

---

## The effect if sucrose on sperm quality of *Osphronemus goramy* two days post-cryopreservation

Abi Abinawanto , Khairani Nurman and Retno Lestari

Genetics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok 16424

### Abstract

Our previous study showed the optimum concentration of DMSO combined with 189M of extender to maintain spermatozoa quality of *Osphronemus goramy*, 24 hours post- cryopreservation. The present study was conducted to investigate the effect of various concentrations of sucrose on sperm quality of *Osphronemus goramy* 48 hours post- cryopreservation. Mature males *Osphronemus goramy* obtained from a private commercial hatchery were brought into laboratory. The ejaculates from a total of three males were collected by hand stripping. The ejaculated semen were diluted with the solvent (sucrose-fish ringer + 10% methanol; 1: 9). Sucrose concentrations which are used in this study were: 0%, 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, respectively. Samples were then equilibrated at 4°C for 10 minutes, and were freezed at -34°C for 48 hours. Thawing was carried out at 40°C for 1–2 min. Based on Anova test, there were significant effect ( $P < 0.05$ ) of various concentrations of sucrose on post-thawed sperm motility, viability, and abnormality, compared to control (0% of sucrose). According to the Tukey test, the concentration of 0.5% of sucrose showed significant difference ( $P < 0.01$ ) on post-thawed motility, viability, and abnormality, respectively. Zero point five percent of sucrose showed the highest post-thawed sperm motility ( $81.62 \pm 4.19$  %), and post-thawed sperm viability ( $82.17 \pm 2.56$  %), and the lowest post-thawed abnormality ( $12.5 \pm 1.52$  %). Our finding showed the optimum concentration of sucrose as extender.

**Keywords:** Cryopreservation, *Osphronemus goramy*, sperm quality, sucrose

### Introduction

Indonesian giant goramy (*Osphronemus goramy*, Lacepede 1801) is one out of 366 endemic fresh water species in Indonesia

(World Conservation Monitoring Centre, 1992) which also has very important economic value (Sunarma *et al.*, 2007). Accordingly, those of

local (endemic) species are getting extinct if exploited uncontrollably. The protection those of local (endemic) species, can be carried out either by *in situ* (re-stocking) or by *ex situ* conservation (cryopreservation). Cryopreservation is a process to maintain genetic material in subzero freezing. The successful of cryopreservation were influenced by cryoprotectant and extender. Some methodologies, development and application of cryopreservation of fish spermatozoa were reported for some species: carp (Withler, 1982; Harvey, 1983; Horvath *et al.*, 2003), rainbow trout (Stoss and Donaldson, 1983) and other salmonids (Harvey and Ashwood-Smith, 1982). The objective of present study was to investigate the effect of sucrose in various concentrations of 0%, 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, respectively, on sperm quality of *Osphronemus goramy* Lacepede, 1801 cryopreserved for 48 hours.

## Material and Methods

### Collection of ejaculated semen

Mature male goramies were obtained from a private commercial hatchery and were brought into laboratory. The ejaculates from a total of 24 male goramies were collected by hand stripping, 12–15 hours after injected intramuscularly with Ovaprim at a 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 (sucrose-fish ringer + 10% methanol; 1: 9) according to Horvath *et al.* (2003). Sucrose concentrations which were used in this study were: 0%, 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, respectively.

### Equilibration and freezing

Samples were then equilibrated at 4°C for 10 minutes, and were freezed at -34°C for 48 hours.

### Post-thawed parameters examined

After thawing by immersing the cryogenic tubes in a water-bath at 40°C for 1–2 min., each sample was evaluated for the following parameters using a light microscope by a digital eye-piece 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 (sperm) color, volume, and pH.

## Results and Discussion

Fresh semen were milky white, pH 8–8.1, and 0.33–0.75 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 spermatozoa motility, viability, abnormality, and spermatozoa concentrations of fresh semen were:  $75.89 \pm 3.65\%$ ,  $83.17 \pm 3.82\%$ ,  $13.33 \pm 2.58\%$ , respectively (Table 1). While post-thawed spermatozoa motility (Table 2) in control (0%) and in various sucrose concentrations of 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, were:  $57.43 \pm 3.68\%$ ,  $65.66 \pm 5.18\%$ ,  $70.42 \pm 4.47\%$ ,  $81.62 \pm 4.19\%$ ,  $78.74 \pm 3.61\%$ , and  $71.11 \pm 3.91\%$ , respectively. Post-thawed spermatozoa viability (Table 2) in control (0%) and various sucrose concentrations of 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, were:  $72.33 \pm 6.06\%$ ,  $74.33 \pm 5.54\%$ ,  $75.50 \pm 4.23\%$ ,  $82.17 \pm 2.56\%$ ,  $81.17 \pm 3.49\%$ , and  $74.17 \pm 5.85\%$ , respectively. On

the other hand, post-thawed spermatozoa abnormality (Table 2) in control (0%) and in various sucrose concentrations of 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%, were:  $19.50 \pm 3.39\%$ ,  $17.67 \pm 2.25\%$ ,  $15.17 \pm 1.47\%$ ,  $12.50 \pm 1.52\%$ ,  $16.17 \pm 2.14\%$ , and  $18.50 \pm 3.21\%$ , respectively. All of the percentages of spermatozoa motility, viability, abnormality, and spermatozoa concentrations are shown in Table 2. Based on Anova test, there were significant effect ( $P < 0.05$ ) of various concentrations of sucrose on post-thawed sperm motility, viability, and abnormality, compared to control (0% of sucrose). According to the Tukey test, the concentration of 0.5% of sucrose showed significant difference ( $P < 0.01$ ) on post-thawed motility, viability, and abnormality, respectively.

**Table 1. Fresh semen (spermatozoa) profile**

Physical-Chemical Characteristics			Microscopically analysis		
Volume (ml)	pH	Color	Motility (%)	Viability (%)	Abnormality (%)
0.45	8	Milky white	71.71	81	17
0.33	8	Milky white	71.48	77	15
0.45	8	Milky white	78.08	83	13
0.6	8.1	Milky white	79.21	84	14
0.75	8	Milky white	75.3	87	10
0.67	8.1	Milky white	79.58	87	11
<b>Mean</b>	0.54	8	75.89	83.17	13.33
<b>(±S.D)</b>	(± 0.16)	(±0.05)	(±3.65)	(±3.82)	(±2.58)

**Table 2. Post-thawed and fresh spermatozoa quality**

Sucrose (%)	Motility (%)	Viability (%)	Abnormality (%)
0	57.43 ± 3.68 <b>a</b>	72.33 ± 6.06 <b>a</b>	19.50 ± 3.39 <b>b</b>
0.1	65.66 ± 5.18 <b>b</b>	74.33 ± 5.54 <b>ab</b>	17.67 ± 2.25 <b>bc</b>
0.3	70.42 ± 4.47 <b>b</b>	75.50 ± 4.23 <b>ab</b>	15.17 ± 1.47 <b>ac</b>
0.5	81.62 ± 4.19 <b>c</b>	82.17 ± 2.56 <b>b</b>	12.50 ± 1.52 <b>a</b>
0.7	78.74 ± 3.61 <b>c</b>	81.17 ± 3.49 <b>b</b>	16.17 ± 2.14 <b>abc</b>
0.9	71.11 ± 3.91 <b>b</b>	74.17 ± 5.85 <b>ab</b>	18.50 ± 3.21 <b>bc</b>
<b>Fresh spermatozoa</b>	<b>75.89±3.65</b>	<b>83.17 ± 3.82</b>	<b>13.33% ± 2.58</b>

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

The effect of 0.5% of sucrose-fish ringer + 10% methanol on the percentage of spermatozoa motility 48 hours post-cryopreservation was higher (81.62%) than our previous study using 189M extender + 13% of DMSO; 24 hours post-cryopreservation (68.58%; Abinawanto *et al.*, in press) and also was higher than those observed in other fish species such as *Brachydanio rerio* (51%, Harvey *et al.* 1982), *Oreochromis mossambicus* (70%, Harvey *et al.* 1983), tilapia's fish (40–80%, Chao *et al.* 1987), *Cyprinus carpio* (55%, Akcay *et al.* 2004), *Osteochiilus hasseltii* (63.33%, Sunarma *et al.*, 2007). In contrast, those of sucrose effect was lower than our another finding using 20% of skim milk + 5% of methanol (24 hours post-cryopreservation) which was shown 83.23% of post-thawed spermatozoa motility (Abinaw-anto *et al.*, unpublished data).

The difference is related to the cryopro-

tectant and extender were used (5% of methanol + 15% of skim milk as conducted by Harvey *et al.* 1982; Harvey *et al.* 1983; Chao *et al.* 1987; 5% of methanol + 20% of skim milk as studied by Abinawanto *et al.* (unpublished data) 13% DMSO + 189M extender; as reported by Abinawanto *et al.* in press; 15% of DMSO; as previously discussed by Akcay *et al.* 2004; Sunarma *et al.*, 2007). The obtained concentration in this study provides an effective comparison between cryopreserved sperm (Harvey *et al.* 1982; Harvey *et al.* 1983; Chao *et al.* 1987; Akcay *et al.*, 2004; Sunarma *et al.*, 2007; Abinawanto *et al.* unpublished data; Abinawanto *et al.* in press).

The combination of 0.5% Sucrose-fish ringer and 10% methanol also maintained the percentage of spermatozoa viability (82.17%). This result (spermatozoa viability) was higher than our previous finding using 189M extender

+ 13% of DMSO; 24 hours post-cryopreservation which was shown only 63.5% (Abinawanto et al. in press) and also was higher compared to *Cyprinus carpio* (20% of, Withler, 1982; 58%, Withler and Morley see Horton and Otto 1976). On the other hand, those of 0.5% of sucrose-fish ringer + 10% of methanol effect on post-thawed viability was comparable with 20% of skim milk + 5% of methanol (81.75%; Abinawanto et al. , unpublished data).

Furthermore, the combination of 0.5% Sucrose-fish ringer and 10% methanol also maintained the percentage of spermatozoa abnormality (12.5%). Those of abnormality was lower than our previous study either using 20% of skim milk + 5% of methanol; 24 hours post- cryopreservation which was shown 26.25% (Abinawanto et al. unpublished data) or using 189M extender + 13% of DMSO which was reported 29% (Abinawanto et al. in press).

Thawing procedures at 40 °C for 1–2 min was effective for 2 ml cryogenic tubes. We chose 40 °C because this was 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 a successful internal cryoprotectant in *Brachydanio rerio* (Harvey et al. 1982); *Oreo-*

*chromis mossambicus* (Harvey et al. 1983); tilapian's fish (Chao et al. 1987); *C. carpio* (Horváth et al., 2003), *Osphronemus goramy* (Abinawanto et al. in press), *Barbon-ymus gonionotus* (Abinawanto et al. unpublished data). 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.

## Conclusion

It is concluded that 0.5% of sucrose showed the highest post-thawed sperm motility (81.62±4.19) %, and post-thawed sperm viability (82.17±2.56) %, and the lowest post-thawed abnormality (12.5±1.52) %.

## Acknowledgement

We address our thanks to University of Indonesia Competitive Research Grant No. 240AT/DRPM-UI/N1.4/2008 for the financial support.

## References

- ✓ Abinawanto, A.; Zuraida, Z.; and Lestari, R. (Unpublished data)The combination effect of 5% of methanol and Skim milk in various concentration on spermatozoa quality of Java Barb (*Barbonymus gonionotus*, Bleeker 1850), 24 hours post-cryopreservation.
- ✓ Abinawanto, A.; Bayu, M. D., Lestari, R. and Sunarma, A. (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 (Indonesian Journal, in press).
- ✓ Akcay, E.; Bozkurt, Y.; Secer, S. and Tekun, N. (2004) Cryopreservation of mirror carp semen. Turkey J. Vet. Anim. Sci., 28: 837–843.
  - ✓ Chao, N-H.; Chao, W-C.; Liu, K-C.; and Liao, I-C. (1987) The properties of tilapia sperm and its cryopreservation. J. Fish Biol., 30(2):107–118.
  - ✓ Harvey, B. (1983) Cryopreservation of *Sarotherodon mossambicus* spermatozoa. Aquacult., 32: 313–320.
  - ✓ Harvey, B. and Ashwood-Smith, M.J. (1982) Cryoprotectant Penetration and Supercooling in the Eggs of Salmonid Fishes. Cryobiology, 19: 29–40.
  - ✓ Harvey, B.; Kelley, R. N.; and Ashwood-Smith, M.J. (1982) Cryopreservation of zebra fish Spermatozoa using methanol. Can. J. Zool., 60: 1860–1870.
  - ✓ He, S. and Woods, C. (2003) The Effect of Osmolality, Cryoprotectant and Equilibration Time on Striped Bass *Morone saxatilis* Sperm Motility. J. of the World Aquacult. Soc., 34 (3): 255–265.
  - ✓ Horton, H.F. and Otto, A.G. (1976) Cryopreservation of Fish Spermatozoa and Ova. J. Fish Resour. Board. Can. , 33: 995–1000.
  - ✓ Horvath, A.; Miskolczi, E. and Urbanyi, B. (2003) Cryopreservation of Common Carp Sperm. Aquatic Liv. Resour. , 16: 457–460.
  - ✓ Stoss, J. and Donaldson, E.M. (1983) Studies on Cryopreservation of Eggs from Rainbow Trout (*Salmo gairdneri*) and Coho Salmon (*Onchorchynchus kisutch*). Aquacult., 31: 51–65.
  - ✓ Sunarma, A.; Hastuti, D.W. and Sistina, Y. (2007) Utilization of Honey as an Extender Combined to Different Cryoprotectant on Sperm Cryopreservation of Nilem (Indonesian Sharkminnow, *Osteochiilus hasseltii* Valenciennes, 1842). Indonesian Aquacult. Conf.: 1–9.
  - ✓ Withler, F.C. (1982) Cryopreservation of Spermatozoa of Some Freshwater Fishes Cultured in South and Southeast Asia. Aquacult., 26: 395–398.
  - ✓ World Conservation Monitoring Centre (1992) *Global biodiversity: Status of the earth's living resources*. Chapman & Hall, London: xviii + 585 pp.