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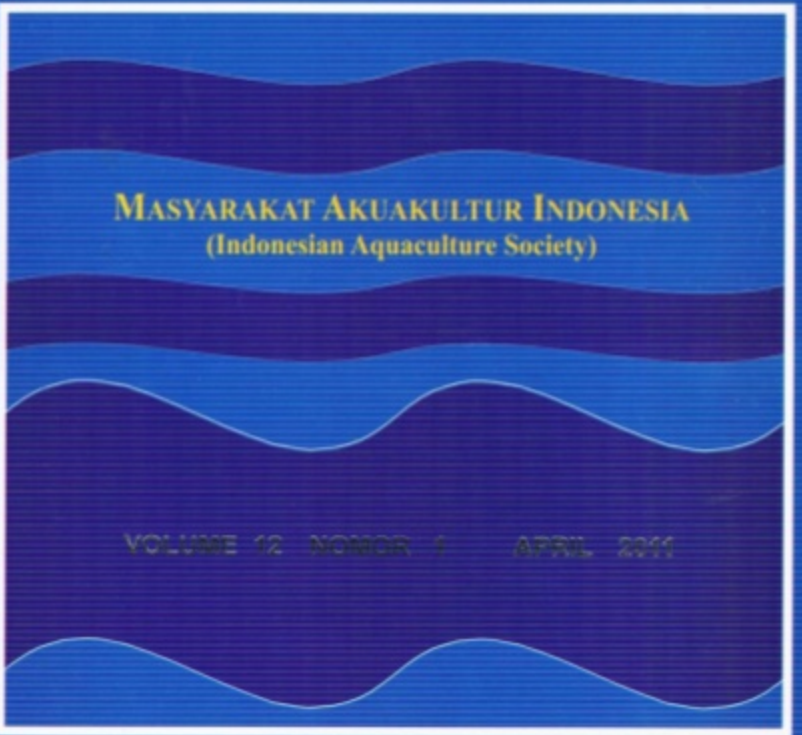

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The Intracellular Cryoprotectant Effects in Preserving Goramy Spermatozoa after Two Days Sub-Zero Freezing

Abinawanto[1], Nisa Fitrianingrum[1], Retno Lestari[1], Agung Sudaryono[2], Rita Rostika[3], and Yushinta Fujaya[4]

[1] Biology Graduate Study Program, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia
[2] Aquaculture Study Program, Diponegoro University, Semarang, Indonesia
[3] Faculty of Fisheries and Marine Science, Padjadjaran University, Bandung, Indonesia
[4]Faculty od Marine Science and Fisheries, Hasanudin University, Makassar, Indonesia
Correspondence to: abinawanto.ms@sci.ui.ac.id

Abstract

Abinawanto Abinawanto, Nisa Fitrianingrum, Retno Lestari, Agung Sudaryono, Rita Rostika, and Yushinta Fujaya. 2015. The Intracellular Cryoprotectant Effects in Preserving Goramy Spermatozoa after Two Days Sub-Zero Freezing, *Aquacultura Indonesiana, 16 (1): 16-21*. The spermatozoa quality of goramy after 2 d sub-zero freezing was examined. The quality of spermatozoa examined included motility, viability, and abnormality. We aimed to determine the optimum concentration of glycerol protecting spermatozoa during preservation. We used 0%, 1%, 3%, 5%, 7%, and 9% of glycerol, respectively. Sperms were diluted by the combination of glycerol and fish ringer (1 part of sperm + 3 part of solvent). The dilute sperms were then equiliberated at 4°C for 45 min, and were freezed at -34°C for 2 d. Thawing was then carried out at 30°C for 2 min. Based on Dunnet test, 5% of glycerol was the optimum concentration maintaining spermatozoa motility (75.95±4.76)%.

Keywords: Glycerol; *Osphronemus goramy;* Spermatozoa motility; Sub-zero freezing; Viability and Abnormality

Introduction

According to Sunarma *et al.* (2007), *Osphronemus goramy* is very important commodity of the local freshwater fish in Indonesia. However, goramy fish aquaculture is still traditionally, and cause declining in genetic material quality (Alam *et al.*, 2002). Accordingly, cryopreservation effort is needed as an alternative method for preserving the quality of genetic materials such as spermatozoa, ovum and embryos under low temperature condition (Fickle *et al.*, 2007). Research on cryopreservation of fish spermatozoa has been reported such as in salmon (Kusuda *et al.*, 2005) and catfish (Urbanyi *et al.*, 1999). Cryoprotectant and extender are needed during cryopreservation. Christensen and Tiersch (2005) were reported that glycerol, Dimethyl Sulphoxide (DMSO), Propilene Glycol, and methanol can be used as the cryoprotectant. While, fish ringer have been used as the extender (Park and Chapman, 2005). Horvarth and Urbanyi (2000) have administered glycerol and fish ringer to preserve spermatozoa of *Clarias gariepinus*. Whereas, Kyoung Ho Kang *et al.* (2004) have used 5%, 10%, 15%, and 20% of glycerol for preserving spermatozoa of *Thamnaconus septentrionalis*. On the other hand, Muchlisin *et al.* (2004) have studied the combination effect of 5%, 10%, and 15% of glycerol and fish ringer on *Mystus nemurus* spermatozoa under low temperature. Accordingly, the studies of cryopreservation using variation of cryoprotectant and extender are very important.

Studies of cryopreserved goramy spermatozoa have been reported. Dimethyl Sulfoxide (DMSO) has been used as the cryoprotectant for preserving goramy spermatozoa in liquid nitrogen for 24 h (Abinawanto *et al.*, 2011). Besides, sucrose could also be used as the extender and cryoprotectant (Abinawanto *et al.*, 2012[a]). It has been reported by Abinawanto *et al.* (2012[b]) that skim milk play an important role as the extracellular cryoprotectant. However, currently lack information is available on the glycerol effect as the intracellular cryoprotectant in preserving goramy spermatozoa after 2 d sub-zero freezing.

The aim of this study was to evaluate the intracellular cryoprotectant effects (motility, viability, and abnormality) at the optimum concentration of glycerol as the cryoprotectant.

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