Effects of cooling and freezing on sperm motility of the endangered fish 
piracanjuba *Brycon orbignyanus* (Characiformes, Characidae)

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Abstract

The effects of extenders and cryoprotectants on sperm motility of the endangered fish piracanjuba (*Brycon orbignyanus*, Characiformes, Characidae) after storage at 4-6°C and at -196°C were evaluated. In Experiment 1, 20 extender-cryoprotectant combinations (5 extenders x 3 cryoprotectants, plus 5 solutions containing only extender without cryoprotectant) were tested. The extenders tested were: 154 mM NaCl, 200 mM NaCl, Saad (mM: 200 NaCl, 30 Tris), coconut water, and Kurokura (mM: 128.4 NaCl; 2.7 KCl; 1.4 CaCl₂; 2.4 NaHCO₃). The cryoprotectants (dimethyl sulphoxide - DMSO, methanol, and methylglycol) were added at 10% of the total volume. One aliquot of semen was kept undiluted and served as a control. Motility was subjectively estimated at 0, 1, and 2 days after cold storage. In Experiment 2, the extender-cryoprotectant combinations that produced motility above 70% on Day 0 (Experiment 1) were selected as freezing media. The amount of egg yolk and semen included in each medium was 5 and 10% of the total volume, respectively. Three 0.5-mL straws for each freezing medium were frozen in nitrogen vapor container (Taylor-Wharton, CP 300) at -170°C and then stored in liquid nitrogen. Straws were thawed in a water bath at 60°C for 8 seconds. Sperm motility one day after cooling was higher in samples diluted in Saad solution (82%), 200 mM NaCl (67%), and 154 mM NaCl - DMSO (77%). Undiluted samples yielded 53% motility. Higher post-thaw sperm motility (66%) was observed in semen cryopreserved in 154 mM NaCl - egg yolk - methylglycol compared to all the other samples. Piracanjuba semen diluted in Saad solution or 200 mM NaCl and stored at 4-6°C for one day or frozen in 154 mM NaCl - yolk - methylglycol maintained most of its sperm motility.

Keywords: semen, cooling, freezing, sperm motility, fish.