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Spermatozoa Quality of Goramy Fish, *Osphronemus goramy* Lacepede, 1801, Twenty Four Hours Post-cryopreservation: The Role of Dimethyl Sulfoxide (DMSO) as a Cryoprotectant

Peranan Dimetil Sulfoksida sebagai Krioprotektan dalam Mempertahankan Kualitas Spermatozoa Ikan Gurami, *Osphronemus goramy* Lacepede, 1801, Dua Puluh Empat Jam Pascakriopreservasi

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Abstract

The objective of the study was to find the optimum concentration of dimethyl sulfoxide (DMSO) among 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively, on sperm quality of *Osphronemus goramy* Lacepede, 1801, twenty four hours post-cryopreservation. Sperm was collected by hand-stripping method, and was put on 2ml of cryotube, and was then diluted by combination of DMSO, and 189M extender. The ratio of sperm and diluent was 1 : 4 according to Horton & Otto (1976). Sample (sperm + diluent) was equlibrated at 4°C for 45 minutes, and was vaporated for 10 minutes, and was then frozen in Liquid Nitrogen for 24 hours. Thawing was carried out at 30°C for 30 seconds. According to Tukey test (P>0.05), 13% of DMSO was showed the highest post-thawed sperm motility (68,58%) and sperm viability (63,5%), and also was showed the lowest post-thawed sperm abnormality (29%), respectively.

Key words: DMSO, 189M extender, cryopreservation, goramy, sperm quality

Abstrak

Penelitian dilakukan dengan tujuan mengetahui pengaruh berbegai konsentrasi DMSO (0%, 5%, 7%, 10%, 13%, 15%, dan 17%) sebagai krioprotektan terhadap kualitas spermatozoa ikan gurami, *Osphronemus goramy* Lacepede, 1801, dua puluh empat jam pascakriopreservasi. Penelitian dilakukan di Balai Besar Pengembangan Budidaya Air Tawar (BBPBAT), Sukabumi. Semen dikoleksi dengan cara pengurutan (*stripping*) dan dievaluasi secara makroskopis (warna, pH, dan volume) dan mikroskopis (persentase motilitas, viabilitas, abnormalitas, dan konsentrasi spermatozoa) baik sebelum maupun sesudah kriopreservasi. Semen diencerkan dengan pengencer yang mengandung ekstender 189M dan krioprotektan DMSO (0%, 5%, 7%, 10%, 13%, 15%, dan 17%), disimpan pada *cryotube* 2 ml dan dikriopreservasi selama 24 jam. Peningkatan konsentrasi DMSO memengaruhi kualitas spermatozoa ikan gurami pascakriopresevasi. Konsentrasi DMSO yang terbaik berdasarkan hasil pengamatan adalah DMSO konsentrasi 13% (P > 0,05) dengan persentase motilitas sebesar 68,58%; abnormalitas sebesar 29%; dan viabilitas sebesar 63,5%.

Kata kunci: DMSO, ekstender 189M, kriopreservasi, ikan gurami, kualitas spermatozoa

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Introduction

Indonesia is one of the two megabiodiversity countries in the world, besides Brazil. About 44 out of 360 species of fresh water fish are endemic in Indonesia (World Conservation Monitoring Centre, 1992). Indonesian giant goramy (*Osphronemus goramy*, Lacepede 1801) is an indigenous species in Indonesian fresh water which also has a very important economic value (Sunarma *et al*., 2007). Those of local (endemic) species

are getting extinct if exploited in uncontrollable. There were two strategies in order to protect those of local (endemic) species, either by *in situ* (re-stocking) or by *ex situ* conservation (cryopreservation). Cryopreservation is a process to maintain genetic material in subzero freezing. The expected result of the cryo- preserved materials was still performed in good physiological function. The successful of cryopreservation were influenced by cryo-protectant and extender. The methodologies, development and application of cryopreservation of fish spermatozoa were reported for some of the species: carps (Withler, 1982; Harvey, 1983; Horvath *et al*., 2003), rainbow trouts (Stoss and Donaldson, 1983) and other salmonids (Harvey and Ashwood-Smith, 1982). This communication presents the effect of dimethyl sulfoxide (DMSO) in various concentration of 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively, on sperm quality of *Osphronemus goramy* Lacepede, 1801 cryopreserved for 24 hours.

Materials and Methods

Collection of Ejaculated Semen

Mature male goramies obtained from a private commercial hatchery were brought into laboratory. The ejaculates from a total of six male goramies were collected by hand stripping, 12−15 hours after injected intra-muscularly with Ovaprim at a single-dose of 0.2 ml/kg body weight according to modification method of Sunarma *et al*., (2007).

Semen Dilution

The ejaculated semen were diluted with the solvent (189M extender + dimethyl sulfoxide (DMSO) in various concentration of 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively ; 1 : 4) according to Withler and Morley (*see* Horton and Otto, 1976) and modification method of Sunarma *et al*., (2007).

Equilibration and Freezing

Samples were stored in 2 ml cryogenic tube, equilibrated at temperature 4°C for 45 minutes according to the modification method of He and Woods (2003). Samples were then vaporized by putting the cryogenic tubes 3 cm from the surface of Liquid Nitrogen, and then were frozen (cryo-preseved) in Liquid Nitrogen for 24 hours.

Post-thawed Parameters Examination

After thawing by immersing the cryogenic tubes in a waterbath at 30°C for 30 sec., each sample was then evaluated for the following parameters using a light microscope with an aid of a digital eye-piece connected to the computer (image driving software; Scopephoto 2.0.4): the percentage of spermatozoa motility, viability, abnormality, and the spermatozoa per mililiter ejaculate (spermatozoa concentration). Some physical and chemical characteristics were also observed, such as: semen color, volume, and pH.

Results and Discussion

Fresh semen were milky white, pH 8−8.5 and 0.7−1.5 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. Most of the abnormal sperm had bigger head. The percentage of spermatozoa motility, viability, abnormality, and spermatozoa concentration of fresh semen were: 82.4 ± 4.93%, 64.5 ± 10.15%, 34.75 ± 9.32%, and (8.51 ± 3.76) x 10^{10} cell/ml, respectively (Table 1). Post-thawed spermatozoa motility in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: 31.55 ± 10.1%, 55.13 ± 8.71%, 62 ± 15.60%, 47.625 ± 3.87%, 68.58 ± 17.98%, 62.83 ± 15.06% and 68.4 ± 7.67%, respectively. Post-thawed spermatozoa viability in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: 27.25 ± 13.96%, 58.5 ± 10.85%, 41 ± 5.23%, 55 ± 6.28%, 63.5 ± 4.93%, 47.5 ± 3.87% and 51.5 ± 12.58%, respectively. On the other hand, post-thawed spermatozoa abnormality in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were:

39.25 ± 5.19%, 44 ± 7.44%, 45.75 ± 14.73%, 30.75 ± 5.91%, 29 ± 2.31%, 41.25 ± 6.45%, and 43.25 ± 8.46%, respectively. While post-thawed spermatozoa concentration in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: (3.01 ± 1.12) x 10^8 cell/ml, (2.83 ± 1.36) x 10^8 cell/ml, (4.24 ± 0.70) x 10^8 cell/ml, (57.56 ± 1.23) x 10^8 cell/ml, (58.64 ± 2.10) x 10^8 cell/ml, (5.59 ± 2.27) x 10^8 cell/ml, and (54.7 ± 2.19) x 10^8 cell/ml respectively. All of the percentage of spermatozoa motility, viability, abnormality, and spermatozoa concentration were shown in Table 2. According to the Kruskal-Wallis test, the highest post-thawed spermatozoa motility was 13% of DMSO (Fig. 1), while the lowest one was 0% of DMSO (control). Most treatment groups (5%, 7%, 10%, 13%, 15%, and 17% of DMSO) were relatively higher and significantly different compared to the control. The highest post-thawed spermatozoa viability was 13% of DMSO (Fig. 2). Post-thawed spermatozoa viability was relatively higher and significantly different also compared to the control. On the other hand, the lowest post-thawed spermatozoa abnormality was 13% of DMSO (Fig. 3). While another treatment groups (5%, 7%, 10%, 15%, and 17% of DMSO) were not significantly different in post-thawed spermatozoa abnormality compared to the control. Accordingly, 13% of DMSO was the optimum concentration that could protect spermatozoa during cryopreservation for 24 hours.

The effect of 13% DMSO on the percentage of spermatozoa motility of goramy 24 hours post-cryopreservation (68.58%) was higher than those observed in other fish species such as *Cyprinus carpio* (55%, Akcay *et al.* 2004), *Osteochiius hasseltii* (63.33%, Sunarma *et al*., 2007). The difference related to DMSO was used (15% of DMSO) as previously discussed by several authors (Akcay *et al.* 2004; Sunarma *et al*., 2007). The obtained concentration in this study provides an effective comparison between cryopreserved sperm (Akcay *et al*., 2004; Sunarma *et al*., 2007). The combination of 13% DMSO and extender 189M also maintained the percentage of spermatozoa viability (63.5%) and that of spermatozoa abnormality (29%), 24 hours postcryopreservation. This result (spermatozoa viability) was higher compared to *Cyprinus carpio* (20% of, Withler, 1982; 58%, Withler and Morley *see* Horton and Otto 1976). Thawing procedures at 40°C for 30 s was effective for 2 ml cryogenic tubes. We choose 40°C because this is easy to achieve using heating devices in our temperature condition.

DMSO as an internal cryoprotectant significantly improved motility of cryopreserved sperm. DMSO was employed as successful internal cryoprotectant in cypriniforms (*C. carpio* – Horváth *et al*., 2003). It was also demonstrated that the protocol of sperm cryopreservation of the carp species is applicable for goramy species, although this is the first protocol for the goramy species evaluated in this study.

Table 1. Fresh semen (sperm) profile.

n	Physical-Chemical Characteristics			Microscopically Analysis				Remarks
	Vol. (ml)	pH	Color	Motility (%)	Viability (%)	Abnormality (%)	Conc. (10^{10})	
1	0.8	8	Milky white	87.2	58	47	5.41	Semen were pooled from 6 males.
2	0.7	8	Milky white	84.6	57	37	5.16	
3	0.8	8.5	Milky white	82.1	79	28	11.17	Semen volume range: 0.1−0.2 ml/male
4	1.5	8	Milky white	75.7	64	27	12.29	n = replication
			X (avg)	82.4	64.5	34.75	8.51	
			SD	4.93	10.15	9.32	3.76	

Table 2. Post-thawed spermatozoa quality.

DMSO	Motility (%)	Viability (%)	Abnormality (%)
0%	31.65 ± 10.1	27.25 ± 13.96	39.25 ± 5.19
5%	55.13 ± 8.71	44 ± 7.44	58.5 ± 10.85
7%	62 ± 15.6	41 ± 5.23	45.75 ± 14.73
10%	47.63 ± 3.87	30.75 ± 5.91	55 ± 6.28
13%	68.58 ± 17.98	63.5 ± 4.93	29± 4.93
15%	62.83 ± 15.06	41.25 ± 6.45	47.5 ± 3.87
17%	68.4 ± 7.67	43.25 ± 8.46	51.5 ± 12.58

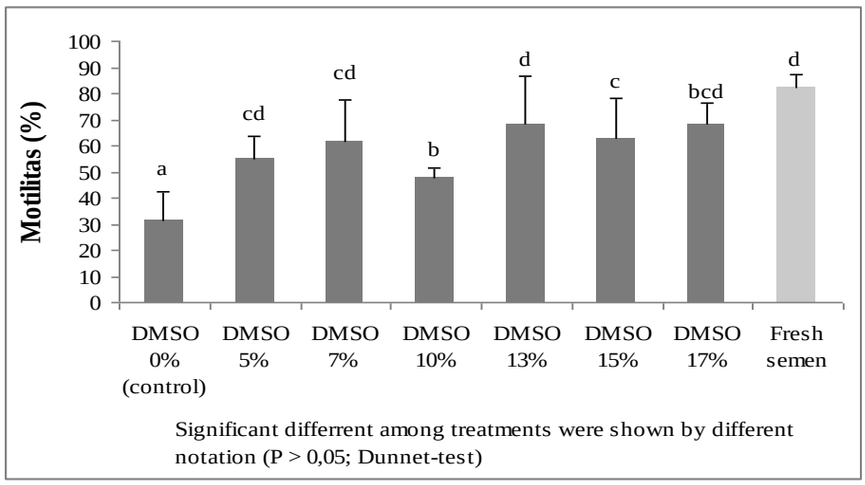

Figure 1. Histogram of Post-thawed spermatozoa motility.

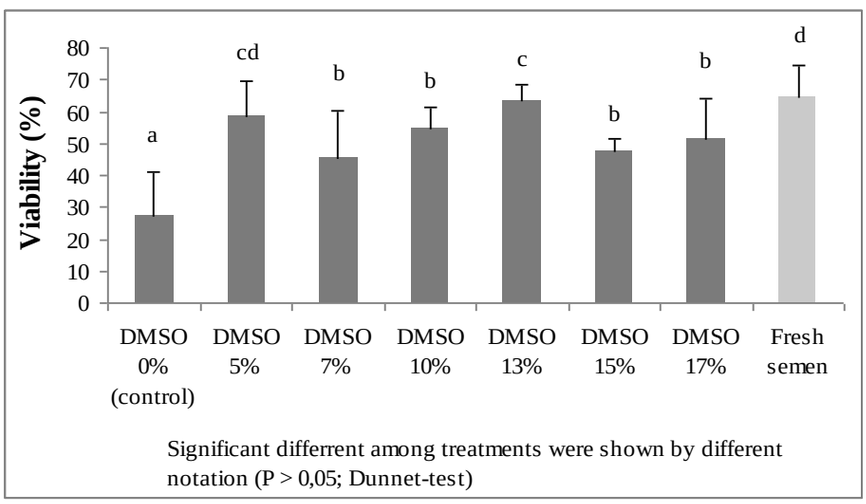

Figure 2. Histogram of Post-thawed spermatozoa viability

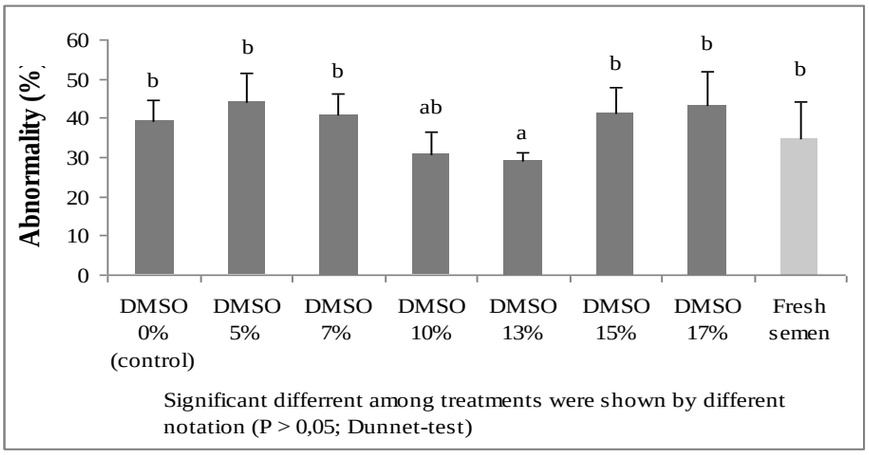

Figure 3. Histogram of Post-thawed spermatozoa abnormality.

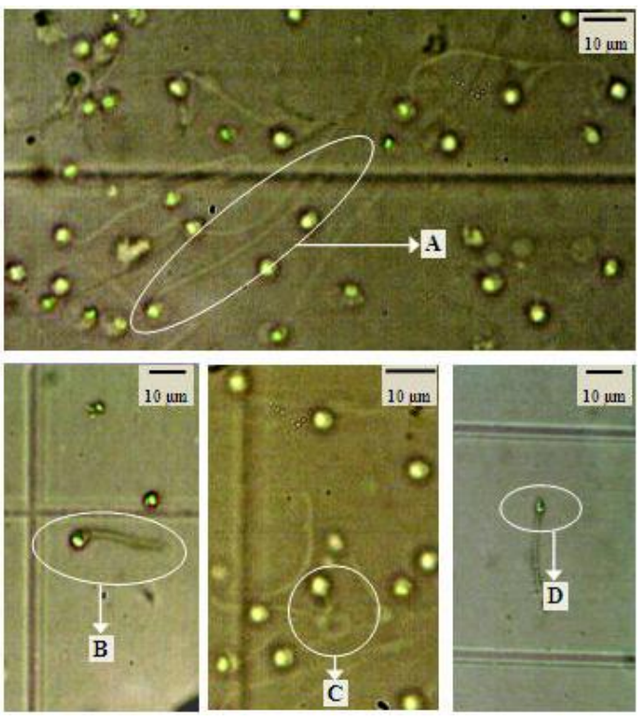

Fig. 4. Spermatozoa morphology.
(A. Normal spermatozoa; B. short tail spermatozoa; C. rolling tail spermatozoa; D. microcephalus).

Conclusion

It is concluded that 13% of DMSO is the optimum concentration as a cryoprotectant that can maintain spermatozoa motility (68.58%) and viability (63.5%), respectively, and protect spermatozoa abnormality (29%).

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