E: ISSN: 2347-5129
P: ISSN: 2349-0506
IJFAS 2014; 2(2): 30-32

www.fisheriesjournal.com
Received: 07-09-2014
Accepted: 30-09-2014

Cheryl Antony
Institute of Fisheries Technology, Tamil Nadu Fisheries University, Ponneri-601 204, Chennai, India.

S. Felix
Institute of Fisheries Technology, Tamil Nadu Fisheries University, Ponneri-601 204, Chennai, India.

K. Ravaneswaran
Institute of Fisheries Technology, Tamil Nadu Fisheries University, Ponneri-601 204, Chennai, India.

Correspondence:
Cheryl Antony
Institute of Fisheries Technology,
Tamil Nadu Fisheries University, Ponneri-601 204,
Chennai, India.

Studies on the milt quality of Common carp (*Cyprinus carpio var communis*) and Koi carp *(Cyprinus carpio var koi*).

Cheryl Antony, S. Felix and K. Ravaneswaran

Abstract
The objective of this study was to determine the quality of milt of the two species of fishes, Common carp (*Cyprinus carpio var communis*) and Koi carp *(Cyprinus carpio var koi*) belonging to the family Cyprinidae. The fishes were reared from fingerling size (2 cm) in the perennial pond system and they attained broodstock sizes (average weight, 300 gm) in a period of four months. The milt was collected by applying mild abdominal pressure. The volume, pH, motility, quality and cryopreservation trials were carried out. The milt samples were diluted with Cortland Medium (pH 7.56) and protected with Glycerol and DMSO (Dimethyl Sulphoxide) were used as cryoprotectants at 5%, 10% and15% concentrations. Comparative studies were also made on the effectiveness of DMSO and Glycerol as cryoprotectants. It was observed that motility rates were higher (60%) for DMSO than Glycerol (40%) at 15% concentration levels. The samples were stored in LN_2 upto 60 days. The post thaw motility rates were observed based on motility rate. The equilibration periods were also studied and it was observed that motility rate was 60% for samples stored using DMSO as cryoprotectant. Among the cryoprotectants used it was also observed that DMSO proved better than Glycerol as cryoprotectant for both the samples.

Keywords: Fish sperm, Cyprinus carpio v communis, Cyprinus carpio v koi, sperm motility, cryoprotectant concentration, equilibration period.

1. Introduction
Intensive aquaculture requires efficient and effective methods in broodstock management and genetic improvement protocols. Cryopreservation of fish spermatophores could play a major role in seed production, genetic management of broodstock and conservation of aquatic resources [4]. Asynchronization of gonadal maturation is one of the major constraints that impede fish breeding. Spermatozoa motility, milt volume and spermatozoa concentration are good indicators for milt quality [3]. Moon *et al.* (2003) [5] reported positive correlation between milt volume and spermatozoa concentration in male flounder, *Platichthys stellatus.* The spermatozoa motility and its duration have great influence on successful fertilisation [7].
The aim of this study was to standardise the protocol for the cryopreservation of milt from common carp and koi carp both belonging to the Family Cyprinidae. This study will pave way for developing a practical, efficient and reproducible protocol with potential for storage studies of milt for further reproductive trials.

2. Materials and Methods
Milt samples were collected from the fishes that were reared in the perennial pond located at TANUVAS campus. The fishes had been stocked in the pond for culture to broodstock fishes and were fed daily with rice bran and groundnut oil cake. Before the collection of milt the fishes were first harvested and stocked in indoor rearing tanks to avoid stress. The fishes selected were from 250 gm to 350 gm in size and the region around the genital opening was wiped free of mucus, urine and faecal matter. Care was taken to avoid polluting the samples by blood by applying mild abdominal pressure while stripping. The milt sample was directly drawn into sterile cryovials and transferred immediately in ice to the laboratory for further trials.

The quality of fresh spermatozoa was determined by placing a small drop of milt (1 µl) on a glass slide and mixed with 100 µl of distilled water to activate the sperm. The motility was observed using a phase contrast microscope (Nikon Inc.) at 100X magnification. The motility was expressed as the percentage of sperm which moved actively. Motility of the spermatozoa was studied by assessing the movement of the spermatozoa with duration of motility.

For each sample the sperm motility was estimated thrice. The cryoprotectants DMSO and Glycerol were both evaluated for this trial. The motility of thawed sperm frozen using DMSO and Glycerol at different percentages and different equilibration rates were studied together with thawing rates.

Table 1: Chemical Composition of Cortland's medium used for cryopreservation of spermatozoa

Chemical	mgm
NaCl	725
$CaCl_2$ H_2O	23
NaH_2 PO_4H_2O	38
$NaHCO_3$	1000
$MgSO_4$ $7H_2O$	23
Glucose	100
Distilled Water	100 ml

3. Results and Discussion

Table 2a: Effect of cryoprotectant level and equilibration time on post thaw motility (%) in common carp *(Cyprinus carpio v. communis*) using DMSO as cryoprotectant

Cryoprotectant (%) DMSO	Equilibration time (min)				
	10	20	30	40	50
5	31.2± 1.67	33.3±1.67	35.1± 1.67	28.2± 1.67	29.5± 1.67
10	31.6± 1.67	31.7± 0.12	29.7± 0.12	26.5± 0.12	27.5± 1.67
15	45.1± 1.67	**55.2**± 1.67	**61.5**± 1.67	40.5± 1.62	41.5± 1.62

Table 2b: Effect of cryoprotectant level and equilibration time on post thaw motility (%) in common carp *(Cyprinus carpio v. communis*) using Glycerol as cryoprotectant.

Cryoprotectant (%) Glycerol	Equilibration time (min)				
	10	20	30	40	50
5	29.8± 1.67	27.6± 1.67	31.7± 1.62	23.9± 1.62	27.6± 1.67
10	32.7± 1.67	31.5± 1.67	33.5± 1.67	24.9± 1.67	25.7± 1.62
15	31.3± 1.67	**39.7**± 1.62	**40.3**± 1.62	32.7± 1.62	33.5± 1.62

Table 3a: Effect of cryoprotectant level and equilibration time on post thaw motility (%) in koi carp *(Cyprinus carpio v. koi*) using DMSO as cryoprotectant

Cryoprotectant (%) DMSO	Equilibration time (min)				
	10	20	30	40	50
5	31.3± 1.62	33.5± 1.62	35.2± 1.67	28.7± 1.62	29.5± 1.62
10	31.5± 1.67	31.7± 1.67	29.3± 1.67	26.2± 1.67	27.5± 1.67
15	45.9± 1.62	**55.5**± 1.62	**61.9**± 1.62	40.3± 1.62	42.1± 1.62

Table 3b: Effect of cryoprotectant level and equilibration time on post thaw motility (%) in koi carp *(Cyprinus carpio v. koi*) using Glycerol as cryoprotectant

Cryoprotectant (%) Glycerol	Equilibration time (min)				
	10	20	30	40	50
5	29.5±1.67	27.5± 1.62	30.7± 1.62	24.9± 1.62	27.5± 1.62
10	32.4± 1.62	30.5± 1.62	32.5± 1.62	25.9± 1.62	25.7± 1.62
15	31.5± 1.62	**38.5**± 1.62	**40.5**± 1.62	32.3± 1.62	33.4± 1.62

Table 3c: Effect of cryoprotectant level on motility rates of cryopreserved milt of Common carp *(Cyprinus carpio v. communis*) and koi carp *(Cyprinus carpio v. koi*)

Species	Cryoprotectant (%)	Motility % in Cryopreserved sample
C. carpio (Common Carp)	DMSO	60%
C. carpio (Common Carp)	Glycerol	40%
C. carpio var koi (Koi carp)	DMSO	60%
C. carpio var koi (Koi carp)	Glycerol	35%

Table 4: Effect of thawing on motility rates of cryopreserved milt of Common carp *(Cyprinus carpio v. communis*) and koi carp *(Cyprinus carpio v. koi*)

Species	Cryoprotectant (%) 15%	Motility % in sample thawed at room temperature	Motility % in sample thawed in water bath (37 °C)
C. carpio (Common Carp)	DMSO	60%	40%
C. carpio var koi (Koi carp)	DMSO	60%	35%

4. Results and Discussion

The post thaw motility of *Cyprinus carpio* (common carp) and *Cyprinus carpio var koi* (koi carp) are presented in Tables II and Table III. The post thaw motility rates presented in Tables II and III also shows the effect on using Glycerol and DMSO as Cryoprotectants. High post thaw motility rates have been observed for the milt samples using DMSO as cryoprotectant. It was also observed that the percentage of cryoprotectant used also influenced post thaw motility where samples containing 15% DMSO and equilibrated upto 30 minutes led to significantly higher rates when compared to 10, 20, 40 and 50 minutes equilibration period. Motility rates were high (61%) in samples stored using DMSO as cryoprotectant than in samples using glycerol as cryoprotectant at an equilibration time of 30 minutes. The decline in the motility duration from the initial value as a generally accepted phenomenon for all the fish spermatozoa under cryopreservation has been studied [8].

There has been many successful cryopreservation attempts in fish spermatozoa and artificial fertilization [10] and motility is the commonly used parameter to evaluate the sperm quality [2].

The motility rate was observed to be 60% in the cryopreserved milt samples thawed at room temperature when using 15% DMSO for both the samples. On thawing the samples using water bath (37 ^{0}C) the motility rates were lower (40% for common carp and 35% in koi carp).The effect of cryoprotectant level (15%) on using DMSO and Glycerol for both the species are given in Table III. It was observed that the motility rates were higher (60%) on using 15% DMSO as cryoprotectant is given in Table IV.

This basal study on cryopreservation of common carp and koi carp milt indicated that DMSO was found to be suitable for the cryopreservation of common carp and koi carp milt samples. Various extenders with varying chemical composition have been successfully used for the cryopreservation of gametes [7, 6]. They had used Cortlands medium as extender. Basavaraja *et al*. 2002 [1] observed that egg yolk citrate produced good post thaw motility and fertilising ability in cryopreserving Indian major carp spermatozoa. Simple freezing and thawing protocols have been used for gene banking of India's culturable species. In this paper the protocols have been standardised for common carp and koi carp.

5. Acknowledgements

The authors wish to place on record their gratitude to the NADP programme for providing funds to run the scheme. We also thank the Vice Chancellor, Tamil Nadu Veterinary and Animal Sciences University, (TANUVAS), for having granted funds from the NADP budget and also The Director, Centre for Animal Health Studies, TANUVAS, for providing the necessary facilities to carry out the study. We also the Director of Research (Animal Sciences), TANUVAS, for facilitating the study.

6. References

1. Basavaraja N, Hegde SN, Akash N, Udupa KS. The fertility of cryopreserved deccan mahseer, Tor khudree (Sykes) spermatozoa. Asian Fisheries Science 2002; 15:193-202.
2. Billard R, Cosson J, Perchec G, Linhart O. Biology of sperm and artificial reproduction in carp. Aquaculture 1995; 124:95-112.
3. Cabrita E, Anel L, Herraez PM. Effect of external cryoprotectants as membrane stabilizers on cryopreserved trout sperm. Theriogenology 2001; 56:623-635.
4. Chao NH, Liao IC. Cryopreservation on finfish and shellfish gametes and embryos. Aquaculture 2001; 197:161-189.
5. Moon SH, Kwon YJ, Lee KJ, Chang JY. Increased plasma 17- hydroxyprogesterone and milt production in response to gonadotropin-releasing hormone against in captive male starry flounder, Platichthys stellatus. Aquaculture 2003; 218:703-716.
6. McAndrew BJ, Rana KJ, Perman DJ. Conservation and preservation of genetic variation in aquatic organisms. In: Recent Advances in Aquaculture Vol. 4, (eds. J.F. Muir and R.J. Roberts). Blackwell Scientific Publications, Oxford 1993, 295-296.
7. Faruk A, Sahinoz E, Dogu Z. A study on the milt quality of *Oncorhynchus mykiss* (Walbaum, 1972) and *Carasobarbus luteus* (heckle, 1843) in Ataturk Dam Lake, Southeastern Turkey, 2007.
8. Muchlisin ZA. Review: Current Status of Extenders and Cryoprotectants on Fish Spermatozoa Cryopreservation. Biodiversitas 2005; 6(1):66-69.
9. Scott AP, Baynes SM. A review of the biology, handling and storage of salmonid spermatozoa. Journal of Fish Biology 1980; 17:703-739.
10. Saad A, Billard R, Theron MC, Hollebeq MG. Short term preservation of carp (*Cyprinus carpio*) semen. Aquaculture 1988; 71:133-150.