Cryobiology 73 (2016) 140–146

Contents lists available at ScienceDirect

Cryobiology

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

Influence of *Aeromonas hydrophila* and *Pseudomonas fluorescens* on motility, viability and morphometry of cryostored silver barb (*Barbodes gonionotus*) sperm

Traimat Boonthai [a, 1], Weerasith Khaopong [b], Jumlong Sangsong [b], Verapong Vuthiphandchai [c], Subuntith Nimrat [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 Aquatic Science, Faculty of Science, Burapha University, Chon Buri, 20131, Thailand
[d] Department of Microbiology and Environmental Science Program, Faculty of Science, Burapha University, Chon Buri, 20131, Thailand

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form
8 August 2016
Accepted 17 August 2016
Available online 19 August 2016

Keywords:
Barbodes gonionotus
Cryopreservation
Sperm quality
Aeromonas hydrophila
Pseudomonas fluorescens
Morphometry

ABSTRACT

This objective of the study was to evaluate the effects of *A. hydrophila* subsp. *hydrophila* and *P. fluorescens* on sperm motility, sperm viability and sperm morphometry of cryopreserved silver barb (*Barbodes gonionotus*) semen and survival of tested bacteria after cryostorage. Semen was diluted in a calcium-free Hank's balanced salt solution (Ca-F HBSS) supplemented with or without 0.25% penicillin-streptomycin (PS) after which *A. hydrophila* subsp. *hydrophila* or *P. fluorescens* was immediately added into extended semen prior to freezing. Extended semen and cryostored semen kept for 20 min, 24 h, 7 d, 14 d and 28 d were assessed for sperm motility, sperm viability, sperm morphometry, survival of challenged bacteria and the relationship between bacteria and sperm. Bacterial-exposed semen with or without 0.25% PS supplementation showed a significant reduction ($P < 0.05$) in sperm motility and viability during a cryostorage of 28 d, compared to semen without bacterial supplementation (control groups). Addition of *A. hydrophila* subsp. *hydrophila* and *P. fluorescens* resulted in a significant ($P < 0.05$) alteration of sperm morphometry of cryopreserved semen, especially flagellum width. The two pathogens were detected at a level of 10^5 CFU ml^{-1} in cryostored semen with or without antibiotic supplementation. There were significant correlations among bacterial number, percentage of sperm motility and viability and flagellum width. In conclusion, the presence of *A. hydrophila* subsp. *hydrophila* and *P. fluorescens* had a deleterious effect on cryopreserved silver barb sperm based on a reduction in sperm motility and viability and alteration of sperm morphometry, especially flagellum width.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Cryopreservation of fish sperm has currently received a great attention for utilization in commercial hatchery and conservation program. Health concern and biosafety issue related with pathogenic contamination of cryopreserved sperm have also received a considerable attention after an emergence of human hepatitis B transmission via cryostored bone marrow transplants [26]. Cryopreservation procedure of fish sperm generally lacks a hygienic condition leading to bacterial contamination of sperm. Semen, a mixture of sperm and seminal plasma, is regarded as an ideal medium for bacterial colonization and proliferation. Bacteria are deleterious to sperm quality, sperm morphology, sperm function resulting in a shortened storage period [11,18,19]. The use of semen containing pathogenic contamination enhances the risks of spreading diseases within and between farms worldwide. To minimize the noxious effects, antibiotics have been supplemented into extender to prevent and/or remove bacterial contaminants

Abbreviation: PS, Penicillin-streptomycin; Ca-F HBSS, Calcium-Free Hank's Balanced Salt Solution; Me_2SO, Dimethyl sulfoxide; PI, Propidium iodide; PBS, phosphate buffer saline.

* Corresponding author.
E-mail addresses: boonthait@gmail.com (T. Boonthai), s.coccus@hotmail.com (W. Khaopong), Jumlong_Sangsong@hotmail.com (J. Sangsong), verapong@buu.ac.th (V. Vuthiphandchai), subunti@buu.ac.th (S. Nimrat).
[1] Present address: Aquatic Animal Health Laboratory, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA.

http://dx.doi.org/10.1016/j.cryobiol.2016.08.008
0011-2240/© 2016 Elsevier Inc. All rights reserved.

[19,29]. However, antibiotics can negatively affect sperm quality of extended semen during cryopreservation [1,2].

A number of studies have examined the influence of bacterial contamination on sperm quality of human and cattle. In general, bacteria create a negative effect that leads to malfunction of male reproduction system including apoptosis, reduction in sperm motility and viability, alteration of sperm morphology and impairment of fertilization and embryonic development [3,6,25,28]. *Aeromonas hydrophila* subsp. *hydrophila* and *Pseudomonas fluorescens* have recently been isolated from fish semen after cryostorage despite antibiotic administration [8]. Despite the need for improving the quality of cryopreserved fish semen used in the sperm bank for aquaculture and conservation, there is limited information how pathogenic bacteria affect on morphology and characteristic of cryopreserved sperm. Without the baseline information on evaluation of cryostored sperm quality and relationship between pathogen and sperm, it is difficult to implement the risk management approaches for fish sperm depositories and hatchery operations. The purpose of this study was to determine the effects of pathogenic bacteria (*A. hydrophila* subsp. *hydrophila and P. fluorescens*) on sperm motility, sperm viability and sperm morphometry of cryostored semen of a high commercial value fish of Thailand, silver barb (*Barbodes gonionotus*), with or without antibiotic supplementation and evaluated survival of the two tested bacteria after cryostorage of semen.

2. Materials and methods

2.1. Broodstock and semen collection

The experimental trials were approved for animal ethics by Burapha University Animal Care and Use Committee. Silver barb broodstocks (175.1 ± 45.4 g body weight) were held in a 10-t tank at the hatchery of Department of Aquatic Science, Burapha University during the spawning season. All broodstocks were fed with a commercial diet (protein > 30%) to sanitation two-time daily. Males were treated with gonadotropin-releasing hormone analogue (Suprefact® nasal, Sanofi-Aventis Deutschland GmbH, Germany) and dopamine antagonist (Motilium-M) at a dosage of 20 μg kg^{-1} and 5 mg kg^{-1} body weight, respectively, to induce spermiation.

After hormonal administration for 12 h, the urogenital areas were rinsed with sterile distilled water and dried with an autoclaved towel. Semen was gently collected using a catheter equipped with a 25-ml syringe, 7 cm in length, internal and external diameter of 2 mm and 3.5 mm, respectively. During semen collection, aseptic technique was implemented to prevent bacterial contamination. Semen with fecal materials and urine contamination were discarded. After collection, freshly collected semen was immediately assessed for percentage of motile sperm. Semen from spermiating males with an excellent sperm quality (≥ 80% sperm motility) was pooled to reduce the variation of sperm quality among individuals and placed onto a dry sterile petri disc under aerobic conditions at 4 ± 2 °C. Pooled semen was used in the experiments within 30 min after collection.

2.2. Bacterial inoculation in semen

Previous study exhibited that 0.25% penicillin-streptomycin (PS) administration was capable of maintaining excellent quality, removal of contaminated bacteria and prolonging the storage period of cryostored sperm of silver barb [8]. However, two types of fish pathogenic bacteria, *A. hydrophila* subsp. *hydrophila* BG19 (Accession no. KF699878) and *P. fluorescens* BG20 (Accession no. KF699879), were isolated based on determination with morphological and biochemical characteristics and 16S rRNA gene sequencing. Therefore, the two pathogens were selected for further study and evaluated their effects on the quality of cryostored silver barb sperm. Bacterial preparation was achieved on a basis of the protocol described by Bar et al. [3] with some modifications. *A. hydrophila* subsp. *hydrophila* BG19 and *P. fluorescens* BG20 were seeded separately in Brain Hearth Infusion broth at 35 °C for 24 h. Bacterial pellets were harvested by centrifugation at 8,228 *g*, 4 °C for 5 min and washed aseptically three-times with Calcium-Free Hank's Balanced Salt Solution (Ca-F HBSS extender). Cell pellets were then re-suspended in the same extender and adjusted to 10^9 CFU ml^{-1} using spectrophotometric method at 580 nm of 1.5 absorbance unit.

Compositions of Ca-F HBSS extender were (g l^{-1}) NaCl (8.89), KCl (0.44), $Na_2HPO_4 \cdot 2H_2O$ (0.13), $NaHCO_3$ (0.39), KH_2PO_4 (0.07), $MgSO_4 \cdot 7H_2O$ (0.22) and Glucose (1.11), pH 7.6 and osmolarity 320 mOsm kg^{-1}. The extender was made approximately one day prior to use and filtered through 0.45-μm membrane filter (Sartorius, Bedford, MA, USA) to remove bacterial contaminants. Pooled semen (≥ 80% sperm motility) was diluted (1:1) with extender. Thereafter, semen solution was divided into six batches according to the treatments of the study. All treatments were accomplished in triplicates as following: T1) extender only, T2) extender with 0.25% PS, T3) extender with *A. hydrophila* subsp. *hydrophila* BG19, T4) extender with *A. hydrophila* subsp. *hydrophila* BG19 and 0.25% PS, T5) extender with *P. fluorescens* BG20, and T6) extender with *P. fluorescens* BG20 and 0.25% PS. Prior to addition of 0.25% PS into extender, bacterial cultures were separately inoculated to a final concentration of 10^5 CFU ml^{-1}. During manipulation, semen solution, antibiotic solution and bacterial suspension were kept on crushed ice.

2.3. Semen cryopreservation

Extended semen of each treatment was frozen using dimethyl sulfoxide (Me_2SO) as a cryoprotectant. Extended semen was gently mixed (1:1) with sterile 20% Me_2SO and held at the room temperature (approximately 25 °C) for 10 min to allow equilibration. Semen solution (0.1 ml) was slowly aspirated into 0.25-ml cryostraws (IMV International Corp., Minneapolis, MN, USA) using micropipette before sealing. Cryostraws containing semen samples were frozen at a cooling rate of 8 °C min^{-1} using a controlled-rate programmable freezer (model 3000, Cryologic Pty., Australia). Semen samples were cooled from the room temperature to 0 °C, then cooled again to a final temperature of −40 °C and followed by a storage in liquid nitrogen tank. After cryogenic storage for 20 min, 24 h, 7 d, 14 d and 28 d, cryopreserved semen samples were thawed at 70 °C for 5 s to evaluate sperm quality and presence of inoculated pathogenic bacteria.

2.4. Assessment of sperm quality

Extended semen and cryopreserved semen of pathogenic bacteria inoculation groups were assessed for the changes in percentages of motile and viable sperm and numbers of *A. hydrophila* and *P. fluorescens*, in comparison with those of the two control groups. Freshly collected semen, extended semen and cryopreserved semen kept for 20 min, 24 h, 7 d, 14 d and 28 d were sampled for determination of sperm motility [30]. Motile sperm were estimated by mixing about 1 μl of sperm samples with distilled water (50 μl). Observation was made immediately within 15 s post-activation under a light microscope (Zeiss model D-7085, Berlin, Germany) at ×400 magnification. Sperm motility was subjectively estimated based on the percentage of forward moving cells as: 0, when no movement or vibrating in place of sperm; 20, when up to 20% cells moving; 40, when up to 40% cells moving; 60,

Download English Version:

https://daneshyari.com/en/article/5530811

Download Persian Version:

https://daneshyari.com/article/5530811

Daneshyari.com