Animal Reproduction Science 166 (2016) 36–46

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

# Effect of antibiotic supplementation on the quality of cryopreserved fish sperm of silver barb (*Barbodes gonionotus*): Sperm motility and viability, bacterial quality and fertilization

Traimat Boonthai[a], Weerasith Khaopong[b], Jumlong Sangsong[b], Subuntith Nimrat[c], Verapong Vuthiphandchai[d,*]

[a] *Biological Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand*
[b] *Environmental Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand*
[c] *Department of Microbiology and Environmental Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand*
[d] *Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri 20131, Thailand*

ARTICLE INFO

*Article history:*
Received 18 April 2015
Received in revised form
10 December 2015
Accepted 4 January 2016
Available online 6 January 2016

*Keywords:*
*Barbodes gonionotus*
Cryopreservation
Sperm
Antibiotic
Bacteria
Fertilization

ABSTRACT

Disease prevention is a key aspect in developing cryopreservation procedures for fish sperm and in improving the reproductive biotechnology for commercially important aquatic species. The purpose of this study was to evaluate the effects of an antibiotic supplementation (0.25% penicillin–streptomycin, PS and 0.25% penicillin–gentamicin, PG) on sperm motility and viability, bacterial profile and fertilization capacity of cryopreserved silver barb (*Barbodes gonionotus*) semen. The experimental protocol involved three treatments: addition of PS alone; addition of PG alone; and no addition of antibiotics (Control). Semen samples were frozen and cryostored for 12-mo. Administration of 0.25% PS significantly ($P < 0.05$) improved sperm motility and viability and reduced ($P < 0.05$) total heterotrophic bacteria, Gram negative bacteria and pseudomonads bacteria in cryopreserved semen. Post-thawed semen treated with 0.25% PS did not contain contaminating bacteria including *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus safensis*, *Aeromonas punctata* subsp. *caviae*, *Serratia plymuthica*, *Pseudomonas azotoformans* and *Pseudomonas* sp. Post-thawed semen supplemented with 0.25% PG showed degenerative changes in motility and viability of sperm. Eggs fertilized with 0.25% PS or antibiotic-free cryopreserved semen had similar fertilization rates, lower ($P < 0.05$) than those of fresh semen. Incorporation of 0.25% PS was suitable for cryopreservation of silver barb semen based on the presence of good quality of post-thawed sperm and elimination of bacterial contaminants: *B. subtilis* subsp. *inaquosorum*, *B. safensis*, *A. punctata* subsp. *caviae*, *Ser. plymuthica*, *P. azotoformans* and *Pseudomonas* sp. This is the first study to investigate the antibiotic effect on the number of bacteria and their profile in cryopreserved semen of fish.



## 1. Introduction

Cryopreservation is widely used for sperm preservation, allowing for long-term cryostorage, conservation of

* Corresponding author. Fax: +66 38 393 491.
*E-mail address:* verapong@buu.ac.th (V. Vuthiphandchai).

http://dx.doi.org/10.1016/j.anireprosci.2016.01.002
0378-4320/

genetic stability and facilitation of artificial insemination (AI) program of cultured and endangered species (Tiersch, 2006). Silver barb (*Barbodes gonionotus*) or locally, Pla Ta Pian Khao, is an important commercial fish in Thailand and Southeast Asia. Its annual production reached 96,100 t in 2008 accounting for 12.1% of total freshwater fish production of Thailand (Department of Fisheries, 2010).

Biosafety of cryopreserved germplasm has received increased attention after emergence of a case of human hepatitis B transmission via bone marrow transplants stored under cryostorage in liquid nitrogen (−196 °C) during 1993–1994 (Tedder et al., 1995). In general, cryopreservation of fish sperm lacks satisfactory sanitation awareness resulting in bacterial contamination during the steps of cryopresevation i.e. collection, processing, storage and transportation (Jenkins, 2000). Therefore, semen is regarded as the potential source of bacterial contamination that deteriorates sperm quality during storage and reduces AI success. Bacterial contamination in semen has been reported to result in diminishing fertilization success in Atlantic salmon, *Salmo salar* (Stoss and Refstie, 1983), sperm quality and fertilization in common carp, *Cyprinus caprio* (Saad et al., 1988) and sperm motility in channel catfish, *Ictalurus punctatus*, (Christensen and Tiersch, 1996). Jenkins and Tiersch (1997) reported that a decline in sperm quality of refrigerated channel catfish sperm increased concomitantly with an increase in the number of contaminating bacteria. Therefore, a reliable technology for minimizing bacterial contamination in fish sperm is needed.

Antibiotic is commonly utilized to prevent and/or inhibit bacterial infections. Several antibiotics, especially aminoglycosides, penicillin and its derivatives, have been used in sperm extenders to control bacterial propagation in refrigerated semen of common carp, *C. caprio* (Saad et al., 1988), Atlantic salmon, *S. salar*, sea trout, *S. trutta* (Stoss and Refstie, 1983), and Nile tilapia, *Oreochromis niloticus* (Segovia et al., 2000). Addition of gentamicin (0.1 mg/mL) was beneficial in chilled storage of Piracanjuba (*B. orbignhyamus*) semen resulting in the maintenance of highly motile sperm and absence of bacterial growth (Viveiros et al., 2010). Although there is currently limited data regarding the effectiveness of antibiotic on cryopreserved semen of fish, information on the optimal use of antibiotic in cryopreservation of fish sperm is needed for establishing sperm repositories. Mixtures of PS have been selectively used for fish semen preservation owing to their lower toxicity, absence of side effects on semen quality and presence of broad-spectrum bacteriocidal activity (DeGraaf and Berlinsky, 2004; Peck et al., 2004; Nimrat et al., 2005, 2006). However, some pathogenic bacteria were isolated after the PS mixture administration presumably due to a resistance to antibiotics (Holcomb et al., 2005). The purpose of this study was to evaluate the effects of antibiotic supplementation (0.25% penicillin–streptomycin, PS and 0.25% penicillin–gentamicin, PG) on sperm motility and viability and the bacterial profile and fertilization efficiency of silver barb semen during cryostorage for 12 mo.

## 2. Materials and methods

### 2.1. Male broodstock management and semen collection

Silver barb broodstock ($N = 24$) were maintained in a rectangular cement tank (10 $m^3$) at the hatchery of Department of Aquatic Science, Burapha University. The experiment was carried out during the spawning season from May to October in 2012. Management practices were carried out following the best standard protocol ethically approved by the Burapha University Animal Care and Use Committee.

Prior to the beginning of the experiment, spermiation was induced in the male broodstock by a single intramuscular injection of a mixture of gonadotropin-releasing hormone analogue (Suprefact® nasal, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) and dopamine antagonist (Motilium-M) dissolved in physiological saline solution at 20 μg/kg and 5 mg/kg body weight, respectively. After 12 h of hormonal administration, the genital aperture and urogenital area were rinsed with sterile distilled water and dried with autoclaved towels. A flexible catheter (7 cm in length, internal and external diameter 2 mm and 3.5 mm, respectively) with an attached 25-mL syringe was gently inserted into the genital aperture for about 1.5–2.0 cm. During collection of semen, an aseptic technique was followed to prevent bacterial contamination. Contamination by fecal matter, urine and blood was avoided by discarding any semen in which this material was observed, in most cases, within the first few drops. Freshly collected semen was labeled and placed in a styrofoam box containing crushed ice. Immediately after collection, the percentage of motile sperm was determined prior to the beginning of cryopreservation. Sperm with good motility (≥80%) was used in this study within 30 min after collection.

### 2.2. Experimental design and cryopreservation

In accordance with the previous study on the effects of final concentration of antibiotic mixture of PS or PG ranging from 0.025% to 0.75% in sperm quality, 0.25% of PS and PG could effectively inhibit contaminated bacteria and extend the storage period of chilled silver barb semen (unpublished data). Therefore, in this study, 0.25% PS and 0.25% PG were selected for supplementation in the semen cryopreservation process. Due to a variation of sperm quality among individuals, semen with good quality (≥80%) from each male broodstock ($N = 24$) was pooled in a pre-chilled sterile petri dish onto crushed ice. Semen was then frozen based on the three treatments as follow: (T1) addition of 0.25% PS (10,000 units/mL penicillin and 10 g/L streptomycin, Invitrogen, Grand Island, NY, USA) into extended semen containing dimethylsulfoxide (DMSO); secondly or (T2) addition of 0.25% PG (10,000 units/mL Penicillin G potassium salt, AppliChem GmbH, Darmstadt, Germany and 40 g/L gentamicin sulfate, Amresco, Solon, OH, USA) into extended semen containing DMSO, and thirdly or (T3) no addition of any antibiotic into extended semen containing DMSO (control group). Calcium-free Hank's Balanced Salt Solution (Ca-F HBSS) as the extender was composed of (g/L) NaCl (8.89), KCl (0.44), $Na_2HPO_4 \cdot 2H_2O$ (0.13), $NaHCO_3$