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ScienceDirect
Theriogenology 74 (2010) 551–556
Theriogenology
www.theriojournal.com

Motility and fertility of the subtropical freshwater fish streaked prochilod (*Prochilodus lineatus*) sperm cryopreserved in powdered coconut water

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Received 6 October 2009; received in revised form 21 March 2010; accepted 21 March 2010

Abstract

Streaked prochilod (*Prochilodus lineatus*) is a freshwater fish inhabiting many South American rivers. The objective was to determine the effectiveness of coconut water (ACP™), combined with methylglycol, as a freezing medium for streaked prochilod sperm. A secondary objective was to compare a computer-assisted sperm analyzer (CASA) system versus subjective microscropic examination as a means of assessing sperm motility. As a control, glucose and methylglycol was used, according to our previous study. Sperm diluted in each medium was loaded into 0.5 mL straws, frozen in liquid nitrogen vapor (in a dry shipper), and stored in liquid nitrogen (-196 °C). Half of the samples were evaluated for sperm motility, both subjectively and with CASA; the remainder were evaluated for fertility. There was no difference ($P > 0.05$) between subjective or CASA assessment of post-thaw sperm motility. Although sperm motility was higher in sperm cryopreserved in ACP™ (85%) than in glucose (75%), cryopreservation in either extender yielded similar fertilization rates (46–48%) and sperm velocities. There were positive correlations ($r = 0.56–0.8$) between all sperm velocities and fertilization rate. In conclusion, streaked prochilod sperm cryopreserved in glucose or ACP™ and methylglycol was fertile, and thus could be used for research or commercial settings. Furthermore, although the CASA system provided objective data regarding sperm motility, in the present study, subjective evaluation of sperm motility was practical and a good indication of sperm quality; it could readily be done by well-trained personnel under field or laboratory conditions.
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Keywords: *Prochilodus lineatus*; Semen cryopreservation; Sperm motility; Fertility; Fish

1. Introduction

The streaked prochilod, *Prochilodus lineatus* (Valenciennes, 1836), is a migratory Characiformes Brazilian fish species, with a large geographical distribution throughout South America, accounting for 50–90% of the total fish biomass in the Paraná river basin [1]. Their detritivorous habit makes streaked prochilod a dominant element in structuring tropical stream community dynamics by sediment processing activities [2]. Streaked prochilod is one of the freshwater fish species with the greatest importance in the Brazilian aquaculture industry, where it is known as curimba, curimbatá, and curimatã. Larvae are used as live food for endangered carnivorous fish species, including piracanjuba (*Brycon orbignyanus*) and jaú (*Zungaro jahu*), whereas adult fish are used for human consumption, mainly in

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0093-691X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.03.018

northeastern Brazil [3]. Brazilian hydroelectric companies breed streaked prochilod for restocking programs. Finally, because artificial reproduction methods are well established and prolificacy is high, the streaked prochilod has been used as a model species for research in fish reproduction.

Sperm cryopreservation is an important technique in fish culture, as it facilitates procedures for artificial reproduction. With viable sperm stored in liquid nitrogen, it is necessary to induce spawning and collect gametes only from females. Cryopreserved sperm may be kept in germplasm banks for an indefinite period, which allows the establishment of breeding programs, eliminates the problem of asynchronous reproductive activity between males and females, and enables maintenance of fewer male broodfish [4]. Prior to freezing, sperm must be diluted in a medium containing an extender and a permeable cryoprotectant agent (CPA). In our previous study, streaked prochilod sperm cryopreserved in a medium containing glucose and methylglycol retained 86–95% motility and 47–83% fertility [3].

During the past decade, researchers at the State University of Ceará (UECE), Brazil, have developed a technology to dehydrate coconut water and produced a stable and standardized powdered coconut water (ACP™), which contains minerals, amino acids, vitamins, carbohydrates, growth factors, phytohormones, and saturated fatty acids [5]. The use of ACP™ as an extender for streaked prochilod sperm extender has undergone preliminarily testing. Although post-thaw sperm quality was limited to a subjective evaluation of sperm motility, results were promising [3].

The objective of this study was to further investigate the effects of ACP™ as an extender for cryopreservation of streaked prochilod sperm. A secondary objective was to compare a CASA system versus subjective microscopic examination as a means of assessing sperm motility. As a control, sperm cryopreserved in glucose and methylglycol was used, according to our previous study [3].

2. Materials and methods

2.1. Fish and sperm collection

All fish were handled in compliance with published guidelines for animal experimentation [6]. Streaked prochilod (*P. lineatus*) males were selected from earthen ponds at the Fish Culture Unit of the Hydroelectric Company of Minas Gerais (CEMIG; Itutinga, MG, Brazil) during the spawning season (December and January). Males with detectable running sperm in response to soft abdominal pressure were given a single dose of carp pituitary extract (Argent Chemical Laboratories, Redmond, WA, USA; 5 mg/kg body weight, IM) and maintained at ~26 °C. Eight hours after treatment, the urogenital papilla was carefully dried, and sperm was hand-stripped directly into test tubes. Sperm collection was carried out at room temperature (27–29 °C), and soon after collection, tubes containing sperm were placed in crushed ice (~5 °C). An aliquot (5 μL) of each sample was placed on a slide and observed with a light microscope (Model L1000, Bioval, Jiangbei, China) at 400 × magnification. Any sperm motility (auto-activation) observed was attributed to urine or water contamination and the sample discarded, as fish sperm in seminal plasma is immotile. In immotile samples (n = 8 males), sperm progressive motility was subjectively estimated (in increments of 5%) immediately after the addition of 25 μL of 0.29% NaCl as an activating agent [3]. Only samples with at least 80% motility were used in the subsequent analysis. Sperm volume and concentration (hemacytometer/Neubauer chamber) were also determined.

2.2. Sperm cryopreservation

Two freezing media, comprising combinations of two extenders and one CPA (methylglycol: $CH_3O(CH_2)_2OH$; Vetec Química Fina Ltda™, Duque de Caxias, RJ, Brazil), were prepared and maintained in crushed ice (~5 °C). Powdered coconut water (ACP™, ACP Serviços Tecnológicos Ltda, ACP Biotecnologia, Fortaleza, Ceará, Brazil; each 100 mL contained: glucose 4.4 g; proteins: 0.37 mg; phosphorus 6.2 mg; potassium 175 mg; calcium: 17.5 mg; magnesium: 8.5 mg; sodium: 10.5 mg; iron 0.06 mg; among other components [7]; pH 7.8; 300 mOsmol) was tested as extender and a commercial 5% glucose solution (Fresenius-Kabi™, Brazil; pH adjusted to 7.6; 277 mOsmol) was used as a control [3]. Sperm samples were diluted in each freezing media (8 males × 2 media) at a final proportion of 10% sperm, 80% extender, and 10% methylglycol. After dilution, sperm samples were immediately aspirated into 0.5 mL straws (n = 6 replicate straws) and frozen in a nitrogen vapor vessel (Cryoporter™ LN_2 *dry vapor shipper*, Cryoport Systems, Brea, CA, USA) at approximately −170 °C, which gives a freezing rate of approximately −35.6 °C/min between +21 °C and −170 °C [8]. Thereafter, cryopreserved sperm was transported 50 km by car from CEMIG to the Animal Sciences Department at the Federal University of Lavras (UFLA), Lavras, MG, Brazil, where straws were transferred to liquid nitrogen (M.V.E. Millenium, XC 20, Chart, Minessota, USA) at −196 °C within 20–24 h for storage.

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