and with fresh or frozen semen at a normal oestrus (Trial 2) were 61, 69, 59 and 19%, respectively.

INTRODUCTION

Artificial insemination (AI) in sheep allows more widespread use of superior sires than can be achieved under natural mating and thus permits more intensive sire selection. Clarke (1975), reviewing the genetic advantages to be gained from this intensive selection, concluded that a large New Zealand breeding scheme using AI would increase selection progress over natural mating by 20 to 30%.

In New Zealand, AI could be used to exploit the superior sires being identified in the various group breeding schemes, the small numbers of imported exotic sires and sires selected for special characters. The aim of the present study was to investigate factors affecting the utilization of fresh diluted or deep-frozen semen in an AI programme.

MATERIALS AND METHODS

TRIAL 1:

Two hundred mixed-age Perendale ewes were treated with intravaginal sponges containing 70 mg MAP (Provera, Upjohn) on March 18. The sponges were removed 15 or 16 days later and at this time each animal was injected intramuscularly with 400 i.u. pregnant mares' serum gonadotrophin. The ewes were run with harnessed teaser rams and were inseminated at the synchronized oestrus regardless of whether or not they were marked by teasers. They were randomly allocated to treatment groups in a factorial design (Table 1. A). The ewes inseminated once were inseminated approximately 54 hours after sponge removal and those inseminated twice at 48 and 60 hours after removal.

The semen was collected in an artificial vagina and diluted 1:4 (semen:diluent) in a milk diluent (Colas *et al.*, 1968). The

TABLE 1: EXPERIMENTAL DESIGN AND PERCENTAGE OF EWES LAMBING AFTER AI WITH FRESH SEMEN

Oestrus and Source of Variance		*No. Ewes Inseminated*	*Percent Lambing*
A. Synchronized oestrus			
Ewe position at AI	— Elevated[1]	94	55
	— Standing	94	66
Semen volume	— 0.25 ml	93	58
	— 0.5 ml	95	63
Inseminator	— A	91	62
	— B	97	60
Method of deposition	— Straw	97	57
	— Pipette	91	65
No. of inseminations	— Once	95	56
	— Twice	93	66
Overall		188	61
B. Return oestrus			
Ewe position at AI	— Elevated[1]	34	71
	— Standing	36	67
Inseminator	— A	36	64
	— B	34	74
Method of deposition	— Straw	36	64
	— Pipette	34	74
Overall		70	69

[1] Animal's hindquarters elevated.

diluted semen (approximately 600×10^6 sperm/ml) was stored for up to 2 hours at 15° C in either Cassou straws (0.25 or 0.5 ml) or covered test-tubes, from which glass inseminating pipettes could be loaded.

Ewes which returned to oestrus 15 to 19 days after the synchronized oestrus were detected by harnessed teaser rams and allocated to the treatment groups shown in Table 1, B. Marked ewes were drafted once daily and inseminated 1 hour later with 0.25 ml of semen diluted 1:4 in a milk diluent.

TRIAL 2:

One hundred and eighty-six mixed-age Romney ewes were inseminated at the second oestrus after sponge removal with either fresh semen (Colas *et al.*, 1968) or deep-frozen semen processed using the procedures described by Visser and Salamon (1974) (V & S) or Colas (1975) (C). The ewes were run with harnessed teaser rams and marked ewes were drafted once daily and inseminated an hour later. Insemination was performed in the hindquarters elevated position with (i) 0.25 ml of fresh semen

diluted 1:4; (ii) 0.25 ml of frozen semen diluted 1:2 (V & S); or (iii) 0.5 ml of frozen semen diluted 1:4 (C).

RESULTS AND DISCUSSION

In Trial 1 the overall percentage of ewes lambing to the synchronized oestrus was 61% (Table 1, A). None of the main treatments was significant but the first order interactions indicated that the advantage of two inseminations was negated if the ewe was stressed by being placed in the elevated position (elevated *vs* standing, 54 *vs* 77%, $P < 0.1$). Ewes inseminated once gave similar results whether inseminated elevated or in the standing position (55 *vs* 56%, respectively). A low volume of semen could be satisfactorily used with pipettes (0.25 *vs* 0.5 ml, 69 *vs* 61%) but the less precise placement of semen with straws meant that better results were obtained with the higher volume (0.25 *vs* 0.5 ml, 48 *vs* 65%, $P < 0.1$). The most efficient use of semen occurred when ewes were inseminated in the standing position with 0.25 ml of semen in pipettes. Under these conditions, 69% of the ewes inseminated once and 82% inseminated twice lambed to the synchronized oestrus. The dose rate was approximately 135×10^6 motile sperm per inseminate; thus one ejaculate could be used to inseminate 20 ewes once or 10 ewes twice.

At the return oestrus, 69% of the ewes lambed following the single insemination of 135×10^6 motile sperm (Table 1, B). No significant effects or interactions were observed.

Overall, 83% of the inseminated ewes lambed. Those lambing to the synchronized oestrus produced 161% lambs and those to the return oestrus 130% lambs.

The results of Trial 2 are presented in Table 2. The lambing result of the ewes receiving fresh semen was satisfactory but results from ewes receiving frozen semen were poor. This poor performance was probably due to the low post-thaw motility of the semen and therefore the low numbers of motile sperm inseminated per ewe ($60\text{-}75 \times 10^6$).

TABLE 2: PERCENTAGE OF EWES LAMBING AFTER AI WITH FRESH QR FROZEN SEMEN

Semen Type	*No. Ewes Inseminated*	*Percent Lambing*
Fresh	73	59
Frozen (V + S)	53	21
Frozen (C)	60	18

Fresh *vs* (V + S plus C); $P < 0.001$.

The development of a successful technique for deep-freezing ram semen is important since it would increase further the utilization of superior sires. It would enable semen to be collected for most of the year, stored and then distributed to AI centres and farmers for insemination when required. Also, successful techniques would allow the import and export of semen and the storage of reference sire material. However, freezing has proved difficult, with only 2 or 3 groups of workers reporting satisfactory experimental results from selected rams and semen. These results have generally been achieved after high numbers of motile sperm were inseminated at a natural or synchronized oestrus. Under these conditions only 3 or 4 ewes would be inseminated per ejaculate.

The decision whether to use AI in New Zealand and whether to use oestrus synchronization as an aid to AI depends on many factors. The results with fresh semen indicate that this technique can be considered and that its economics should be assessed for various sheep improvement schemes.

The use of AI remains limited because of difficulties encountered in the storage of semen. The current Ruakura research programme aims to extend the fertilizable life of liquid semen beyond 16 hours and to improve the results from deep-frozen semen.

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