

Cryopreservation of Filefish (*Thamnaconus septentrionalis*) Sperm

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The present study examined the possibility of long term storage, by cryopreservation in liquid nitrogen, of the sperm of Filefish (*Thamnaconus septentrionalis*), and the changes in motility, survival rate and ultrastructure of the sperm after freezing and thawing. The sperm was collected by stripping and stored on ice until experiments. For selection of the immobilizing solution, diluted artificial seawater (ASW) of 20, 30 and 40% were tested. The sperm motility was significantly inhibited in 30% ASW, and restored entirely after 100% ASW was added again. Two cryoprotectants, dimethyl sulfoxide (Me₂SO) and glycerol, were added to 30% ASW to formulate the extenders at the concentrations between 5 to 20% by volume for freezing. The sperm was diluted at the ratio of 1:6 with the extenders, inserted into 0.5ml plastic straws and frozen at a freezing rate of 50°C/min to -100°C after equilibration for 10 min at room temperature, followed by plunging into liquid nitrogen. The straws were thawed in a 30°C water bath for 15 sec. The highest post-thawed sperm motility and survival rate were obtained with 5% glycerol. Afterward, the effect of different freezing rates was examined using 5% glycerol as a cryoprotectant, and the rate of 20°C/min to -80°C showed the best result. Some ultrastructural changes of sperm, such as the detachment of plasmatic and nuclear membranes, destruction of mitochondria, were observed after cryopreservation. Morphological normality of the sperm in 5% glycerol frozen at the ratio of 10°C/min to -80°C was better than that of others.

Key words) *Filefish, Thamnaconus septentrionalis, Sperm, Cryopreservation*