Effect of sperm dose, diluent type and timing of insemination on pregnancy rates in sheep

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ABSTRACT

Low sperm numbers and ambient temperature storage are two important factors in the successful AI system for dairy cattle in New Zealand. Similar technologies with ram semen are required before the establishment of a similar AI system within the NZ sheep industry is feasible. A trial involving a total of 1200 Romney ewes was conducted in April 1997 to examine the effects of inseminate dose (50, 25, or 5 x 10^6 sperm) of semen held for up to 9 h at 20°C in two diluents (RSD-1 and Tris + egg yolk). In the first half of the trial ewes were laparoscopically inseminated with semen from Finn rams at set times (49 and 56 h) after CIDR removal, while in the second half of the trial ewes were inseminated with semen from Romney rams following the detection of oestrus, after CIDR removal, resulting in insemination times of 26, 44 and 49 h post removal. Returns to service in the cycle following AI were recorded and ewes were scanned for pregnancy 50 days post AI.

There was a significant difference between ram breeds in pregnancy rate (Finn 63.2 % v Romney 50.2 % ; $P<0.05$). There were no effects of inseminate dose nor of diluent. Time to onset of oestrus was affected by ewe live weight ($P<0.05$) with the heavier ewes showing a trend for an earlier onset but there was no overall effect of ewe weight on fertility. The effect of time to onset was reflected in an effect of time from CIDR removal to AI in the second half of the trial, with ewes AI'd within 30h of CIDR removal having a 10% lower fertility.

These results indicate that acceptable fertility can be achieved with semen dose rates 5 to 10 fold less than normal, thereby increasing the number of inseminations available per ejaculate. Improved synchrony methods seem imperative to obtaining better fertility from AI.

Key words: Sperm dose, diluent, time of AI, pregnancy.

INTRODUCTION

Rapid genetic progress within the New Zealand Sheep Industry will be dependent on the availability of effective and efficient techniques for Artificial Insemination (AI). This will be even more essential for the rapid dissemination of identified superior genotypes once 'marker-assisted selection' is more readily available. The very efficient and successful system of genetic improvement and large scale AI in New Zealand Dairy Industry is a possible model. This system depends on two core semen technologies which enable the use of very dilute fresh semen, held at ambient temperatures. These technologies enable large numbers (1,000's) of inseminations to be made from a single ejaculate from intensively selected (high BW) bulls while current ram semen technology will only allow small numbers (10's) of inseminations per ejaculate, although the difference between species in the total number of sperm per ejaculate is only a factor of 2x. The ability to use low sperm doses in sheep AI is an essential pre-requisite for the extensive use of "special rams". The current experiment examined the effect on the pregnancy rate of ewes, of sperm dose, diluent type and timing of AI, with semen held at 20°C for periods up to 9 hrs.

METHODS

Animals: Ewes: Romney ewes (1,200) were allocated to treatment sub-groups on the basis of age and liveweight. Ewes were grazed in two flocks of 600 on similar pasture levels.

Rams: Four Finn (4-tooth) and four Romney (2-tooth) rams selected for performance traits were trained for semen collection by artificial vagina prior at the commencement of the trial.

Oestrus synchronisation: All ewes were treated with CIDR-G™ devices (InterAg, Hamilton, New Zealand) for a period of 13-14 days to synchronise the onset of oestrus. All ewes were run with harnessed vasectomised rams and were examined twice daily at 0730 h and 1730 h for presence of crayon marks.

Trial Design and Conduct: The trial design was essentially a (3 x 2 x 2 x 2 x 2) factorial design, which was modified by management constraints. Three inseminate doses (50, 25 and 5 x 10^6 sperm) of semen were used in two diluents RSD-1 (Upreti, *et al*., 1995) and Tris + egg yolk [EY] (Evans and Maxwell, 1987) modified to have an EY level of only 5%. Semen from two breeds of ram (Finn and Romney) were used but for management reasons this was at two separate times in the trial (1st half vs 2nd half). Two times of insemination were chosen (48 and 56 h after CIDR removal). All ewes allocated to these subgroups were AI'd after CIDR removal independent of tupping marks and inseminations were performed by two experienced inseminators. The inseminations were spread evenly over 8 days (2 groups of 4 days separated by a 2 day interval). Due to the pattern of onset of oestrus observed in the first four days of the trial the timing of AI for the second four days was

altered. In this half of the trial ewes were inseminated on detection of oestrus [i.e. ewes 'crayon marked' at 1730 h were AI'd next morning (0900 h) while ewes marked at 0730 h were AI'd that afternoon (1600 h)] , resulting in insemination times of 26, 44 and 49 h post removal. Ewes not detected in oestrus by 44 h after CIDR removal were also inseminated at 49 h.

Semen collection: Each day at 0700 h ejaculates from two rams of a breed were collected, evaluated and pooled at 30°C. The semen was then divided into two and serially diluted with the two diluents to the required concentrations. Semen was then cooled to 20°C and packaged into 0.25 ml straws containing the required sperm dosage. The straws were then held at 20°C in a portable incubator until used for inseminations at 0930 - 1100 h and 1500 - 1630 h that day.

Inseminations: Ewes were inseminated laparoscopically (Killeen and Caffrey, 1982) following sedation with 0.4 ml "Rompun".

Pregnancy measures: Initial conception rates were determined by detection of 'returns to service' from harnessed rams introduced 10 days after AI and subsequently by ultrasonic scanning at day 50 and also from lambing records.

Statistical analysis: The percentage ewes pregnant was analysed by fitting models using the generalised linear model procedure for binomial data in the 'Genstat' statistical package.

RESULTS

The individual ewe live-weights ranged from 76 to 40 kg with the means for those AI'd with Romney semen being 53.6 kg and for those AI'd with Finn semen being 51.0 kg.

Pregnancy rate: There was no significant effect of semen dose ($x10^6$) on pregnancy rate (**50** = 57.7 ± 6.3%, **25** = 59.1 ± 6.2% and **5** = 53.3 ± 6.5%) nor was there an effect of diluent (RSD-1 = 57.9 ± 6.1% vs Tris + EY = 55.7 ± 6.1%). There was no effect of inseminator (A= 57.9 ± 6.1% and B = 55.7 ± 6.1%. Ewe age and live-weight had no effect on pregnancy rate. There was no interaction of the above factors with ram breed, day of AI, or with each other.

There was a significant effect of ram breed (Finn 63.2 ± 6.7% vs Romney 50.2 ± 6.2%) and a significant interaction between ram breed and day of insemination (Table 1). The Finn semen showed no difference between days while the Romney semen was significantly lower on the first day and gradually improved over the insemination period.

TABLE 1: Interaction of ram breed and day of insemination on percentage of ewes pregnant.

Day	Finn	Romney
1	63.3 ± 8.0	38.8 ± 7.2
2	62.6 ± 8.1	46.7 ± 7.9
3	60.0 ± 8.2	52.2 ± 7.5
4	66.8 ± 7.7	63.3 ± 8.1
Total	63.2 ± 6.7	50.2 ± 6.2

The interval from CIDR removal until AI tended to an effect with those inseminated at 44 h after removal (64.4 ± 6.6%) being slightly higher than those at the other times (26 h = 52.8 ± 9.2%, 49 h = 56.0 ± 6.1% and 55 h = 53.6 ± 7.1%).

Removal of the animals which failed to exhibit oestrus from the analysis had no effect on the significance of the comparisons.

Time of onset of oestrus: The time of onset of oestrus after CIDR (Table2) indicated that those in heat within 24h of CIDR removal (25% of ewes) and those not detected by time of AI (10% of ewes) had fertility levels 10-15% lower than the other ewes but this was not statistically significant. This effect was apparent in both weeks of the trial and appears independent of the insemination protocol.

The pattern of oestrous onset was influenced by ewe live-weight ($P<0.05$) with a higher proportion of the heavier ewes exhibiting oestrus early.

TABLE 2: Pattern of tupping after CIDR removal .

Ram Breed	Tup interval*	No. Ewes	% of flock	%pregnant
Finn	24	160	26.8	55.0
	33	231	38.7	63.2
	48	101	16.9	67.3
	not tupped	105	17.6	53.3
Romney	18	141	22.7	42.6
	27	268	43.1	56.7
	42	174	28.0	49.4
	not tupped	39	6.3	43.6

* Interval from CIDR out to tupping (h)

DISCUSSION

The lack of any sperm dose effect in these results highlight the ability to use much lower doses of fresh semen via laparoscopic AI than is currently the practice and suggest that further trials be conducted to determine the lower limits of the number of sperm needed for insemination. The successful use of 5×10^6 sperm per inseminate means that about 600 ewes can be inseminated with a single ejaculate of semen of average volume and concentration. Coupled with the ability to keep this semen viable for up to 9 h when held at 20°C provides the opportunity to dispatch large numbers of fresh semen doses from a central location. The use of low doses of motile sperm via laparoscopic AI has previously been reported (Maxwell, 1986) although doses below 20 $x10^6$ resulted in lowered fertility.

The lack of a diluent effect indicates that RSD-1 is as an effective a diluent as the more commonly used Tris-EY diluent for use with fresh semen and confirms the results of laboratory storage trials (unpublished data).

The difference in fertility between breeds of ram is most likely due to subtle effects of semen quality as the possible effects of confounding by time (maxium of 6

days) and insemination system (detection v time) were accounted for in the model used for analysis. Semen evaluations performed immediately after collection and dilution showed no differences between breeds or days. However, evaluations performed post-insemination (i.e. 10-11 h after collection and dilution) showed a tendency for a greater decline in the percentage of live sperm on days 1 and 2 of use of the Romney semen.

Laparoscopic AI is most efficient if the throughput of ewes can be predetermined using oestrus synchronisation and fixed time insemination. There was no difference between AI at 48 or 56 h in the first week of the trial using fixed times, while insemination 44 h after CIDR removal was the best when inseminations were performed on detection of oestrus . The time of onset of oestrus after CIDR removal was earlier than that predicted from previous work (Smith *et al*., 1991). The premature onset of oestrus following CIDR treatment and the associated lower fertility with this early onset presents a significant problem for the large scale use of sheep AI and indicates the need for changes in the synchronisation method or the CIDR device. The earlier onset in the heavier ewes is supported by previous data (Smith *et al*., 1991) and most probably reflects the increased rate of metabolism of progesterone.

Further research should investigate the use of even lower semen doses per insemination and means of improving the precision of oestrous synchronisation.

ACKNOWLEDGEMENTS

To Peter Moore and the staff at Whatawhata Research Station for their assistance in the conduct of the trial. This work was supported by NZMRDC project (91MT 66/3.2) and NSOF funding.

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