A.I. OF SHEEP WITH FROZEN SEMEN

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SUMMARY

Two experiments are described in which deep-frozen ram semen was used for artificial breeding. In Trial 1, natural mating and A.I. with fresh semen or frozen semen were compared at first and second cycles after synchronization of oestrus. Overall conception at second cycle was higher than at first cycle. But the results from frozen semen were much lower than the other methods of mating at both cycles. In Trial 2, A.I. was done with fresh or frozen semen at two sites (cervix or uterus). Fresh semen gave higher conception than frozen semen and uterine insemination was superior to cervical insemination.

INTRODUCTION

The development of large-scale breeding schemes and the identification of superior rams, coupled with the introduction of the various exotic breeds of sheep to New Zealand, has produced a demand for a practical system of artificial breeding in sheep.

Practical techniques for the artificial insemination of sheep have been available for many years (Emmens and Robinson, 1962). However, the application of such techniques has been limited by the lack of suitable methods for the preservation and storage of ram semen. Recently there have been reports of successful insemination with deep-frozen ram semen (Salamon, 1972; Colas and Brice, 1970).

The practical application of artificial breeding in sheep in New Zealand depends on the use of frozen semen from selected rams in conjunction with the synchronization of oestrus in ewes.

This paper presents the results of two trials in which various mating methods are compared at the first and subsequent oestrus following synchronization with progestogens.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Rams

Ten entire Romney rams of proven libido were used for the natural mating groups. A further 6 Romney rams were trained for

semen collection and provided both the frozen and fresh semen. Twenty-five vasectomized Romney rams were used for detection of oestrus in the insemination groups.

TRIAL 1

Treatments and Design

Three types of mating (natural mating, A.I. with fresh semen, A.I. with frozen semen) were carried out at the first and second cycles following synchronization of oestrus. The trial was designed as a 3 × 2 factorial and involved 600 four-tooth and older Romney ewes.

Method of Synchronization

All ewes were treated with intravaginal sponges (Robinson, 1965) containing 50 mg M.A.P. for a period of 14 days. For ease of handling the synchronization was staggered so that 50 ewes from each of the six groups were mated in one week and the remaining 50 from each group a fortnight later.

Detection of Oestrus

All rams were fitted with sire-sine harnesses and crayons and ewes were inspected at 0600 and 1800 h daily. Matings in the entire-ram groups were recorded similarly.

Collection and Storage of Semen

Semen was collected by means of an artificial vagina. Immediately on collection the semen was placed in a water bath at 37°C, and the volume, motility and density of the sample determined.

Ejaculates of good initial motility were pooled and then diluted 1:4 (semen:diluent v/v) with a diluent containing 300 mM *tris*—27.75 mM glucose—94.7 mM citric acid. The diluted semen contained 12% (v/v) egg yolk and 4% (v/v) glycerol (Salamon and Visser, 1972) and was processed and frozen as described by Visser and Salamon (1973).

Frozen semen was reconcentrated by thawing the pellets in test tubes containing a solution of *tris* (300 mM)—fructose (55.5 mM)—citric acid (94.7 mM) held at 37°C (thawing dilution 1:2 pellets:thawing solution v/v). The thawed semen was centrifuged at 1000 × g for 10 min and the supernatant removed to obtain

a cell concentration of 1.0×10^9 motile sperm/ml. Only that semen showing initial post-thawing motility above 30% was used.

The fresh semen was collected and assessed in a similar fashion. Ejaculates of good motility were pooled and the pooled semen used for insemination.

Inseminations

Ewes were inseminated at 0800 and 2000 h, being 14 to 15 hours after drafting. Ewes were inseminated with 0.05 to 0.1 ml of fresh semen or 0.2 ml of frozen semen so that each received about 200×10^6 motile spermatozoa. Ewes were carried through to lambing to determine conception results.

TRIAL 2

Treatments and Design

This trial compared type of semen (fresh vs frozen) inseminated either via cervix (normal) or via uterine horn (surgical) to ewes at 2nd oestrus following synchronization. A 2×2 factorial design was used involving 200 Romney ewes four-tooth and older.

Collection and Storage of Semen

General management was as in Trial 1. Unlike Trial 1, the frozen semen was not reconcentrated. Pooled semen was diluted 1:2 with a diluent containing 360 mM *tris*—33.3 mM glucose—113.7 mM citric acid. The diluted semen contained 12% egg yolk and 4% glycerol and processed and frozen as before. The pellets were thawed into dry test tubes at 37°C. Only semen with an initial post thawing motility above 30% was used. The pooled ejaculates of fresh semen was diluted 1:2 with the same diluent prior to insemination. Ewes were inseminated with either 0.2 ml cervically or 0.1 ml into each uterine horn via a mid-ventral laparotomy.

Seventy-two hours after insemination all ewes were subjected to laparotomy and ova recovered by flushing the Fallopian tubes with sterile sheep serum. Recovered ova were examined and apparent normal cleavage was used as a criterion of fertilization.

RESULTS

TRIAL 1

The results are summarized in Table 1.

TABLE 1: EFFECT OF MATING TYPE AND CYCLE ON LAMBING PERCENTAGE

Cycle and Mating Type	*Mated*	*No. Ewes Lambed*	*%*
1st Cycle:			
Natural	87	36	41.4
A.I. (Fresh)	100	46	46.0
A.I. (Frozen)	90	1	1.1
Sub-total	277	83	30.0
2nd Cycle:			
Natural	100	84	84.0
A.I. (Fresh)	100	67	67.0
A.I. (Frozen)	72	5	6.7
Sub-total	272	156	57.4
Sub-totals:			
Natural	187	120	64.2
A.I. (Fresh)	200	113	56.5
A.I. (Frozen)	162	6	3.7
Total	549	239	43.5

Conception rates were all higher following mating at the 2nd oestrus than at the 1st oestrus after synchronization (30 vs 57.4% $P < 0.001$). There was also a significant effect of type of mating. There was no difference in conception rate between natural mating and A.I. with fresh semen while that following A.I. with frozen semen was much lower (64.2% vs 56.5% vs 3.7% $P < 0.001$). There was a significant ($P < 0.01$) interaction between type of mating and cycle number. This was due mainly to the higher response to natural mating at the 2nd oestrus following sponge withdrawal.

TRIAL 2

Table 2 presents the results for both egg recovery and fertilization. There were no significant interactions.

(a) *Egg Recovery*

There was a significant effect of type of insemination on the proportion of ewes from which eggs were recovered. Fewer ewes yielded eggs following surgical insemination than following normal cervical insemination (72.7% vs 87.0% $P < 0.01$).

TABLE 2: EFFECT OF SEMEN TREATMENT AND INSEMINATION TYPE ON FERTILIZATION RATE

Semen Type	*Type of Insemination*	*No. Ewes Inseminated*	*Yielding Eggs*	*(%)*	*Yielding Fertilized Eggs*	*(%)*
Fresh	Normal	50	39	(78.0)	22	(56.4)
	Surgical	40	28	(70.0)	23	(82.1)
Sub-total		90	67	(74.4)	45	(67.2)
Frozen	Normal	50	48	(96.0)	7	(14.6)
	Surgical	48	36	(75.0)	17	(47.2)
Sub-total		98	84	(85.7)	24	(28.6)
Sub-totals						
	Normal	100	87	(87.0)	29	(33.3)
	Surgical	88	64	(72.7)	40	(62.5)
Totals		188	151	(80.3)	69	(45.7)

There was also a significant effect of semen type on rate of egg recovery with more ewes yielding eggs following insemination with frozen semen (fresh 74.4% vs frozen 85.7% $P < 0.05$).

(b) *Fertilization*

There was a higher fertilization rate following surgical insemination (62.5%) than following normal insemination (33.3%) ($P < 0.001$). Semen type also produced a significant effect on level of fertilization with a higher level being obtained with fresh semen (fresh 67.2% vs frozen 28.6% $P < 0.001$).

DISCUSSION

The low fertility obtained with frozen semen is in agreement with that obtained by many other workers. However, it is markedly lower than that reported by Visser and Salamon (1973; 1974) who used the same technique of semen freezing. This discrepancy is difficult to explain. It is unlikely to be due to insemination technique as the levels of fertility obtained with fresh semen in Trial 1 are in the acceptable range (Robinson *et al.*, 1967). In addition, the post-thawing motility recorded in these trials is comparable to that obtained by Salamon and co-workers

although there was a slightly higher incidence of acrosomal damage (S. Salamon, pers. comm.).

Similar problems with repeatability of conception levels with a range of various freezing techniques has been experienced in other centres (M. Barlow, pers. comm.).

The lower levels of fertility obtained following mating at the first synchronized oestrus confirm a large number of reports of depressed fertility following synchronization with progestogens. Recent reports from French workers (Colas *et al.*, 1973; Colas, 1974) have demonstrated that this problem can be overcome.

The results of Trial 2 indicate that a large proportion of the lowered fertility with frozen semen can be explained in terms of reduced sperm transport. However, it is obvious that a problem does exist with the fertilizing ability of the frozen semen. The results obtained in this trial contrast with those of Lightfoot and Salamon (1970) who obtained fertilization levels of 46 and 91% following insertion of frozen semen into cervix and uterus, respectively. Similarly, they obtained levels of 70 and 87% with fresh semen.

The lower fertilization levels obtained with the fresh semen are probably best explained by the composition of the diluent used. In the majority of reports which compare fresh and frozen semen, different diluents have been used for each semen type. In this experiment the same diluent was used for the fresh as well as the frozen semen. The results indicate that the diluent used was not completely suitable probably owing to the presence of glycerol (necessary for freezing) (Sanford *et al.*, 1972).

The effect of route of insemination and rate of egg recovery observed is similar to that reported by Killeen and Moore (1971) and Trounson and Moore (1974). The handling of the reproductive tract at about the time of ovulation obviously results in rapid transport of ova through the tract.

The significant effect of semen type on egg recovery rate is most difficult to explain as it is predominantly due to the lower than normal recovery rate following normal insemination with fresh semen.

The disappointing results obtained in these trials, however, should not lead to a dismissal of the use of A.I. with frozen ram semen as the recent and consistently high levels reported by Salamon and Visser (1974) and by Colas (1974) indicate the considerable potential of the technique.

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