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Cryopreservation of Pike Perch Sperm in Hatchery Conditions

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Abstract

Experiments were carried out on pike perch (*Sander lucioperca* L.) to find a method for stripping sperm without contamination by urine, to determine sperm concentration, and to fertilize large batches of eggs with cryopreserved sperm. Induced spawning technology for pike perch, an important predator fish species in Hungarian and central-eastern European pond aquaculture, has been developed only recently. Sperm cryopreservation can be an important tool for several reasons including the simplification of hatchery work and long-term preservation of genomes of males with high genetic value. In the present study, sperm was stripped without urine contamination, improving sperm quality, and large amounts of eggs were fertilized using 0.25 ml sperm, resulting in hatching percentages of 55±3% for 30-g batches of eggs and 87% for a 50-g batch.

Introduction

Pike perch (*Sander lucioperca* L) is one of the highest priced farmed predator fish species in Hungary. Several studies have been published on induced spawning in this species (Schlumberger and Proteau, 1996; Zakes, 2007). Hatchery propagation results in more efficient fry production than traditional pond spawning where broodfish spawn in nests.

Cryopreservation of sperm ensures long-term conservation of male gametes. It also enables reducing the number of males in the broodstock so that farmers can concentrate on the more problematic females. So far, the few studies published on cryopreservation of percid sperm involve the North American yellow perch (*Perca flavescens*; Ciereszko et al., 1993), the walleye (*S. vitreus*; Moore, 1987), and the European species of perch (*Perca fluviatilis*; Horvath and Urbanyi, 2001) and zingel (*Zingel zingel*; Keresztessy et al., 2003). The results of these studies, however, are not sufficient for developing a general method that can be applied to pike perch sperm.

In our earlier study, the effects of extenders, cryoprotectants, and sperm dilution ratios were tested on sperm of pike perch and Volga pike perch (*S. volgensis*), a closely related species (Bokor et al., 2007). Motility of

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fresh sperm in these experiments was 45±30% and the highest hatch rate (41±22%) was observed using a glucose-based extender and 10% methanol at a dilution ratio of 1:1 as the cryoprotectant. However, contamination of sperm with urine affected the post-thaw motility and fertilizing capacity of the cryopreserved sperm by its activation of the spermatozoa.

The objective of the present study was to apply sperm cryopreservation technology in hatchery conditions and to experiment with fertilizing large amounts of eggs with the frozen sperm.

Materials and Methods

The study was conducted at the hatchery of a commercial fish farm (Attalai Hal, Kft, Attala, Hungary) in April 2007. All gametes used in the experiment originated from the broodstock of this enterprise.

Males and females were treated according to hatchery protocols for routine commercial spawning. Individuals of both sexes were given 4 mg carp pituitary per kg body weight approximately 72 hours prior to the planned stripping. The hormone was administered intraperitoneally in a single dose. Before stripping, males were anesthetized in a solution of clove oil (*Syzygium aromaticum* L., 1 aliquot added to 1 l water). Fish were removed from the anesthetic solution, laid on a sponge mattress, and the genital area was wiped dry. Urine-free stripping of sperm was attempted using several methods. The most effective was the introduction of an elastic silicone catheter (internal diameter 1 mm, outside diameter 1.5 mm) into the sperm duct of the fish. Sperm was stripped through this catheter.

Motility of fresh sperm was estimated by diluting 1 µl of sperm in 39 µl of hatchery water on a microscope slide and viewing it under a light microscope (Zeiss Laboval, Carl Zeiss, Jena, GDR) at 200x magnification. Sperm concentration was calculated at 1000x dilution (in the extender described below) using a Burker-type hemocytometer at 200x magnification.

Sperm from three males was used in the fertilization experiment. Sperm was diluted in a 1:1 ratio with an extender composed of 350 mM glucose and 30 mM Tris. The pH of the extender was set at 8.0 using concentrated HCl. Methanol was used as the cryoprotectant at a final concentration of 10% v/v. Diluted sperm was loaded into 0.5-ml straws and frozen in liquid nitrogen vapor in a styrofoam box. The straws were placed on a 3-cm high styrofoam frame floating on the surface of the liquid nitrogen. Freezing time was 3 min, after which the straws were plunged directly into liquid nitrogen. The straws were stored in canister storage dewars (BIO 20, Statebourne Cryogenics, Washington Tyne & Wear, UK) until use one week later. Straws were thawed in a 40°C water bath for 13 s. Motility after thawing was estimated as described above.

Females were checked on an hourly basis before the planned stripping. When eggs appeared in the genital orifice after gentle abdominal pressure the individual was assumed to be ready for stripping. Females were removed from their tanks and anesthetized as described for males. Eggs were stripped into a dry plastic bowl by gentle abdominal pressure. Eggs were distributed into three batches each of 10 g and 30 g and a single batch of 50 g for fertilization. One straw of thawed sperm was added to each of the seven batches and gametes were activated by approximately 5 ml of hatchery water. Eggs fertilized with freshly stripped sperm were used as a control. Eggs were allowed to harden in a Woynarich salt-urea solution (0.4% NaCl, 0.3% urea) for 90 min which also reduced stickiness of the eggs. A tannic acid solution (0.5%) was used for final elimination of stickiness and hardening of the egg envelope. Eggs were washed in this solution twice for 20 s, then incubated in 7-l Zug-type hatching jars. The percent hatched was calculated.

Motility of fresh and thawed sperm and hatch rates for 10-g and 30-g batches of eggs were compared using a two-sample *t* test at $p \leq 0.05$.

Results

The motility of the fresh pike perch sperm was 63±10%. Sperm concentration was 1.8571± 0.1538 x 10^{10} per ml and the number of eggs

per gram was 1367±54. Since each batch was fertilized with one 0.5-ml straw of diluted sperm (1:1), the sperm:egg ratio was 3.396 x 10^5 spermatozoa per egg in 10-g batches of eggs, 1.132 x 10^5 spermatozoa per egg in 30-g batches of eggs, and 6.792 x 10^4 sperm cells per egg in the 50-g batch. Post-thaw motility of cryopreserved sperm was 53±5% and no significant difference was detected between motility percentages before and after freezing (p = 0.1135).

The ratio of hatched larvae was 47±4% for the 10-g batches and 55±3% for the 30-g batches. The difference between the two hatch percentages was not quite significant although the result of the t test (p = 0.05701) was very close to the significance level. Surprisingly, the single 50-g batch of eggs yielded a hatch percentage of 87%. The percentage for the control was 61±45%.

Discussion

Motility of fresh as well as cryopreserved sperm in the present study was higher than that (45±30%) reported earlier by our group (Bokor et al., 2007). Also, there was much less variation in sperm motility percentages of different individuals compared to earlier results. This can, in part, be attributed to successful stripping of the sperm without contamination with urine. The lack of a significant difference between motility percentages of fresh and cryopreserved sperm supports this assumption.

Compared to our earlier report, improvement was also observed in the hatch rate. Our present results also show that higher volumes of eggs can be used for fertilization with a single straw of thawed sperm.

Hatch percentages increased as the amount of eggs fertilized by one straw of sperm increased. We observed that egg batches of different volumes displayed different behaviors in the hatching jars. Batches of 10 g slightly stuck together in a single clutch, while batches of 30 g were arranged in several smaller clutches, and eggs in the single batch of 50 g freely rolled on each other. Although only one batch of 50 g was fertilized due to a shortage of eggs, results suggest that the use of higher egg volumes for fertilization results in better hatch results. So far, there have been few studies on the effect of egg volume on incubation success. Egg volume was weakly but positively correlated with the hatch length of fry in medaka (*Oryzias latipes*), representing a developmental advantage (Teather et al., 2000). In our study, the results can be partly attributed to the toxicity of the cryoprotectant since the lower egg volumes were exposed to relatively higher methanol concentrations than the higher. The lower sperm to egg ratio in the larger egg batches did not seem to affect results, suggesting that sperm was used in excess in all cases and the fertilization-limiting sperm:egg ratio was not reached. Also, clumped eggs in the 10-g batches were more sensitive to the possible lack of oxygen inside the clumps than the more separated eggs in the larger batches.

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