Artificial insemination in sheep—comparison of storage time, dose rate and insemination technique

T.G. HARVEY[1], D.L. JOHNSON[2], R.L. BAKER[2], B.K. TRUST[1] AND B.C. THOMSON[1]

ABSTRACT

Trials undertaken at Rotomahana in 1984 and 1985 examined factors involved in sheep AI and provided a link between Rotomahana and a commercial AI programme. Semen collected and diluted by the New Zealand Dairy Board gave similar conception rates (46%) to semen collected at Rotomahana (42%). Results in 1984 indicated that there was a small but non-significant decrease in conception rate with semen storage time up to 15 hours (5 h 68%, 10 h 61%, 15 h 57%). In 1985 there was a highly significant difference between 5 and 20 h storage (46% *v* 25%) and this result was consistent over ewe ages and dose rate.

In 1985, 200×10^6 sperm dose was compared to 100×10^6 dose in 3 subtrials. The overall conception rates were 45.7% and 37.5% respectively (P 0.05).

In 1984 diluted semen stored in glass and inseminated using a fine glass pipette gave a higher, but not significant, conception rate than semen stored in mini straws and inseminated using a standard cattle pistollet (66% *v* 58%). In 1985 all semen was stored in glass and inseminated with either a glass pipette (conception rate of 49.8%) or a cattle pistollet (43.2%). This difference was non-significant, although there was a significant interaction of insemination equipment with dose rate.

A significantly higher conception rate was achieved for cervical (52.8%) than blind insemination (29%) when ewes were inseminated 'on time' in 1985 and this effect was consistent over dose rate.

There was no difference in conception rate when 'on-oestrus' insemination was compared to 'on-time' (63% *v* 61%). All first-order interactions among the effects of storage time, insemination method and 'on-oestrus' *v* 'on-time' were non-significant in the 1984 trial.

Keywords Sheep; AI; synchronisation; semen; CIDR; reproduction.

INTRODUCTION

Major advances in the use of artificial insemination (AI) in sheep have been achieved around the world in the last 5 years. The developments have been not only in the techniques and the methods of semen handling, but also in the development of breeding programmes using AI for genetic improvement (Clarke *et al.*, 1984). Australia has greatly increased the number of ewe inseminations over the last 5 years with 83 000 ewes being inseminated in 1984 (Maxwell, 1985). The use of sire referencing is being developed and expanded in recorded flocks in Western Australia since the development and use of intra-uterine insemination and frozen semen (Lewer, 1984).

It is generally accepted that conception rates of above 50% are required for commercial AI. Conception rates considerably lower than this (33% to 39%) have been achieved by some commercial operations using the controlled internal drug releasing device (CIDR) for synchronisation. These low conception rates and the high costs involved are still the major obstacles to developing an AI service within this country.

Trials undertaken in 1984 and 1985 at Rotomahana looked at some of the factors influencing conception rates in sheep AI and provided a link between Rotomahana and a commercial AI programme.

MATERIALS AND METHODS

Trial Design (1984)

The 1984 trial was a 3×2^2 factorial design, involving 248 3-year old ewes. The trial ran for 5 days with ewes being inseminated between 1100 and 1300 h daily with semen diluted to 200×10^6 sperm/dose. The factors investigated in the trial were:

1. Time of insemination. Ewes were either inseminated 51 to 53 h after CIDR removal (on-time) irrespective of whether or not they showed oestrus, or inseminated only when oestrus was detected (on-oestrus).
2. Storage time of semen. Semen was stored at ambient temperature for 5, 10 or 15 h after collection and then inseminated between 1100 h and 1300 h.
3. Insemination equipment. Semen was either stored in glass test tubes and inseminated using a fine glass pipette or was stored in 0.25 ml mini straws and inseminated using a standard cattle pistolett.

Ewes were pre-allocated to 16 rams of 4 breeds; Southdown, Suffolk, Border Leicester and English Leicester.

Trial Design (1985)

The 1985 trial involved 3 different subtrials spread over 3 weekly periods with each subtrial being a 2x2 factorial design (Table 1). A common treatment over

[1] Rotomahana Research Station, R.D.3, Rotorua

[2] Ruakura Animal Research Station, Ministry of Agriculture and Fisheries, Hamilton.

all 3 subtrials was a comparison of semen diluted to $200x10^6$ sperm/dose *v* $100x10^6$ sperm/dose. The other factors investigated were insemination of semen in glass pipettes *v* plastic straws (week 1, 255 ewes), storage of semen for 5 *v* 20 h (week 2, 541 ewes) and blind *v* cervical insemination (week 3, 172 ewes).

The 968 ewes inseminated in 1985 (Table 1) were all inseminated 'on-time' and were 2-, 3- and 4-year old ewes. These ewes were mated to 20 rams of 5 breeds; Southdown, Suffolk, Hampshire, English Leicester and Lincoln.

TABLE 1 Design of the 1985 AI trial and number of ewes inseminated.

Sperm (millions)	Week 1		Week 2		Week 3	
	Glass	Straw	5h	20h	Blind	Cervical
100	47	45	165	109	42	43
200	83	80	162	105	44	43

Experimental Procedures

Ewes from the Booroola Merino crossbreeding trial at Rotomahana Research Station were used in both years. All ewes were synchronised using CIDR devices and were inseminated cervically at 51 to 53 hours for 'on-time' treatments (1984 and 1985) or at the first oestrus after CIDR removal for 'on-oestrus' treatments (1984). In an attempt to stimulate onset of natural oestrus (Meyer, 1979), vasectomised rams were run with all ewes for 4 weeks before CIDR insertion in 1984 and with only 2-tooth ewes in 1985. All ewes in both years were run with vasectomised harnessed rams for 48 h after CIDR removal. Ewes were inseminated whether or not they had been marked by the vasectomised rams for the 'on-time' insemination. For the 'on-oestrus' treatments, ewes were inseminated only when oestrus was detected. Ewes were re-introduced to vasectomised rams 4 days after insemination. At the end of the AI trial ewes were mob-mated in breed groups for 21 days. Ewes that lambed 148 (± 5) days after insemination were accredited to AI conceptions.

All ewes were inseminated with 0.25 ml of diluted semen. Semen was collected using an artificial vagina, monitored and evaluated for concentration, motility and density (Harvey *et al.*, 1984). Semen was then diluted to $800x10^6$/ml or $400x10^6$/ml with skim milk. Diluted semen was used within 5 hours of collection except where ewes were allocated to treatments requiring 10, 15 or 20 h semen storage when special semen collections were made. All semen was stored at 32° before dilution and then stored at ambient temperature (8 to 18° C). As ewes were pre-allocated to specific sires, no semen was pooled and all live semen was used, irrespective of density and motility scores.

Statistical Analysis

Conception rate to AI was treated as a binomial trait and analysed using generalised linear models (Nelder and Wedderburn, 1972) with the logit link function.

The 1984 trial was analysed using a model including time of insemination, storage time and insemination equipment as main effects, all first-order interactions among these 3 main effects and day of insemination as another main effect.

The three 1985 weekly sub-trials were each analysed separately, the model for week 1 included day of insemination (2), ewe age, dose rate, insemination equipment and all first-order interactions between these effects but excluding day of insemination. A third day of insemination was excluded from this analysis because poor quality semen collected from some rams resulted in very low conception rates and was not used uniformly over the other treatments.

The model for week 2 included day of insemination (4), ewe age, dose rate, storage time and first-order interactions as in week 1.

In week 3 there was just one insemination day and the model included ewe age, dose rate, insemination method and all first-order interactions.

RESULTS

Storage time in 1984 indicated that there was a linear, but non-significant decrease in conception rate (5 h 68%, 10 h 61%, 15 h 57%). The linear trend on the logit scale was −0.26±0.17. In 1985 there was a highly significant difference between 5 h and 20 h storage (46% *v* 25%, logit difference 0.98±0.26), and there was no interaction with ewe age or dose rate.

In 1984, insemination carried out using a fine glass pipette gave a higher, but non-significant, conception rate than semen inseminated using a cattle straw pistollet (66% *v* 58%). In 1985 the trial (week 1) was modified with the semen being held in glass for 5 h and then inseminated with either a glass pipette or a cattle pistollet. Averaged over both semen dose rates there was a small but non-significant, advantage in conception rate from insemination with glass pipettes (49.8%) *v* insemination with cattle straws (43.2%). There was however an interaction ($P<0.10$) of dose rate with insemination equipment (Table 2). At the $100x10^6$ dose rate, conception rate was higher with glass pipettes.

TABLE 2 Effect of equipment and sperm dose rate on conception rate (Week 1—1985).

Sperm (millions)	Glass	Straw	Average
100	41.0 ± 7.3	46.8 ± 7.5	43.9
200	58.6 ± 5.4	39.5 ± 5.6	49.1
Average	49.8	43.2	

The conception rate was consistently higher for the 200 million sperm dose than the 100 million dose in all 3 weeks and the difference was significant pooled over the 3 weekly sub-trials (Table 3). Averaged over all 3 sub-trials the conception rates were 45.7% *v* 37.5% for 200 *v* 100 million sperm doses.

There was no significant difference between on-oestrus and on-time insemination (63% *v* 61%) in 1984.

The cervical *v* blind results in 1985 (Table 4) show a significantly higher conception rate for cervical insemination (52.8%) than blind insemination (29.1%) with no interaction with semen dose rates.

The difference in conception rate among ewe ages was significant ($P<0.001$) when pooled over the 3 sub-trials (Table 5).

TABLE 3 Effect of sperm dose rate on conception rate (1985).

Sperm (millions)	Week 1	2	3	Weighted average
100	43.9±5.2	33.1±3.0	32.2±5.0	37.5
200	49.2±3.9	38.4±4.0	49.7±5.0	45.7
Difference: 200-100 (logit scale)	0.22±0.27	0.25±0.18	0.79±0.33*	0.34±.14*

TABLE 4 Effect of insemination method and dose rate on conception rate (Week 3—1985).

Sperm (millions)	Insemination method Blind	Cervical	Average
100	19.8	44.6	32.1
200	38.4	61.0	49.7
Average	29.1	52.8	

TABLE 5 Comparison of AI conception rate by ewe age.

Ewe age	Week 1	2	3	Average
2	49.7±5.1	45.4±3.3	41.3±5.9	45.5
3	43.9±5.1	26.9±3.3	34.8±5.7	35.2
4	45.8±6.4	32.2±4.4	41.3±5.9	39.8
Significance	ns	***	ns	***

DISCUSSION

In 1985 a new design of hogget CIDR was used in the 2-tooth ewes. Compared to a ewe CIDR for 3- and 4-year old ewes, this new hogget CIDR was easier to insert and surprisingly the 2-tooth ewes had a higher conception rate than the older ewes. The ewe CIDRs supplied in 1985 had Cetiol A added which may explain some of the decrease in conception rate of the ewes in 1985. However, lack of ewe condition and of flushing may also have contributed (Ainsworth and Downey, 1984).

Because of other experimental requirements, vasectomised rams were run for 4 weeks with all ewes before CIDR insertion in 1983 and 1984 and with only the 2-tooth ewes in 1985 and this may have stimulated oestrous activity. This practice has not been undertaken in the industry.

To provide a link between the industry and research at Rotomahana, Perendale and Coopworth semen was supplied form Awahuri LIA Station and used at Rotomahana. There was only a small, non-significant, difference in conception rate between LIA semen (46%) compared to Rotomahana semen (42%) at Rotomahana. Also to provide a link, Rotomahana staff inseminated ewes in the industry in 1984 with little difference in conception rates between the Rotomahana and the LIA technicians. With the elimination of operator and semen from the model, the only major outstanding factor to explain the differences between Rotomahana and some commercial results in 1983 and 1984, and the poor result overall in 1985, is synchronisation.

The genetic gain possible through AI is affected by the number of ewes inseminated by a single ram and therefore dose is an important factor (Clarke *et al.*, 1984). The majority of overseas programmes using cervical insemination, inseminate at 400 million sperm in a 0.25 ml dose (MLC Report, 1982). At Rotomahana a 200 million dose was used in 1983 and 1984.

Very satisfactory AI results can be achieved with 100×10^6 dose rate in non-synchronised ewes. However, semen transportation is affected by progesterone and 200×10^6 may be the minimum dose for CIDR synchronised ewes (Langford and Marcus, 1982).

Because of the technical training required for cervical insemination, there is an interest in the industry for the use of blind insemination where the diluted semen is blindly injected into the vagina rather than carefully placed at the os of the cervix. Good results with blind insemination have been achieved at non-synchronised oestrus (Tervit *et al.*, 1984). Results were very low at Rotomahana for blind insemination, especially at 100×10^6 dose rate. If blind insemination is to be effective, insemination with higher sperm numbers seems to be required.

The inclusion of Cetiol A in the ewe CIDR does not fully explain the lower conception rate at Rotomahana in 1985 or in the industry. The effect that use of vasectomised rams had in the 1983 and 1984 trials is unknown and requires future research.

The effectiveness of CIDRs to give a tight synchronisation suitable for on-time insemination except under optimum conditions must be questioned and studied in the future. The genetic advantages offered by sire referencing and the use of AI (Clarke *et at.*, 1984) should not be overlooked and hopefully New Zealand can learn and expand in this field.

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