FERTILITY OF RAM SPERMATOZOA FOLLOWING PELLET FREEZING ON DRY ICE AT −79 AND −140°C*

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The fertility obtained with ram semen pellet frozen on dry ice (−79°C) (Lightfoot and Salamon 1970; Salamon and Lightfoot 1970) has stimulated further investigation. Information was also needed on fertility of ram spermatozoa pelleted at a temperature below −79°C (Salamon 1970) and this communication presents the results of two experiments and treatment comparisons for each.

Methods

Semen was collected from five mature Merino rams by artificial vagina. Ejaculates of good initial motility were pooled and diluted 1 : 4 at 30°C with a diluent consisting of 166·5 mM raffinose, 68 mM sodium citrate, 15% (v/v) egg yolk, and 5% (v/v) glycerol. The diluted semen was cooled to 5°C over a period of 1·5 hr and held at that temperature for an additional 1 hr before pelleting (0·40 ml) on dry ice at either −79°C (expts. 1 and 2; Nagase and Niwa 1964) or on dry ice cooled to −140°C (expt. 2) as described by Salamon (1970). In the second experiment one-half of the diluted and cooled semen was frozen at −79°C and the other half at −140°C. The frozen pellets were stored in liquid nitrogen for 5–8 weeks before use.

For insemination three pellets were thawed in separate test tubes containing 1·20 ml of glucose (44·4 mM)–citrate (72·8 mM) held in a water-bath at 37°C (thawing dilution 1 : 3, pellet : thawing solution, v/v). Following thawing the semen was collected into centrifuge tubes (at 37°C), centrifuged at 1000 *g* for 10 min, and the supernatant discarded to obtain a concentration of $1 \cdot 5–1 \cdot 6 \times 10^9$ motile spermatozoa per millilitre. The volume of inseminate of both thawed reconcentrated and fresh semen was 0·1 ml.

Fresh semen was collected from the same five rams from which ejaculates were obtained for freezing. Ejaculates from individual rams were pooled and diluted with yolk–glucose–citrate diluent [15%(v/v)–44·4 mM–80·6 mM] to cell concentration of $1 \cdot 5–1 \cdot 6 \times 10^9$ per millilitre.

Mature Merino ewes were used and they were inseminated either at the second oestrus after synchronization with intravaginal sponges (expt. 1; Robinson 1965) or during a natural oestrous cycle (expt. 2). Oestrous ewes were detected using vasectomized rams previously tested by electro-ejaculation, and after each drafting were randomly allocated into treatment groups. The ewes were drafted at 0800 and 1800 hr (expt. 1) or only once daily at 0800 hr (expt. 2). The ewes drafted in the evening were inseminated at 0900–1100 hr the following day with the morning's draft ewes and the second insemination was performed at 1900–2030 hr. In the second experiment single and double inseminations were carried out at 0900–1000 and 1900–2000 hr respectively. Frozen–thawed and fresh semen (expt. 1), or semen pelleted at both temperatures (expt. 2) were used on each day of insemination, and both single and double inseminations were carried out.

Lambing was determined by udder examination on yarding 160 days after the last date of insemination. The significance of differences between the proportion of ewes lambing in treatment groups was tested by χ^2.

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Results and Discussion

Results for the first experiment are given in Table 1. There was a marked difference ($\chi^2_{(1)} = 33{\cdot}68$; $P < 0{\cdot}001$) in the fertility of frozen and fresh semen.

TABLE 1

LAMBING FOLLOWING INSEMINATION WITH FROZEN–THAWED AND FRESH–DILUTED SEMEN

No. of Inseminations	No. of Ewes Inseminated*	No. Lambing*	No. of Ewes Inseminated†	No. Lambing†
		Frozen–thawed semen		
1‡	77	33 (42·9%)	80	41 (51·3%)
2§	79	47 (59·5%)	82	48 (58·5%)
Totals	156	80 (51·3%)	162	89 (54·9%)
		Fresh–diluted semen		
1‡	68	47 (69·1%)	70	53 (75·7%)
2§	70	55 (78·6%)	68	55 (80·9%)
Totals	138	102 (73·9%)	138	108 (78·3%)

* Ewes drafted 1–2 hr before first insemination (0800 hr).

† Ewes drafted 15–17 hr before first insemination (1800 hr).

‡ Ewes inseminated at 0900–1100 hr.

§ Ewes inseminated at 0900–1100 and 1900–2030 hr.

Double insemination resulted in a significant increase in lambing ($\chi^2_{(1)} = 5{\cdot}90$; $P < 0{\cdot}02$) regardless of semen treatment. No significant interactions were revealed, nevertheless the variations in response to both single and double inseminations with their timings in relation to the stage of oestrus can be regarded as being of practical usefulness. It indicates that single insemination is preferable during the late rather than early part of oestrus, and that most gain from double insemination could be obtained when the first is performed at early oestrus. A similar response has been observed in a previous investigation (Salamon and Lightfoot 1970).

TABLE 2

LAMBING FOLLOWING INSEMINATION WITH SEMEN PELLET FROZEN AT −79 AND −140°C

Temperature of Pelleting (°C)	One Insemination		Two Inseminations		Overall	
	No. of Ewes Inseminated	No. Lambing	No. of Ewes Inseminated	No. Lambing	No. of Ewes Inseminated	No. Lambing
− 79	93	38 (40·9%)	98	54 (55·1%)	191	92 (48·2%)
−140	92	36 (39·1%)	95	48 (50·5%)	187	84 (44·9%)
Overall	185	74 (40·0%)	193	102 (52·8%)	378	176 (46·6%)

In the second experiment spermatozoa frozen at −79°C and −140°C showed similar survival on thawing, and the resultant lambing rates following insemination with the two "types" of semen were also comparable (Table 2). Double insemination

increased the proportion of ewes lambing ($\chi^2_{(1)} = 6{\cdot}27$; $P < 0{\cdot}02$). There was no significant interaction.

The fertility results with pelleted ram semen in the present experiments could be considered as satisfactory. Undoubtedly, most contributing factors were the relatively high concentration of motile spermatozoa in the inseminate and careful cervical insemination (Salamon and Lightfoot 1970). A possible additional factor could have been the careful handling of ewes during the insemination to reduce stress to a minimum.

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