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FERTILITY OF FROZEN RABBIT SEMEN

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Summary. Rabbit semen was extended in a tris–yolk–12·5% DMSO extender and frozen in liquid nitrogen vapour or on a 'dry ice' block after glycerol had been added. No differences ($P > 0{\cdot}10$) were found between inseminations with liquid semen and semen frozen in pellets with regard to the number of young born or the pregnancy rate. Other methods produced significantly ($P < 0{\cdot}05$) fewer young. Significantly ($P < 0{\cdot}05$) more young were born to does inseminated 5 hr after they received the ovulating hormone.

Rabbit semen has been frozen with differing results (Smith & Polge, 1950; Fox, 1961; Fox & Burdick, 1963; Sawada & Chang, 1964; Wales & O'Shea, 1968; O'Shea & Wales, 1969). The fertility of frozen rabbit semen in the earlier reports was low. Since the ovulation time in the rabbit is known, it is a good laboratory animal in which to study the handling of spermatozoa *in vitro*.

Two ejaculates were collected within 20 min once weekly from fourteeen White Vienna male rabbits, using an artificial vagina. Semen volume and motility per ejaculate were recorded and those having 50% or more progressive motility were pooled. The pooled semen was diluted 1:4 with a tris–yolk dimethyl sulphoxide (DMSO) extender. The diluter was a slight modification of the tris–yolk extender reported by Steinbach & Foote (1967), having the following composition: 3·028 g tris-hydroxymethylaminomethane ($C_4H_{11}NO_3$), 1·675 g citric acid ($C_6H_8O_7.H_2O$), 1·250 g D-glucose ($C_6H_{12}O_6$), 15 ml DMSO and twice distilled water to make a total volume of 100 ml. To the 100 ml were added 20 ml egg yolk and 120,000 units each of penicillin and streptomycin. The pH was adjusted to 6·7 using citric acid. The sperm concentration of the extended semen was determined with a haemocytometer. The extended semen was divided into two parts and handled as follows: one part was placed in a 5° C cool room for 6 to 8 hr until it was inseminated (liquid semen); the other part was cooled in a 5° C cool room for 1 to 3 hr and glycerol (6% of the extender volume) was added 30 min before freezing. The extended semen containing glycerol was placed in three different forms of container: 2·5-ml plastic ampoules, 0·3 × 0·4 × 20 cm coiled polyvinylchloride tubing and 0·5-ml plastic straws, and frozen in liquid nitrogen vapour for 6 to 10 min or frozen as 0·15-ml pellets in holes on a 'dry ice' block for 10 min (frozen semen). The frozen semen was stored in liquid nitrogen until used for the inseminations.

The semen in the ampoules, tubing and straws was thawed in a 40° C water

bath for 40 to 60 sec. The pellets were placed in a plastic bag and similarly thawed. Motilities were recorded after thawing and the semen divided into 0·5- or 0·6-ml insemination portions.

Each White Vienna doe inseminated was injected intravenously with 0·5 mg luteinizing hormone (PLH, Armour)/kg body weight to induce ovulation. Two trials were conducted, involving 339 inseminations. In the first trial, 223 does were artificially inseminated with 0·3 ml liquid or 0·5 ml frozen semen containing a minimum of 15×10^6 motile spermatozoa immediately following the LH injection. The kindling results were analysed using the analysis of variance and the Chi square test.

The results of insemination with liquid semen compared to semen frozen in ampoules and tubing are given in Table 1. Significantly ($P<0·005$) more young were produced from liquid semen. The percentage of does kindling was also higher ($P<0·005$, $\chi^2 = 42·4$, 1 d.f.). No difference ($P>0·10$) was found between semen frozen in ampoules versus tubing.

TABLE 1

COMPARISON OF VARIOUS FREEZING METHODS

Semen treatment	*Freezing method*	*No. of does kindling/ total insem.* (%)	*Total no. of young*	*No. of young/ doe insem.*	*No. of young/ doe kindling*
Liquid		35/52 (67·3)	226	4·3	6·5
Frozen	Ampoule	11/37 (29·8)	38	1·0	3·5
	Tubing	24/134 (17·9)	82	0·6	3·4

In the second trial, 116 does were artificially inseminated with 0·3 ml liquid semen or 0·6 ml frozen semen containing a minimum of 12×10^6 motile spermatozoa. After thawing, the frozen semen samples had motilities ranging from 25 to 50%. LH was injected in fifty-seven does 5 hr before the insemination while the remaining fifty-nine does were injected just before insemination.

The number of young resulting from the insemination of semen frozen in tubings, straws or as pellets was compared with the number resulting from the insemination of liquid semen. The results and significances are given in Table 2. No significant difference ($P>0·10$) was found between liquid and frozen pelleted semen with regard to the number of young born or the number of does kindling.

Does inseminated 5 hr after the LH injection produced significantly more ($P<0·05$) young than the comparable animals inseminated immediately following the ovulating injection.

The method of freezing semen was shown to have an influence on the fertilizing ability of the spermatozoa. Freezing rabbit semen as pellets was easily accomplished and gave the only comparable results to liquid semen in the number of young born. The maternal environment appeared to have an influence on the number of young born as more young were produced when the

TABLE 2

COMPARISON OF VARIOUS FREEZING METHODS AND INSEMINATION TIMES

Semen treatment	*Freezing method*	*Time between PLH inject. and insem (hr)*	*No. of does kindling/ total insem. (%)*	*Total no. of young*	*No. of young/ doe insem.*	*No. of young/ doe kindling*
Liquid		5	13/20 (65·0)	96	4·8	7·4
		0	10/20 (50·0)	56	2·8	5·6
		Total	23/40 (57·5)	152	3·8[a]	6·6
Frozen	Pellets	5	10/19 (52·6)	71	3·7	7·1
		0	7/17 (41·2)	45	2·6	6·4
		Total	17/36 (47·2)	116	3·2[a,b]	6·8
	Tubing	5	5/12 (41·7)	25	2·1	5·0
		0	3/12 (25·0)	11	0·9	3·7
		Total	8/24 (33·3)	36	1·5[b,c]	4·5
	Straws	5	2/8 (25·0)	9	1·1	4·5
		0	1/8 (12·5)	6	0·8	6·0
		Total	3/16 (18·8)	15	0·9[c]	5·0

[a,b,c] Means with the same superscript are not significantly different ($P<0·05$).

spermatozoa were in the reproductive tract for approximately 5 to 7 hr versus the normal 10 to 12 hr. This is in agreement with the work of Maurer, Whitener & Foote (1969) which showed that the highest percentage of cleaved ova were found when does were inseminated 5 hr after the ovulating injection.

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