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Comparison of growth performance between cryopreserved and fresh sperm-originated fry of *Barbonymus gonionotus*

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Abstract

This experiment was conducted to compare the growth performance of silver barb fry produced from cryopreserved sperm with those produced from fresh sperm. Cryopreserved sperm used for fry production was preserved with three extenders, Alsever's solution, urea egg-yolk, egg-yolk citrate solution and one cryoprotectant, DMSO. Cryodiluents were prepared by mixing the cryoprotectants at 10% concentration of the extender (% v/v). Fry produced with fresh sperm was considered as control. For comparing the growth, 60 fry of 15 day-old for each treatment of both cryopreserved and control groups were stocked to glass aquarium (50 cm x 30 cm x 28 cm) and reared them for ten weeks. Growth of fry in terms of length and weight increment in both cryopreserved and control groups were measured weekly. The growth pattern was more or less similar for all the treatments and there was no significant difference (P>0.05) between them. The survival rate of fry produced from cryopreserved sperm was 82.3% and that from fresh sperm 88%, and there was no significant difference (P>0.05) between two groups. It is therefore, concluded that the use of cryopreserved sperm does not impair survival and growth of fry.

Keywords: Silver barb, Cryopreservation, Growth, Survival

Introduction

Bangladesh is enriched with endemic fish resources including 260 freshwater fishes and 24 prawn species; 475 marine fishes and 36 shrimp species (DoF, 2005). Fish provides 63% of total animal protein to the people of Bangladesh. The fish production has been increased in last two decades through aquaculture but it is still unable to cope with the increasing demand of 150 million people. In order to further enhance the total fish production, 15 exotic species including silver barb have been introduced and cultured in Bangladesh (Ali, 1998).

Silver barb (*Barbonymus gonionotus*), locally known as Thai sarputi was introduced in Bangladesh from Thailand in 1977. It is a fast growing freshwater fish species and its seed production technology has been developed (Hussain *et al.,* 1987). It has better growth and production over the local sarputi. This species attains sexual maturity within a year. It may attain a weight of 150-200g within 6 months and becomes ready for harvesting (Siddiqui and Chowdhury, 1996). However, the production of fish has been declined over the years due to poor quality of seed. As an exotic fish, the stock of pure silver barb is limited and even its purity is becoming vulnerable day by day due to unwanted interferences such as inbreeding, negative selection and hybridization with local sarputi. Therefore, it is important to conserve and improve the original pure stocks, and cryopreservation of gametes could be considered as an important tool in this regard.

Protocols for cryopreservation of sperm of silver barb have been developed in Bangladesh. Production of fry using cryopreserved sperm has become successful and this production technique could be applied to commercial hatcheries. However, before recommending the cryopreservation techniques to be used in hatcheries, it is important to check the survival and growth rate of fry produced by cryopreserved sperm. Therefore, the purpose of this work was to compare the survival and growth rate of fry derived from cryopreserved sperm with that those from fresh sperm during their first three months of life.

Materials and Methods

The brood fish were collected from different sources and reared in the ponds at the vicinity of the Fisheries Faculty premises. The cryopreservation work was conducted in the Genetics Laboratory of the Faculty of Fisheries. Breeding trials were carried out in the Mini Hatchery and Wet Laboratory of the Department of Fisheries Biology and Genetics of the Faculty.

Sperm preservation

Selection of mature male fish and induction for milt collection: Mature males were selected on the basis of their desired phenotypic characteristics. Before selecting male fish, a gentle pressure was applied on abdomen to remove some milt which indicated the mature one. Selected males were brought to the cistern from the pond for conditioning before 6-8 h of hormone treatment. The brood fish were induced using PG extract after conditioning for at least 6-8 h. with previously prepared. The dose administered to the male fish was 2.0 mg/kg body wt. The PG solution was injected intramuscularly behind the pectoral fin. The spawners were handled very carefully during the whole operation.

Milt collection and sperm quality evaluation: Induced males were placed on a piece of soft foam and the genital area was cleaned with absorbent paper to remove excess moisture, urine, gut extrudes and mucus. Gentle pressure was applied on abdomen to remove some milt to avoid any contamination with urine. When the milt looked concentrated and creamy white, 5 ml glass tubes were held against the tip of the genital papilla to collect the milt. Watery or bloody milt was discarded. The collected milt was stored on ice. The quality of sperm in each tube was checked under the microscope at X10 and X40 magnifications taking about 1-2 µl of milt on a glass slide. Samples containing >80% motile sperm were used for cryopreservation.

Preparation of milt for cooling: Collected milt samples were diluted with cryodiluents at different ratios depending on the type of extender. In this experiment, three extenders Alsever's solution, urea egg-yolk, egg-yolk citrate solution and one cryoprotectant, DMSO were used. Cryodiluents were prepared by adding 10% cryoprotectant to 90% extender (% v/v). The milt sample was diluted with the cryodiluents at the ratio of 1:9 for Alsever's soultion and 1:4 for urea egg-yolk and egg-yolk citrate. The diluted milt was taken into the plastic straws with the help of micropipette carefully without allowing any air bubble inside the straw and the free end of the straws was heat-sealed. The straws were placed into the cryochamber and cooled, in computer-controlled freezer (CL-3300) following the two-steps freezing protocol, where samples were cooled to -4°C at a decreasing rate of 4°C per min, and then to -80°C at a decreasing rate of 10°C per min. Samples were removed from the cryochamber and loaded into the canisters and placed into liquid nitrogen (-196°C) for long-term preservation.

Production of fry

Collection, fertilization and hatching of eggs: The matured females were induced using PG extract at the dose of 5-6 mg/kg body wt. Eggs were collected from female by stripping into a plastic bowl immediately after ovulation. Stripped eggs were divided into four batches where each batch contained approximately 1200 eggs. Three batches of eggs were fertilized with cryopreserved sperm (sperm preserved with three different cryodiluents) and one batch was fertilized with fresh sperm as control. The frozen straws, after thawing at room temperature for about 30-60 sec, were cut at both ends. Each batch of eggs was fertilized with thawed sperm from 15 straws where each straw contained 230 µl of diluted milt.

After insemination, 5-10 ml of 0.9% NaCl solution was added to the egg mass-milt and mixed with feather for about 1 min where 0.9% NaCl solution was used as activating solution. Then the fertilized eggs were washed carefully 3 to 4 times with tap water and transferred into marked incubation jars. A continuous movement of the eggs was maintained in the incubation jar through flushing supply water. After 6 h of fertilization, some eggs were collected from all jars (eggs fertilized with cryopreserved and fresh sperm) and observed the progress of cell division under microscope. After 18-24 h of fertilization when the eggs were hatched, hatchlings were counted and transferred to the plastic bowls and reared.

Growth and survival rate

After absorption of yolk sac when the hatchlings started feeding, hard boiled egg-yolk was supplied. Planktons collected from ponds were also supplied to the fry. For comparing the growth between the fry produced with cryopreserved and fresh sperm, 60 fry of 15 day-old for each treatment were stocked to each glass aquarium (50 cm x 30 cm x 28 cm) and reared them for ten weeks. Each of the treatments had two replications. The fry were fed twice a day with a supplementary feed comprising 50% fish meal and 50% rice bran. In addition, planktons were provided to the aquarium regularly. The length and weight of fry were measured at seven days intervals throughout the experiment. The aquaria were cleaned each morning and evening before providing feed. Additional oxygen was provided to the aquarium water through aerators. Mortality of fry was also recorded daily.

Statistical analyses: Data were analyzed by using computer software package (MSTAT) and one-way ANOVA followed by Duncan multiple range test (DMRT).

Results and Discussion

The survival rate of fry of silver barb produced with cryopreserved and fresh sperm was calculated at each week of the rearing period. At the end of the experiment, i.e. at 10th week of the rearing, the survival rate of fry produced from cryopreserved sperm was 82.3% and that from fresh sperm 88% (Fig. 1). There was no significant difference (P>0.05) on survival between two groups. Similar result was reported by Chereguini *et al.* (2002) who found no decrease in survival rates with 1-year old young turbot (*Scophthalmus maximus* L.) produced from thawed sperm.

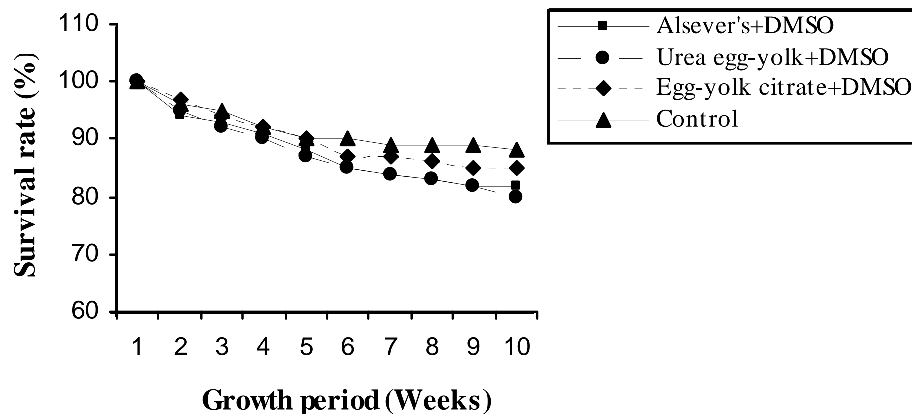

Fig.1. Survival rate (%) of *B. gonionotus* fry derived from artificial fertilization with fresh and cryopreserved sperm

The mean values of length and weight of the two groups of fry measured weekly are shown in Figs. 2 and 3. The statistical analyses showed no significant differences (P>0.05) either in length or in weight among the fry produced with cryopreserved and fresh sperm. Conversely, when compared the growth of fry produced from sperm preserved with different cryodiluents (sperm preserved with three different cryodiluents, Alsever's + DMSO, urea egg-yolk + DMSO, egg-yolk citrate + DMSO), no significant differences (P>.05) either in length or weight were found among them. Chao *et al.* (1987) reported that tilapia *Oreochromis hornorum,* juveniles produced from thawed sperm showed similar growth with the control group, reaching 800g after 18 months. Similarly, Tiersch *et al.* (1994) found no growth difference of channel catfish, *Ictalurus punctatus* fry produced with cryopreserved sperm or untreated sperm at any age (2-12). Sarder *et al.* (2007) reported no significant growth difference between the fry of common carp (*C. carpio*) produced from cryopreserved and fresh sperm in 11 weeks rearing period. In contrast, significant differences were obtained between length and weight of African catfish *Clarias gariepinus*, fry produced from cryopreserved and fresh sperm and these differences might be attributed to variable environment in tanks (Van der *et al.*, 1998).

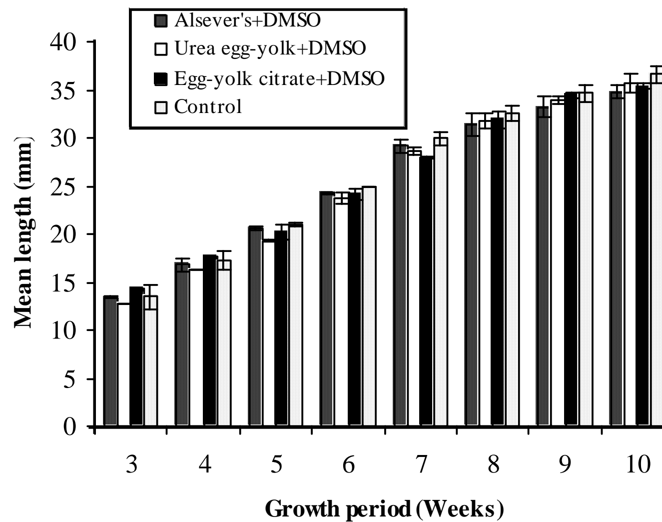

Fig. 2. Length (mm) of *B. gonionotus* fry derived from artificial fertilization with fresh and cryopreserved sperm.

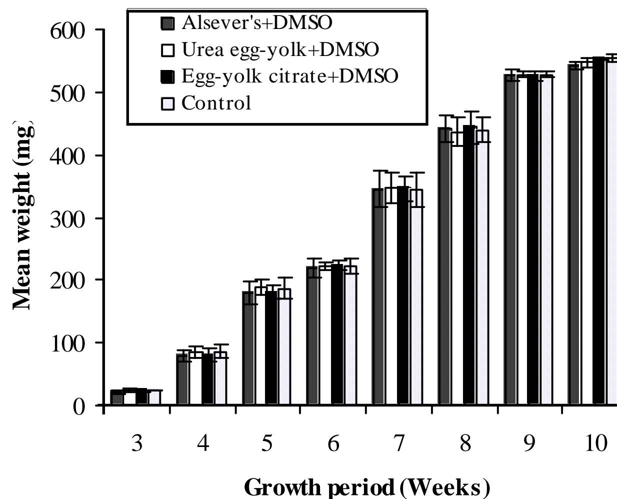

Fig. 3. Weight (mg) of *B. gonionotus* fry derived from artificial fertilization with fresh and cryopreserved sperm.

In the present study, the survival, growth and quality of silver barb fry produced with cryopreserved sperm were similar to those produced from fresh sperm. There was no negative impact of cryopreservation on survival and growth of fry. Therefore, it is expected that this finding will open a new avenue to apply the cryopreservation techniques as a useful tool for production and raising of silver barb for commercial purposes.

Acknowledgement

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