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The insemination of sheep with fresh or frozen semen

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ABSTRACT

Ewes were inseminated in 1982 and 1983 at the second oestrus after synchronisation. They received fresh or frozen semen deposited once or twice, either into the cervix, blind in the vagina, onto the ovaries or into the uterine horn lumen. Very poor results occurred after ovarian insemination. More ewes lambed when inseminated with fresh compared to frozen semen, when inseminated twice rather than once, and for 1983, the ranking of insemination positions (best to worst) was intra-uterine, blind, cervix. Ewes lambing after insemination with frozen semen in 1982 produced fewer multiples than those inseminated with fresh semen. A single blind insemination of fresh semen gave a lambing rate (64%) only slightly lower than for cervical deposition (69%) and the intra-uterine technique performed well with both fresh and frozen sperm (83% and 38%, respectively). Both these techniques should be considered for artificial insemination.

Keywords Sheep; artificial insemination; semen; endoscope; pregnancy; cervix; vagina

INTRODUCTION

The value of artificial insemination (AI) for increasing breeding progress through more intensive sire selection is well documented (Tervit et al., 1978). Despite this knowledge and the availability of regimes utilising fresh semen, the technique is seldom used in the New Zealand sheep industry. This is primarily because relatively few animals are inseminated from each ejaculate collected, the technique is labour intensive and technically demanding, and poor results are generally achieved with frozen semen.

This paper describes recent trials aimed at simplifying the insemination techniques involved, increasing the number of ewes inseminated per ejaculate and assessing the pregnancy rate from frozen semen.

MATERIALS AND METHODS

Trials were conducted at Wairakei Research Station during April/May in 1982 and 1983. Each year 400 mature Perendale ewes were treated for 14 to 16 days with intravaginal sponges impregnated with 70 mg MAP (Medroxyprogesterone acetate, Upjohn). The synchronised oestrus was detected with harnessed vasectomised rams (ram:cwc, 10:100) and the ewes inseminated at the subsequent return oestrus. To detect the return oestrus the ewes were run with vasectomised rams and examined for mating marks twice daily at 8 am and 5 pm.

Number and timing of inseminations

Ewes to be inseminated once were drafted daily at 8 am and inseminated 1 h later (an average of 13 h after onset). Ewes to be inseminated twice and first detected in oestrus at 8 am, were inseminated at 9 am and again at 9 pm on the day of detection while ewes first detected at 5 pm were inseminated at 9 pm on the day of detection and again at 9 am the following day (an average of 8.5 and 20.5 h after onset at first and second inseminations, respectively).

Semen processing

Fresh: Ejaculates from Perendale rams were collected in artificial vaginas, pooled and diluted in milk (Colas et al., 1968) to 900, 450 or 40 x 10^6 sperm/ml. The majority of the semen was then loaded into 0.5 ml Cassou straws and all semen was held at room temperature until insemination.

Frozen: Pooled ejaculates were diluted in a Tris-based diluent to 900 or 80 x 10^6 sperm/ml and then frozen in 0.5 ml straws in liquid nitrogen. Representative straws were thawed shortly after freezing and incubated for 3 h at 39°C. Samples were rejected if less than 45% of the sperm were motile immediately after thawing and less than 20% were motile at 3 h.
Insemination techniques

During the 2 years 4 insemination techniques were investigated.

1) Cervix: The cervix was viewed through a lighted speculum and fresh or frozen semen deposited as far as possible through the folds using a Cassou gun.

2) Blind: The vulva lips were parted and a Cassou gun passed as far as possible into the vagina. No speculum was used.

3) Ovarian: Each ewe was tranquilised with Rompun (Bayer), loaded onto an endoscopy cradle, and the abdominal cavity inflated with compressed air. The reproductive tract was viewed through an endoscope and 0.25 ml fresh or frozen semen deposited onto each ovary through a Cassou gun.

4) Intra-uterine: Each ewe was tranquilised, the abdomen inflated with carbon dioxide, the reproductive tract viewed through an endoscope and 0.03 to 0.04 ml fresh or frozen semen deposited through glass pipettes into the lumen of each uterine horn.

Lambing dates and numbers of lambs born were recorded for all ewes.

RESULTS

The percentage of inseminated ewes which lambed, and percentage lambed ewes producing multiples for the treatments in 1982 are shown in Table 1. Lambing percentages following the deposition of low numbers of sperm onto each ovary were very poor and these treatments were not included in the data analysis. For the other treatments, a very satisfactory result was achieved with fresh semen and the result with frozen semen, although inferior to that of fresh deposited in the cervix \( (P<0.001) \), was encouraging. More of the animals inseminated twice lambed than those inseminated once \( (P<0.05) \). With fresh semen, there tended to be an effect of position semen was deposited \( (P<0.05) \).

TABLE 1 Lambing performance of ewes inseminated in 1982.

<table>
<thead>
<tr>
<th>Semen Type</th>
<th>Position deposited</th>
<th>No. of inseminations</th>
<th>Total no. of sperm inseminated ( \times 10^6 )</th>
<th>Ewes lambing%</th>
<th>Lambed ewes with multiples%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>Cervix</td>
<td>1</td>
<td>225</td>
<td>84.1</td>
<td>29.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>450</td>
<td>82.9</td>
<td>41.1</td>
</tr>
<tr>
<td></td>
<td>Blind</td>
<td>1</td>
<td>225</td>
<td>61.9</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>450</td>
<td>83.3</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>1</td>
<td>20</td>
<td>10.3</td>
<td>50.0</td>
</tr>
<tr>
<td>Frozen</td>
<td>Cervix</td>
<td>1</td>
<td>450</td>
<td>38.6</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>900</td>
<td>51.2</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>1</td>
<td>40</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td>52.1</td>
<td>34.3</td>
</tr>
</tbody>
</table>

TABLE 2 Lambing performance of ewes inseminated in 1983.

<table>
<thead>
<tr>
<th>Semen Type</th>
<th>Position deposited</th>
<th>No. of inseminations</th>
<th>Total no. of sperm inseminated ( \times 10^6 )</th>
<th>Ewes lambing%</th>
<th>Lambed ewes with multiples%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>Cervix</td>
<td>1</td>
<td>225</td>
<td>55.3</td>
<td>15.4</td>
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<tr>
<td></td>
<td>Blind</td>
<td>1</td>
<td>225</td>
<td>65.4</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>450</td>
<td>77.4</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>450</td>
<td>74.4</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>Intra-uterine</td>
<td>1</td>
<td>30</td>
<td>82.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Frozen</td>
<td>Cervix</td>
<td>2</td>
<td>900</td>
<td>17.0</td>
<td>12.5</td>
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<tr>
<td></td>
<td>Blind</td>
<td>2</td>
<td>900</td>
<td>22.7</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Intra-uterine</td>
<td>1</td>
<td>60</td>
<td>37.8</td>
<td>21.4</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td>54.7</td>
<td>14.1</td>
</tr>
</tbody>
</table>
to lamb when inseminated twice with $225 \times 10^6$ sperm or once with $450 \times 10^6$ than when inseminated once with the low concentration ($P < 0.10$). The percent of lambing ewes producing multiples was not affected by any of the factors examined.

**DISCUSSION**

Blind insemination of ewes once with $225 \times 10^6$ fresh sperm gave lambing rates of around 64%. This is only slightly lower than the average for cervical insemination (69%). Since the blind technique is faster, easier and involves less specialised ewe handling facilities, it should be considered by any commercial group considering inseminating at a natural oestrus. The performance of the technique at a synchronised oestrus is untested but will be the subject of future research. Doubling the number of fresh sperm inseminated blind (either by 2 inseminations or a single high-concentration insemination) improved lambing rates and gave results very similar to that achieved after double cervical insemination. However the high lambing rate (about 80%) is achieved at the expense of the number of ewes inseminated per ejaculate.

The poor performance of ewes inseminated onto the ovaries agrees with Australian results (I. D. Killeen, pers. comm.) and could be due to insufficient sperm inseminated or entering the fimbria or to an adverse effect of the technique on ovary/egg/oviduct relationships.

The intra-uterine technique performed well for both fresh and frozen semen. It is relatively easy to perform and, most importantly, substantially increases (by a factor of 7 and 15 for fresh and frozen semen, respectively) the number of ewes inseminated per ejaculate. Its major drawback is that it requires an endoscope (about $3500) and ancillary equipment. The equipment can however be used to determine ovulation rates and so could be worthwhile for any group breeding scheme considering artificial insemination and ovulation recording. The technique makes the use of frozen semen feasible and it is being used for this purpose in Australia. Lambing rates of about 50% are achieved (Killeen et al., 1982; Maxwell et al., 1983) and although the result in the present trial is lower than this, it should improve when better quality frozen sperm is used. The intra-uterine technique could also be useful where maximum use is to be made of fresh semen from a small number of rams.

The variable result achieved in the 2 years from frozen semen was disappointing. The same processing system was used in both years and the semen had similar bench motilities. A factor which could have contributed to the variation was the introduction of 4 new rams in 1983.

The observation that ewes that lamb after insemination with frozen semen produce fewer multiples than those inseminated with fresh semen is not new (e.g., Langford et al., 1979). We can offer no obvious explanation for the interaction observed in 1982 between semen type and whether animals were first observed in oestrus at an am or pm mating.

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**REFERENCES**


