DISTRIBUTION OF FROZEN-THAWED SPERMATOZOA IN THE REPRODUCTIVE TRACT OF GILTS AT DIFFERENT TIME INTERVALS AFTER INSEMINATION

S. EINARSSON AND S. VIRING

*Department of Obstetrics and Gynaecology and
Department of Clinical Biochemistry, Royal Veterinary College,
S-104 05 Stockholm 50, Sweden*

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Summary. The distribution of spermatozoa in the uterus and oviducts in twelve gilts was determined 1 and 4 hr, respectively, after insemination of 10^{10} deep-frozen spermatozoa thawed in seminal plasma or in TESNaK-glucose buffer. The numbers found in the oviducts 4 hr after insemination were markedly higher when the spermatozoa were thawed in seminal plasma. Different theories are advanced concerning the mode of action of the seminal plasma in this respect.

A normal fertilization rate was reported by Polge, Salamon & Wilmot (1970) for boar spermatozoa after deep freezing followed by deposition of the thawed spermatozoa directly into the oviducts by surgery. No fertilization took place when insemination was performed by way of the cervix, a finding which is in agreement with the those of Crabo & Einarsson (1971) and Crabo, Einarsson, Lamm, Soosalu & Viring (1972) when using TESNaK (Crabo, Brown & Graham, 1972) –glucose buffer as thawing fluid. Satisfactory conception rates, however, were obtained by Crabo & Einarsson (1971), by Crabo, Einarsson, Lamm, Soosalu & Viring (1972) and by Einarsson, Swenssou & Viring (1972) on insemination by way of the cervix after thawing and dilution of the deep-frozen spermatozoa in boar seminal plasma. From these results, it is evident that some factor in the seminal plasma is of importance for conception after insemination with deep-frozen spermatozoa. To elucidate whether the seminal plasma affects the transport of spermatozoa and/or is of significance for the fertility of frozen spermatozoa, the following experiment was performed.

Twelve sexually mature gilts were used and the onset of oestrus was taken as the time at which the gilts first stood to permit a boar (with hypoplastic testicles) to mount. The gilts were allocated to four experimental groups, A, B, C and D. Each gilt was inseminated once about 24 hr after the onset of oestrus. All gilts were inseminated with 10^{10} spermatozoa collected from two fertile boars and deep frozen by the method described by Crabo & Einarsson (1971) and by Crabo, Einarsson, Lamm, Soosalu & Viring (1972). Thawing of frozen spermatozoa was performed in 70 ml fresh seminal plasma obtained from a boar with totally hypoplastic testicles, or in 70 ml TESNaK–glucose buffer

(TESNaK buffer with 10% isotonic glucose). Sperm motility after thawing was about 30% (25 to 40%).

Group A: three gilts were inseminated with frozen spermatozoa thawed in seminal plasma. The gilts were slaughtered 1 hr after insemination.

Group B: three gilts were inseminated with frozen spermatozoa thawed in TESNaK-glucose buffer. The gilts were slaughtered 1 hr after insemination.

Group C: three gilts were inseminated with frozen spermatozoa thawed in seminal plasma. The gilts were slaughtered 4 hr after insemination.

Group D: three gilts were inseminated with frozen spermatozoa thawed in TESNaK-glucose buffer. The gilts were slaughtered 4 hr after insemination.

Immediately after slaughter, the reproductive organs were removed. Clamps were placed in various positions, dividing the oviducts into two approximately equal parts and each uterine horn into three parts, the length of the anterior part being 5 cm and the other two being of equal size. Each region was flushed

Table 1. Recovery of spermatozoa from the reproductive tracts of gilts 1 hr after insemination of frozen-thawed spermatozoa

Segment of reproductive tract	Mean number of spermatozoa recovered ($\times 10^6$) $\pm$ S.E.	
	Group A	Group B
Uterine horns (right and left)		
Posterior part	423.700 $\pm$ 225.600	1303.700 $\pm$ 730.500
Middle part	310.500 $\pm$ 96.800	1268.800 $\pm$ 609.000
Anterior part (5 cm)	31.000 $\pm$ 23.600	55.600 $\pm$ 15.500
Oviducts (right and left)		
Posterior half	0.020 $\pm$ 0.010	0.150 $\pm$ 0.130
Anterior half	0.150 $\pm$ 0.130	0.140 $\pm$ 0.130
Ratio between numbers of spermatozoa found in oviducts and in anterior 5 cm of uterine horns	0.010 $\pm$ 0.006	0.005 $\pm$ 0.004

Spermatozoa were thawed in seminal plasma (Group A) or in TESNaK-glucose buffer (Group B). Each group contained three gilts.

with a measured volume of isotonic saline. The washings from the oviducts were concentrated 1:5 by centrifugation before counts were carried out. The sperm concentration was determined in a Bürker haemocytometer. The total number of spermatozoa in each region was calculated.

The results are presented in Tables 1 and 2. There was no significant difference in the number of spermatozoa recovered from the oviducts of gilts in Groups A and B (Table 1). The distribution of spermatozoa within the uterine parts was also approximately the same but, on average, more spermatozoa were recovered from the uterine parts of the gilts in Group B. The ratio between the numbers of spermatozoa recovered from the oviducts and from the anterior 5 cm of the uterine horns was somewhat lower for gilts in Group B than in Group A. The number of spermatozoa found in the oviducts 4 hr after insemination was markedly lower when the spermatozoa were thawed in TESNaK-glucose buffer than when they were thawed in seminal plasma (Table 2). The very low

numbers of spermatozoa found in the oviducts of the gilts in Group D are in agreement with the findings of Schefels, Biegert & Leidl (1971). In the uterine horns too, except for the anterior 5 cm, the numbers of spermatozoa found were markedly lower for gilts belonging to Group D than to Group C. The ratio between the numbers of spermatozoa found in the oviducts and in the anterior 5 cm of uterine horns was lower for gilts in Group D than in Group C.

Deep-frozen spermatozoa thawed in TESNaK-glucose buffer disappeared more rapidly and thus appear to survive for a shorter time in the uterus than spermatozoa thawed in seminal plasma. This may correlate with the observations by Bedford (1965) that damaged or dead spermatozoa disappear more rapidly from the uterus by phagocytosis than intact spermatozoa. A difference in survival time was also found in *in-vitro* studies of deep-frozen boar spermatozoa thawed in TESNaK-glucose buffer and in seminal plasma, respectively, and preserved at 37° C for at least 4 hr (Einarsson, Soosalu, Swenssou & Viring,

Table 2. Recovery of spermatozoa from the reproductive tracts of gilts 4 hr after insemination of frozen-thawed spermatozoa

Segment of reproductive tract	Mean number of spermatozoa recovered ($\times 10^6$) $\pm$ S.E.	
	Group C	Group D
Uterine horns (right and left)		
Posterior part	269.600 $\pm$ 268.500	7.400 $\pm$ 7.400
Middle part	25.800 $\pm$ 25.400	0.200 $\pm$ 0.200
Anterior part (5 cm)	0.300 $\pm$ 0.100	0.100 $\pm$ 0.040
Oviducts (right and left)		
Posterior half	0.020 $\pm$ 0.001	0.004 $\pm$ 0.001
Anterior half	0.030 $\pm$ 0.030	0
Ratio between numbers of spermatozoa found in oviducts and in anterior 5 cm of uterine horns	0.148 $\pm$ 0.061	0.056 $\pm$ 0.028

Spermatozoa were thawed in seminal plasma (Group C) or in TESNaK-glucose buffer (Group D). Each group contained three gilts.

1972). Obviously, some factor in the seminal plasma is of significance for the survival and fertility of deep-frozen spermatozoa during and after thawing and dilution. The possible contribution of substances of a protein nature in the seminal plasma to the maintenance of the motility and fertilizing capacity of the spermatozoa is being studied.

Labelled albumin, suspended in boar seminal plasma, remains attached to the surface of ejaculated boar spermatozoa (Einarsson, 1971). The mode of action of the seminal plasma protein, therefore, might be to give the spermatozoa a protective coating during thawing and dilution.

When an adequate volume of semen is inseminated into gilts, the number of spermatozoa reaching the oviducts depends on the sperm concentration (Baker, Dziuk & Norton, 1968). Rigby (1966) found a direct correlation between the number of spermatozoa at the uterotubal junction and the number of spermatozoa in the corresponding oviduct. In the present study, the ratio

between the number of spermatozoa found in the oviducts and in the anterior 5 cm of the uterine horns was lower for gilts belonging to Groups B and D than for gilts in groups A and C. This might be due to a higher percentage of dead spermatozoa in the anterior 5 cm of uterine horns in the former groups. Although the initial transport of dead spermatozoa into the oviducts is as rapid as that of live spermatozoa (First, Short, Peters & Stratman, 1968), it has been established that fewer dead spermatozoa enter the oviducts (Baker & Degen, 1972).

Another possibility is that the seminal plasma itself affects the transport of spermatozoa into the oviducts. The effect of boar seminal plasma on uterine motility has been studied (Chury, Kozumplik & Simunek, 1968; Zerobin, 1968), but there appears to be no published report concerning the effect on sperm transport into the oviducts of female swine.

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