THE USE OF FORMALIN IN THE STORAGE OF RAM SEMEN

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SUMMARY

A calcium-free Krebs Ringer solution, supplemented with 1 mg/ml glucose, with or without 0.025% formalin, was used to dilute freshly collected ram semen on a 1:l basis. The treated semen was inseminated into the cervix of Merino ewes either within 60 minutes of collection or after storage at 5°C for 24 h. Although the Ringer-formalin diluent always caused complete immobilisation of the spermatozoa, 43% and 17% of the ewes lambed as a consequence of insemination with the immobilised spermatozoa either within 60 minutes or after 24 h respectively. Ewes inseminated with semen diluted with milk or Ringer diluent alone had lambing rates of 31% and 37% respectively. Both the source of semen and the day of insemination caused significant variations in ewe fertility.

INTRODUCTION

Dott and Foster (1975) showed that formalin preserved the integrity of the cell membranes of spermatozoa and Dott et al. (1976) reported that four out of five ewes became pregnant after surgical insemination with formalin-treated semen directly into the uterus.

Fairnie (1979) indicated that the absence of a simple and reliable system for the short or long term storage of ram semen was a major limitation to greater use of artificial insemination (A.I.) in sheep in W.A. Recent experiments at the University of Queensland indicated that formalin-treated semen could be used to successfully inseminate ewes after immobilisation of the spermatozoa with formalin diluents (T.D.Glover, personal communication).

This paper decribes an experiment in which formalin-treated semen was used in an A.I. programme on a flock of Merino ewes held on a commercial wool producing property near Kojonup, W.A., over five days in February 1982.

MATERIALS AND METHODS

Approximately 2000 mature Merino ewes were shorn and then treated with intravaginal sponges containing progesterone ("Repromap", Upjohn Pty. Ltd.) in January 1982. The sponges *were* removed 12 or 14 days later, and 20 testosterone-treated "teaser" wethers added to the flock to stimulate ovarian activity. Eighteen days after the first sponges were removed the "teasers" were fitted with "Sire-Sine" harnesses and raddles, and put back with the ewes to detect oestrous behaviour. Unexpected rain and unseasonal cold weather in the intervening period lead to the death of 600 of these ewes and only 401 of the survivors were detected in oestrus during the period of the experiment.

The routine used for A.I. was that described by Fairnie and Wales (1982) with the following modifications : the ewes were checked twice daily for rump marks left by the "teasers", and ewes detected in oestrus were set aside and inseminated 12 hours later.

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The semen used was collected from one of the four groups of five rams. After pooling of the semen, and evaluation for concentration using a calibrated colorimeter, and motility under a microscope, the semen was subjected to one of four treatments :

(a) diluted with heat-treated reconstituted powdered cows-milk
(b) diluted with Ringer solution
(c) diluted with Ringer-formalin solution
(d) as for (c) but stored at 5°C for 24 hours.

The Ringer solution used was a calcium-free Krebs Ringer phosphate solution supplemented with 1 mg/ml glucose. The formalin concentration was 0.025% in the Ringer diluent which, when used on a 1:1 basis, provided a final formalin concentration of 0.0125%.

All ewes were inseminated with 200 million motile spermatozoa and the immobilised spermatozoa were inseminated without being washed free of the formalin. The volume of diluted semen inseminated varied according to the concentration and motility of spermatozoa in the particular pool of ejaculates collected.

Each of the inseminated ewes was examined for udder activity as evidence of having lambed, three weeks after the expected date of lambing. The data were examined by chi-square analysis of contingency tables as described by Ostle (1972), using a split plot technique to gauge the significance of treatment effects in which the treatment x experiment interaction was used as the error factor.

RESULTS

Table 1 shows the percentage of ewes lambing as a result of insemination with each of the four semen treatments.

TABLE 1 Lambing rate of ewes following insemination with spermatozoa immobilised with formalin-Ringer diluent, or extended with other diluents

Treatment	Diluent	Time of A.I.	Number of ewes inseminated	% ewes lambing
(a)	Milk	Immediate	96	31
(b)	Ringer	Immediate	103	37
(c)	Ringer-Formalin	Immediate	107	43
(d)	Ringer-Formalin	After 24 hr at 5°C	95	17

Table 2 shows the results after chi-square analysis of all the data in which the various effects - semen treatment, source of semen, and day of A.I. - are separated.

TABLE 2 Chi-square analysis of the lambing data from ewes inseminated with spermatozoa immobilised with Ringer-formalin solution, or extended with other diluents.

Source of Variation	X^2	d.f.	P
Overall	66.24	41	0.01
Semen treatment			
(c) v. (d)	16.92	1	0.001
other treatments	3.21	2	N.S.
Between ram groups	8.83	3	0.05
Between days	12.92	4	0.025
Interaction	24.36	31	N.S.

(N.S. = not significant at 0.10)

Table 2 shows that there was a significant effect on fertility of holding Ringer-formalin immobilised spermatozoa for 24h. However, there was no significant difference between the semen treatments, but there were significant effects due to the source of the semen (i.e. between ram groups), and the day on which A.I. occurred.

DISCUSSION

Although the overall fertility of inseminated ewes was lower than normally expected in W.A. (Fairnie and Wales 1982) this was most likely to have been due to the severe stress of the unseasonally'cold and wet weather. Notwithstanding this unseasonal environmental effect, the fertility of ewes in treatment group (c) was as good as that with conventional diluents and indicated that immobilisation of spermatozoa with a Ringer-formalin solution does not impair their ability to move through the cervix of oestrous ewes to the uterine tubes and achieve fertilisation with ova.

Storage of immobilised spermatozoa for 24h at 5°C did lead to fewer ewes lambing following insemination. This effect may have been due to either the length of storage or the temperature of storage. Dott and Foster (1975) showed that the integrity of the cell membranes of immobilised spermatozoa was maintained for periods as long as 96h at 4°C or "room temperature", described as being between 19° and 25°C, but showed signs of deterioration when kept at 40°C for 48h. They were equivocal as to whether motility could then be restored by washing the inmobilised spermatozoa.

Osinowo et al. (1982) *were* able to restore motility to ram spermatozoa immobilised with Ringer-formalin diluent for six hours, by washing them with phosphate buffered saline. However they showed that whilst eosin uptake was unaffected by length of storage (the measure of cell membrane integrity used by Dott and Foster 1975), it was affected by the temperature of storage, being higher at 5° and 25°C than at 15°C. They found that motility of the washed spermatozoa declined sharply after six hours of storage, an observation which may explain the differences between groups (c) and (d).

The experiment reported here was conducted under field conditions in which the diurnal variation in ambient temperature can be as wide as 10° to 40°C.

Hence, in planning the experiment it was decided to first keep the diluted spermatozoa in a water bath at 30°C. Cooling to 5°C in treatment (d) was carried out without the use of sophisticated laboratory equipment nor with any protectants for spermatozoa against too rapid chilling (Colas and Courot 1979), but it was a gradual process over a period of several hours.

The variations in ewe fertility that are related to the source of the semen and the day of A.I. have been observed previously in trials in which ewes were inseminated at a fixed time following synchronisation of ovulation with Cloprostenol (Fairnie et al. 1976). In the current experiment, ewes were synchronised with progesterone intravaginal sponges. However, one normal oestrous cycle elapsed between removal of the sponges and A.I. Thus it would seem that factors other than the synchronising agent are affecting the fertility of ewes and rams on a daily basis. Very few experiments reported in the literature comment on whether day-to-day variations occur in flock fertility, and perhaps this phenomenon should be examined further.

ACKNOWLEDGEMENTS

This work was supported by the Wool Research Trust Fund. In addition we would like to thank Mr. Robert O'Halloran, "Cherry Tree Pool", Kojonup, W.A., for the use of his sheep and other resources, and Messrs. J.P. Carroll and P.J. Wales for their valuable technical assistance.

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