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Short-term Storage and Cryopreservation of Trumpet shell, *Charonia lampas sauliae* sperm

Kyoung Ho Kang and Jae Min Kim
Division of Marine Technology, Chonnam National University, Yeosu 550-749, Korea

Introduction

In seed production artificial insemination requires a large quantity of good quality semen. Collection and storage of good quality semen for future use may improve the convenience of artificial insemination and reduces stress to male broodstock caused by repeated semen sampling, which reduces semen quality (Yao et al., 2000).

At present cryopreservation of sperm has been well established for many years in many fish species, such as salmonid, tilapia *Oreochromis mossambicus*, carp *Cyprinus carpio* and catfish *Silurus asotus* (Rana, 1995), but only in a limited number of shellfish, e.g., several commercially important species, hard clam *Meretrix lusoria*, oyster *Crassostrea gigas*, small abalone *Haliotis diversicolor* (Chao, 1996; Chao &Liao, 2001; Paniagua-Chavez & Tiersch, 2001). Since procedures for sperm cryopreservation are generally species specific, the present study investigated the effect of various diluents on sperm motility during cold storage, optimized methods of cryopreservation of *Chaonia lampas sualiae* sperm for the cryoprotectant and freezing rate, equilibration time, and dilution ratio, in terms of the motility and survival rate of post-thawed sperm.

Materials and methods

Two cryoprotectants, Me_2SO and glycerol, were added to 100% ASW to formulate the extenders at the concentrations between 5 and 20% by volume for freezing. The sperm was diluted 1:3 with the extenders. The diluted sperm was placed into 0.5-ml plastic straws, equilibrated for 8 min at room temperature, and then were cooled in a programmable freezer at various freezing procedures before transfer into liquid nitrogen: (a) 50℃ /min to -80℃ (b) 30℃/min to -80℃ (c) 10℃/min to -80℃. The straws were thawed in a 30℃ water bath for 15 sec. On thawing, the motility of post-thawing sperm was evaluated. The survival rate was also estimated by the eosin-nigrosin staining technique.

According to results of above trial, 15% Me_2SO and 10% glycerol were selected to perform the trial of equilibration time. Sperm diluted 1:3 with 15% Me_2SO and 10% glycerol, respectively was equilibrated 2, 8, and 15 min at room temperature, and then were cooled at freezing procedure (b): 30℃/min to -80℃. After thawing in the 30℃ water bath, the motility and survival rate of post-sperm were examined.

A range of dilution ratio from 1:1 to 1: 19 (sperm volume to cryoprotectant volume) was tested by using 15% Me_2SO as cryoprotectant, 8 min of equilibration, and freezing procedure (b): 30℃/min to -80℃.

Results

In the present study, attempts were made to preserve *Charonia lampas sauliae* sperm in at 4℃. Cryopreservation procedures were optimized for various cryoprotectants and freezing rates, equilibration times and dilution ratios. During short-term storage, the motility of undiluted sperm was extended for 6 days of cold storage. The survival rate of undiluted sperm was maintained at a high level accordingly. After cryopreservation, the highest motility and survival rate were obtained in 15% Me_2SO using a freezing rate of 30℃/min. The motility and survival rate of post-thawing sperm did not show significant difference after 8 and 15 min equilibration using 15% Me_2SO as cryoprotectant, which were significantly higher than

those of 2 min equilibration. Comparisons of motility and survival rate between treatments pooled by dilution ratio showed that the effect of 1:1 ratio (sperm volume to cryoprotectant volume) was best. There was no difference between 1:3 and 1:5, and other ratios were significantly worse.

Reference

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, 361-375

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