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Brief Communication

A NEW THAWING FLUID FOR DEEP FROZEN BOAR SPERMATOZOA*

Since 1971 satisfactory fertility results with intracervical insemination of deep frozen boar spermatozoa have been reported by several workers (c.f. *Pursel & Johnson* 1974, *Larsson & Einarsson* 1975).

The method of *Crabo & Einarsson* (1971) has yielded good pregnancy rates and good litter size (cf. *Einarsson et al.* 1974). This method involves thawing of the deep frozen spermatozoa in seminal plasma. *Larsson & Einarsson* (1975) showed that thawing in protein-free seminal plasma yielded the same pregnancy rate and the same litter size as thawing in whole seminal plasma. In this work a relationship was demonstrated between acrosome morphology, GOT release and fertility of the frozen and thawed spermatozoa.

In 1973 *Brooks & Mann* showed that boar spermatozoa preferably utilize pyruvate as energy source. After cold shock the preference for pyruvate was increased.

It has been suggested that some factor in the seminal plasma is of importance for the fertilizing capacity of the deep frozen boar spermatozoa (*Einarsson* 1971). As the same fertility rate has been obtained with spermatozoa thawed in protein-free seminal plasma as in whole plasma, this factor is likely to be of a non-protein nature. A new thawing fluid based on the electrolyte composition of seminal plasma was therefore composed and tested. This fluid was given the name "OLEP". Its composition will be presented in a coming paper. In this preliminary report the following data can be given: pH and osmotic pressure are equivalent to those of seminal plasma, ionic strength and electrolyte composition are mainly the same as in seminal plasma. Pyruvate and fructose are used as energy sources.

In the laboratory tests performed, the post thawing characteristics of deep frozen boar spermatozoa thawed in OLEP have been equal to those of spermatozoa thawed in seminal plasma.

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In a fertility test OLEP was compared with seminal plasma as thawing fluid. OLEP was tested with and without the addition of a membrane protecting agent (EDTA — disodium ethylenediamine tetraacetate).

Five ejaculates from each of three boars (one Swedish Landrace and two Swedish Yorkshire boars) were deep frozen according to the method of *Crabo & Einarsson*. From each ejaculate spermatozoa for insemination were thawed in each of the thawing solutions used (seminal plasma, OLEP, OLEP + EDTA). The seminal plasma was obtained from the sperm poor fractions of ejaculates of the three boars. These fractions were centrifuged for elimination of the spermatozoa. After addition of benzylpenicillin the seminal plasma was kept at —20°C until used. The spermatozoa thawed in seminal plasma derived from the same boar which supplied the seminal plasma.

Forty-five crossbred (Swedish Landrace × Swedish Yorkshire) gilts were inseminated twice (at ab. 16 hrs. interval) during their second or third naturally occurring heat period. The insemination dose contained 6×10^9 spermatozoa added to 70 ml thawing fluid.

The gilts were slaughtered four weeks after insemination and the numbers of foetuses and of corpora lutea (c.l.) were recorded.

Two of the gilts were excluded from the experiment after slaughter due to malformation of their genital tracts.

The results of the trial are summarized in Table 1.

Table 1. Pregnancy rate, litter size and ratio of foetuses to corpora lutea (c.l.) after insemination of deep frozen boar spermatozoa from five ejaculates of three boars thawed in three different thawing fluids.

Thawing fluid:	Seminal plasma				OLEP				OLEP + EDTA			
Boar:	172	388	1164	Total	172	388	1164	Total	172	388	1164	Total
Number of gilts inseminated	5	5	5	15	5	5	4	14	5	4	5	14
Number of gilts pregnant	2	5	1	8	2	4	3	9	1	4	3	8
Mean litter size	5.5	10	9	8.8	8.5	11.3	7	9.2	5	8.3	4.7	6.5
Ratio foetuses to c.l.	0.42	0.76	0.56	0.65	0.63	0.87	0.62	0.72	0.42	0.73	0.43	0.58

From the results it is evident that thawing in OLEP has yielded as good results as thawing in seminal plasma. Addition of EDTA to OLEP has not improved the results.

The pregnancy rate throughout the trial was somewhat lower than reported in earlier trials by us. It is, however, apparent that one of the boars (172) has consistently given a very low pregnancy rate. The litter sizes after thawing in seminal plasma and in OLEP are satisfactory. The ratio of foetuses to c.l. is on average high with spermatozoa thawed in OLEP. As this ratio is the best measure of the fertilizing ability of the spermatozoa, it is interesting to observe that the variation between boars in this respect is not as high as the variation in litter sizes.

The results indicate that the electrolyte composition, the pH and the osmolarity of seminal plasma are important factors in maintaining the fertilizing ability of frozen boar spermatozoa after thawing.

Further studies are being performed to compare OLEP and seminal plasma and to evaluate the freezability of spermatozoa from different boars.

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