FERTILITY OF RAM SPERMATOZOA FROZEN-STORED FOR 5 YEARS

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Summary. Fertility results for ram semen frozen-stored in liquid nitrogen for 5 years ('experimental') and for 2 weeks ('control') are presented. Lambing results for semen stored for 5 years and for the 'control' semen were 52.9% (37/70) and 54.4% (37/68) respectively. The mean lambing rates following single and double inseminations were 49.3% (34/69) and 58.0% (40/69).

The fertilizing capacity of frozen-stored bull semen can be maintained for several years (Mixner & Wiggin, 1964; Mixner, 1968; Cassou, 1972). To examine the effect of long-term storage on the fertility of ram semen, a semen bank has been laid down, and the first stage of the fertility test conducted after 3 years of storage has been reported (Salamon, 1972). This communication presents the results for the second stage of the test using semen after 5 years of storage, and 'control' semen stored for 2 weeks.

The processing and freezing in a raffinose–citrate–yolk–glycerol diluent was similar for both 5-year and 2-week-old semen, and has been already described (Salamon, 1971, 1972). The 'control' semen was collected from five Merino rams of known fertility which belonged to the same stud from which the sires supplying the 5-year-old semen bank originated.

The frozen pellets were thawed in test-tubes containing 210 mM-inositol/40 mM-sodium citrate solution and held in a water-bath at 37°C (thawing dilution 1:2, pellets : thawing solution, v/v). The thawed semen was centrifuged at 1000 g for 10 min and the supernatant was removed to obtain a cell concentration of 1.7 to 1.9×10^9 motile spermatozoa/ml (total number of cells: 3.4 to 3.8×10^9).

Semen smears, stained and cleared according to the technique described by Dott & Foster (1972), were prepared from uncentrifuged and centrifuged semen and the integrity of the spermatozoa was examined under a phase-contrast microscope using an interference filter (Leitz Wetzlar).

For insemination, a volume of 0.1 ml reconcentrated semen was used. Mature Merino ewes were inseminated at the second oestrus after synchronization with intravaginal sponges (Robinson, 1965). The flock was 'run' with vasectomized rams previously tested by electroejaculation, and the ewes detected in oestrus were removed twice daily (08.00 and 18.00 hours). The inseminations were

carried out 12 to 14 hr (single insemination) or 12 to 14 hr and 23 to 25 hr (double insemination) after removal from the flock. The oestrous ewes were allocated randomly into treatment groups. Single and double inseminations using semen of both ages were performed on each day.

The lambing results are presented in Table 1. There was no difference between the fertility of semen frozen-stored for 2 weeks and that of semen frozen-stored for 5 years. The average of 52.9% (37/70) lambing for the 5-year-old semen was similar to that obtained with semen stored for 3 years (52.9%, 91/172) in the same bank (Salamon, 1972). There was no significant difference between the mean fertility for single and double inseminations (49.3 versus 58.0%). Semen of the two ages responded differently to the number of inseminations; nevertheless, the interaction was not significant ($\chi^2_{(1)} = 1.89$; $0.10 < P < 0.20$). The indistinguishable results for single and double inseminations can be attributed to the fact that they were performed within the optimum time range after detection of oestrus (Salamon & Lightfoot, 1970; Salamon, 1971, 1972; Schindler & Amir, 1973).

Table 1. Lambing results following insemination with frozen-thawed semen

<i>Period of storage</i>	<i>No. of inseminations</i>	<i>No. of ewes inseminated</i>	<i>No. lambing</i>	<i>% Lambing</i>
2 weeks	1	34	19	55.9
	2	34	18	52.9
5 years	1	35	15	42.9
	2	35	22	62.9
Overall total and mean		138	74	53.6

There was no decrease in the proportion of surviving spermatozoa during the 5 years of storage, as judged by the comparison of sperm recovery upon thawing in the present test with cell recovery data recorded during the previous years.

Examination of semen smears revealed no difference in the proportion of spermatozoa with undamaged acrosomes for the 2-week and 5-year-old semen (41 and 40%). Although for this comparison the 'old' and 'new' semen originated from different rams, it may safely be presumed that enhancement of cell damage did not occur during the prolonged period of storage in liquid nitrogen.

After centrifugation of the thawed semen, there was only slight or no decline in motility (45 to 50%), but the proportion of spermatozoa with intact acrosomes decreased for semen of both ages by an average of 20%. Thus, the numbers of spermatozoa with undamaged acrosomes in 0.1 ml of reconcentrated semen were 110.5 to 125.5×10^6 of which an apparently sufficient proportion reached the site of fertilization. The use of an 'inseminating race' which ensured minimal stress to the ewes could also have been a contributory factor.

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