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Effect of very low sperm doses on pregnancy rates after AI 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. A critical evaluation of these factors with ram semen is required before the establishment of a similar AI system within the NZ sheep industry is feasible. A trial involving a total of 1262 Romney ewes was conducted in April 1998 to examine the effects of inseminate dose (25, 5, 1 and 0.5 x 10^6 sperm) of semen held for up to 9 h at 20°C in two diluents (RSD-1 and RSD-8). Ewes, synchronised with 3 types of CIDR devices were laparoscopically AI'ed with semen from either a Finn or a Romney ram at set times (49 or 56h) after CIDR removal. AI was spread over 4 days in each of two consecutive weeks. Rams were used on each alternative day. Ewes were checked for return to service to determine pregnancy.

There was a significant ($P<0.001$) difference between rams in pregnancy rate (Finn =35.6 and Romney =52.5 %). There was an effect ($P<0.001$) of inseminate dose (25 = 55.5 ± 2.6 %; 5 = 45.2 ± 2.6 %; 1 = 37.2 ± 2.6 % and 0.5 = 37.5 ± 2.6 %). However there was a suggestion of an overall dose by ram interaction with the Romney semen maintaining higher fertility at lower dose levels. There were no effects of inseminator or of diluent. Time from CIDR removal to oestrus had a significant ($P<0.05$) effect. Time to onset of oestrus was affected by CIDR type. While there was no overall effect of CIDR type on fertility, there was a significant ($P<0.05$) interaction between sperm dose and CIDR type. These results indicate that acceptable (>50%) fertility can be achieved with sperm dose rates as low as 1.0x10^6 if semen quality is satisfactory thus increasing the number of inseminates per ejaculate 20-50 fold. Improved synchrony methods seem imperative to obtaining better fertility from AI.

Keyword: 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 that involve the use of very dilute fresh semen, held at ambient temperatures. This enables large numbers (1,000's) of inseminations to be made from a single ejaculate, from intensively selected (high BW) bulls. 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 very 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 (1344) were allocated to treatment sub-groups on the basis of age and liveweight. Ewes were grazed in two flocks (of about 650) on similar pasture levels.

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

Trial Design and Conduct

The design of the trial was a 4x2x2x2x2x3 factorial with n=7 and N= 1344. A total of 82 ewes on which complete information was not collected were removed from the final data base used for statistical analysis, for various reasons such as death, loss of weight, loss of CIDR device, loss of ear tag.

Dose of semen

Four doses of sperm (25.0 v 5.0 v 1.0 v 0.5 x10^6 sperm) were inseminated.

Diluent

Two diluents [RSD-1 (Upreti, *et al*., 1995) and RSD-8 - a simplified medium] were used.

Rams

Semen from either a Finn or a Romney ram was collected by artificial vagina.

Time of insemination

Two times of insemination were chosen (48 and 56 h after CIDR removal). All ewes allocated to these sub-groups were AI'd after CIDR removal independent of tupping marks.

Inseminators

Two experienced inseminators performed the inseminations.

Oestrus synchronisation

All ewes were treated with one of three different

types of CIDR device for 14 days to synchronise oestrus (1x CIDR-G, 2x CIDR-Gs, 1x Experimental type CIDR 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.

Semen collection

Each day at 0700 h an ejaculate from one ram was collected. 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. AI was spread over 4 days in each of two consecutive weeks and the individual rams were used on each alternative day.

Inseminations

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

Pregnancy measures

Conception rates were determined by detection of 'returns to service' using harnessed rams introduced 10 days after AI and recorded 3 times a week for a period of 21 days.

Statistical analysis

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

RESULTS

Liveweight

The mean and range of live-weights for the ewes mated to the Finn ram were 48.2 kg (42.0 to 66.6) and for those mated to the Romney ram 50.1 kg (41.0 to 73.0).

Pregnancy rate

The overall pregnancy rate was 44.1 ± 1.3%. (mean ± se).

Ewe age and live-weight had no effect on pregnancy rate and there was no interaction between these and any other factors. There was a significant (P<0.001) difference between the two rams (Romney = 52.5 ± 1.9 % v Finn = 35.6 ± 1.8 %). There was also a significant (P<0.001) overall effect of semen dose (25 x10^6 = 55.5 ± 2.6 %; 5 x10^6 = 45.2 ± 2.6 %; 1 x10^6 = 37.2 ± 2.6 % and 0.5 x10^6 = 37.5 ± 25.6 %) but this was different for the two rams with the drop off point at a much lower concentration for the Romney ram than for the Finn ram (Table 1). There was a significant (P<0.001) interaction between ram and day of experiment with the Romney ram performing much better in the second week of the trial but not the Finn ram (Table 2). There were no significant effects of diluent (RSD-1 = 44.5 ± 1.8 % and RSD-8 = 43.6 ± 1.8 %) nor of inseminator (JFS = 44.6 ± 1.8 % and JP = 43.5 ± 1.8 %). However there was a significant (P<0.01) interaction between Inseminator and Ram with JP performing relatively worse with the Finn semen but relatively better with the Romney semen.

TABLE 1: Effect of different doses of sperm inseminated on pregnancy rate (%).

Dose (x10^6)	Romney	Finn
25.0	59.7 %	50.9 %
5.0	53.3 %	36.7 %
1.0	50.6 %	23.9 %
0.5	43.0 %	30.9 %
Total	52.5 %	35.6 %

TABLE 2: Effect of ram by day of collection on pregnancy rate (%).

Day of Collection	Finn	Romney
Week 1 - Day 1	35.4 ± 3.5	
Week 1 - Day 2		31.6 ± 3.7
Week 1 - Day 3	28.6 ± 3.6	
Week 1 - Day 4		30.3 ± 3.7
Week 2 - Day 1	35.6 ± 3.7	
Week 2 - Day 2		73.6 ± 3.7
Week 2 - Day 3	42.9 ± 3.6	
Week 2 - Day 4		75.3 ± 3.8

There was a significant (P< 0.05) effect of time of insemination (am 49h = 45.0 ± 2.3 % and p.m. 56h = 38.8 ± 2.3 %).

While there was no significant overall effect of CIDR type on pregnancy (2xG = 45.0 ± 2.2 %, 1xG = 45.6 ± 2.2 % and Experimental = 41.5 ± 2.3 %), there was an indication (P= 0.1) of an interaction between CIDR type and semen dose, with the 2xG treatment being better than the other treatments at the low semen doses and the Experimental CIDR being generally poorer (Table 3).

TABLE 3: Interaction between sperm dose and CIDR type on pregnancy rate (%)..

Semen Dose	2xG	1xG	Experimental
0.5	41.9 ± 4.6	33.4 ± 4.4	37.1 ± 4.5
1.0	40.7 ± 4.5	34.8 ± 4.4	36.0 ± 4.5
5.0	39.0 ± 4.4	56.4 ± 4.5	40.1 ± 4.5
25.0	58.6 ± 4.5	58.0 ± 4.4	52.9 ± 4.6

Time to onset of oestrus had an effect on pregnancy rate, with the ewes exhibiting onset by 8 h having a much lower rate (23.8%) than animals showing oestrus later (mean 44.3%). There appeared to be an interaction (P<0.1) between CIDR type and time to onset of oestrus, with the Experimental CIDR being lower in the pregnancy rates at the 22 and 32 h times (Table 4).

TABLE 4: Interaction between time to onset of oestrus and CIDR type on pregnancy rate (%).

Time to onset of oestrus (hrs after CIDR removal)	2xG	1xG	Experimental
8	16.7	46.2	9.1
22	52.6	52.0	44.6
32	49.4	42.2	33.3
46	40.7	44.3	40.8
Not Tupped at AI	46.8	42.3	48.2

There was also an interesting (non-significant) indication of CIDR type influencing the extent of the difference in pregnancy rate between am and pm AI times, with the Experimental CIDR having the largest difference (Table 5).

TABLE 5: Effect of CIDR type on pregnancy rate (%) at different times of AI.

CIDR type	AI am	AI pm	Difference (am-pm)
2xG	47.6	42.2	5.4
1xG	45.9	42.9	3.0
1x Experimental	46.0	35.1	10.9

Time of onset of oestrus

There was an effect of CIDR type on 'time to onset of oestrus after removal' with type 2xG showing a trend for a delay in onset compared to the other two types (Table 6). Overall CIDR retention rate was 98.8% with no effect of CIDR type.

TABLE 6: Percentage of ewes exhibiting oestrus at various times after CIDR removal.

CIDR Type	8h	22h	32h	46h	No Tup
2xG	4.33%	4.57%	20.91%	21.88%	48.32%
1xG	3.02%	17.44%	31.40%	16.28%	31.86%
Experimental	2.64%	19.95%	33.17%	11.78%	32.45%
Total	3.33%	14.03%	28.53%	16.64%	37.48%

DISCUSSION

These results support the findings of a previous trial (Smith *et al*., 1998) and extend them.

The effect of sperm dose levels is apparent and the minimum level for acceptable results (>50% pregnancy rate) can be quite low (i.e. 1 x 10^6 sperm per inseminate) provided the quality of the semen used is satisfactory. While the visual motility of both the Romney and Finn semen was acceptable at time of collection and dilution the post AI measures (at 11hr post collection) of motility and viability were poorer for the Finn semen. This under-performance of the Finn semen was the major reason for the low overall fertility level. This is not likely to be a breed effect but rather an individual ram difference as the results of the previous trial showed the opposite breed ranking.

This is one of the very few reported examples of semen quality by sperm dose effects on fertility. These results mean that if semen quality is good then very large numbers of ewes (up to 3,000) can be inseminated from 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, in that work, doses below 20 x10^6 resulted in lowered fertility. The extension of semen to 1 x 10^6 sperm per inseminate is the same level of sperm utilisation currently achieved in the Dairy industry with bull semen. However, if this magnitude of semen extension is to be used, then there is a urgent need to establish measures of differences in quality of semen, and its ability to withstand dilution to the low concentrations, prior to the performance of insemination rather than the post-insemination differences recorded in the present trial.

While 'semen quality' may explain the overall ram and ram by dose differences seen the marked differences between the first and second week in the performance of the Romney semen is difficult to explain as semen quality changes of that magnitude were not evident.

The lack of difference between the two diluents is encouraging as the RSD-8 diluent is a much simplified diluent compared to RSD-1 and will be considerably cheaper and easier to prepare. The laboratory tests on these diluents indicate similar ability to maintain sperm *in vitro*.

The overall result for the two inseminators was similar and this was consistent with results from other trials involving these inseminators. The interaction between inseminator and ram is difficult to explain. While a poorer performance of one inseminator with the inferior quality sperm could indicate a less adequate insemination technique this does not fit the achievement of higher fertility by the same inseminator with the better semen.

The effect of time of insemination (i.e. am vs p.m.) could indicate an effect of deterioration of semen quality with duration of storage and/or a better synchronisation of AI time with onset of oestrus. The fact that the difference (am -p.m.) was greater for the lower sperm doses and that time to onset of oestrus showed no consistent pattern in the (am - p.m.) difference, would suggest that semen quality was the major cause of the effect.

Laparoscopic AI is most efficient if the throughput of ewes can be predetermined using oestrus synchronisation and fixed time insemination. The effect of a very early onset of oestrus is somewhat more marked in this trial than in the previous trial but the effect of 'not detected in oestrus' before AI is less so, possibly due to the delay in onset of ewes treated with type 2xG CIDR's. Ewes treated with this type of CIDR definitely had a later onset of oestrus than those treated with the other types. This delay in onset is undoubtedly due to the significantly higher levels of plasma progesterone present at time of removal in the 2xG type CIDR ewes (data not presented).

While there was no overall effect of CIDR type on pregnancy rate, the interaction of CIDR type and sperm dose indicated that the 2xG type was superior at the low sperm doses.

This effect is an important finding in that quite often effects of this nature (i.e. time to onset of oestrus or synchronisation device effects) are masked by the use of excessive amounts of semen.

The poorer performance of ewes treated with the Experimental CIDR that exhibited early onset of oestrus times and the greater drop in fertility with pm AI after this treatment is probably also a reflection of the lower (although non-significant) progesterone levels following this treatment.

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. These present results indicate that the development of a CIDR device that produces higher levels of plasma progesterone at the end of treatment is likely to enhance the conception rates following AI at a synchronised oestrus.

While the overall results and the low sperm dose responses are encouraging findings further work is required to extend the time of storage of semen at 20°C and to develop more precise synchronisation techniques and more cost effective insemination methods.

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