Characteristics of Cryopreserved Spermatozoa from a Holstein-Friesian Bull Thawed at Different Temperature

Yoshiaki HAYASHI

Graduate School for International Development and Cooperation, Hiroshima University,
Higashi-Hiroshima 739-8529, Japan
E-mail: yoshiha@hiroshima-u.ac.jp

Naoki ISOBE

Graduate School of Biosphere Science, Hiroshima University,
Higashi-Hiroshima 739-8528, Japan
E-mail: niso@hiroshima-u.ac.jp

Abstract

The present study was undertaken to examine the viability, motility and morphology of spermatozoa that were frozen-thawed at different temperature followed by the storage at 4ºC until 10 hours under the condition of tropical area. The frozen semen derived from a Holstein-Friesian bull with the egg-yolk-base extender was thawed at 23ºC or 37ºC for 30 seconds or 15 seconds, respectively, and kept at 4ºC. Viability, motility and damage of acrosomal cap in sperm were assessed every 2 hours after thawing. The sperm viability just after the refrigeration at 4ºC was not different between the groups. Besides, only 5% difference was found in the viability between the groups from 2 to 10 hours after thawing. On the other hand, the sperm had a fast progressive movement until 6 hours post-thawing. Only 2% or less sperms showed the damage of acrosomal cap in both groups. This value did not rise with keeping the semen at 4ºC until 10 hours in both groups. In conclusion, the thawing at 23ºC for 30 seconds does not have detrimental effects on the viability, motility, and morphological change of sperm. This suggests that the semen thawed at 23ºC can keep the quality at 4ºC for 6 hours.

1. Introduction

High viability and motility of spermatozoa are important factors for successful artificial insemination (AI) because the significant correlation between the post-thawing sperm viability and the subsequent conception rate has been reported (Linford et al., 1976; Correa et al., 1997). Various factors, such as type of extender, concentration of glycerol, method of semen packing, cooling rate and semen handling during cryopreservation procedure, affect the post-thaw motility of sperm (Rodriguez et al., 1975; Robbins et al., 1976). Experimental conditions, such as available facilities, tools and chemicals, vary among countries and areas (Thibier and Wagner, 2002; Vishwanath, 2003). Thus, the methods to freeze

and thaw spermatozoa should be examined in each country and area.

Egg yolk has been used as the main component of extender for freezing bull semen due to its availability even in tropical countries. Frozen semen with egg-yolk-base extender is generally thawed at 37ºC for 15 seconds. However, the semen is dissolved at below 37ºC for 30 seconds in some tropical areas where are not facilitated to prepare 37ºC. Because the thawing of frozen semen usually takes 30 seconds at the temperature less than 37ºC. Besides, the effect of lower temperature (<37ºC) for thawing semen on the subsequent motility of sperm has not been examined yet.

The number of liquid nitrogen tank for storage of the frozen semen is shortage in the developing countries of the tropics. Thus, most semen straws have to been thawed at the collection site and kept it in a cold water until insemination. However, no information shows the changes of sperm characteristics in process of time during the storage in a cold water.

Hence, the present study was conducted to report the viability, motility and the morphological change in cryopreserved spermatozoa thawed at 23ºC for 30 seconds and 37ºC for 15 seconds, and kept at 4ºC for contribution to the AI activities under the tropical condition.

2. Materials and Methods

2. 1. Collection and freezing procedure of semen

This study was conducted at the Mikolongwe Veterinary Station in Malawi. The Holstein-Friesian bull used in the study was 7 years old and 830 kg. Ejaculate from the bull was collected with the aid of an artificial vagina. Just after the collection, the ejaculate was evaluated for volume, color, pH, rate of motile sperms and sperm concentration. The semen was extended in 10 ml of extender supplemented with 20.0% egg yolk, 1.6% sodium citrate, 1.4% glucose, 1.0 mg/ml streptomycin and 1000 IU/ml penicillin. The extended semen was transferred into a sterile tube at 37ºC followed by the cooling at 4ºC over 2-hour equilibration period. The final concentration (60 million sperms/ml) was made by addition of the extender containing 14% glycerol. The extended semen was loaded into 0.5 ml straws and frozen in liquid nitrogen vapor.

2.2. Thawing of frozen semen and evaluations of sperm characteristics

The semen straw was subjected to a water bath at 23ºC or 37ºC for 30 seconds or 15 seconds, respectively. The temperature of 23ºC was the average temperature of tap/well water in this study area. The thawing time of 30 seconds was a prevalent duration for dissolving the frozen semen at the temperature below 37ºC. Following the semen was thawed, the straw was transferred to a thermos flask to keep temperature at 4ºC.

Viability, motility and acrosomal change of spermatozoa were assessed at every 2 hours after thawing until 10 hours. Sperm viability was defined as the rate of moving sperms. The motility of sperm was estimated by the modified method of Correa et al. (1996). Sperm motility was graded as follows: Grade 1, slow progressive movement; Grade 2, fast progressive movement. Sperm viability and motility were evaluated, using a microscope equipped with differential interference contrast optic at 200 × magnification and a thermal stage at 37ºC, within 90 seconds after the semen fixation on the stage. For observation of sperm acrosomal cap damage, sperms were stained with a 10% solution of Giemsa for 2 hours following the smear of sperms on the slide glass. All sperms in the field selected randomly were evaluated under the light microscopy at 400 × magnification until 500 spermatozoa were examined.

3. Results and Discussion

The evaluations of ejaculation are shown in Table 1. All parameters of semen characteristics were considered as normal. Spermatozoa viability just after the refrigeration at 4ºC was not different between the groups (Table 2). Besides, only 5% difference was found in the viability between the groups from 2 to 10 hours post-thawing. On the other hand, the sperm had a fast progressive movement until 6 hours after thawing. This suggests that neither normal (37ºC) nor lower temperature (23ºC) for thawing the sperm has detrimental effects on the subsequent viability and motility under the storage at 4ºC until 6 hours. The viability just after thawing in this study was 35%. This viability was comparable to the results in the other reports (Rodriguez et al., 1975; Brown et al., 1991).

Only 2% or less spermatozoa showed the damage of acrosomal cap in both groups (Table 3). This

Table 1. Evaluations of semen just after ejaculation from bull

Volume (ml)	5.5
Color	Pale white
pH	6.6
Sperm viability (%)	65
Sperm motility grade	2*
Sperm concentration (sperms/ml)	455 million

* 2: fast progressive movement

Table 2. Viability and motility of sperm after thawing at 23ºC for 30 seconds and 37ºC for 15 seconds

Time after thawing (hour)	23ºC for 30 seconds		37ºC for 15 seconds	
	Viability (%)	Motility*	Viability (%)	Motility*
0	35	2	35	2
2	25	2	30	2
4	20	2	25	2
6	20	2	25	2
8	20	1	25	1
10	15	1	20	1

* 1: slow progressive movement, 2: fast progressive movement

Table 3. Percentage of sperm with damaged acrosomal cap after thawing at 23ºC for 30 seconds and 37ºC for 15 seconds

Time after thawing (hour)	23ºC for 30 seconds	37ºC for 15 seconds
0	1.6	1.2
2	2.0	1.0
4	0.8	1.0
6	1.6	0.4
8	1.8	0.6
10	0.8	0.4

rate did not rise with keeping the semen at 4ºC until 10 hours in both groups. These results indicated that most acrosomal caps were not damaged at least for 10 hours under this experimental condition. However, the acrosomal damage was reported to occur in sperm thawed at less than 37ºC (Robbins et al., 1976; Senger et al., 1976; Pace et al., 1981; Correa et al., 1996; DeJarnette et al., 2000). In these cases, the thawed semen was incubated at 37ºC at least for 60 minutes and evaluated the rate of damaged sperm. Additionally, the latent sperm injury was reported to be apparent when sperm was incubated at 37ºC for 3 hours (DeJarnette et al., 2000). Thus, the low rate of damaged acrosomal cap in this study was possibly due to the absence of incubation at 37ºC after thawing. If the thawed semen in this study was incubated at 37ºC, the acrosomal damage might have been occurred in higher rates.

In conclusion, there is no difference in the viability, motility, acrosomal change of sperm at 4ºC until 6 hours between sperms thawed at 23ºC for 30 seconds and 37ºC for 15 seconds. This suggests that the frozen semen thawed at 23ºC can be kept the quality until 6 hours post-thawing. This finding must extend the AI activities using the frozen semen and contribute to the improvement of livestock industry in developing countries of the tropics.

References

Brown, D.W., et al. (1991), Effect of group thawing on post-thaw viability of bovine spermatozoa packaged in .5-milliliter french straws, *Journal of Animal Science*, 69, 2303-2309.

Correa, J.R., et al. (1996), Thawing and processing of cryopreserved bovine spermatozoa at various temperatures and their effects on sperm viability, osmotic shock and sperm membrane functional integrity, *Theriogenology*, 46, 413-420.

Correa, J.R., et al. (1997), Relationships among frozen-thawed sperm characteristics assessed via the routine semen analysis, sperm functional tests and fertility of bulls in an artificial insemination program, *Theriogenology*, 48, 721-731.

DeJarnette, J.M., et al. (2000), Effects of pre- and post-thaw thermal insults on viability characteristics of cryopreserved bovine semen, *Theriogenology*, 53, 1225-1238.

Linford, E., et al. (1976), The relationship between semen evaluation methods and fertility in the bull, *Journal of Reproduction and Fertility*, 47, 283-291.

Pace, M.M., et al. (1981), Effect of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of bovine spermatozoa packaged in .5-ml french straws, *Journal of Animal Science*, 53, 693-701.

Robbins, R.K., et al. (1976), Influence of freeze rate, thaw rate and glycerol level on acrosomal retention and survival of bovine spermatozoa frozen in French straw, *Journal of Animal Science*, 42, 145-154.

Rodriguez, O.L., et al. (1975), Effect of rates of freezing, thawing and level of glycerol on the survival of bovine spermatozoa in straws, *Journal of Animal Science*, 41, 129-136.

Senger, P.L., et al. (1976), Effect of thawing rate and post thaw temperature on motility and acrosomal maintenance in bovine semen frozen in plastic straws, *Journal of Animal Science*, 42, 932-936.

Thibier, M. and H.G. Wagner. 2002. World statistics for artificial insemination in cattle, *Livestock Production Science*, 74, 203-212.

Vishwanath, R. 2003. Artificial insemination: the state of the art, *Theriogenology*, 59, 571-584.