



## Cryopreservation of tambaqui semen in 4-mL straws: influence of thawing time and temperature on sperm quality

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The development of semen cryopreservation protocols in containers larger than the traditional 0.5-mL French straws it is necessary because it optimizes packaging, freezing and thawing samples, besides facilitating the management during the process of artificial fertilization in species that exhibit high prolificacy, as is the case of tambaqui. Effective containers need to have a high surface/volume ratio in order to facilitate freezing and thawing at uniform rates. However, as semen container characteristics influence freezing and thawing rates, species specific studies are needed to develop specific protocols. Thus, the objective of this study was to evaluate the influence of thawing time and temperature on the tambaqui semen quality cryopreserved in 4.0-mL straw. Semen samples (N=4) were diluted in freezing solution (5% glucose solution, 10% methylglycol, 5% egg yolk), stored 4.0-mL straw, frozen in liquid nitrogen vapor at -175°C and transferred to a cryogenic container at -196°C. The straw was thawed in a water bath at 30°C for 50 s (T1) or 80 s (T2) and 60°C for 25 s (T3) or 40 s (T4). After thawing, the sperm kinetics (total motility-TM; progressive motility-PM; curvilinear velocity-VCL; straight line velocity-VSL and average path velocity-VAP) and viability was evaluated. For sperm kinetic analysis, thawed semen samples were activated with 230 mOsm NaHCO<sub>3</sub> solution. Aliquots of three microliters were transferred to Makler™ counting chamber and analyzed with a phase contrast microscope equipped with a video camera which generated 100 frames/s. Video recording began 10 seconds after activation and the images were analyzed by the software Sperm Class Analyzer™ (SCA™, Microptics). Sperm viability analysis was performed in an epifluorescent microscope after incubation of the thawed semen with propidium iodide and SYBR-14. The sperm from each sample (n = 200 cells) was grouped into two subpopulations based on the presence of red or green fluorescence, corresponding to cells with damaged or intact plasma membranes. Sperm velocity and viability presented no significant difference (p > 0.05) among the four thawing tested (VCL: 63-71 μm s<sup>-1</sup>; VSL: 30-47 μm s<sup>-1</sup>; VAP: 41-58 μm s<sup>-1</sup>; Viability: 48-58%). The progressive motility was similar in T2 and T3 (20%) which were superior to T1 (15%) and T4 (13%). However, total motility had significantly higher (p < 0.05) only in T3 when compared to other treatments (T1, T2 and T4). Semen samples presented 56% of total sperm motility in T3 and 49%, 49% and 46% in T1, T2 and T4, respectively. The results obtained in this study indicate that 4.0 mL straws have great potential for use in tambaqui semen cryopreservation when thawed at 60°C for 25 s.

**Keywords:** *Colossoma macropomum*; fish; sperm kinetics; macrotube; thawing velocity.

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