12

Marine Fish Sperm Cryopreservation and Quality Evaluation in Sperm Structure and Function

Qing Hua Liu, Zhi Zhong Xiao, Shi Hong Xu,
Dao Yuan Ma, Yong Shuang Xiao and Jun Li
Center of Biotechnology R&D, Institute of Oceanology,
Chinese Academy of Sciences, Qingdao
PR China

1. Introduction

Long-term storage of sperm in liquid nitrogen is a valuable technique for genetic resources preservation (Kopeika et al. 2007). The research on fish sperm cryopreservation has achieved great advances since the first successful sperm cryopreservation in herring (Blaxter 1953). It provides many benefits such as ease of global germplasm shipping and supply (Tiersch et al. 2004), selective breeding and hybridization with desirable characteristics (Henderson-Arzapalo et al. 1994), and conservation of genetic diversity (Van der Walt et al. 1993; Tiersch et al. 2000; Ohta et al. 2001). Furthermore, a frozen sperm bank could maintain the continuous and stable supply of gametes for hatchery seed production or laboratory experimentation. Because of the advantages of this technique, fish sperm of over 200 freshwater and 40 marine species have been cryopreserved successfully (Gwo 2000).

Most of fish sperm cryopreservation researches have focused on freshwater species such as cyprinids (Babiak et al. 1997; Lahnsteiner et al. 2000), salmonoids (Conget et al. 1996; Cabrita et al. 2001), catfishes (Christensen and Tiersch 1997; Viveiros et al. 2000) and loach (Kopeika 2003a, b; Dzuba & Kopeika 2002). In recent years, with the rapid development of marine fish aquaculture, some experiments on germplasm cryopreservation have also been conducted in marine fish species, especially the great commercial value ones such as red seabream (Liu, et al. 2006；Liu, et al. 2007a，b；Liu, et al. 2010 a，b) turbot (Dréanno et al. 1997; Chen et al. 2004), flounder (Richardson et al. 1999; Zhang et al. 2003), and halibut (Billard et al. 1993).

Damage to sperm morphology and function usually occurs during the process of freezing and thawing. Cellular damage may greatly decrease motility, impair velocity, and reduce fertilizing capacity, even lead to DNA strand breakage or mutation (Dréanno et al, 1997; Lahnsteiner et al, 1996a; Warnecke & Pluta 2003; Kopeika et al, 2004). Although motility and fertilizing capacity are usually assessed in frozen-thawed sperm, these methods have limitations. Many factors affect the validity of these assessments, including subjectivity, microscope performance, the quality of eggs, and fertilization protocols. Some new

technologies have been used in fish sperm quality analysis, such as computer-assisted sperm analysis (CASA), being used to objectively evaluate sperm motility (Lahnsteiner et al., 1996b; Lahnsteiner et al., 1998; Kime et al., 1996) ; Electron microscopy, being used to detect cryodamage (ultrastructural changes) in frozen-thawed sperm (Zhang et al, 2003, He &Woods 2004); In addition, flow cytometry of fluorescent-stained sperm have been used in mammals (Graham et al, 1990; Gravance et al, 2001) and turkeys (Donoghue et al ,1995), providing rapid, precise information regarding the viability of thousands of individual sperm. In recent years, flow cytometry has also been successfully used to assess both fresh and cryopreserved fish sperm (Ogier de Baulny et al, 1999; Segovia et al, 2000).

Red seabream is one of the most commercially important marine fish species for aquaculture in China. However, the decline of wild red seabream population has occurred due to over fishing and marine pollution in recent years. The use of cryopreserved sperm can provide an efficient method to increase its genetic population size and to help maintain genetic diversity. The aims of this study were to establish efficient methods for cryopreservation of red seabream sperm with 2-mL cryovials and to objectively measure the post-thaw sperm motility characteristics by means of CASA, to evaluate the post-thaw sperm fertilization capacity, and the cryodamage by electron microscopy and flow cytometry.

2. Sperm cryopreservation and quality evaluation

2.1 Materials and methods

2.1.1 Gametes collection

Naturally matured fishes were obtained from Qingdao hatchery during the spawning season (From the middle of March to the end of May). Twenty males and 10 females (3 kg to 4 kg individually, 10 years old) were cultivated in a 20-m3 concrete rearing pond with flow-through seawater and fed daily with cooked meat of bay mussel, *Mytilus edulis*. Prior to handling, males were firstly anesthetized in a 0.003% eugenol bath. Sperm was collected into petri dishes by gently hand-stripping the abdomen of the ripe males. Extreme care was taken to avoid the contamination of sperm with seawater, blood, urine and feces. The percentage of motile spermatozoa was checked with a Nikon-YS-100 light microscope (Nikon Corporation, Tokyo, Japan) at 250 × magnification. Sperm with motility > 85% was kept on crushed ice and transported to the laboratory for further use. Eggs were collected by abdominal pressure of the females at the time of ovulation. Good eggs were slightly yellowish, translucent and round-shaped. Eggs for fertilization trials were collected only from one female.

2.1.2 General procedure for sperm freezing and thawing

Sperm were diluted in Cortland extenders (Liu et al, 2006) containing DMSO with different concentrations (6–24% DMSO). After mixing thoroughly, 1.6 ml sperm was placed into 2-ml cryovials. The cryovials were transferred into a Kryo-360-1.7 programmable freezer (Planer Plc. Middlesex, UK), equilibrated for five minutes at 0°C, and frozen from 0 to −150°C at a cooling rate of 20°C min^{-1}, then plunged into liquid nitrogen for storage. The frozen sperm were thawed in 40°C water bath after being

preserved in liquid nitrogen for one month. After that, the thawed sperm was evaluated for motility and fertilizing capacity.

2.1.3 Sperm motion characteristics analysis by using CASA

Sperm motion characteristics were assessed by using a computer-assisted sperm motion analysis system (CASAS-QH-III, Tsinghua Tongfang Inc., Beijing, China) at room temperature (18°C to 20°C). The method for computer-assisted sperm motion analysis was described in Liu et al (2007b). The designation of the motility status was based on the level of the average path velocity (VAP). Sperm with average path velocity <5 μm s^{-1} were considered immotile, with average path velocity >20 μm s^{-1} were defined as motile, and 5–20 μm s^{-1} as locally motile. Therefore, in the present study sperm motility includes the percentage of local motile sperm and motile sperm. Motility and velocity of fresh and post-thaw sperm were quantitatively recorded by CASA immediately 10 s after activation, and changes of motility of post-thaw sperm frozen with 15% DMSO were observed every 30 s.

2.1.4 Sperm fertilization and hatching experiments

Fertilization capacity of post-thaw sperm frozen with DMSO (6–24% DMSO) was evaluated. The optimized sperm to egg ratio of 500:1 was selected for the following fertilization trials (Li et al., 2006). The artificial fertilization method was described in detail in Liu et al (2007b). Fertilization rates were evaluated within 6–8 h after insemination by counting the percentage of gastrula-stage embryos in relation to the total number of eggs used. Forty-eight hours after fertilization, the number of hatched larvae was counted in each experiment. The hatching rates were calculated as the percentage of hatched larvae in relation to the total number of eggs used in each experiment.

2.1.5 Ultrastructure

Prior to scanning electron microscopy, sperm were fixed in 2.5% glutaraldehyde diluted in PBS (pH 7.6), dehydrated in a series of increasing concentrations of ethanol, critical-point dried, evaporated with gold, and examined with a scanning electron microscope (KYKY-2800B; KYKY Technology Development Ltd., Beijing, China) For transmission electron microscopy, spermwas prefixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epon 812. Ultrathin sections were prepared, counterstained with 2%uranyl acetate followed by lead citrate, and examined with a transmission electron microscope (HITACHI H- 7000; Hitachi Ltd., Tokyo, Japan), and the number of sperm with various categories (normal, slightly damaged, and seriously damaged sperm) of cryodamage was determined. One-hundred sperm were randomly selected for observation each time; this was repeated three times on different sections (total of 300 frozen-thawed sperm for each male).

2.1.6 Rhodamine 123, propidium iodide and flow cytometry

The staining method used was described in (Liu et al. 2007a). An aliquot of mixed fresh or frozen-thawed sperm with 15%DMSO was incubated for 20 min (in the dark, temperature 4 °C) with 5 mg/mL of Rhodamine 123 (Rh123, Sigma Chemical Co., St. Louis, MO, USA).

Thereafter, sperm were incubated for 45 min in 1.5 mL of Cortland extender. With this staining method, only cells with functional mitochondria were stained, due to the negative potential of the inner membrane of the mitochondria. Samples were diluted and counterstained with 5 mg/mL of propidium iodide (PI, Sigma Chemical Co.). After 10 min, sperm samples were analyzed with flow cytometry (FACSvantage SE flow cytometer; Becton Dickinson, Mountain View, CA, USA) as previously described for trout sperm (Ogier de Baulny, et al, 1997). Sperm populations were identified according to their relative red and green fluorescence (staining with PI and Rh123, respectively). Sperm with red (stained with PI) DNA were interpreted as having a damaged plasma membrane, whereas those that were green (stained with Rh123) were interpreted as having intact mitochondrial function. Sperm that were only red (damaged plasma membrane and lacking mitochondrial function), were localized in Region 1, whereas those that were only green (intact membrane and functional mitochondria), were localized in Region 3. Sperm with both red and green fluorescence (damaged plasma membrane and functional mitochondria) were localized in Region 4, and those with no staining (intact plasma membrane, but no mitochondrial activity) were localized in Region 2.

2.1.7 Statistical analysis

To determine the effects of cryopreservation on sperm motility, fertilization capacity, structure and function, a paired-sample t-test was used to compare fresh versus frozen-thawed sperm. All statistical analyses were performed with SPSS Version 11.0 software (SPSS Inc. Chicago, IL, USA) and $P < 0.05$ was considered significant. All data were expressed as mean±S.D.

2.2 Results

2.2.1 Post-thaw sperm viability

The influence of cryopreservation on sperm motility and velocity was shown in Table 1. Percentages of motile post-thaw sperm frozen with 12–21% DMSO were higher than those with 6% DMSO, 9% DMSO and 24% DMSO. However, the procedure of cryopreservation has no significant ($P>0.05$) influence on the motile sperm velocity 10 s after activation compared with fresh sperm. In addition, the post-thaw sperm frozen with 12-21% DMSO showed similar types of straight trajectories.

Cryoprotectant (%)	Motility parameters		
	Locally motile (%)	Motile (%)	Velocity (μm s^{-1})
Fresh sperm	22.0±7.7	64.7±14.2 c	113.1±10.6 a
6% DMSO	16.6±4.6	26.8±11.4 a	89.1±15.0 a
9% DMSO	21.3±7.2	40.3±9.1 ab	91.9±13.5 a
12% DMSO	17.8±10.6	61.6±8.5 c	95.2±12.3 a
15% DMSO	20.4±6.1	64.8±8.7 c	99.3±11.6 a
18% DMSO	21.8±3.9	62.9±6.2 c	97.7±15.2 a
21% DMSO	16.4±4.3	60.8±5.4 c	90.1±12.3 a
24% DMSO	16.6±7.9	55.7±9.2 bc	95.7±8.9 a

Table 1. The influence of cryopreservation on sperm motility and velocity in *P. major*

The motion characteristics of fresh and post-thaw sperm were evaluated by using computer-assisted sperm analysis 10 s after activation. This table shows the percentages of locally motile (VAP range from 5 to 20μm s-1) and motile sperm (VAP > 20μm s-1) as well as their velocity (VAP) for fresh and post-thaw sperm. Values superscripted by the same letter are not significantly different (P>0.05, n=5).

The effect of time after activations on post-thaw sperm motility was shown in Fig. 1. The percentages of total motile sperm of both fresh (87. 2 ± 6.1%) and post-thaw sperm (81.9 ± 6.6%) frozen with 15% DMSO were not (P>0.05) different significantly 10 s after activation. However, 30 s after activation the percentage of total motile post-thaw sperm (72.3 ± 6.3%) was (P<0.05) lower than that of fresh sperm (82.7 ± 7.2%). Sixty seconds after activation, the percentage of post-thaw sperm motility drastically reduced to 38.7 ± 13.2%.

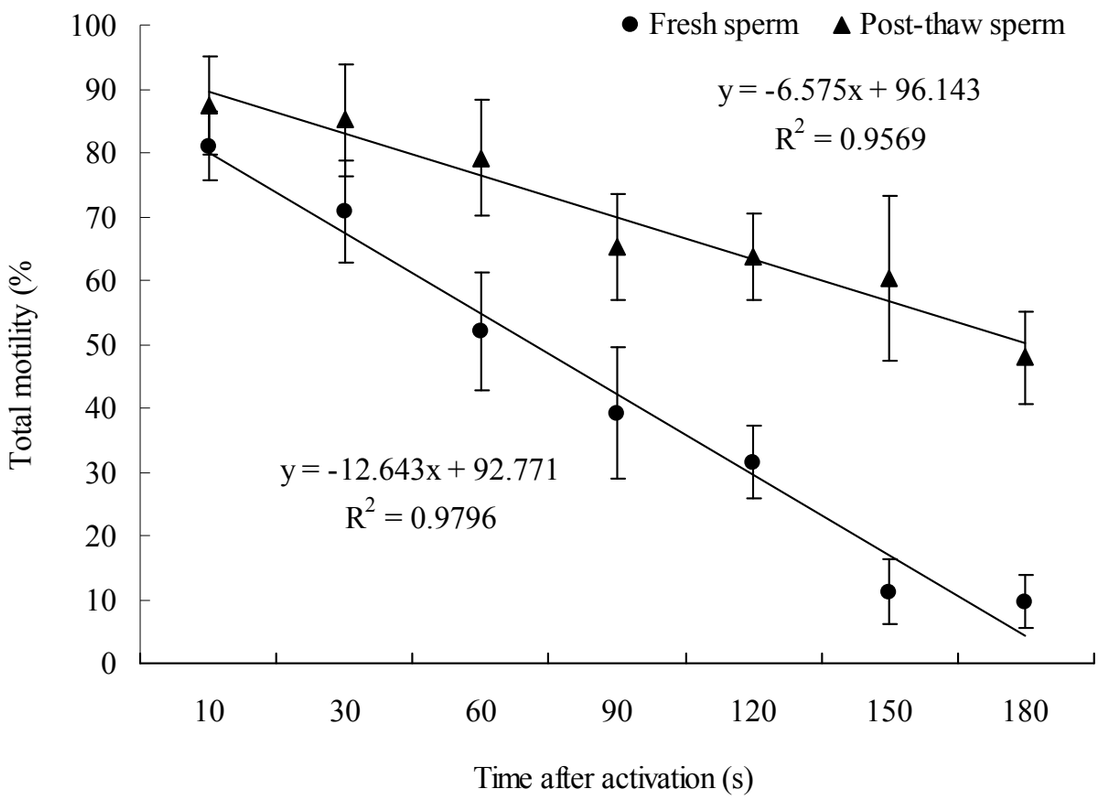


Fig. 1. The influence of time after activation on the motility of fresh and post-thaw sperm in P. major. Ten seconds after activation, the total motilities of fresh and post-thaw sperm frozen with 15% DMSO were observed every 30 s using computer-assisted sperm analysis system. This figure describes the evolution of the total motilities of fresh (▲) and post-thaw sperm (●) after activation respectively (n =5).

2.2.2 Post-thaw sperm fertilizing capacity and hatchability

Fertilization rates and hatching rates of fresh and post-thaw sperm were shown in Fig. 2. The fertilization rates and hatching rates were similar for fresh and post-thaw sperm frozen

with 12–21% DMSO. However, lower (P<0.05) fertilizing capacity of post-thaw sperm frozen with 6% DMSO, 9% DMSO and 24% DMSO were observed. In addition, the percentages of motile of post-thaw sperm and fertilization rates showed a high positive linear regression (r = 0.876). Similarly, the percentages of motile spermatozoa and hatching rates of post-thaw sperm showed a high positive linear regression (r = 0.878).

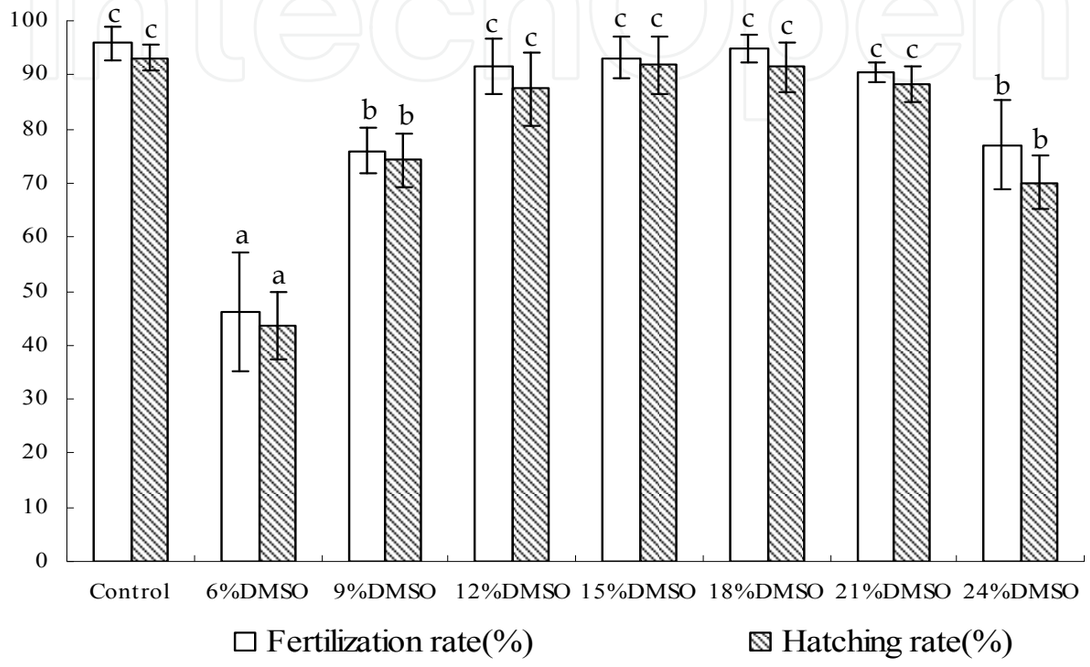


Fig. 2. Fertilization rates and hatching rates of fresh and post-thaw sperm in P. major. Cryopreserved sperm was thawed and activated for the artificial fertilization with sperm to egg ratio 500:1. This figure describes the fertilization rates and hatching rates of post-thaw sperm frozen with 6-24% DMSO. ☐ For fertilization rates of fresh and post-thaw sperm; ▨ For hatching rates of fresh and post-thaw sperm. Columns marked with the same letter are not significantly different (P>0.05, n=5).

2.2.3 Sperm ultrastructure

Ultrastructure of fresh and intact frozen-thawed red seabream sperm are shown in Fig. 1. These sperm had a head, midpiece, and tail. The head was ovoid and contained the nucleus and centriolar complex; the latter consisted of two centrioles. The midpiece was approximately cylindrical and contained mitochondria. The flagellum consisted of nine peripheral doublets and two central microtubules; the axoneme was a typical 9 + 2 structure (Fig. 3 A, B). The proportion of fresh sperm with normal morphology was 77.8 ± 5.6%, whereas after cryopreservation, 63.0 ± 7.2% of the sperm had normal morphology (Fig. 3 C), 20.7 ± 3.1% were partly damaged (e.g. swelling or rupture of head, midpiece and tail region, as shown in Fig. 3 D, as well as damage to mitochondria). Furthermore, 16.4 ± 4.2% were

severely damaged; the plasma membranes was completely ruptured and only nuclei, mitochondria, or some fragments of cellular organelles were found (Fig.3 E).

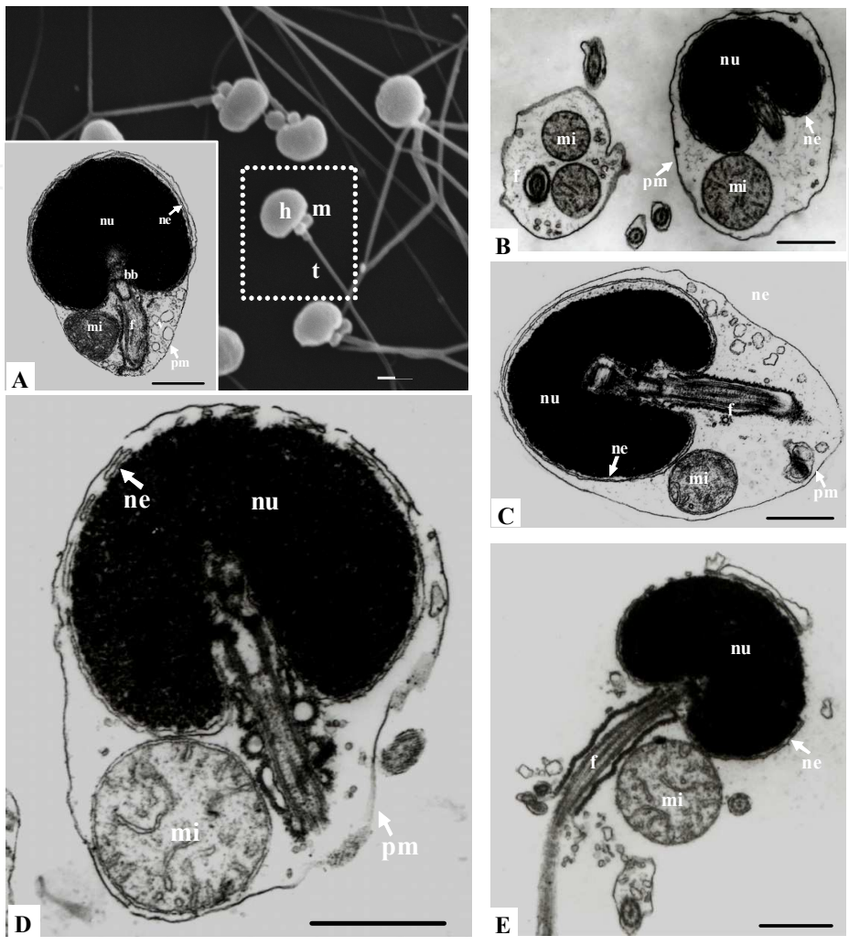


Fig. 3. The morphology and ultrastructure of fresh and normal post-thaw spermatozoa of red seabream. (A) Total view of fresh spermatozoa and the internal structure of head and mid-piece of fresh spermatozoa. (B) Flagellum of fresh spermatozoa. (C) Unchanged spermatozoa cryopreserved with 15% DMSO. (D) Partly damaged spermatozoa. (E) Completely damaged plasmalemma and nuclear envelop. (h, head; m , mid-piece; t, tail region. nu, nucleus; ne, nuclear envelope; bb, basal body; mi, mitochondrion; pm, plasmalemma; f, flagellum; v, vacuole). Scale bar = 0.5 μm.

2.2.4 Fluorescent staining and flow cytometry

Sperm populations were localized into four distinct regions according to their relative green and red fluorescence after staining with PI and Rh123 (Fig. 4). For fresh sperm, 83.9% had an intact membrane and functional mitochondria, 5.1% had nonfunctional mitochondria, 9.8% had nonfunctional mitochondria, and 1.2% had both a damaged membrane and nonfunctional mitochondria; whereas for frozen-thawed sperm, the percentages of sperm localized in four regions were 74.8%(Region 3), 12.7%(Region 4), 9.9% (Region 2), and 2.6%(Region 1), respectively.

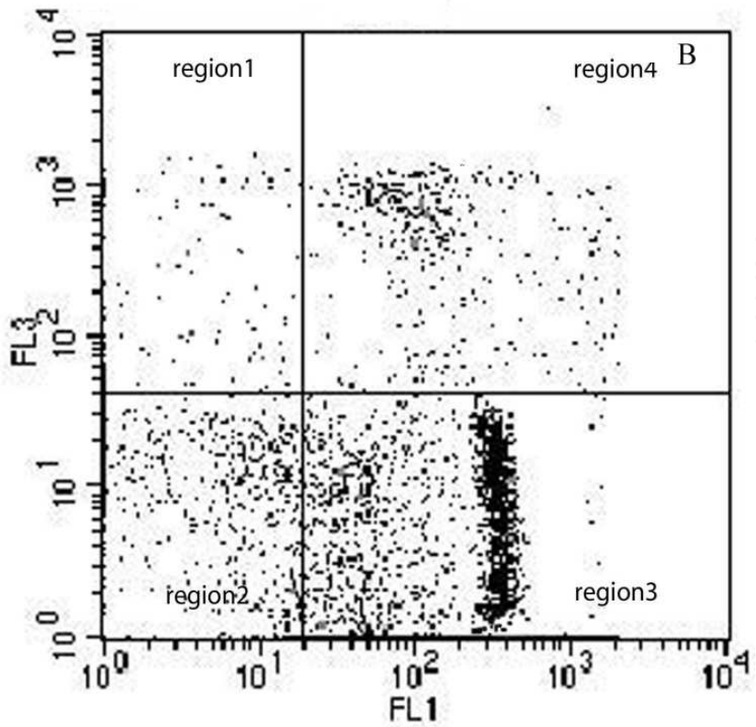


Fig. 4. Flow cytometric dot plots of spermatozoa of red seabream after cryopreservation. Region 1, sperm with a damaged plasma membrane but normal mitochondrial function. Region 2, sperm with an intact plasma membrane but lacking mitochondrial function. Region 3, sperm with an intact plasma membrane and functional mitochondria. Region 4, sperm with a damaged plasma membrane and functional mitochondria.

2.3 Discussion

Motility is an important characteristic for estimating the quality of fresh as well as cryopresrved sperm (Lahnsteiner et al., 1996a). In this study, the freezing-thawing process did not significantly change the main motility pattern and swimming velocity of motile sperm 10 s after activation, and the progressive linear motion was still the dominant pattern. Moreover, for the sperm cryopreserved with 12–21% DMSO, the freezing-thawing process also didn't significantly influence their motility and motility pattern, although it significantly reduced their motility period. However, different results were obtained from the sperm cryopreservation of turbot (Dréanno et al., 1997), which the percentage of motile post-thaw sperm was significantly lower than that obtained from fresh sperm while the velocity and the duration of motion were not significantly modified.

No significant difference in the fertilization rates and hatching rates were observed between sperm cryopreserved with 12–21% DMSO and fresh sperm. However, Lahnsteiner et al. (2003) reported that in cyprinids sperm, the post-thaw fertilization ratios obtained with sperm to egg ratios of $1.3–2.6\times10^6$:1 did not reach that of the fresh sperm. Similar results have also been reported in turbot (Chen et al., 2004; Suquet et al., 1998) and flounder (Zhang et al., 2003). These may be due to the species specific or un-ideal protocols used in sperm cryopreservation. In this study, for the post-thaw sperm a high positive correlation was

observed between the percentage of motile sperm and fertilizing capacity. This was consistent to the results that obtained from turbot (Dréanno et al., 1999), common carp (Linhart et al., 2000) and African Catfish (Rurangwa et al., 2001).

In the present study, the data from ultrastructural investigation and flow cytometric analysis demonstrates that more that 60% of post- sperm were normal in morphology and mitochondrial function. These results further confirmed the high performance of the protocols established for red seabream. In addition, the high fertilization capacity of post-thaw sperm implies that some of the slightly damaged spermatozoa can still fertilize eggs and develop into larvae. However, it remains to be determined whether the larvae from cryopreserved sperm develop into healthy adults.

During the process of cooling, freezing and thawing, spermatozoa are subjected to a series of damages (Oehninger et al., 2000). In ultrastructural investigation, we found 20.7 ± 3.1% were slightly damaged in some way and 16.4 ± 4.2% were severely damaged. One of the causes may be the ice crystal formation during the freezing process and some researchers agree that intracellular ice formation is the major injury mechanism at rapid cooling rates (Toner et al.,1993; Chao & Liao 2001). Other causes of cryodamages include pH fluctuation, cold shock, osmometric effect, and cryoprotectant toxicity (Chao & Liao 2001). The swelling and rupture of the plasmalemma after thawing may be due to the damage to the unit membrane which is very sensitive to freezing and thawing (Lahnsteiner et al., 1992). Similar morphological changes were reported in post-thaw sperm of ocean pout (Yao et al., 2000), rainbow trout (Lahnsteiner et al., 1996c), and atlantic croaker (Gwo et al., 1991). For example, in grayling sperm (Lahnsteiner et al., 1992), a marked decrease in sperm quality was observed, about 40% to 50% of the spermatozoa were completely damaged, 30% to 40% changed and only 10–20% showed an intact morphology. In this study, flow cytometric analysis, based on membrane integrity and mitochondrial function, was used to assess post-thaw sperm quality. After double staining with Rh123 and PI, we found 74.8% of post-thaw sperm showed membrane integrity and mitochondrial function. In rainbow trout (Ogier de Baulny et al., 1997), the plasma membrane and mitochondrial function were better protected with 10% DMSO.

Plasma membrane integrity and mitochondrial function are the two most important attributes for fertilizing an egg. The damage to membrane integrity and mitochondrial function could destabilize the sperm membrane and affect mitochondrial energy metabolism, thereby affecting spermatozoa viability. However, what interested us most is that although about 30% of spermatozoa were damaged in some way or even totally ruptured, the fertilization capacity of post-thaw sperm were not affected significantly in the standardized artificial fertilization experiment (Oehninger et al., 2000). Three hypotheses can be considered. The first hypotheses is that the sperm that survived freezing-thawing with normal morphology and mitochondrial function as shown in Fig. 3 and Fig. 4 region 3 should be similar to fresh sperm in fertilization capacity. The second is that the sperm cryopreserved with the established method could provide adequate numbers of motile spermatozoa with normal sperm parameters to fertilize the eggs in artificial fertilization experiment. The third is that the process of freezing-thawing may result in a population of partially damaged yet motile spermatozoa, which can fertilize eggs and develop into larva normally. Such a population usually exhibits a certain degree of plasma and mitochondrial membrane leakiness as shown in Fig. 3 and Fig. 4 region 2, 4.

3. Conclusion

In conclusion, the fertilizing capacity and egg hatchability were not significantly reduced by the post-thaw sperm treated with 12-21%DMSO, although the post-thaw sperm quality was influenced during the freezing and thaw process in motility, ultrastructure and mitochondrial function. The cryopreservation protocol used for red seabream sperm should be of great value for the establishment of sperm banks and assessment of ultrastructure and flow cytometry facilitated identification of damaged sperm; However, the exact nature of cryodamage to fish sperm are not yet fully understood. Sperm motility, structure integrity and mitochondrion function were damaged with different extent, although the fertilization capacity of cryopreserved sperm was not changed. There are many questions need to answer, how does the cryodamage reduce the sperm motility duration? If the cryodamages influence the gene expression and the embryo and larvae development? how to improve the post-thaw sperm quality by optimize the cryopreservation method?

4. Acknowledgment

This study was funded by the State 863 High-Technology R&D Project of China (2003AA603510 and 2004AA603310), the Knowledge Innovation Program of the Institute of Oceanology, Chinese Academy of Sciences (Y02507101Q), The National Natural Science Foundation of China (NSFC) (41076100 an31072212) and the earmarked fund for Modern Agro-industry Technology Research System (nycytx-50).

5. References

Babiak, I.; Glogowski, J.; Brzuska, E. & Adamek, J. (1997). Cryopreservation of sperm of common carp, Cyprinus carpio L. Aquaculture Research 28(7): 567–571.

Billard, R.; Cosson, J. & Crim, L. W. (1993). Motility of fresh and aged halibut sperm. Aquatic Living Resources 6(1):67–75.

Blaxter, J. H. S. (1953). Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature 172(4391):1189–1190.

Cabrita, E.; Robles, V.; Alvarez, R. & Herráez, M. P. (2001). Cryopreservation of rainbow trout sperm in large volume straws: application to large scale fertilization. Aquaculture 201(3-4):301–314.

Chao, N. H. & Liao, I,C. (2001). Cryopreservation of finfish and shellfish gametes and embryos. Aquaculture 197(1-4):161–189.

Chen, S. L.; Ji, X. S.; Yu, G. C.; Tian, Y. S. & Sha, Z. X. (2004). Cryopreservation of sperm from turbot (Scophthalmus maximus) and application to large-scale fertilization. Aquaculture 236(1-4):547–556.

Chen, S. L.; Ji, X. S.; Yu, G. C.; Tian, Y. S.& Sha, Z. X. (2004). Cryopreservation of sperm from turbot (Scophthalmus maximus) and application to large-scale fertilization. Aquaculture 236(1-4):547–56.

Christensen, J. M. & Tiersch, T. R. (1997). Cryopreservation of channel catfish spermatozoa: effect of cryoprotectant, straw size, and formulation of extender. Theriogenology 47(3):639–645.

Conget, P.; Ferna´ndez, M.; Herrera, G. & Minguell, J. J. (1996). Cryopreservation of rainbow trout (Oncorhynchus mykiss) spermatozoa using programmable freezing. Aquaculture 143(3-4):319–329.

Donoghue, A. M.; Garner, D. L.; Donoghue, D. J. & Johnson, L. A. (1995). Viability assessment of turkey sperm using fluorescent staining and flow cytometry. Poult Sci 74(7):1191–1200.

Dréanno, C.; Suquet, M.; Quemener, L.; Cosson, J.; Fierville, F.; Normant, Y. & Billard, R. (1997). Cryopreservation of turbot (Scophthalmus Maximus) spermatozoa. Theriogenology 48(4):589–603.

Dzuba, B. B.; Kopeika, E. F.（2002）Relationship between the changes in cellular volume of fish spermatozoa and their cryoresistance. Cryo Letters 23 (6):353-60.

Graham, J. K.; Kunze, E. & Hammerstedt, R. H. (1990). Analysis of sperm cell viability, acrosoma integrity and mitoxhondrial function using flow cytometry. Biol Reprod 43(1):55–64.

Gravance, C. G.; Garner, D. L.; Miller, M. G. & Berger, T. (2001). Fluorescent probes and flow cytometry to assess rat sperm integrity and mitochondrial function. Reprod Toxicol 15(1):5–10.

Gwo, J. C. (2000). Cryopreservation of sperm of some marine fishes. In: T. R. Tiersch & P. M. Mazik , (Ed.), Cryopreservation in aquatic species, World Aquaculture Society, Baton Rouge, Louisiana, USA, pp. 138–60.

Gwo, J. C. 2000. Cryopreservation of sperm of some marine fishes. in: T. R. Tiersch & P. M. Mazik, editors. Cryopreservation in aquatic species. World Aquaculture Society, Baton Rouge, Louisiana, USA, pp. 138–160

Gwo, J. C.; Strawn, K.; Longnecker, M. T. & Arnold, C. R. (1991). Cryopreservation of Atlantic croaker spermatozoa. Aquaculture 94(4):355–375.

He, S. & Woods, III. L. C. (2004). Changes in motility, ultrastructure, and fertilization capacity of striped bassMorone saxatilis spermatozoa following cryopreservation. Aquaculture 236(1-4):677–686.

Henderson-Arzapalo, A.; Colura, R. L. & Maciorowski, A. F. (1994). A comparison of black drum, red drum, and their hybrid in saltwater pond culture. Journal of the World Aquaculture Society 25(2):289–296.

Kime, D. E.; Ebrahimi, M.; Nysten, K.; Roelants, I.; Rurangwa, E.; Moore, H. D. M. & Ollevier, F. (1996). Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals. Aquatic Toxicology 36(1-4): 223–227.

Kopeika, E.; Kopeika, J.; Zhang, T.（2007）Cryopreservation of fish sperm. Methods Mol Biol.;368:203-17.

Kopeika, J., Kopeika, E.; Zhang, T.; Rawson, D. M. (2003a) Studies on the toxicity of dimethyl sulfoxide, ethylene glycol, methanol and glycerol to loach (Misgurnus fossilis) sperm and the effect on subsequent embryo development. Cryo Letters. 24(6):365-74.

Kopeika, J., Kopeika, E.; Zhang, T.; Rawson, D. M.; Holt, W. V.（2003b）Detrimental effects of cryopreservation of loach (Misgurnus fossilis) sperm on subsequent embryo development are reversed by incubating fertilised eggs in caffeine. Cryobiology46(1):43-52.

Kopeika, J.; Kopeika, E.; Zhang, T.; Rawson, D. M.; Holt, W. V. （2004） Effect of DNA repair inhibitor (3-aminobenzamide) on genetic stability of loach (Misgurnus fossilis) embryos derived from cryopreserved sperm. Theriogenology 61(9):1661-73.

Lahnsteiner, F.; Berger, B. & Weismann, T. (2003). Effects of media, fertilization technique, extender, straw volume, and sperm to egg ratio on hatchability of cyprinid embryos, using cryopreserved semen. Theriogenology 60(5):829–841.

Lahnsteiner, F.; Berger, B.; Horvath, A.; Urbanyi, B. & Weismann, T. (2000). Cryopreservation of spermatozoa in cyprindid fishes. Theriogenology 54(9):1477–1498.

Lahnsteiner, F.; Berger, B.; Weismann, T. & Patzner, R.A. (1996a). Physiological and biochemical determination of rainbow trout, Oncorhynchus mykiss semen quality for cryopreservation. J Appl Aquacult;6:47–73.

Lahnsteiner, F.; Berger, B.; Weismann, T. & Patzner, R.A. (1998). Determination of semen quality of the rainbow trout, Oncorhynchus mykiss, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 163(1-2):163–181.

Lahnsteiner, F.; Berger, B.; Wiesmann, T. & Patzner, R. A. (1996c). Changes in morphology, physiology, metabolism, and fertilization capacity of rainbow trout semen following cryopreservation. Prog Fish Cult 58(3):149–59.

Lahnsteiner, F.; Weismann, T. & Patzner, R. A. (1992). Fine structural changes in spermatozoa of the grayling, Thymallus thymallus (Pisces:-Teleostein), during routine cryopreservation. Aquaculture 103(1):73–84.

Lahnsteiner, F.; Weismann, T. & Patzner, R.A. (1996b). Cryopreservation of semen of the grayling (Thymallus thymallus) and the Danube salmon (Hucho hucho). Aquaculture 144(1-3):265–274.

Linhart, O.; Rodina, M. & Cosson, J. (2000). Cryopreservation of sperm in common carp Cyprinus carpio: sperm motility and hatching success of embryos. Cryobiology 41(3):241–250

Liu, Q. H.; Chen, Y. K.; Xiao, Z. Z.& Li, J. (2010a). Effect of storage time and cryoprotectant concentration on the quality of cryopreserved sperm in red seabream (Pagrus major). Aquaculture research. 41(9):e89-e95.

Liu, Q. H.; Chen, Y. K.; Xiao, Z. Z.; Li, J. & Xu, S. H. (2010b). Effect of long-term cryopreservation on physiological characteristics, antioxidant activities and lipid peroxidation of red seabream (Pagrus major) sperm. Cryobiology 61(2):189-193.

Liu, Q. H.; Li, J.; Xiao, Z. Z., Ding, F. H.; Yu, D. D. & Xu, X. Z. (2007b). Use of computer-assisted sperm analysis (CASA) to evaluate the quality of cryopreserved sperm in red seabream (Pagrus major). Aquaculture 263(1-4):20–25.

Liu, Q. H.; Li, J.; Zhang S. C.; Ding, F. H.; Xu, X. Z.; Xiao, Z. Z. & Xu, S. H. (2006). An Efficient Methodology for Cryopreservation of spermatozoa of Red Seabream, Pagrus major, with 2-mL Cryovials. Journal of the world aquaculture society 37(3):289-297.

Liu, Q. H.; Li, J.; Zhang, S. C.; Xiao, Z. Z.; Ding, F. H.; Yu, D. D. & Xu, X. Z. (2007a). Flow cytometry and ultrastructure of cryopreserved red seabream (Pagrus major) sperm. Theriogenology 67(6):1168–1174.

Oehninger, S.; Duru, N. K.; Srisombut, C. & Morshedi, M. (2000). Assessment of sperm cryodamage and strategies to improve outcome. Mol Cell Endocrinol 169(1-2):3–10.

Ogier de Baulny, B.; Labbe´, C. & Maisse G. (1999). Membrane integrity, mitochondrial function, ATP content, and motility of the European catfish (Silurus glanis) testicular spermatozoa after freezing with different cryoprotectants. Cryobiology 39(2):177–184.

Ogier de Baulny, B.; Le Vern, Y.; Kerboeuf, D. & Maisse, G. (1997). Flow cytometric evaluation of mitochondrial function and membrane integrity in fresh and cryopreserved rainbow trout (Oncorhynchus mykiss) spermatozoa. Cryobiology 34(2):141–149.

Ohta, H.; Kawamura, K.; Unuma, T. & Takegoshi, Y. (2001). Cryopreservation of the sperm of the Japanese bitterling. Journal of Fish Biology 58(3):670–681.

Richardson, G. F.; Wilson, C. E.; Crim, L.W. & Yao, Z. (1999). Cryopreservation of yellowtail flounder (Pleuronectes ferrugineus) semen in large straws. Aquaculture 174(1-2):89–94.

Rurangwa, E.; Volckaert, F. A. M.; Huyskens, G.; Kime, D. E. & Ollevier, F. (2001). Quality control of refrigerated and cryopreserved semen using computer-assisted sperm analysis (CASA), viable staining and standardized fertilisation in African catfish (Clarias gariepinus). Theriogenology 55(3):751–769.

Segovia, M.; Jenkins, J. A.; Paniagua-Chavez, C. & Tiersch, T. R. (2000). Flow cytometric evaluation of antibiotic effects on viability and mitochondrial function of refrigerated spermatozoa of Nile Tilapia. Theriogenology 53(7):1489–1499.

Suquet, M.; Dréanno, C.; Petton, B.; Norman, Y.; Omnes, M. H. & Billard, R. (1998). Long-term effect of the cryopreservaton of turbot (Psetta maxima) spermatozoa. Aquatic Living Resources 11(1):45–48.

Tiersch, T. R.; Figiel, C. R.; Jr.; Wayman, W. R.; Williamson, J. H.; Carmichael, G. J. & Gorman, O. T. (2000). Cryopreservation of sperm of the endangered razorback sucker. In: T. R. Tiersch & P. M. Mazik, (Ed.), Cryopreservation in aquatic species. World Aquaculture Society, Baton Rouge, Louisiana, USA, Pages 117—122

Tiersch, T. R.; Wayman, W. R.; Skapura, D. P.; Neidig, C. L. & Grier, H. J. (2004). Transport and cryopreservation of sperm of the common snook, Centropomus undecimalis (Bloch). Aquaculture Research 35(3):278–288.

Toner, M.; Cravalho, E. G. & Karel, M. (1993). Cellular response of mouse oocytes to freezing stress: prediction of intracellular ice formation. J Biomech Eng 115(2):169–174.

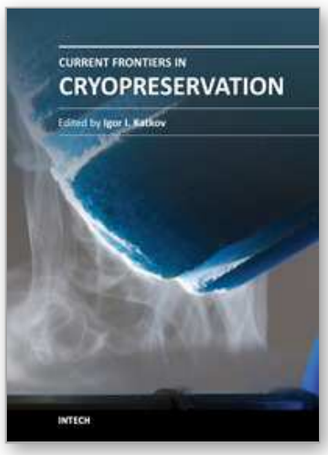
Van derWalt, L. D.; Van der Bank, F. H. & G. J. Steyn. (1993). The suitability of using cryopreservation of spermatozoa for the conservation of genetic diversity in African catfish (Clarias Gariepinus). Comparative Biochemistry Physiology 106(2):313–318.

Viveiros, A. T. M.; So, N. & Komen, J. (2000). Sperm cryopreservation of African catfish, Clarias garepinus: cryoprotectants, freezing rates and sperm:egg dilution ratio. Theriogenology 54(9):1395–1408.

Warnecke, D. & Pluta, H. J. (2003). Motility and fertility capacity of frozen/thawed common carp (Cyprinus carpio L.) sperm using dimethyl-acetamide as the main cryoprotectant. Aquaculture 215(1-4):167–85.

Yao, Z.; Crim, L. W.; Richardson, G. F. & Emerson, C. J. (2000). Motility, fertility and ultrastructural changes of ocean pout (Macrozoarces americanus L.) sperm after cryopreservation. Aquaculture 181(3-4):361–375.

Zhang, Y. Z.; Zhang, S. C.; Liu, X. Z.; Xu, Y. Y.; Wang, C. L.; Sawant, M. S.; Li, J. & Chen, S. L. (2003). Cryopreservation of flounder (Paralichthys olivaceus) sperm with a practical methodology. Theriogenology 60(5):989–996.



Current Frontiers in Cryopreservation

Edited by Prof. Igor Katkov

ISBN 978-953-51-0302-8
Hard cover, 462 pages
Publisher InTech
Published online 14, March, 2012
Published in print edition March, 2012

Almost a decade has passed since the last textbook on the science of cryobiology, Life in the Frozen State, was published. Recently, there have been some serious tectonic shifts in cryobiology which were perhaps not seen on the surface but will have a profound effect on both the future of cryobiology and the development of new cryopreservation methods. We feel that it is time to revise the previous paradigms and dogmas, discuss the conceptually new cryobiological ideas, and introduce the recently emerged practical protocols for cryopreservation. The present books, "Current Frontiers in Cryobiology" and "Current Frontiers in Cryopreservation" will serve the purpose. This is a global effort by scientists from 27 countries from all continents and we hope it will be interesting to a wide audience.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Qing Hua Liu, Zhi Zhong Xiao, Shi Hong Xu, Dao Yuan Ma, Yong Shuang Xiao and Jun Li (2012). Marine Fish Sperm Cryopreservation and Quality Evaluation in Sperm Structure and Function, Current Frontiers in Cryopreservation, Prof. Igor Katkov (Ed.), ISBN: 978-953-51-0302-8, InTech, Available from: http://www.intechopen.com/books/current-frontiers-in-cryopreservation/marine-fish-sperm-cryopreservation-and-cryodamage-evaluation-in-sperm-viability-structure-antioxidan

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen