# Fertility of inactivated ram sperm

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**ABSTRACT**

Ram sperm inactivated by centrifuging through a Ficoll solution were stored for 3 days at room temperature. A high proportion (80%) of the sperm was motile after reactivation with a milk diluent but compared to fresh diluted semen, the sperm gave a low pregnancy rate (6% *v* 60%).

## INTRODUCTION

The benefits of artificial insemination (AI) in sheep are well recognised and satisfactory techniques are available for AI with diluted fresh semen and raw semen (Tervit *et al.*, 1978; Meyer and Harvey, 1981). These techniques are however limited by the short time the semen can be stored between collection and use. Techniques for long-term storage of semen are needed. The ultimate technique is deep-frozen semen but, despite numerous reports of satisfactory post-thaw survival of frozen semen, few workers have obtained acceptable conception rates after cervical insemination (Visser and Salamon, 1974; Colas, 1975). Similarly, there are numerous reports on chilled storage of semen and although the motility of sperm can be maintained for several days, the lambing rate following AI declines with increasing duration of storage. Satisfactory results are not always achieved after as little as 1 day's storage (Salamon *et al.*, 1979). The present study investigates the fertility of ram sperm stored for 3 days, inactivated after centrifugation through a Ficoll-containing medium.

## MATERIALS AND METHODS

**Semen Processing:**

Inactivated Semen: The procedure used was essentially that developed by the N.Z. Dairy Board for bull semen (Shannon *et al.*, 1982). Ejaculates from 4 to 6 mixed breed rams were collected as rapidly as possible at approximately 8, 10 a.m. and 2 p.m. on May 8, 1981. Individual ejaculates were then inactivated by centrifuging twice through a 7% Ficoll solution (W/V; Ficoll, mol. wt. 400 000, Sigma Chemicals/14 G buffer; Shannon, 1964). After the second centrifugation the sperm fractions from 2 to 3 rams were pooled and diluted in 14 G to 20 × $10^6$ sperm/ml. After all ejaculates were inactivated, the sperm were pooled and stored for 3 days at room temperature on the bench.

Reactivation took place on May 11 by centrifuging the sperm for 10 min at 1200 *g*. The sperm plugs were then combined and diluted to 600 × $10^6$ sperm/ml with a milk diluent (Colas *et al.*, 1968) and the diluted sperm stored at room temperature for 30 to 90 min until insemination.

Fresh Semen: Ejaculates from 2 to 3 rams were pooled and then diluted to 600 × $10^6$ sperm/ml with the milk diluent. The semen was then held at room temperature for 30 to 90 min until insemination.

Ewe Treatment: One hundred ewes were treated on April 24 with intravaginal sponges containing 70 mg MAP (Provera, Upjohn). The sponges were removed 15 days later and at this time each ewe was injected intramuscularly with 200 i.u. PMS gonadotrophin (Gradient Labs., Australia). The ewes were run with harnessed teaser rams and were inseminated either once (54 h after sponge removal) or twice (48 and 60 h after sponge removal) with either reactivated sperm or fresh semen. The ewes stood on an elevated platform and received 0.25 ml of semen through plastic pipettes. All ewes were slaughtered approximately 60 days post AI and their reproductive tracts examined for the presence of foetuses.

## RESULTS AND DISCUSSION

The sperm washed through the Ficoll-containing solution were effectively inactivated since, when they were examined during storage on the bench, less than 1% showed forward motion although 50 to 70% showed very slow tail movement. In agreement with Harrison (1976), there was no evidence that the inactivation technique had caused any marked damage to the sperm.

Within 30 min of reactivation, 80% of the sperm were progressively motile and definite wave motion was present. The speed of movement was however much slower than in fresh semen diluted to the same concentration in the same diluent. A further 5% of the reactivated sperm showed slow tail movement with no forward motion and the remainder were immotile. Approximately 10% of the reactivated

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sperm had curved tails. This abnormality has been observed in previous reactivation experiments but the incidence was always lower (0 to 5%) than in the present trial.

The inactivation/reactivation procedure used was inefficient in terms of sperm recovery. Only 73% of the original sperm population was recovered after the double centrifugation/inactivation procedure and only 49% of these sperm were recovered after the centrifugation/reactivation step. Thus overall, only 36% of the original sperm population was available for insemination. Harrison (1976) reported a high sperm recovery efficiency (82 to 100%) after a single centrifugation in Ficoll but did not present data for 2 centrifugations. It is possible that our sperm recovery efficiency could be improved by adopting the 2 stage centrifugation procedure of Harrison (1976) and by varying centrifuge speeds and times.

**TABLE 1** Percentage of ewes pregnant after AI

| Semen type | No. inseminations | No. ewes inseminated | Percent ewes pregnant |
|---|---|---|---|
| Inactivated | 1 | 23 | 9 |
| | 2 | 25 | 4 |
| Fresh | 1 | 20 | 50 |
| | 2 | 22 | 68 |

Few of the ewes receiving inactivated sperm were pregnant (Table 1). The pregnancy rate from fresh semen was higher than from inactivated sperm ($P<0.001$) and was similar to that previously reported for this type of semen (Tervit *et al.*, 1978). There was no effect of number of inseminations nor any interaction between semen type and number of inseminations.

As used here, inactivated sperm was similar to frozen and chilled semen in its ability to show good motility post treatment but disappointing results after insemination. The reasons for the poor fertility are not known but could be the result of poor survival *in vivo* or loss of the ability to fertilise. The sperm certainly moved relatively slowly after reactivation and it is possible that this weak motility could be due to the lack of suitable forward-motility protein in the milk diluent used for reactivation. Seminal plasma and other diluents may be more effective in stimulating motility and this, together with the fertilising ability of the sperm, should be the subject of further experimentation.

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