Practical Procedure of Sperm Cryopreservation of the Bar-tailed Flathead Platyecephalus indicus

By Do Hyung Kim, Il Keun Kong, Sung Ju Rha, Ji Won Yun, Kyeong Ho Han and Kang Hee Kho

Department of Aquatic Biology, College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu 550-749, Korea
1Department of Animal Science, College of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea
2Fisheries Science Institute, Chonnam National University, Yeosu 550-749, Korea

ABSTRACT This study was conducted to investigate protocol standardization for cryopreservation spermatozoa of the bar-tailed flathead Platyecephalus indicus. The suitability of the cryoprotectants, dimethyl sulphoxide (DMSO), glycerol and methanol were tested against three freezing rates and three thawing temperatures. DMSO and glycerol gave significantly higher motile index and survival rates than methanol. Among the freezing rates, freezing at a height of 2 cm above LN2 surface for 10 min−1 gave higher motile index and survival rates. In terms of best thawing temperature, 20°C obtained the highest motility.

Key words: Platyecephalus indicus, bar-tailed flathead, spermatozoa, cryopreservation, cryoprotectants

INTRODUCTION

Cryopreservation is a process where biological samples, such as cells or tissues, are preserved by cooling to sub-zero temperatures. It is an effective method for long-term storage of sperm and used in breeding strategies of numerous animal species (Gañán et al., 2009; Anzar et al., 2010; Hu et al., 2010). Preservation of spermatozoa at low temperatures was initially reported by Polge et al. (1949), where in a brief article they stated that the addition of 20% glycerol to the freezing process achieved high survival rates for human and fowl sperm after thawing. Cryopreservation offers benefits in aquaculture and experimental studies related to protecting stocks from extinction due to sudden disease outbreaks, natural disasters, or anthropogenic factors by making top-quality gamete and larvae available year-round, and providing greater ease in conducting selective breeding for disease resistance, preserving desirable characteristics and establishing gene banks (Chang et al., 1997; Chang, 1998; Bart, 2000; Chao and Liao, 2001). Cryopreservation techniques of fish sperm is well established in many species, including puffer, sea bass, mandarin fish, cod, zebra-fish and carp (Mounib, 1978; Gwo et al., 1993; Babiak et al., 1995; Fauvel et al., 1998; Lihnart et al., 2000; Ding et al., 2009; Jinga et al., 2009), but only in a limited number of shellfish such as several commercially important species, abalone, mud crab, Mytilus galloprovincialis, oyster, marine shrimp (Jeyalecticum and Subramonian, 1989; Tsai and Chao, 1994; McFadden, 1995; Adams et al., 2004; Kawamoto et al., 2007; Vuthiphandchai et al., 2007; Matteo et al., 2009).

Bar-tailed is one of the most important commercial species in the Korean fisheries industry (Yoon, 2008). However, there remains a paucity of data on standardized procedure for cryopreservation of sperm. The present study attempted to identify an optimized method of sperm cryopreservation with various cryoprotectants at different freezing rates and thawing rates. Sperm motility and survival rates were assessed post-thawing in order to decide on the best procedure.

MATERIALS AND METHODS

Bar-tailed flatheads were captured from shallow areas near Yeosu city, Jeonnam province, Korea in July 2008
Table 1. Motility index in relation to percentage of sperm with rapid, vigorous and forward movement

<table>
<thead>
<tr>
<th>Motility index</th>
<th>5.0</th>
<th>4.5</th>
<th>4.0</th>
<th>3.5</th>
<th>3.0</th>
<th>2.5</th>
<th>2.0</th>
<th>1.5</th>
<th>1.0</th>
<th>0.5</th>
<th>0.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward sperm (%)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>65</td>
<td>50</td>
<td>30</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

and transported to the laboratory. Semen was collected by abdominal stripping and stored in polyethylene tubes on crushed ice until use.

1. Motility estimation

Percentage of sperm exhibiting rapid, vigorous and forward movement was evaluated under a microscope by diluting sperm samples in Artificial SeaWater (ASW; 423.00 mM NaCl, 9.00 mM KCl, 9.27 mM CaCl₂, 22.94 mM MgCl₂, 2.114 mM NaHCO₃, 10 mM HEPES-pH 7.8) at a ratio of 1:1000 (Table 1). Samples with high motility were kept on crushed ice until use for the experiments outlined below.

2. Cryopreservation of sperm

DMSO (dimethyl sulfoxide), glycerol or Methanol was added to 36% ASW to formulate the extenders at concentrations between 5 and 15% of total volume. Sperm was diluted 1:3 with the extenders. The diluted sperm was inserted into 0.5 mL plastic straw and frozen at different freezing temperatures according to the method of Bouysson and Chupin (1982). The straws were then placed in a polystyrene box at 2 mm, 4 mm and 6 mm above liquid nitrogen. The straws were thawed in a 30°C water bath for 2 min. Survival rate was also estimated by the eosin-nigrosin staining technique.

To determine the optimum thawing temperature, frozen tubes were thawed in a water bath at different temperatures of 10°C, 20°C or 30°C.

RESULTS AND DISCUSSION

On contact with ASW, sperm became immediately activated and reached maximum motility. Vigorous forward movement was constant for about 10 sec, after which sperm lost total motility.

1. Cryoprotectant trial

Motility and survival rate using different cryoprotectants are shown in Fig. 1. The highest post-thawed sperm motile index 4. and survival rate (70%) were obtained with glycerol and DMSO, respectively, while sperm motility with methanol was below motile index 1.5.

2. Freezing trial

Results of cryoprotectants trial indicated that 10% glycerol was most effective, hence it was selected for the freezing trials. The best result (motility: motile index 4; survival rate: 75%) was achieved when freezing depth (the 2 cm above the surface of liquid nitrogen) was used (Fig. 2).

3. Thawing trial

The survival rates and motile index of sperm cells thawed at different temperatures are shown in Fig. 3. Motility at 20°C was significantly higher than those of other treatments (p<0.05). Spermatozoa from teleost fish may be stored successfully in liquid form for short periods, or by cryopreservation for longer periods (Chang et al., 1997; Chang et al., 1999; Daly et al., 2008; Yasui et al., 2009; Cabrita et al.,...