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Cryopreservation of sperm of common carp, *Cyprinus carpio* and silver barb, *Barbonymus gonionotus* for genetically improved seed production

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Abstract
Experiments were conducted to develop and standardize the protocols for cryopreservation of sperm of common carp, *Cyprinus carpio* and also for using the cryopreserved sperm for fertilization of eggs. Nine extender solutions as Alsever's solution, kurokura-1, kurokura-2, urea egg-yolk, egg-yolk citrate, 0.6% glucose, 0.9% NaCl, M^a and M^b , and five cryoprotectants namely ethanol, methanol, dimethylsulfoxide (DMSO), dimethylamine (DMA) and glycerol were tested. The cryoprotectants were mixed at 10% concentration of the extenders (v/v) to make the cryodiluents. Milt and cryodiluents were mixed at a ratio of 1:9 for Alsever's solution, kurokura-1, kurokura-2, 0.6% glucose and 0.9% NaCl, 1:4 for urea egg-yolk, egg-yolk citrate, M^a and M^b. Among the cryodiluents Alsever's solution mixed with either ethanol or methanol was found to be suitable and it produced more than 90% and 80% spermatozoan motility at equilibrium and post-thaw periods, respectively. Kurokura-1 and kurokura-2 when mixed with the same cryoprotectants showed good spermatozoan motility at equilibrium period (80-90%) but the motility was reduced (30-55%) at post-thaw state. Other extenders did not produce acceptable sperm-motility and in some cases the frozen milt became clotted. Different dilution ratios (1:1, 1:2, 1:4, 1:5, 1:7, 1:9, 1:12, 1:15, 1:20) were formulated for obtaining a suitable milt dilution, the dilution ratio of 1: 9 (milt : cryodiluent) demonstrated the highest post-thaw spermatozoan motility (80%) in Alserver's solution. The optimum concentration of cryoprotectants in the cryodiluents was determined, 10% concentration level was found to be effective to produce the highest number of spermatozoan motility in comparison to the other concentrations (5%, 15%, 20% 30%). Sperm preserved with the cryodiluent Alsever's solution along with either methanol or ethanol was found to be effective to fertilize eggs and produce hatchlings. The hatching rates ranged between 1.48% and 14.76%, compare to control. The fish produced through use of cryopreserved sperm and normal sperm were found to grow well and no significant ($P<0.05$) growth difference was observed between them. In case of silver barb, *Barbonymus gonionotus*, sperm tested against six extenders such as egg-yolk citrate, urea-egg-yolk, kurokura-1, kurokura-2, 0.9% NaCl and modified fish ringer (MFR) solution. Cryoprotectants used were the same as those of *C. carpio*. Milt was diluted with the cryodiluent at a ratio of 1:4 for egg-yolk citrate and urea-egg-yolk, 1:5 for kurokura-1 and 1:9 for 0.9% NaCl, MFR and kurokura-2. The cryoprotectant

concentration was maintained at 10% of the extender (v/v) in all the cases. Among the extenders, egg-yolk citrate and urea-egg-yolk mixed with 10% DMSO, methanol and ethanol produced 50% post-thaw spermatozoan motility, whereas DMA and glycerol provided only 10% motility. Trials on milt dilution ratio and cryoprotectant concentration are being conducted. Fertilization trials are also underway.

Key words: Cryopreservation, Sperm banking, Common carp, Silver barb

Research findings

- Preliminary optimization of the protocols for cryopreservation of sperms of common carp and silver barb was done.
- Alsever's solution mixed with either 10% methanol or ethanol was found to be the best for common carp sperm preservation and produced 80% post-thaw active spermatozoa. However, the presence of 10% DMA and DMSO in Alsever's solution produced only 40% and 25% spermatozoan motility, respectively. The milt samples having glycerol became clotted in most cases.
- The fertilization of eggs with the cryopreserved sperm was successful and the hatching rate of 1.48% to 14.76% was obtained.
- The fish produced by cryopreserved sperm performed well and there was no significant deviation from the growth rate that was obtain4ed from the fish produced by using normal sperm.
- Egg-yolk citrate and urea-egg-yolk mixed with 10% DMSO, methanol and ethanol exhibited 50% post-thaw motility of silver barb spermatozoa, whereas DMA and glycerol provided only 10% motility (Fig. 1).

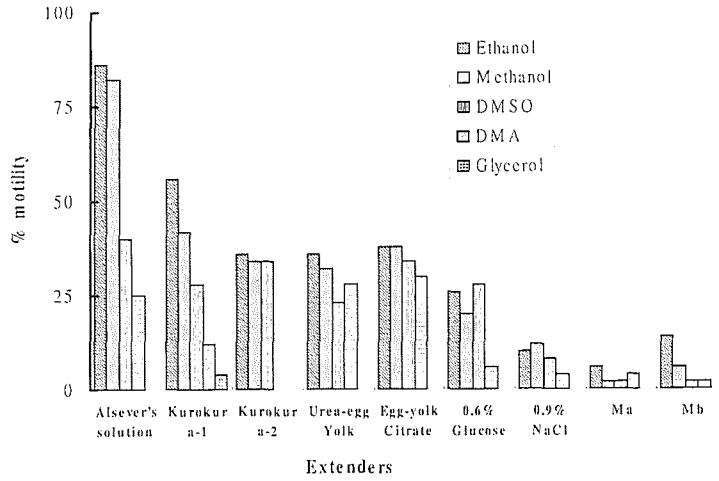

Fig. 1. Post-thaw motility of sperm preserved with different combinations of extenders and cryoprotectants.

Policy implications

- The cryopreservation techniques should be optimized and adopted by the government as well as by the private entrepreneurs. Department of Fisheries (DoF) can take a pioneer role to disseminate the optimized techniques.
- Unwanted inter-specific hybridization mostly resulted from asynchronous breeding times or shortage of breeding partners and inbreeding can be effectively reduced through cryopreservation technique.
- As quality seed production entirely depends on good genetic materials (sperm and ovum), cryopreservation technique can potentially help to preserve and supply of quality sperms from outbred and genetically improved brood stock.
- To maintain a sustainable supply of quality sperm, regional cryogenic gene banks need to be established. Government and private entrepreneurs can take initiative to set up such gene banks at convenient places in the country.

Livelihood implications

The unexpected growth performance of fish mostly resulted from negative selection, inbreeding and inter-specific hybridization in the hatcheries have become a serious constraint to quality seed production in Bangladesh. Cryopreservation of sperm can potentially resolve the problem as it will facilitate to use good sperm and its timely supply to the users. Establishment of cryogenic gene bank and practice of cryopreservation techniques in the selected hatcheries will obviously improve the livelihood of the farmers and the hatchery operators in a number of ways. Fish farmers can get their desired fish seeds produced by cryopreserved sperm in the hatcheries. The hatchery operators, on the other hand will be economically benefited by producing more female brood stocks instead of investing their valuable resources and time for male brood stock production.