BRIEF COMMUNICATION

Separating dead from live sperm in thawed frozen merino semen does not enhance conception rate

G.M. VAN REENEN AND J.F.T. GRIFFIN[1]

Aspiring Animal Services Ltd, Ballantyne Road, R.D. 2, Wanaka, New Zealand.

Keywords: Sperm; Semen; Merino; Frozen; Separation; Density Gradient; Artificial Insemination.

INTRODUCTION

Conception rates following artificial insemination (A.I.) with frozen semen in sheep are frequently lower than when fresh semen is used. Shannon and Curson (1972) demonstrated that an amino acid oxidase becomes active following the death of bovine sperm. Hydrogen Peroxide, a metabolic product of the enzyme, is known to be toxic to remaining live sperm. The deleterious effects of the aromatic amino acid oxidation is further exacerbated by components of egg yolk which is used as a cryoprotectant for the freezing of mammalian semen. Various methods have been developed to minimise the detrimental effect of amino acid oxidation in bovine semen such as the displacement of dissolved oxygen from diluents, addition of catalase enzyme and chelating agents and the removal of seminal plasma components using dialysis membranes.

This paper records our attempt to determine the effect of physically removing dead sperm from thawed semen prior to A.I., under field conditions, on the conception rate of merino ewes.

MATERIALS AND METHODS

A technique used to separate mammalian leucocytes from whole blood was modified and adapted to separate live from dead sperm in thawed frozen ram semen.

Prior to the trial, semen from two, four year old merino rams was collected by artificial vagina, pooled and deep frozen in pellet form on dry ice using Salomon's Tris, Egg Yolk, Citric Acid, Glycerol formula.

Density gradient columns for sperm separation were prepared using two layers of 65% and 90% isosmotic Percoll in 50ml polyethylene Greiner Labortechnik disposable centrifuge tubes. The Percoll fractions were diluted with the Tris semen diluent buffer to a final concentration of 300 m Osmoles/L.

Three 0.2ml deep frozen semen pellets were thawed in thin walled borosilicate glass tubes and layered on top of each column. Tubes were then spun in a Hettich Universal model 1200 centrifuge at 600 r.p.m. for 6 minutes then at 1200 r.p.m. for 20 minutes. Supernatant layers of diluent and dead sperm were removed by aspiration using a short form Pasteur pipette.

The sperm concentration in the subnatant layer was adjusted to 100 million sperm /ml using Tris based diluent buffer. All procedures were carried out at 30°C.

Treatment group ewes were inseminated with 0.25 mls of the subnatant solution into each uterine horn. Control group ewes were inseminated with 0.25 mls of thawed untreated semen which had live sperm density adjusted to 100 million / ml using Tris buffer diluent.

Five hundred and four two tooth ewes on a commercial property in Central Otago were synchronised by inserting intravaginal CIDR G type devices for thirteen days. Sixteen days following CIDR removal 16 vasectomised merino rams were introduced to the flock. A sticky soluble dye was daily painted on the briskets of the teaser rams. Marked ewes were drafted off at 12 hour intervals and randomly allocated to treatment or control group and identified.

All ewes were grazed together on pasture throughout gestation and separated into control and treatment groups one week prior to lambing.

RESULTS

Of the 504 ewes initially programmed, 313 were marked as showing oestrus by the teaser rams during the three day insemination period. Conception rate and lambing percentages are summarised in table 1.

TABLE 1: Conception and lambing rates in ewes treated with separated and untreated semen.

	Control (Conventional I.U. insemination)	Treatment (Separated Sperm)
No ewes inseminated	162	151
No conceiving to AI	72 (44%)	70 (46%)
No lambs born	75 (46%)	73 (48%)

No significant difference in either conception rate or lamb drop was detectable between treatment and control group ewes.

DISCUSSION

The very high proportion of ewes not cycling was attributed to drought conditions in the three months prior to commencement of the trial. This resulted in body weights being 5 kg lower than normal.

[1]Department of Microbiology University of Otago, P.O. Box 6384, Dunedin North, New Zealand.

Results of this trial suggest that the deleterious effects of large numbers of dead sperm present in thawed frozen ram semen do not have a significant influence on conception rate of merino ewes following intrauterine A.I.

In this experiment no further attempt was made to remove the Percoll from the separated sperm. This was because the method was devised as a relatively simple system which could be readily applied in on-farm situations. The trial was designed to test the potential of the technique under such conditions.

Further washing of the sperm, though not complex or difficult under controlled laboratory conditions, would severely restrict the usefulness of the technique in the field and would slow dramatically the hourly throughput of ewes.

Any deleterious effect of centifugation, handling and temperature fluctuations was not assessed or accounted for during the trial.

ACKNOWLEDGEMENTS

Alison Law, John Cook, James Barr and Raymond Miller for assistance with field work. Livestock Improvement Corporation, N.Z. Dairy Board for provision of CIDRS

REFERENCES

Shannon, P. and Curson, B. 1972 Toxic effect and action of dead sperm on diluted bovine semen. *Journal Dairy Science* **55:** 614 - 620.

Shannon, P. and Curson, B.1982. Kinetics of the aromatic L amino acid oxidase from dead bovine spermatozoa and the effect of catalase on fertility of diluted bovine semen stored at 5°C and ambient temperatures. *Journal Reprod. Fert.*, **64:** 463 - 467.