Sains Indonesia Vol. 10, No.2 (2005) 11-13

The Cryoprotectant Effect of Dimethyl Formamide on Spermatozoa Quality of Carp Fish *Cyprinus carpio* race Majalaya 24 hours Post-Cryopreservation

Narista Pramandhani[1], Yanuarso Eddy Hedianto[2], Retno Lestari[1], Abinawanto[1]

[1]Dept. of Biology, FMIPA-UI, Kampus UI Depok 16424
[2]Environmental Engineering Lab., Puspiptek, BPPT Serpong
nawanto@ui.edu

Abstract

The effect of Dimethyl Formamide (DMF) as a cryoprotectant on spermatozoa quality of carp fish *Cyprinus carpio* race Majalaya 24 hours post-cryopreservation was studied. This study was conducted in the Environmental Engineering Laboratory, Puspiptek, Serpong. Sperm was collected by the hand stripping method and were immediately diluted with extender [6] and cryoprotectant (DMF). The final concentrations of DMF were 0, 3.75, 7.5, and 11.25%, respectively. Sperm were frozen in liquid nitrogen for 24 hours. All sperm were re-examined after thawing for motility, viability, and abnormality. The highest percentage of the motility and the viability of preserved sperm was showed by the DMF 7.5%. On the other hand the lowest percentage of the abnormality of preserved sperm was demonstrated by the DMF 7.5% also. Accordingly, DMF 7.5% is the optimum levels that could preserved spermatozoa still in a good quality 24 hours post-cryopreservation.

Keywords: Spermatozoa quality, Cyprinus carpio race Majalaya, cryopreservation, Dimethyl Formamide

1. INTRODUCTION

Indonesia is one of the megabiodiversity country in the world [1]. About 44 out of 360 species of the freshwater fishes are endemic [2]. Those of endemic (local) species are getting extinct. Accordingly, those of endemic species must be protected to avoid the extinction. The conservation effort can be carried out by in situ or ex-situ conservation (for example by cryopreservation) [3]. Cryopreservation is a process to keep genetic materials under the liquid nitrogen [4]. The expectation result of the cryopreserved materials are still performed good physiological function [4]. Cryopreservation has several benefits such as stock protection, a stable supply of sperm for optimal utilization in hatchery production and laboratory experiments, easy stock transportation, and improvement in selective breeding and gene transfer [4]. Some factors are involved in cryopreservation procedure, such as, cryoprotectant, extender, equilibration, and thawing [4]. Cryoprotectants are needed to protect the spermatozoa from cold and hot shock treatments, and also prevent cell dehydration during equilibration, freezing, and thawing [4]. Extenders are chemicals needed for sperm dilution to produce large volume of diluted sperm [4]. Carp fish, *Cyprinus carpio* race Majalaya is one of the local carp, widely cultured in Indonesia [5]. When breeding, the genealogy of individual fish is given much consideration, and pairs of fish are carefully matched [6]. However, it is often difficult to obtain a specific sexually mature male and female fish at the same time under favourable conditions for breeding [6]. This difficulty can be resolved by artificial fertilization using cryopreserved sperm [7]. The aim of this study was to evaluate the cryoprotectant effect of Dimethyl Formamide (DMF) on the sperm quality of carp fish *Cyprinus carpio* race Majalaya, 24 hours post-cryopreservation

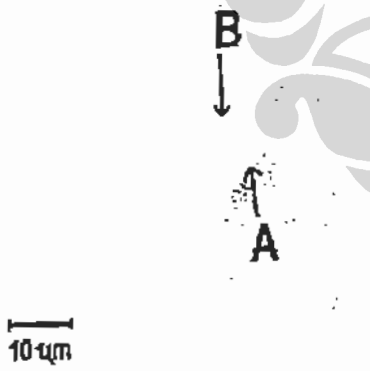
2. MATERIALS AND METHODS

Sperm was collected by the hand stripping method and was examined for motility, viability, and abnormality under the microscope. The samples were immediately diluted with extender [6] and cryoprotectant (DMF). The final concentrations of DMF were 0, 3.75, 7.5, and 11.25%, respectively. Straw-type polyethylene tubes were used as containers

for diluted sperm. Equilibration were carried out at 0-2° C for 60-180 min. Sperm were frozen by liquid nitrogen gas in a methanol-dry ice bath for 15 min. After freezing, all straws were placed in liquid nitrogen for 24 hours. All sperm were re-examined after thawing.

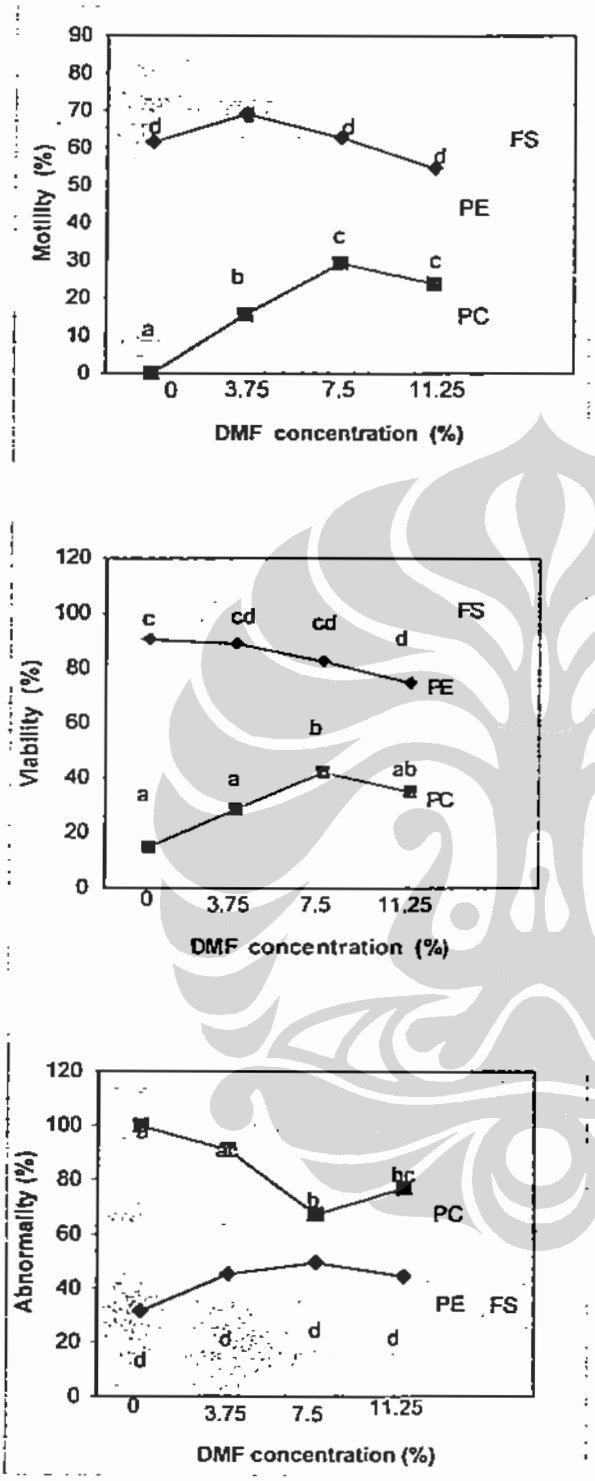
3. RESULTS AND DISCUSSION

Motility data showed that preserved sperm in DMF 0, 3.75, 7.5, and 11.25% at 24 hours post cryopreservation were 0, 15.57, 29.23, and 23.87%, respectively compared with 82.64% obtained with fresh sperm. The highest percentage of the motility of preserved sperm was showed by the DMF 7.5%. Further, the viability data was demonstrated by the preserved sperm in DMF 0, 3.75, 7.5, and 11.25% at 24 hours post-cryopreservation were 15.17, 28.33, 35.15, and 42.17%, respectively compared with 96.17% obtained with fresh sperm. The highest percentage level of the viability was also showed by the DMF 7.5%. On the other hand, abnormality data showed that preserved sperm in DMF 0, 3.75, 7.5, and 11.25% at 24 hours post-cryopreservation were 99.67, 90.83, 67.17, and 77%, respectively, compared with 21% obtained with fresh sperm. The lowest percentage level of the abnormality was demonstrated by the DMF 7.5%.



Spermatozoa viability
A. Non-viable spermatozoa
B. Viable spermatozoa

Morphology of spermatozoa
A. Normal spermatozoa;
B. Head abnormality (a category abnormality)
C. Tail abnormality (b category abnormality)
D. Head and tail abnormality (c category abnormality)



Percentage of spermatozoa motility, viability, and abnormality of carp fish at the post-equilibration (PE), post-cryopreservation (PC), and under fresh sperm (FS) condition.

4. CONCLUSIONS

DMF 7.5% is the optimum levels that could preserved sperm of the carp fish *Cyprinus carpio* race Majalaya 24 hours post-cryopreservation

ACKNOWLEDGMENTS

We would like to thanks to Dr. Yanuarso Eddy Hedianto for providing a grant. Special thank to staff at the Environmental Engineering Lab., Puspiptek – BPPT, Serpong for their encouragement and assistance.

REFERENCES

[1] World Conservation Monitoring Centre, Global Biodiversity: Status of The earth's living resources, Chapman & Hall, London. (1992). xviii + 585 pp

[2] B.G.M. Jamieson, Fish Evolution and systematics: Evidence from spermatozoa, Cambridge University Press, Cambridge, (1991), xiv + 319 pp.

[3] J.H. Gibbons. Technologies to maintain biological diversities. Congress of the United States. Washington, (1987), vii + 398 pp.

[4] J. Stoss, Fish gamete preservation and spermatozoa physiology, *In:* W.S. Hoar, D.J. Randall, and E.M. Donaldson (eds), Fish physiology 9B, Academic Press, Orlando, (1983), 303-339.

[5] Direktorat Jenderal Perikanan, Pengembangan prasarana perikanan dalam Bangkajang II, *Dalam: Prosiding Forum II Perikanan*, Puslitbang Perikanan, Jakarta, (1992) 231-235

[6] H. Kurokura, R. Hirano, M. Tomita, M. Iwahashi, Cryopreservation of carp sperm, Aquaculture 37 (1984) 267-273

[7] G.J. Steyn and H.J. Van Vuren, The fertilizing capacity of cryopreserved sharptooth (*Clarias gariepinus*) sperm, Aquaculture 63, (1987), 187-193