Fertility and Survival of Frozen Boar Spermatozoa Stored in Aluminum Packaging Containers

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Liquid semen stored at 15°C has been practically used for the artificial insemination of pig. The current technique, however, can hardly maintain high fertility of spermatozoa for more than one or two days. Therefore, it is impossible to practice artificial insemination systematically and effectively with transported semen for wide areas.

The author conducted experiments on frozen boar semen to contribute to the improvement of pig by making the long storage of boar semen and effective use of transported semen for large areas possible. In 1969, the authors succeeded in obtaining seven litters out of 12 sows by inseminating frozen boar semen stored in liquid nitrogen at −196°C for one to 154 days[10),11),12)]. The study is now under way continuously.

Experimental methods

The semen was collected by the glove hand technique from 4 boars of Middle Yorkshires, two of Landraces and one Berkshire mounted on a dummy sow, and only the sperm rich fraction of the semen was filtered out with a piece of gauze, and then the semen was transferred into a semen bottle pre-warmed at 35°C.

The diluent (EGT) (RD-1) used for this experiment was prepared as follows:

EGT-10(A): Into a cylinder, 180 ml of distilled solution containing glucose, fructose and inositol at the percentages of 5.0, 0.5 and 0.5 respectively was poured and 20 ml of egg yolk was added and mixed. The cylinder containing the solution mixed with egg yolk was left still in a refrigerator at 5°C for more than 10 hours, and the supernatant material was used for the experiments. The pH of the supernatant was adjusted to 7.4 with tris (hydroxymethyl) aminomethane and citric acid.

EGT-10(B): A diluent made by adding and mixing 7 ml of glycerol to 93 ml of EGT-10(A).

RD-1: 2 ml of egg yolk was added to 98 ml of EGT-10(A) solution without egg yolk, and poured into a cylinder to be mixed. The cylinder containing the egg yolk mixture was left to stand in a refrigerator at 5°C for more than 10 hours, and then the supernatant material was taken. TPD (Thyamine propyldisulfide hydrochloride) (2 mg/1000 ml), Potassium Penicillin G (1000 IU/ml) and 10 ml of phosphate buffer solution (pH 7.4) diluted 10 times with distilled water were added to 90 ml of the supernant material.

Experiment 1. Effect of rapid freezing

The semen equilibrated with glycerol for three to four hours was divided into two parts, A and B, and every 4 ml of each were sealed into containers made of aluminum pack.

The semen (A) was frozen rapidly by lowering temperature from 5°C to −100°C within 2.5 to 3 minutes.

The semen (B) was treated with slow freezing, that is, the temperature was lowered slowly with solid CO_2 and alcohol at the velocity of 1 to 2°C per minute from 5°C to −20°C and

3°C per minute from −20°C to −70°C.

Motility of spermatozoa was examined after the semen was thawed by placing the semen container in hot water of 45°C.

Experiment 2. Effect of glycerol equilibration

The semen diluted (a second dilution) with EGT-10(B) was frozen rapidly in the aluminum pack after the glycerol equilibration for 0.5 to 20 hours. The frozen semen was thawed with 45°C hot water and then the effect of duration of equilibration on the motility of spermatozoa was determined.

Experiment 3. Viability of frozen boar spermatozoa

The semen contained in an aluminum pack was frozen rapidly with liquid nitrogen vapor and then stored in liquid nitrogen for 900 days. The viability of the spermatozoa was determined during the term of storage at fixed intervals until the 900th day.

Experiment 4. Semen extension and fertility test

The sperm rich fraction of semen was diluted 1.5 to 2.0 times with EGT-10(A) at 27 to 30°C (first dilution). Then EGT-10(B) was added to the semen by means of the dropping method or separation method (four to five times at 10 minute interval) with a gradual lowering of temperature of the diluted semen (second dilution). In this second dilution, EGT-10(B) was added at a rate 1:1 (equal volume) to the diluted (first dilution) semen. Consequently, the final dilution rate was three to four times v/v.

The diluted semen was put in a polyethylene beaker containing 300 ml of 25°C water and was equilibrated with glycerol at 5°C in a refrigerator. The temperature of the semen was gradually lowered to 5°C during three to four hours.

After the glycerol equilibration for three to four hours, every 4 to 5 ml of the semen was put into aluminum packaging containers which were then sealed up.

The semen at a state of thin film in the aluminum pack was rapidly frozen with liquid nitrogen vapor and stored in liquid nitrogen.

In the fertility test, eight sows and 12 gilts were inseminated. The frozen semen was thawed in 43 to 45°C hot water and transferred into semen bottles and then rediluted with RD-1 at a rate of 1 : 1 in volume. The semen was kept at 25–30°C until insemination, and was used for insemination within 20 minutes after thawing.

On an average, 66.7 ml (sows) or 62.1 ml (gilts) of semen which contains 2.4×10^9 (sows) or 2.2×10^9 (gilts) of motile spermatozoa was inseminated. The semen was inseminated once or twice per one oestrus via the cervix with a spiral tip insemination catheter.

Results and discussions

A high percentage of motile spermatozoa was recognized with the semen contained in an aluminum packaging semen container and frozen rapidly in liquid nitrogen vapor.

Table 1 shows survival of motile spermatozoa after the rapid freezing in comparison with slow freezing. After the rapid freezing of

Table 1. Effect of rapid freezing on percentage of motile boar spermatozoa after freeze-thawing

Freezing	No. Semen	Motile spermatozoa (%)				Recovery (%)
		Pre-freezing		Post-thawing		
		Range	Average	Range	Average	
Rapid freezing	15	55~85	72.0	25~60	47.7**	66.3**
Slow freezing	15	55~85	72.0	1~40	19.2	26.7

**= $P < 0.01$

semen, the percentage of motile spermatozoa was 25 to 60% (av. 47.7%) and the percentage of recovery was 66.3%. The survival percentage of spermatozoa in the rapid freezing was higher than that of slow freezing.

In early studies on frozen boar semen, it was reported that slow freezing gives good results[1),5),6)]. In recent studies, however, rapid freezing renders good effect on the survival of spermatozoa[2),4),7),9)] in the same way as the results of this experiment.

Fig. 1 shows the effect of glycerol equilibration on the motility of spermatozoa after freeze-thawing. The semen equilibrated for three to six hours showed a high percentage of motile spermatozoa. The optimum time for the glycerol equilibration of boar semen was three to six hours, and the percentage of motile spermatozoa after freeze-thawing was 45 to 60%.

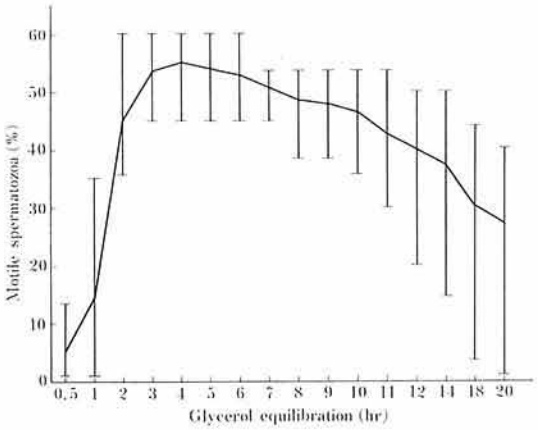

Fig. 1. Effect of glycerol equilibration on percentage of motile spermatozoa after freeze-thawing

In early studies on the effect of the glycerol equilibration of frozen boar semen, it was reported that no significant difference was observed with the duration of treatment, short time or 24 hours[5)]. Afterward, however, it was reported that the glycerol equilibration in a short time, such as 2.5 or 6 hours, gives best effect on the motility of spermatozoa after freeze-thawing[8),9)].

The viability of spermatozoa in the semen which was stored at −196°C up to 900 days was examined during the storing terms. As shown in Table 2, no difference in survival was recognized with different period of storage. Thus, it was proved that the frozen boar semen stored in liquid nitrogen at −196°C is comparable to a long storage of frozen bull semen, which is already an established technique.

The semen stored for 4 to 532 days was inseminated to eight sows and 12 gilts in a fertility test. Seven pregnancies out of eight sows and seven pregnancies out of 12 gilts were obtained and the percentage of pregnancy (CR) was 87.5 and 58.3% respectively. From 12 sows and gilts (2 aborted at 64 and 92 days), 98 piglings were farrowed.

Table 2. Percentage of motile boar spermatozoa stored in liquid nitrogen (−196°C)

Semen	Motile spermatozoa (%)						
	1 day	30~400	401~500	501~600	601~700	701~800	801~900
No. 1	50	50	—	—	—	—	—
2	55	50	—	—	—	—	—
3	45	45	—	—	—	—	—
4	60	60	—	—	—	—	—
5	50	50	—	—	—	—	—
6	55	50	50	—	—	—	—
7	50	50	50	—	—	—	—
8	40	40	40	40	40	40	40
9	50	50	50	50	50	50	50
10	65	60	60	60	60	60	60
11	55	55	55	50	55	50	50

Table 3. Fertility of sows and gilts inseminated with frozen boar semen

Swine	No. of pregnant	Term stored (day)	Volume of semen inseminated (ml. av.)	No. of sperm inseminated ($\times 10^9$)	% pregnant	No. in litter (av.)
Sow	7	4～532	66.7	2.4	87.5 (7/8)	10.2
Gilt	7	5～510	62.1	2.2	58.3 (7/12)	5.8

The longest record of storage of frozen semen which could give satisfactory conception and farrowing was 510 days in this experiment :10 piglings were farrowed from a gilt.

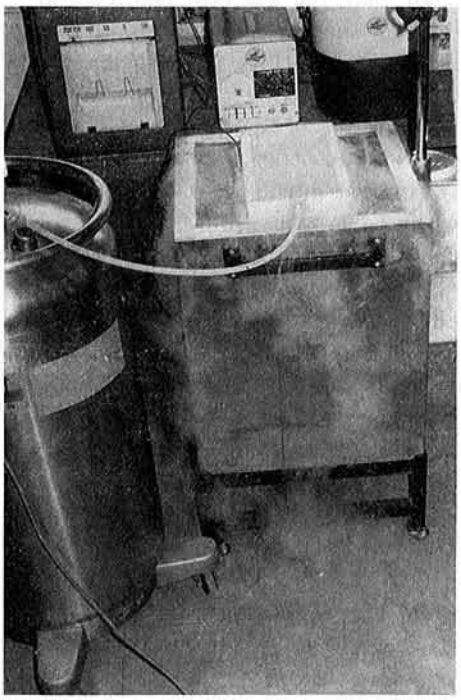

Plate 1. Freezing of boar semen with liquid nitrogen vapor

In foreign countries, Hoffman (1959) reported one litter from 11 insemination in early days[6]. After 1971, cases of conception in fertility tests were reported by Graham (1971)[3),4)], Pursel (1971)[7),8)] in U.S.A. and by others in Europe and Australia[2),9)].

In the future, the fertility test on a large scale and experiments on freezability of individual boar will be needed.

Summary

Experiments of rapid freezing of boar semen using the semen container made of aluminum pack in the vapor of liquid nitrogen were carried out. Semen was collected from 4 Middle Yorkshires, 1 Berkshire and 2 Landraces. The semen was stored at −196°C in liquid nitrogen and motility of spermatozoa before freezing and after thawing was examined. Fertility of frozen boar semen was also tested. The results obtained are as follows:

1) Survival of spermatozoa after a rapid freezing using aluminum packaging semen container with the vapor of liquid nitrogen

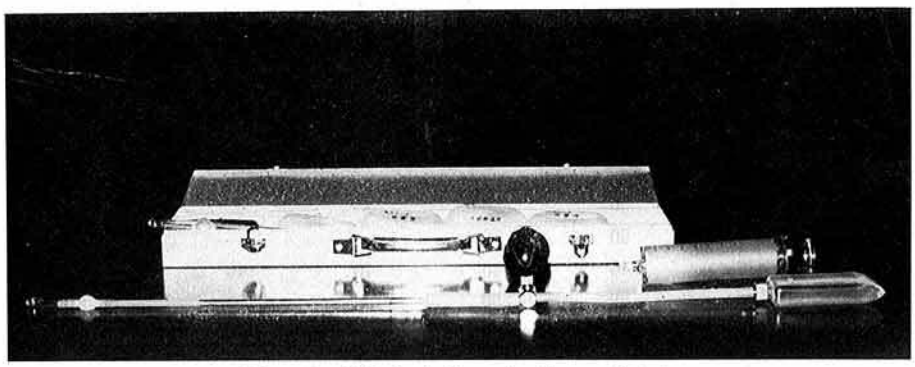

Plate 2. Spiral tip insemination cathetel

was 25%–60%; with an average of 57.7%. Rapid freezing was superior to slow freezing.

2) Within a range of 0.5–20 hours, the semen equilibrated for 3–6 hours gave high percentage of motile spermatozoa.

3) Good survival and high percentage (40–60%) of motile spermatozoa was shown after storage in liquid nitrogen up to 900 days.

4) The semen stored for 4 to 532 days at −196°C was inseminated to 8 sows and 12 gilts. The results were 14 conceived and 96 piglings were farrowed out of 12 sows and gilts (2 aborted). The longest record of storage with satisfactory conception and farrowing in sows and gilts was 510 days and a record-setting gilt has farrowed a litter of 10 piglings.

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