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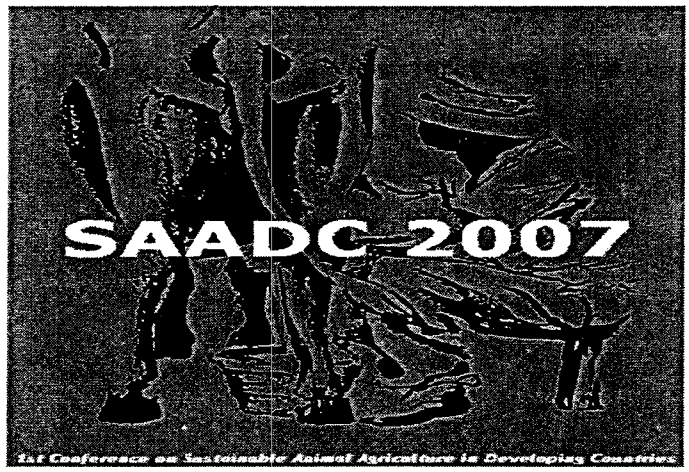

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Effects of Equilibration Times on the Fertilization Rate of Cryopreserved Striped Catfish, *Pangasius hypophthalmus* (Sauvage, 1878) Sperm

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Summary

This is the first study to report the effects of equilibration times on the cryopreservation of striped catfish, *Pangasius hypophthalmus* sperm. Four equilibration times (5, 10, 20 and 40 min) with three treatments (10%DMSO+0.9%NaCl, 10%DMA+C-FHBSS and 5% MeOH+0.9%NaCl), at one-step freezing rate (10 °C min^{-1}) were investigated. Sperm were stored for 3 weeks in a liquid nitrogen container. They were airthawed at room temperature, and fertilization rate with different equilibration times were assessed. The highest fertilization rate of 60% was achieved with the highest time of exposure (40 min) when tested with DMSO treatment. Fertilization rates among the four equilibration times of cryopreserved sperm were significantly lower than the controls ($p<0.05$), except when tested with DMSO treatment and MeOH treatment, at 5 min, fertilization rates were not significant different with the control. Based on the results of this study, the equilibration times at 5, 10, 20 or 40 min could be used to cryopreserve of *Pangasius hypophthalmus* when using DMSO, DMA or MeOH treatment.

Keywords: equilibration time, cryopreservation, sperm, striped catfish, Pangasius hypophthalmus

Introduction

Post-thaw fertilization rate of frozen-thawed fish spermatozoa depends on the type and concentration of the cryoprotectants and the time of exposure before freezing. Most cryoprotectants act on the lipids of cell membranes during the freezing process to protect cell damaged by affecting the size and the shape of ice crystal, which form during the freezing and thawing process (Rana, 1995). Horvath & Urbanyi (2000) reported that increasing in concentration and equilibration time reduced post-thaw motility rate to zero, when tested with glycerol. Poor motility rate may be the result of the type of cryoprotectant or the time of exposure before freezing. This was similar to Linhart et al. (2005) who reported that the cause of low hatching rate of European catfish, *Silurus glanis* may be due to the higher of exposure time. However, they found that equilibration time had no effect on velocity of spermatozoa or on percentage of post-thawed sperm motility. In addition, Babiak et al. (2001) found that the addition of egg yolk to extender did not affect the fertilization ability of frozen sperm and 10-min equilibration of diluted sperm before freezing significantly decreased the fertilization rate of rainbow trout, *Oncorhynchus mykiss* sperm. Most studies were concerned with the success of the type and concentration of the cryoprotectants and not the time of exposure before freezing. The present study was proposed to examine the effect of equilibration times (5, 10, 20, and 40 min) on cryopreservation of *Pangasius hypophthalmus* sperm using the treatments have shown promising result fertilization rates to cryopreserve sperm of *P. hypophthalmus* (Kwantong & Bart, 2003).

Materials and Methods

Experimental broodfish

Mature *P. hypophthalmus* (70 males and 50 females) with a mean weight of 3.3 Kg $fish^{-1}$ were held in an earthen pond at the Suranaree University of Technology (SUT) in Thailand. They were fed once a day with 30% protein commercial catfish pellet feed. Experimental fish were not fed for 6-12 h prior to sperm and egg collection. Luteinizing hormone releasing hormone analogue (LHRHa; Suprefact and domperidone (Motilium) were used to induce spermiation and ovulation. Dose injections were followed Kwantong & Bart (2003).

Effect of equilibration times on cryopreservation of Pangasius hypophthalmus

Cryopreservation method used in this study (extenders, cryoprotectants and freezing rates) was based on the excellent results in cryopreservation of *P. hypophthalmus* sperm Kwantong & Bart (2003). These treatments were: 1) DMSO(10%)+NaCl(0.9%), 2) DMA(10%)+C-F HBSS), and 3) MeOH(5%)+NaCl(0.9%). Sperm was diluted with C-F HBSS or 0.9% NaCl at sperm: extender ratio of 1: 3. The three cryoprotectants (DMSO, DMA and MeOH) were mixed separately with diluted sperm in 1.5 mL Eppendorf tubes and the mixture (240 µL), loaded into 250 µL straws and finally sealed with a heated hemostat. The effects of equilibration time on cryopreservation at 5, 10, 20 and 40 min were investigated. A freezer control (CL 3300) with a Cryogenesis, version 4, for Windows (Cryologic, Pty Ltd., Australia, 1998 and 1999) was used to regulate the rate of freezing. Temperature was lowered at 10 °C min^{-1} until the samples reached -80 °C, the samples were plunged into a liquid nitrogen container for 3 weeks, and fertilization rate was assessed.

Fertilization trials

Egg preparation and method for fertilization procedures used in this study were similar to in the previous described by Kwantong & Bart (2003). Good quality eggs with pale yellow color were used to fertilize 200 eggs $batch^{-1}$. Each batch was placed in a glass Petri dish with three replications per treatment. The frozen straws with respective equilibration period were airthawed at 25 °C and the contents were poured onto 200 eggs $batch^{-1}$. At the same time, fresh sperm were used to fertilize with the 200 eggs $batch^{-1}$ as control. The percentage of fertilized eggs was determined at the gastrula stage (7 h after fertilization). Fertilization rate among four exposure times in each treatment were analyzed with one way ANOVA at $\alpha = 0.05$ using SPSS for Windows. Significant differences among four exposure times were compared using the Duncan Multiple Range test.

Results and Discussions

The Effects of equilibration times on the fertilization rate

The highest fertilization rate of 60% (88% of control) was achieved with 10% DMSO and 0.9% NaCl, at 40- min exposure time. This was not significant difference from the control (Table 1). Equilibration times at 5, 10, 20 and 40 min were not effect fertilization rates when tested with DMSO, DMA or MeOH treatment. Fertilization rates among an equilibration times were significantly lower than the control, except when tested with DMSO treatment and MeOH treatment, at 5- min, fertilization rates were not significant with the control. These results were similar to Huang et al. (2004) who

reported that the motility rate of green sword tail, *Xiphophorus helleri* was not significant different among an equilibration time of 10, 20 and 30 min. However, the present studied are different than those reported by Linhart et al. (2005) who found that the hatching rate of European catfish, *Silurus glanis* decreased when increased the higher of exposure time to 20- min. In addition, Babiak et al. (2001) reported that 10-min equilibration of diluted sperm before freezing significantly decreased the fertilization rate of rainbow trout, *Oncorhynchus mykiss* sperm.

Table 1. Mean percent (± SE) fertilization rates of striped catfish, Pangasius hypophthalmus sperm in four different equilibration times 5, 10, 20 and 40 min obtained with three treatments (10%DMSO + 0.9%NaCl, 10%DMA + C-F HBSS and 5%MeOH + 0.9%NaCl), at one-step freezing procedures (10 $^{\circ}$Cmin^{-1}) were investigated.

Equilibration time	Treatment 10%DMSO + 0.9%NaCl	10%DMA + C-F HBSS	5%MeOH + 0.9%NaCl
5 min	57.05±1.13^{a} (83.73)	36.15±2.95^{b} (53.06)	46.41±2.10^{abc} (68.12)
10 min	44.03±8.16^{a} (64.62)	34.30±3.91^{b} (50.35)	40.37±5.59^{bc} (59.25)
20 min	57.51±8.19^{a} (84.41)	43.68±1.73^{b} (64.11)	39.01±4.21^{bc} (57.25)
40 min	60.00±0.43^{a} (88.07)	39.42±5.39^{b} (57.86)	32.70±2.52^{c} (48.00)
Control (fresh sperm)	68.13±3.32^{a}	68.13±3.32^{a}	68.13±3.32^{a}

Different letter in each column was significantly different at $p<0.05$ (ANOVA, Duncan test).
The values in the parentheses represent percent of control.

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