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Brief Communication

ON THE FERTILITY AND SURVIVAL OF DEEP FROZEN BOAR SPERMATOZOA THAWED IN SKIM MILK*

Satisfactory conception rates of deep frozen boar spermatozoa were obtained, with insemination by way of the cervix, after thawing the deep frozen spermatozoa in boar seminal plasma, both in preliminary trials (*Crabo & Einarsson* 1971, *Crabo et al.* 1972 b) and in a large field trial (*Einarsson et al.* 1972). Fertility with pellet frozen boar spermatozoa, thawed without dilution, was reported by *Graham et al.* (1971 a, b) and *Pursel & Johnson* (1971).

The purpose of the present investigation was to test the possibility of substituting skim milk for seminal plasma as thawing fluid.

The sperm-rich fraction of the semen was collected from two fertile boars of Swedish Landrace and Swedish Yorkshire breed. The semen was frozen according to a method described by *Crabo et al.* (1972 b). The extender used was a TESNaK**-glucose-egg yolk buffer (70 %, 10 % and 20 % respectively) to which 2.5 % glycerol was added before the freezing. Thawing was performed according to *Crabo et al.* (1972 b).

T r i a l I. Thawing was performed in 70 ml skim milk heated at 92°C for 10 min. (in four cases with 10 % egg yolk added). Eleven gilts were inseminated by way of the cervix, twice during heat, with 100 ml of semen containing about 2.5×10^9 motile spermatozoa.

T r i a l I I. Equal numbers of pellet frozen boar spermatozoa from four freezings were thawed in a measured volume of TES-NaK-glucose buffer, seminal plasma obtained from a boar with totally hypoplastic testicles, and skim milk, respectively. After thawing the semen was kept in a water bath at 37°C for 5—6 hrs. Sperm motility was controlled at regular intervals.

Out of 11 inseminated gilts nine conceived (81.8 %). The post-mortem findings in the genital tract of the nine pregnant gilts,

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** *Crabo et al.* (1972 a).

Table 1. Fertility data of nine gilts inseminated with pellet frozen semen thawed in skim milk (in two cases with 10 % egg yolk added).

Thawing fluid	Day of slaughter	Number of corpora lutea	Number of embryos	Number of dead or undeveloped embryos
skim milk + egg yolk	31	4	2	0
—	30	11	5	1
skim milk	32	14	12	0
—	29	14	3	0
—	29	13	4	1
—	46	16	14	0
—	44	14	3	0
—	40	10	3	0
—	39	14	8	2
Mean		12.2	6.0	0.4

slaughtered between 29 and 46 days after insemination, are presented in Table 1. The average number of embryos per gilt is lower than when using seminal plasma as thawing fluid (*Crabo & Einarsson*). As is evident from Table 2 the post-thawing motility was better when the frozen spermatozoa were thawed in skim milk than in TESNaK-glucose buffer, both as regards initial motility and motility after 4 hrs. storage. Obviously some factor in the skim milk is of significance for survival and fertility of deep frozen boar spermatozoa at thawing and dilution. Probably substances of a proteinic nature from the skim milk (e.g. casein) protect the frozen spermatozoa by coating during thawing and dilution, as was suggested for boar seminal plasma (*Einarsson & Viring* 1972). The average low number of embryos recovered from the slaughtered gilts might be due to insufficient number

Table 2. Post-thawing motility (m. ± s) of deep frozen boar spermatozoa at different storage times (37°C).

Thawing fluid	Storage time (hrs.)					
	0	1	2	3	4	5—6
TESNaK-glucose buffer	20 ± 4	20 ± 4	14 ± 2	10 ± 0	4 ± 2	0
seminal plasma	34 ± 4	34 ± 4	25 ± 3	8 ± 3	5 ± 2	0
skim milk	50 ± 4	50 ± 4	48 ± 3	38 ± 3	15 ± 5	0

of spermatozoa reaching the fertilization site in the oviducts. The present results with good fertility but low litter size are in agreement with recently reported results by *Pursel & Johnson* and *Richter & Liedicke* (1972). Two possible explanations of the better fertility results when using seminal plasma than skim milk as thawing fluid are:

1. Better protection effect of the spermatozoa in the oviducts of inseminated gilts.
2. Better transport of the spermatozoa into the oviducts of inseminated gilts.

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REFERENCES

Crabo, B. & S. Einarsson: Fertility of deep frozen boar spermatozoa. Acta vet. scand. 1971, *12*, 125—127.

Crabo, B., K. I. Brown & E. F. Graham: J. Animal Sci. 1972 a. In press.

Crabo, B., S. Einarsson, A.-M. Lamm, O. Soosalu & S. Viring: Studies on the fertility of deep frozen boar spermatozoa. Proc. VIIth Int. Congr. Animal Reprod., München 1972 b. In press.

Einarsson, S. & S. Viring: Distribution of frozen-thawed spermatozoa in the reproductive tract of gilts at different intervals after insemination. In manus, 1972.

Einarsson, S., S. Swensson & S. Viring: To be published 1972.

Graham, E. F., A. H. J. Rajamannan, M. K. L. Schmehl, M. Maki-Laurila & R. E. Bower: Preliminary report on procedure and rationale for freezing boar semen. A. I. Dig. 1971 a, *19*, no. 1, 12—14.

Graham, E. F., A. H. J. Rajamannan, M. K. L. Schmehl, M. Maki-Laurila & R. E. Bower: Fertility studies with frozen boar spermatozoa. A. I. Dig. 1971 b, *19*, no. 6, 6—8.

Pursel, V. G. & L. A. Johnson: Fertilizing capacity of frozen boar spermatozoa. J. Animal Sci. 1971, *33*, 1162, Abstr. 101.

Richter, L. & A. Liedicke: The use of deep-frozen sperm in pigs. I.P.V.S. 2nd Congr., Hannover 1972, 136.

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