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Sperm cryopreservation of Japanese eel, *Anguilla japonica*

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Abstract

In the present study, methods for the cryopreservation of *Anguilla japonica* spermatozoa were examined. Spermatozoa were collected from artificially matured males and incubated in Japanese eel K30 artificial seminal plasma (K30 ASP) before experiments. Sperm motility was investigated using computer-assisted sperm analysis (CASA). 10% and 15% MeOH as cryoprotectant was the most successful cryoprotectant with percentage of the intial motility of 59.7 ± 12.1%; the combination of 5% MeOH and 5% DMA was also viable. DMSO was unsuitable as a cryoprotectant with K30 ASP as it showed no cryopreservation properties and was toxic to sperm, causing sperm to be immotile immediately after dilution. Japanese eel spermatozoa had a narrow range of optimal cooling rates (6.3~28.6 °C min^{-1}) and immersion temperatures of -40~-70 ^{o}C were effective. The different fetal bovine serum (FBS) concentration in extender, temperature of milt before cooling, dilution rates (3~100 times) and equilibrium time showed no significant difference. The results indicate that the type of extender media greatly influenced the suitability of cryoprotectant. The establishment of a protocol to cryopreserved Japanese eel sperm will benefit the artificial seed production of Japanese eel and provide an important tool for genetic and breeding studies.

Keywords: Cryopreservation, sperm, aquaculture, Japanese eel, *Anguilla japonica*

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1. Introduction

The Japanese eel *Anguilla japonica* is an important aquaculture fish species in Japan (Ohta et al., 1997). The culture of this species is dependent on the capture of wild elvers. In the last 50 years, over-exploitation has led to a critical decrease of the annual harvest of this species