

## EFFECT OF FOLLICULAR FLUID ON MOTILITY OF THAWED BOAR SPERMATOZOA

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### Abstract

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Boar spermatozoa in volumes of 0.1 ml, 2 ml and 5 ml were pellet-frozen on solid carbon dioxide, and, in volumes of 2 ml, 5 ml and 10 ml were frozen in form of cylinders in evaporation of liquid nitrogen. After 1–6 months of storage, follicular fluid from sow ovaria was added to a part of thawed ejaculates, and, motility of spermatozoa kept at 38.5 °C was evaluated. Motility of spermatozoa frozen in 10 ml was significantly lower than that of spermatozoa frozen in 0.1 ml volume. About 30 % of thawed spermatozoa showed straight movement. After 6 hours, their motility decreased to 7 % in average (2–10 %), and to 12 % (9–15 %) if follicular fluid has been added. In non-frozen ejaculates kept under identical conditions, 48 % of spermatozoa showed motility. Following insemination with frozen-thawed spermatozoa, 7 gilts from 16 fell pregnant (43.75 %). The comparatively low survival of thawed spermatozoa might be an explanation for low fertility rate in inseminated sows.

*Boars, deep-freezing of spermatozoa, volume, survival of spermatozoa, fertility rate.*

Hammer and Williams (1964), Foley and Williams (1967), Grotjan et al. (1974) and others demonstrated recently that follicular fluid as well as secretion from oviducts supported motility and respiration of spermatozoa. Simultaneously it has been pointed out that follicular fluid from one species agglutinates spermatozoa of another species. If, nevertheless, for example bovine follicular fluid has been heated, the toxic factor was destroyed, and, elevated respiration and motility could be observed even after addition of boar spermatozoa (Yanagimachi 1969; Grotjan et al. 1975).

The effect of follicular fluid has not yet been studied with frozen-thawed spermatozoa. Since their vitality after thawing is limited, we tried to prolong motility of boar spermatozoa by addition of follicular fluid from ovaria and endometrial secretion from sows slaughtered during the follicular phase of sexual cycle.

### Materials and Methods

The second fraction of ejaculate from 4 Landrace boars obtained by manual method was used for freezing of spermatozoa. The ejaculates were kept at room temperature for 1 hour and then diluted 1 : 1 by one of the two tested solutions described in Table 1. The diluted ejaculates were poured into special jars with ground neck and an outlet through the stopper. Air was removed from the jars by a vacuum pump and replaced by hydrogen. After keeping it in hydrogen atmosphere at +5 °C for 3 hours, the diluted ejaculates were placed for 20 minutes in a water bath containing lumps of ice (Konovov 1975). Then, volumes of 0.1 ml, 2 ml and 5 ml were exposed to freezing to pellets on solid carbon dioxide, or volumes of 2 ml, 5 ml and 10 ml to cylinders formed from aluminium foil in evaporation of liquid nitrogen. Frozen spermatozoa were kept in liquid nitrogen for 1–6 months.

Table 1  
Composition of diluents

	Diluent No. 1	Diluent No. 3
Sucrose	60.0 g	60.0 g
Glucose	6.0 g	—
Fructose	—	6.0 g
ADTA	12.0 g	4.0 g
TRIS	—	4.0 g
Calcium monoxide	0.6 g	—
Magnesium oxide	0.4 g	—
Citric acid	—	2.5 g
Sodium citrate	2.0 g	—
Glycerol	40.0 ml	40.0 ml
Yolk	50.0 ml	50.0 ml
Distilled water	—	—
ad	1,000.0 ml	1,000.0 ml

Table 2  
Motility of spermatozoa after thawing

Volume in ml	Diluent No. 1						Diluent No. 3					
	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>
% of moving spermatozoa	36 ± 6.75	35 ± 7.63	29 ± 7.74	35 ± 7.63	30 ± 5.45	19 ± 7.12	34 ± 6.66	30 ± 5.24	29 ± 6.87	34 ± 6.41	30 ± 7.50	19 ± 8.10

+ Frozen in evaporation of liquid nitrogen

Pellets were thawed on a teflon pan at 42 °C. One part of follicular fluid was added to 2 parts of thawed spermatozoa, and, the test tubes containing samples were placed in a water bath at 38.5 °C (± 0.5 °C).

Follicular fluid from ovaria or endometrial secretion from sows killed in the course of follicular phase of sexual cycle was centrifuged, the supernatant drawn off, frozen and stored at -20 °C. Survival of spermatozoa was microscopically examined in the course of 6 hours, and, spermatozoa with progressive motion only were considered alive. This way of examination was applied for each volume and form of frozen ejaculate under study.

## Results

Solution No. 1 (Tab. 2) seemed to have the best composition, though the difference between both solutions tested was not statistically significant. Evaluation of the effect of various volumes used for freezing made it obvious that there was no difference in motility of spermatozoa as far as volumes 0.1 ml, 2.0 ml and 5.0 ml were concerned. The motility of spermatozoa frozen in 0.1 ml was significantly higher than in 10 ml. Results concerning motility are summarized in Table 2, their statistical evaluation in Tab. 3. There was virtually no difference between volumes 0.1 ml and 2 ml, while in 5 ml the motility of spermatozoa was decreased, although not to a significant degree. Practical implications concern not only the

period of time requested for freezing the insemination dose, but, the whole processing of large amounts of spermatozoa.

Disregarding the volume frozen, thawed spermatozoa survived a considerably shorter period of time than spermatozoa in freshly diluted ejaculate. After 6 hours in a water bath at 38.5 °C, not more than 10 % of thawed spermatozoa showed

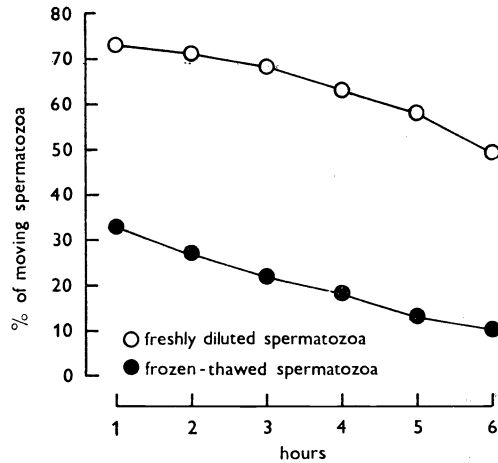


Fig. 1.

Motility of spermatozoa in the course of 6 hours in water bath at 38.5 °C.

progressive movement, while an average of 48 % was registered with freshly diluted spermatozoa (Fig. 1).

The effect of follicular fluid on the rate of moving spermatozoa was studied under the same conditions. Follicular fluid influenced both the number and the duration of motility. With progress of time, comparatively more motile spermatozoa were present in samples with follicular fluid so that they differed by 3–6 % from control samples at final evaluation. The results are in agreement with the findings described by Foley et al. (1967), Grotjan et al. (1974), and others.

Table 3

Statistical evaluation of effect of frozen-thawed volume on motility of spermatozoa

Volume of spermatozoa		Criteria compared	Critical values	Significance	n
Diluent No. 1	0.1 ml: 2 ml		0.5064	0	11
	0.1 ml: 5 ml		1.1503	0	11
	0.1 ml: 2 ml <sup>+</sup>		0.4249	0	11
	0.1 ml: 5 ml <sup>+</sup>		1.3684	0	11
	0.1 ml: 10 ml		5.7518	P = 0.01 <sup>++</sup>	11
Diluent No. 3	0.1 ml: 2 ml		1.0122	0	11
	0.1 ml: 5 ml		1.1650	0	11
	0.1 ml: 2 ml <sup>+</sup>		0	0	11
	0.1 ml: 5 ml <sup>+</sup>		1.6265	0	11
	0.1 ml: 10 ml		6.6769	P = 0.01 <sup>++</sup>	11

<sup>+</sup> Frozen in evaporation of liquid nitrogen  
<sup>++</sup> Advantage for 0.1 ml

Table 4  
Motility of spermatozoa 6 hours after thawing

		Diluent No. 1						Diluent No. 3						n
Volume in ml	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>	11	
Motility in %	8 ±5.3	9 ±6.6	4 ±3.5	10 ±9.8	7 ±4.6	6 ±4.3	9 ±2.1	10 ±3.8	6 ±3.5	8 ±4.2	7 ±4.7	4 ±3.9	11	

+ Frozen in evaporation of liquid nitrogen

Table 5  
Motility of spermatozoa with follicular fluid added, 6 hours after thawing

		Diluent No. 1						Diluent No. 3						n
Volume in ml	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>	0.1	2.0	5.0	2.0 <sup>+</sup>	5.0 <sup>+</sup>	10.0 <sup>+</sup>	11	
Motility in %	13 ±4.9	14 ±4.9	8 ±3.1	14 ±4.5	12 ±4.8	9 ±3.5	12 ±3.6	15 ±4.1	11 ±2.9	13 ±5.2	12 ±5.6	10 ±4.7	11	

+ Frozen in evaporation of liquid nitrogen

A favourable effect could be generally observed in every sample with follicular fluid (Tab. 4 and 5). The difference in motility of spermatozoa was of statistical significance in 66.6 % of volume categories. The real difference in motility, ranging from 1 % to 13 % after 6 hours in water bath at 38.5 °C, does not appear to be ample. Understandably, follicular fluid can act only upon spermatozoa not damaged by freezing to an extent which would influence their metabolic processes. Table 6 shows that follicular fluid was less efficient with the diluent No. 1. More examination will be needed to elucidate this fact, since without addition of follicular fluid, diluent No. 1 appeared to be slightly superior to diluent No. 3.

Conclusively it is to be stated that boar spermatozoa frozen and thawed in the described way can survive a comparatively short period of time only. An average of 7 % (4–10) spermatozoa survived 6 hours at 38.5 °C. The value increased to 12 % (8–15) if follicular fluid was added. The results indicate that if insemination with frozen-thawed spermatozoa is carried out 10–12 hours prior to ovulation, there is almost no chance of fertilization though conditions in the oviducts decidedly are more pertinent than in our experiments with follicular fluid. Our findings differ from those by Pursel et al. (1975), who reported that deep-frozen spermatozoa maintained their fertility for at least 20 hours after thawing. An explanation for the different results might be the difference in composition of diluents and freezing techniques. In spite of the above-mentioned differences results are close to those of Polge et al. (1970), who counted 83.5 % of fertile eggs after surgical administration of spermatozoa into oviducts, while merely 2.8 % after administration into uterine horns of spermatozoa with a motility of 30 %. In the course of testing survival of spermatozoa without addition of follicular fluid, 16 gilts were inseminated and 7 of them (43.75 %) became pregnant. This insemination rate indicates a short-time survival and consequently fertilizing ability of frozen-thawed boar spermatozoa. Conception is achieved only after insemination carried out shortly prior to ovulation.

### **Vliv folikulární tekutiny na motilitu spermií kance po rozmražení**

Ejakulát kance byl zmrazován v objemu 0,1 ml, 2 ml, 5 ml ve formě pelet na suchém ledu CO<sub>2</sub> a v objemu 2 ml, 5 ml a 10 ml ve formě válečků v parách tekutého dusíku. Po 1 až 6měsíčním uchování byl hodnocen pohyb spermií po rozmražení a jejich držení při teplotě 38,5 °C i po přidání folikulární tekutiny z vaječníků prasnic. Bylo zjištěno statisticky významné snížení pohyblivosti spermií ve vzorcích mražených v objemu 10 ml, oproti vzorkům mraženým v objemu 0,1 ml. Po rozmražení byl zjišťován přímočarý pohyb asi u 30 % spermií. Za 6 hodin klesl pohyb tak, že byl zjišťován v průměru jen u 7 % spermií (2–10) a ve vzorcích s folikulární tekutinou byl zjišťován v průměru u 12 % spermií (9–15 %). U nemraženého ejakulátu, drženého za stejných podmínek, byla zjišťována pohyblivost u 48 % spermií. Z 16 prasniček inseminovaných zmrazeným semenem bez folikulární tekutiny zabřezlo 7, tj. 43,75 %. Relativně nízká přežitelnost spermií kance po rozmražení může být příčinou slabších výsledků v zabřeznutí prasnic.

### Влияние фолликулярной жидкости на подвижность сперм боровов после разморозения

Проводилось размораживание эякулята борова в объеме 0,1 мл, 2 мл, 5 мл формой дражирования на сухом льде CO<sub>2</sub> и в объеме 2 мл, 5 мл и 10 мл в форме валиков в паре жидкого азота. После хранения в течение 1—6 месяцев проводилась оценка движения сперм после разморозения и их устойчивости при температуре 38,5 °С и после добавления фолликулярной жидкости яичников свиноматок. Было выявлено статистически значимое понижение подвижности сперм в образцах, замораживаемых в объеме 10 мл, по сравнению с образцами, самораживаемыми в объеме 0,1 мл. После разморозения было выявлено прямолинейное движение около 30 % сперм. Спустя 6 часов понизилось движение настолько, что оно имело место лишь у 7 % сперм (2—10) и в пробах с фолликулярной жидкостью было движение установлено лишь у 12 % сперм (9—15 %). У хранимого в одинаковых условиях незамороженного эякулята была установлена подвижность в случае 48 % сперм. Из 16 свиноматок, оплодотворенных замороженными спермами, стало беременными 7, т. е. 43,75 %. Относительная низкая живучесть сперм борова после разморозения может быть причиной не особо удовлетворительных результатов забеременения свиноматок.

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