Field scale evaluation of semen diluents for cervical AI in sheep: An attempt at technology transfer

J.F. SMITH, J. ASPINALL[1], J.E. OLIVER, G. MURRAY, J.K. SMITH AND G.C. UPRETI

AgResearch Dairy and Beef Division, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

ABSTRACT

A new semen diluent RSD-1 has been developed which considerably enhances the storage life of ram semen, (at 15°C) when assessed by measurements of the percent sperm motile in the laboratory. This enhanced storage life could potentially overcome the current time constraints on the use of fresh ram semen (within 12 h of collection) when held at 15°C for AI. Field trials were conducted on commercial farms to compare the new diluent with the standard diluent (milk), with semen being cervically inseminated on the day of collection (6-12h storage) or the next day (30-36 storage). Trial 1 in 1992 involved 1127 ewes inseminated with semen from 8 rams on 10 farms in Otago-Southland. Trial 2 (1993) involved 1617 ewes, 16 rams and 11 farms and Trial 3 (1994) 1319 ewes, 10 rams, and 9 farms. There were significant effects on pregnancy rate, after cervical insemination, of diluent type ($P<0.01$), with milk being better than RSD-1 (29.6% vs 19.4%), and of storage time ($P<0.001$) with the day of collection being better than one day later (35.7% vs 13.3%). These overall differences were small compared to those between farms (11 to 44%, $P<0.001$) and between rams (10 to 38%, $P<0.01$).

In February 1994 an additional pre-season trial was conducted on a research station involving 535 ewes insminated cervically with semen from 9 rams in which minor modifications to the RSD-1 diluent were compared to the milk diluent. In this trial there was a significant effect on pregnancy rate after insemination of oestrous status ($P<0.01$) at time of AI (oestrus 57.3% vs not oestrus 37.9%) and of rams ($P<0.001$; 17 to 60%) but no significant effects of diluent nor of storage time.

These results show that the beneficial effects of the new (RSD-1) diluent as assessed by maintenance of percent sperm motile in the laboratory are not reflected in enhanced conception rates with cervical insemination. Thus re-evaluation of the techniques used for the determination of sperm viability are required. Also the major effects of rams and their interactions with diluent type and storage time on conception rates that were shown in these trials require further investigation.

Keywords: semen diluents; cervical AI; rams; field trials; storage time.

INTRODUCTION

Rates of genetic improvement and dissemination of superior genotypes in the New Zealand sheep flock could be increased through greater use of AI technology. In particular the use of AI to increase the degree of sire referencing could markedly increase the rate of genetic improvement through increased selection pressure. However, widespread adoption of AI is currently limited by the cost of insemination using intra-uterine laparoscopic techniques, lower levels of pregnancy obtained with cervical techniques and constraints on the availability of fresh semen from selected rams. Thus an increased uptake of AI technology is dependent on finding an improved system of fresh semen storage (beyond 24 hr) and improved pregnancy rates with cervical insemination. Reviewers of the limitations on the use of sheep AI in Australia have reached similar conclusions (Abbott, 1994; Windsor and van Bueren, 1994).

A recently developed synthetic diluent for ram semen RSD-1 (Upreti *et al,* 1995) has enabled the maintenance of motility (visual assessment of percentage motile) of semen incubated at 38°C (sheep body temperature) to be markedly prolonged compared to that for the standard milk diluent. Trials using the laparoscopic insemination technique have indicated that this prolonged in vitro motility does not increase conception rates (Smith *et al.,* 1993; Smith *et al.,* 1995). However as the success of insemination via the cervical technique is much more dependent on sperm motility it was decided to monitor (on both research and commercial farms) the effect of the new diluent on conception rates after cervical insemination.

MATERIALS AND METHODS

The trial was conducted over 3 years (1992, 1993, 1994).

Farms and Inseminator

Commercial farms were selected, on the basis of the owners' willingness to take part in the trial, from clients of the Woodlands Research Station AB centre. In 1992 10 farms involving 1,264 ewes participated. In 1993, 6 farms from the previous year and 5 new farms participated with 1,632 ewes involved, while in 1994 a total of 9 farms (3 for the first time) and 1,270 ewes were involved. All inseminations were conducted between the last week of March and the first week of May in each year.

On all farms, except Farm #1, insemination was performed using the cervical technique either by the farmers themselves or by staff of the Woodlands AB centre. All inseminators had been trained at courses conducted by the Woodlands centre. Intra-uterine technique via laparoscopy was performed by two experienced inseminators in each year on Farm #1 which served as a reference farm.

[1]AgResearch, Woodlands Research Station, Private Bag, Invercargill, New Zealand.

In February 1994 a pre-season trial was conducted on a research station (Flock House) involving 535 ewes inseminated cervically by two experienced operators.

Rams

The choice of rams was that of the farmers involved and was from those animals stationed at the Woodlands AB centre for provision of fresh semen for commercial AI programmes. Eight rams were used in the first year, 16 (including 3 of those previously used) in the second year and 10 (of which 1 was used in both previous years) in the third year. The breeds represented were Romney, Coopworth, Texel and Texel x Coopworth. In the 1994 February trial semen was collected from 9 (Dorset x Romney) rams at Ruakura Research Centre, processed and transported to Palmerston North for insemination.

Semen treatments

Two diluents were used, being the standard milk diluent and a synthetic diluent RSD-1 (Upreti *et al.*, 1995). Semen was collected - diluted and cooled to 15°C and was then either dispatched on the day of collection (Day 0) or held at 15°C for 24 hours and dispatched the day following collection (Day 1). On each farm semen from the same ram in both diluents and both storage times was used. However as dispatch to and insemination on most farms occurred on only one or two days then the same ejaculate was not always represented in all four treatments. In the 1994 February trial in addition to the milk diluent, semen was diluted in a milk+RSD-1 (50:50) diluent, in RSD-1 + CMC (2.0% w/v carboxymethyl cellulose) or RSD-3 + CMC (RSD-1 minus glutamate and fumerate and with 5mM oxaloacetate added). The dilution, cooling and storage processes were as described above. The inseminations in this trial were spread over 4 days and most of the ejaculates were represented in all treatments.

Oestrous synchrony

Four systems of oestrus control were employed depending on farmer preference. These were (a) natural detection of oestrus ewes from a large pool by harnessed teaser rams; (b) synchronised with CIDR (TM) type G devices (InterAg, Hamilton) and inseminated upon detection at the synchronised oestrus; (c) synchronised but detected at the second oestrus following CIDR removal, and (d) synchronised and inseminated on a time basis (approx. 56 hr) following CIDR removal. In the 1994 February trial all ewes were inseminated on a time basis and the oestrous status of the ewe (presence or absence of a tup mark) at time of insemination recorded.

Pregnancy status. On most farms pregnancy to insemination was determined by means of real time ultrasonic scanning at 50-60 days post-insemination. On 3 farms return to service data was used.

Analysis of data. The percentage of ewes inseminated that were diagnosed as pregnant was analysed using the residual maximum likelihood (REML) procedure in the Genstat statistical package. Farms and rams were entered as random effects while year, diluent, storage time, breed of ram and oestrous control system were entered as fixed effects. The number of ewes inseminated from one ejaculate x treatment combination was used as a weighting factor. Data for farm #1 and for the 1994 February trial were analysed separately.

RESULTS

The pregnancy rates for farm #1 (laparoscopic AI) were consistently higher than those for the remainder (cervical AI) of the farms in all years (Table 1). Within farm #1 there were significant effects of years ($P<0.01$), rams ($P<0.001$; Figure 1) and day of storage ($P<0.001$) but not for diluent type. Semen used on the day of collection was superior to that used the day after collection.

TABLE 1: Effects of year, diluent and day of storage on percentage of ewes pregnant for farm #1 (laparoscopic AI) and the remainder of the farms (cervical AI). Values are the adjusted means ± standard error of difference, the number of ewes inseminated is shown in parenthesis.

Variable		Farm #1	Farms #2 to #19
Year	1992	75.5 ± 8.6 (169)	36.0 ± 4.2 (958)
	1993	59.1 ± 8.6 (228)	23.2 ± 4.2 (1389)
	1994	64.4 ± 8.6 (176)	14.3 ± 4.2 (1143)
Diluent	Milk	68.7 ± 4.4 (293)	29.6 ± 1.6 (1750)
	RSD-1	63.9 ± 4.4 (277)	19.4 ± 1.6 (1740)
Storage time	Day 0	73.8 ± 4.4 (285)	35.7 ± 1.7 (1869)
	Day 1	58.9 ± 4.4 (285)	13.3 ± 1.7 (1621)
Overall		66.3 ± 7.7 (570)	24.5 ± 5.1 (3490)

FIGURE 1: Effect of ram on the pregnancy rate of ewes inseminated by a laparoscopic technique on Farm #1 over 3 years. Values are adjusted means (± sem).

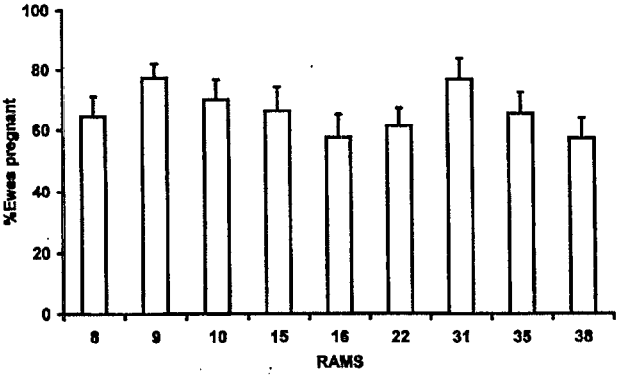
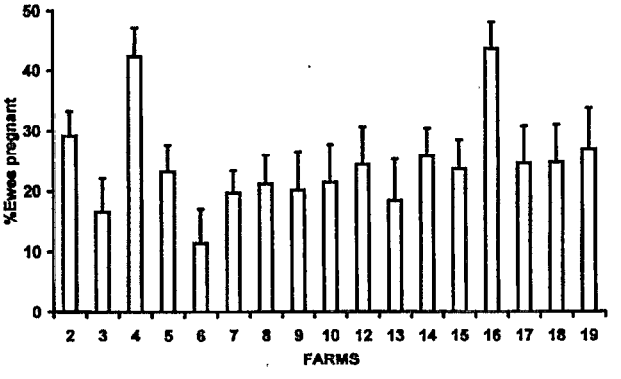


FIGURE 2: Variation between farms in the pregnancy rate of ewes cervically inseminated over 3 years. Values are adjusted means (± sem).



On the farms that used cervical insemination there were significant (P<0.001) differences in pregnancy rates between farms, rams, diluent, and day of storage (Table 1 and Figures 2 and 3). Milk was superior to RSD-1 and D 0 was better than D 1. There were no significant effects of method of oestrous control nor of ram breed.

In the 1994 February pre-season trial the overall percentage of ewes that were pregnant to AI was 44.5%. There was a significant (P<0.01) effect of oestrous status at time of AI (oestrous 51.3%, not oestrous 37.9%) and of rams (17 to 60%) (P<0.001) but no significant effects of diluent nor of storage time (Table 2).

DISCUSSION

This series of on-farm trials highlight the beneficial effect of intra-uterine compared to cervical insemination techniques. In addition to the improved pregnancy rates there is also a 4-fold increase in efficiency of sperm utilisation because of the lower concentration needed for intra-uterine insemination. The results for intra-uterine insemination on farm #1 were consistent with those obtained in other studies (Smith *et al.*, 1995) and show that semen stored at 15°C for over 24h can be successfully used.

Pregnancy rates with cervical insemination were disappointingly low. Large between-farm and between-ram effects are consistent with other reports. However the decline in pregnancy rate over time is difficult to explain especially as it occurred on those farms that participated in all 3 years. It may represent subtle undetected changes in the procedures for semen handling over the period of the trial, or equally be due to changes in environmental factors influencing either or both ram and ewe fertility.

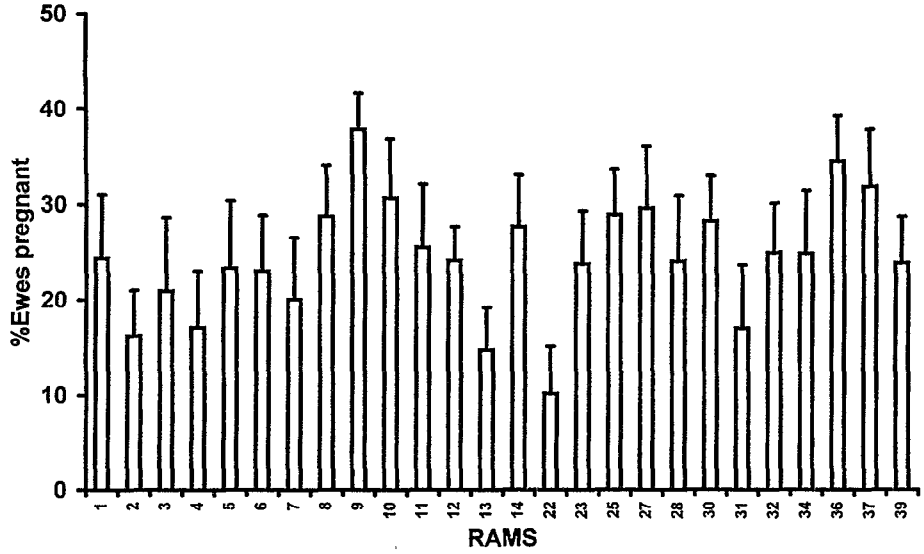
The difference between the 1994 February experiment pregnancy rates and the main trial in 1994 can possibly be explained by differences in location (flock etc) and that different rams were used. The results from this pre-season experiment and those from individual farms in some years illustrates that acceptable results can be obtained with the cervical technique using these diluents. However, variation in pregnancy rates and the large range of factors that had significantly affected these illustrates the problems encountered in technology transfer of systems that are not completely robust. Low numbers of ewes inseminated by the participating farmers each year raises the question of inseminator proficiency: for successful adoption of the technology use of professional inseminators should be considered.

The failure of the RSD-1 diluent to prove superior to the standard milk diluent is indicative that maintenance of sperm motility (percentage sperm motile) upon incubation is not an adequate measure of sperm viability. This disassociation of maintenance of percent motile and fertility of ram sperm has been a widespread problem (Maxwell and Salamon, 1993) and underlines the urgent need for improved in vitro measures of sperm viability.

TABLE 2: Effect of diluent, storage time and oestrous status on the pregnancy rate of ewes cervically inseminated with diluted semen in the 1994 February pre-season trial.
Values presented are the adjusted means (n = number of ewes AI ed)

	Oestrous status					
	Oestrus		not Oestrous		Total	
Diluent						
Milk	62.3	(61)	39.4	(71)	50.0	(132)
RSD-1 + Milk	51.4	(70)	35.7	(70)	43.6	(140)
RSD-1 + CMC	50.7	(71)	40.4	(57)	46.1	(128)
RSD-3 + CMC	41.0	(61)	36.5	(74)	38.5	(135)
Storage time						
Day 0	54.8	(124)	40.4	(141)	47.2	(265)
Day 1	48.2	(139)	35.1	(131)	41.8	(270)
Overall	51.3	(263)	37.9	(272)	44.9	(535)

FIGURE 3: Variation between rams on the pregnancy rate of ewes cervically inseminated over 3 years. Values are adjusted means (± sem).



Large between-ram differences in pregnancy rates despite all semen satisfying stringent criteria of density and motility at the time of processing highlight the need to investigate the contribution of the composition of the seminal plasma to fertility.

ACKNOWLEDGEMENTS

To Mrs E. Lewis and Mrs B. Henderson of the AgResearch Woodlands AB centre for semen collection, processing and dispatch. To the participating farmers and flock owners; J. McEwan, Woodlands; K. Knowler, Invercargill; L. Wilson, Winton; C. Miller, Invercargill; G. Smith, Wyndham; P. Black, Riverton; D. Lyders, Outram; G. Black, Riverton; R. Glover, Te Anau; R. Preston, Winton; C. Reid, Outram; T. Knight, Woodville; N. Beck, Otautau; G. Shaw, Balclutha; N. Morrison, Clinton; N. Ede, Invercargill; C. Halder, Invercargill; K. Hocking, Invercargill; I. Crean, Invercargill. To G. Shackell and I. Scott for inseminations on farm #1. To J. Parr for insemination in the 1994 February experiment, to Mrs S. Beaumont, Mrs J. Oliver and C. Harris for collection and processing of semen at Ruakura, and to Ms A. Death and staff at Flock House for assistance with that trial. To D.M. Duganzich for statistical analysis of the data. The research was funded by the New Zealand Meat Research and Development Council (Project 91 MT 66/3.2).

REFERENCES

Abbott, K.A. 1994. Cost-benefit evaluation of artificial insemination for genetic improvement of wool-producing sheep. *Australian Veterinary Journal* **71:** 353-360.

Maxwell, W.M.C. and Salamon, S. 1993. Liquid storage of ram semen: *A review. Reproduction, Fertility and Development* **5:** 613-638.

Smith, J.F.; Asher, G.W.; Briggs, R.M.; Morrow, C.J.; Murray, G.R.; Oliver, J.E.; Parr, J.; Veldhuizen, F.A. and Upreti, G.C. 1993. Effect of diluent and storage time on pregnancy rate in ewes after intrauterine insemination. *Proceedings of the New Zealand Society of Animal Production* **53:** 295-298.

Smith, J.F.; Parr, J.; Murray, G.; Oliver, J.; Beaumont, S. and Upreti, G.C. 1995. Effect of diluent type and storage time on the pregnancy rate of ewes inseminated laparoscopically with chilled ram semen. *Proceedings of the New Zealand Society of Animal Production.* **55:** 245-247.

Upreti, G.C.; Oliver, J.E.; Duganzich, D.M.; Munday, R. and Smith, J.F. 1995. Development of a chemically defined ram semen diluent (RSD-1). *Animal Reproduction Science* **37:** 143-157.

Windsor, D.P. and van Bueren, M. 1994. Potential benefits of artificial insemination with frozen semen to the Australian Wool Industry. *Wool Technology and Sheep Breeding* **42:** 253-262.