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# Cryopreservation of Java Barb (*Barbonymus gonionotus*) Using Egg Yolk as a Cryoprotectant

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**Abstract:** The aim of this study was to inversitage the effect of egg yolk as a cryoprotectant on spermatozoa quality of *Barbonymus* gonionotus, twenty four hours after sub-zero freezing. The ejaculates from a total of three males were diluted with the glucose-base extender + 10% methanol + egg yolk. Egg yolk concentration which was used in this study were: 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, respectively. Samples were then equilibrated at 4°C for 10 minutes and were freezed at -34°C for 24 hours. Thawing was carried out at 40°C for 30 seconds. Based on Anova test, there were significant effect ($P<0.01$) of various concentration of egg yolk on post-thaw sperm motility and viability, but not on post-thaw abnormality, compared to control (0% of egg yolk). According to the Tukey test, the concentration of 15% of egg yolk showed significant difference ($P<0.01$) on post-thaw motility and viability, respectively. Fifteen percent of egg yolk showed the highest post-thaw sperm motility (96.10±3.31) and sperm viability (85.50±3.11), respectively.

**Key words:** *Barbonymus gonionotus* · Cryopreservation · Egg Yolk · Spermatozoa

## INTRODUCTION

Indonesia is one of the two mega biodiversity countries in the world, besides Brazil. About 44 out of 360 species of freshwater fish are endemic in Indonesia [1]. Indonesian *Barbonymus gonionotus* is an indigenous species in Indonesian fresh water which also has very important economic value [2]. Those of endemic (local) species are getting extinct if exploited in uncontrollable. One out of two strategies in order to protect is by *ex situ* conservation (cryopreservation). Cryopreservation is a process to maintain genetic material in subzero freezing. The successful cryopreservation influenced by cryoprotectant and extender. Some methodologies, development and application of cryopreservation of fish spermatozoa were reported for species: *Osphronemus goramy* [3-5], *Barbonymus gonionotus* [6], carp [7-9], rainbow trout [10] and other salmonids [11]. The objective of present study is to investigate the effect of egg yolk in various concentration of 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, respectively, on spermatozoa quality of *Barbonymus gonionotus* Bleeker, 1850 cryopreserved for 24 hours.

## MATERIALS AND METHODS

### Materials

**Collection of Ejaculated Semen:** Mature male of *Barbonymus gonionotus* obtained from a private commercial hatchery were brought into laboratory. The ejaculates from a total of four males were collected by hand stripping.

**Semen Dilution:** The ejaculated semen were diluted with the glucose-base extender + 10% methanol according to Jodun *et al.*[12]. Egg yolk concentration used in this study were: 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, respectively.

**Equilibration and Freezing:** Samples were then equilibrated at 4°C for 10 minutes and were freezed at -34°C for 24 hours.

**Post-Thaw Parameters Examined:** Thawing was carried out at 40°C for 30 seconds. After thawing, each sample was then evaluated for the following parameters using a light microscope with the aid of a digital eye-piece

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connected to the computer (image driving software; Scopephoto 2.0.4): the percentage of spermatozoa motility, viability and abnormality. Some physical and chemical characteristics were also observed, such as: semen) color, volume and pH.

## RESULTS

Fresh semen were milky white, pH 8.2 and 0.55—0.96 ml of volume per ejaculate.. The viable or motile sperm showed green color (transparent) on the sperm head, while the non-viable sperm showed pink or red color on the sperm head (data not shown). Many variations of abnormal spermatozoa were shown such as big head, microcephalus, rolling tail and short tail (data not shown). The percentage of sperm motility, viability and abnormality of fresh semen were: 91.66%, 82.75% and 16%, respectively (Table 1). While post-thaw spermatozoa motiliy in control (0%) and in various egg yolk concentration of 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, were: 80.20±2.34, 86.08±3.90, 88.33±1.98, 93.25±1.29, 92.63±1.17, 93.22±4.31, 96.10±3.31 and 91.33±2.63, respectively. Post-thaw spermatozoa viability in control (0%) and various egg yolk concentration of 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, were: 68.50±4.80, 75.00±10.23, 69.25±3.30, 75.75±2.63, 80.25±1.71, 73.25±3.40, 85.50±3.11, 78.25±5.74, respectively. On the other hand, post-thaw spermatozoa abnormality in control (0%) and various egg yolk concentration of 0%, 5%, 7%, 9%, 11%, 13%, 15% and 17%, were: 23.00±2.16, 17.25±3.50, 15.75±5.44, 17.50±3.11, 21.00±3.56, 14.00±4.55, 16.00±2.94, 16.25±4.11, respectively. All of the percentage of spermatozoa motility, viability, abnormality and spermatozoa concentration were shown in Table 2. Based on Anova test, there were significant effect ($P<0.01$) of various concentration of egg yolk on post-thaw sperm motility, viability and abnormality, compared to control (0% of egg yolk). According to the Tukey test, the concentration of 15%% of egg yolk showed significant difference ($P<0.01$) on post-thaw motility, viability and, abnormality, respectively.

## DISCUSSION

The effect of 15% of egg yolk-glucose + 10% methanol on the percentage of spermatozoa motility 24 hours post-cryopreservation was higher (96.10%) than our previous study using 6% of glucose + 10% of methanol (87.68%) [13], or using 20% of skim milk + 5% of methanol ( 83.23%) [6] and also was higher than those observed in other fish species such as *Osphronemus goramy* [3-5], *Brachydanio rerio* (51%) [14], *Oreochromis mossambicus* (70%) [10], tilapian's fish (40-80%) [15], *Cyprinus carpio* (55%) [16] and *Osteochiius hasseltii* (63.33%) [2]. Cryoprotectant and extender are two out of some factors cause the differences of spermatozoa quality after sub-zero freezing, such as 5% of methanol + 15% of skim milk [10,15, 17], 5% of methanol + 20% of skim milk [6], 13% DMSO + 189M extender [3] and 15% of DMSO [2, 18]. This study provides an effective concentration

Table 1: Fresh semen (spermatozoa) profile

| | Physical-Chemical Characteristics | | | Microscopically analysis | | |
|---|---|---|---|---|---|---|
| n | Volume (ml) | pH | Color | Motility (%) | Viability (%) | Abnormality (%) |
| 1 | 1.09 | 7.9 | Milky white | 97.20 | 88 | 19 |
| 2 | 0.93 | 7.9 | Milky white | 91.00 | 81 | 17 |
| 3 | 0.87 | 7.9 | Milky white | 83.81 | 76 | 13 |
| 4 | 0.96 | 7.9 | Milky white | 94.63 | 86 | 15 |
| avg | 0.96 | 7.9 | Milky white | 91.66 | 82.75 | 16.00 |
| stdev | 0.09 | - | | 5.82 | 5.38 | 2.58 |

Table 2: Post-thaw and fresh spermatozoa quality

| Egg Yolk (%) | Motility (%) | Viability (%) | Abnormality (%) |
|---|---|---|---|
| 0 | 80.20±2.34a | 68.50±4.80a | 23.00±2.16a |
| 5 | 86.08±3.90a | 75.00±10.23a | 17.25±3.50a |
| 7 | 88.33±1.98b | 69.25±3.30ab | 15.75±5.44a |
| 9 | 93.25±1.29b | 75.75±2.63a | 17.50±3.11a |
| 11 | 92.63±1.17b | 80.25±1.71a | 21.00±3.56a |
| 13 | 93.22±4.31b | 73.25±3.40a | 14.00±4.55a |
| 15 | 96.10±3.31b | 85.50±3.11b | 16.00±2.94a |
| 17 | 91.33±2.63b | 78.25±5.74a | 16.25±4.11a |

Different letters in each column show significant difference ($P<0.01$)

between cryopreserved sperm. The combination of 15% of egg yolk-glucose + 10% methanol was also maintained the percentage of spermatozoa viability (85.50%). This result (spermatozoa viability) was higher than our previous finding using 6% of glucose + 10% of methanol (77.25%) [13] or using 20% of skim milk + 5% of methanol (81.75%) [6]. Besides, the viability was also higher compared to other species like, *Osphronemus goramy* (63.5%) [3], 82.17% [4],84%;[5], as well as *Cyprinus carpio* (20%) [7] and (58%) [18]. Furthermore, the combination of 15% Egg yolk-glucose + 10% methanol was also maintained the percentage of spermatozoa abnormality (16%). Those of abnormality was lower than our previous study using 6% of glucose + 10% of methanol (45%) [13] as well as using 20% of skim milk + 5% of methanol (26.25%) [6]. The spermatozoa abnormality in this study (16%) was also lower compared to other species like, *Osphronemus goramy* (29%) [3]. But, relatively higher compared to another our previous study using 0.5% of sucrose + 10% of methanol which was shown 12.5% of abnormality [4] or 14% of abnormality if using 15% skim milk-fish ringer + 10% methanol [5]. Thawing procedures at 30°C for 30 sec was effective for 2 ml of cryogenic tubes. We choose 30°C because this is easy to achieve using heating devices in our temperature conditions. Methanol as an internal cryoprotectant significantly improved motility of cryopreserved sperm. Methanol was employed as successful internal cryoprotectant in *Brachydanio rerio* [17]; *Oreochromis mossambicus* [8]; tilapian's fish [15]; *C. carpio* [9]; *Osphronemus goramy* [3-5]; *Barbonymus gonionotus* [6, 13]. It was also demonstrated that the protocol of sperm cryopreservation of the carp species is applicable for *Barbonymus gonionotus* (Java barb) species, although this is the first protocol for the Java barb species evaluated in this study.

## CONCLUSIONS

The combination of 15% of egg yolk + 10% methanol showed the highest post-thaw sperm motility (96.10±3.31)% and post-thaw sperm viability (85.50±3.11)% and the lowest post-thaw abnormality (16.00±2.94)%.

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## REFERENCES

1. World Conservation Monitoring Centre, 1992. Global biodiversity: Status of the earth's living resources, Chapman & Hall, London.
2. Sunarma, A., D.W. Hastuti, Y. andSistina, 2007. Utilization of Honey asan extender combined to differentcryoprotectant on sperm cryopreservation of nilem (Indonesian Sharkminnow, Osteochiius hasseltii Valenciennes, 1842), Indonesian Aquacult. Conf, pp: 1-9.
3. Abinawanto, M. D. Bayu, R. Lestari and A. Sunarma, 2011. Spermatozoa quality of goramy fish, Osphronemus goramy Lacepede, 1801 twenty four hours post-cryopreservation: The role of dimethyl sulfoxide (DMSO) as a cryoprotectant. Biota, 16: 10-15.
4. Abinawanto K. Nurman and R. Lestari, 2012a. The effect of Sucrose on sperm quality of Osphronemus goramy two daysPost-cryopreservation. J. Agricult. Sci. Tech. B, 2: 204-207.
5. Abinawanto I. Anindita and R. Lestari, 2012b. Cryopreservation of spermatozoa of Osphronemus goramy fish using skim milk. J. Eng. Innov. Tech., 2(5): 3.
6. Zuraida, 2009. The combination effectof 5% of methanol and Skim milk in various concentration on spermatozoa quality of Java Barb (Barbonymus gonionotus, Bleeker 1850), 24hours post-cryopreservation. Undergraduate thesis. University of Indonesia.
7. Withler, F.C., 1982. Cryopreservation of spermatozoa of some freshwater fishes cultured in South and Southeast Asia. Aquacult., 26: 395-398.
8. Harvey, B., 1983. Cryopreservation of Sarotherodon mossambicus spermatozoa. Aquacult., 32: 313-320.
9. Horvath, A., E. Miskolczi and B. Urbanyi, 2003. Cryopreservation of common carp sperm.. Aquatic Liv. Resourc., 16: 457-460.
10. Stoss, J. and E.M. Donaldson, 1983. Studies on cryopreservation of eggs from rainbow trout (Salmo gairdneri) and coho salmon (Onchorchynchus kisutch). Aquacult., 31: 51-65.
11. Harvey, B. and M.J. Ashwood-Smith, 1982. Cryoprotectant penetration and supercooling in the eggs of salmonid fishes. Cryobiology, 19: 29-40.
12. Jodun, W.A., K. King and P. Farrell, 2006. Methanol and egg yolk ascryoprotectants for atlantic salmonspermatozoa. North America J. Aquacult., 69: 36-40.

13. Fadhillah, 2009. The effect of glucose in various concentrations on spermquality of Barbonymus gonionotus (Bleeker, 1850) 24 hourspostcryopreservation. Undergraduatethesis. University of Indonesia.
14. He, S. and C. Woods, 2003. The effect of osmolality, cryoprotectant and equlibration time on striped bassMorone saxatilis sperm motility, J. of the World Aquacult. Soc., 34(3): 255-265.
15. Chao, N.H., W.C. Chao, K.C. Liuand and I.C. Liao, 1987. The properties of tilapia sperm and its cryopreservation J. Fish Biol., 30(2): 107-118.
16. Akcay. E., Y. Bozkurt, S. Secer and N. Tekun, 2004. Cryopreservation of mirror carp semen. Turkey J. Vet. Anim. Sci., 28: 837-843.
17. Harvey, B., R.N. Kelley and M.J. Ashwood-Smith, 1982. Cryopreservation of zebra fish Spermatozoa using methanol. Can. J. Zool., 60: 1860-1870.
18. Horton, H.F. and A.G. Otto, 1976. Cryopreservation of fish spermatozoa and ova. J. Fish Resourc. Board. Can., 33: 995-1000.