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A comparative study of various extenders of milkfish, *Chanos chanos* (Forsskal) sperm preservation

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To develop the technique of milkfish sperm preservation using various extenders in liquid (0-4°C) and frozen (−196°C) storage conditions, mature milkfish (male) were captured by fish traps off Tigbauan and Hamtik (in Panay Is.) during the spawning season (March–June). The newly caught males were injected with "Durandon Forte" (a combination of 30 mg testosterone propionate, 60 mg testosterone phenylpropinate, 60 mg testosterone insocaproate and 100 mg testosterone decanoate in 1 ml of oily solution) or human chorionic gonadotropin (A.P.L., Ayerst) to stimulate seminal thinning and consequently to increase the quantity of milt (Juario *et al.,* in press). The males were anaesthetized and stripped of milt several days after the hormone treatment. The milt collected from each individual was used separately in the experiments.

Four chemical extenders in seven different concentrations were prepared. They were potassium chloride (KCL), sodium chloride (NaCl), glucose, sodium citrate, Ringer's solution (containing 0.23M NaCl, 0.008 M KCl, 0.002M $MgCl_2.6H_2O$, and 0.002M $NaHCO_3$), cow serum and milkfish serum. Five concentrations of each of the first four substances were tested at a range of 0.10 to 0.30 M, 0.10 to 0.30 M, 0.10 to 0.50 M and 0.050 to 0.125 M, respectively.

Sperm motility was induced by dilution of treated sperm in excess seawater ($34^o/_{oo}$). Two arbitrary scoring systems were used to assess sperm motility.

1) A system modified from Kurokura (1979): the motility of sperm is expressed on a 6 point scale as follows:

Score	*% Motile sperm*
0	0
1	≤ 1%
2	1 – 5%
3	5 – 30%
4	30 – 70%
5	70 – 100%

2) A system modified form Mounib (1978): the vitality of sperm is expressed as:

Score	*Vitality*
1	feeble
2	weak
3	strong
4	very strong

Motility of sperm refers to the percentage of spermatozoa which are motile. Vitality of motile sperm is expressed descriptively. A sperm sample containing more than 30% motile spermatozoa, which also show strong vitality, were considered to have "good motility."

Sperm response to different concentrations of the extenders is shown in Table 1. Sperm started to show motility in 0.20M KCl, 0.15M NaCl, 0.30M glucose and 0.05M sodium citrate, respectively. These findings are similar to those for other marine fish *Mylio macrocephalus, Limanda yokohamae,* and *Anguilla japonicus* (Kurokura, 1979), *Mugil cephalus* and *Valamugil seheli* (Hara, unpublished data).

Table 1. The reaction of fresh milkfish sperm to chemical extenders at various concentrations

Extender Medium	Reaction of concentration (M)						
KCl	0.05	0.10	0.15	0.20	0.25	0.30	0.35
	–	–	–	±	+	+	+
NaCl	0.05	0.10	0.15	0.20	0.25	0.30	0.35
	–	–	±	±	+	+	+
Glucose	0.10	0.20	0.30	0.40	0.50	0.60	0.70
	–	–	±	+	+	+	+
Sodium citrate	0.025	0.050	0.075	0.100	0.125	0.150	0.175
	–	±	+	+	+	+	+

(–) : no reaction in all 3 replicates

(+) : reaction in all 3 replicates

(±) : reaction shown in 1-2 replicates, not in others

The motility of milkfish sperm after various durations of liquid storage is presented in Table 2. Undiluted sperm retained a low percentage of active sperm, scoring between 2.0 and 2.7 for 1-3 days; so did the sperm diluted with KCl and glucose. The motilities of liquid preserved sperm in the KCl extender ranging between 0.10 and 0.30 M, were between 2.7 and 3.0 in the first day and decreased notably to between 1.0 and 1.7 after the second day of preservation. Similar results were observed in glucose as extender. Motility was extended to 10 to 11 days

storage in milkfish serum; 4-5 days in cow serum, and 3-5 days in Ringer's solution and NaCl. High motility of 4.7 was observed up to 4 days in milkfish serum and the motilities of 3.3-4.0 for another two days. The high motilities of 3.7-4.0 were only observed for two days in cow serum, Ringer's solution, and 0.15M NaCl. The results using sodium citrate as an extender were unsatisfactory.

While Chao *et al.* (1975) found that 6, 10 and 12% glucose and 1 and 2% sodium citrate were good extenders for grey mullet sperm stored at 5°C, it was found in this study that they are not so for milkfish sperm storage. Tseng and Hsiao (1979) also reported the use of glucose as a diluent for fresh milkfish sperm for artificial insemination claiming that it made the sperm vital. In this study, however, it was found that milkfish serum is superior to all the other extenders examined.

Table 2. Mean motility of milkfish sperm in liquid storage at 0-4°C in various extenders and concentrations. (Mean vitality is in parentheses).

Extender	Concentration (M)	Motility scores by days of storage											
		1	2	3	4	5	6	7	8	9	10	11	12
KCl	0.10	2.7 (2.3)	1.0 (1.3)	1.0** (1.0)	0								
	0.15	2.7 (2.3)	1.3 (1.3)	0									
	0.20	2.7 (2.3)	1.3 (1.3)	1.0* (1.0)	0								
	0.25	3.0 (3.3)	1.7 (1.3)	1.0 (1.0)	0								
	0.30	2.7 (3.0)	1.7 (1.3)	1.0** (1.0)	0								
NaCl	0.10	3.7 (3.7)	3.3 (3.0)	2.3 (3.0)	1.0** (3.0)	0							
	0.15	4.0 (4.0)	3.7 (3.7)	2.3 (3.0)	1.0 (1.5)	0							
	0.20	3.7 (4.0)	3.3 (3.7	2.7 (3.0)	1.0** (1.0)	1.0 (1.0)	0						
	0.25	3.3 (3.6)	2.3 (3.0)	1.7 (2.3)	1.0* (2.0)	0							
	0.30	3.3 (3.6)	2.7 (3.0)	1.7 (2.3)	1.0** (1.0)	0							
Glucose	0.10	1.0 (3.0)	1.0 (1.3)	1.0** (1.0)	0								
	0.20	2.3 (3.3)	1.7 (2.7)	0									
	0.30	3.0 (3.7)	2.7 (2.7)	0									

Extender	Concentration (M)	Motility scores by days of storage											
		1	2	3	4	5	6	7	8	9	10	11	12
	0.40	3.3	2.3	0									
		(3.7)	(2.7)										
	0.50	3.0	2.3	0									
		(3.7)	(2.7)										
Sodium	0.050	2.0	1.0	0									
Citrate		(1.0)	(1.0)										
	0.075	2.0*	0										
		(1.0)											
	0.100	2.0**	0										
		(1.0)											
	0.125	0											
	0.150	0											
Ringer's		4.0	3.7	3.3	1.5*	1.0*	0						
solution		(4.0)	(3.3)	(3.0)	(2.5)	(2.0)							
Cow serum		4.0	4.0	3.5	2.0	1.0*	0						
		(4.0)	(4.0)	(3.5)	(3.0)	(2.0)							
Milkfish		4.7	4.7	4.7	4.7	4.0	3.3	2.0	1.7	1.0	1.0	1.0*	0
serum		(4.0)	(4.0)	(3.7)	(3.7)	(3.7)	(3.3	(3.0)	(3.0)	(2.0)	(1.3)	(1.5)	
Control		2.7	2.0*	2.0*	0								
(Undiluted sperm)		(3.3)	(2.5)	(3.0)									

Legend: * — the given motility score is the mean of 2 replicates, one sample had stopped being motile. ** — the given motility score is that of a single sample.

The results of the fertilization tests using sperm diluted with milkfish serum are shown in Table 3 (samples 1-4). They indicate that fertility was maintained even after 5 days of storage. Fertilization rates of 6.7-18.6% were observed. The other extenders, which showed low motility (Table 2) were excluded from this fertility study.

Again it was found that milkfish serum was the best of the 7 extenders tested, in terms of motility and fertility of sperm after a long period of storage at 0-4°C. This is probably because milkfish serum is isotonic with milkfish sperm. Similarly, mullet serum was found to be the best extender for mullet (*M. cephalus*) sperm (Hara, unpublished).

Post-thawing motility of cryopreserved sperm in various extenders is shown in Table 4. These extenders were the ones found effective in liquid storage. The results indicated that milkfish sperm diluted with either milkfish or cow serum showed higher motility, proving chemical extenders unsatisfactory. Cryopreserved sperm showed good fertilizing capacity in all the various extenders tested, averaging 67.5% for milkfish serum, 60.5% for 0.40M glucose, 58.0% for 0.15M NaCl, 41.2% for Ringer's solution and 31.9% for cow serum.

Table 3. Results of fertilization tests using liquid preserved and cryopreserved milkfish sperm in various extenders.

Sample No.	Conditions and extenders		Fertilization (%)
A.	Liquid preservation (0-4°C; 5 days)		
1	milkfish serum	1:5	16.7
2	-do-	1:10	18.6
3	-do-	1:20	18.0
4	-do-	1:40	6.7
B.	Cryopreservation (−196°C; 4-5 days)		
5	milkfish serum		67.9
6	-do-		67.1
7	0.15 M NaCl		62.1
8	-do-		53.9
9	0.40 M glucose		50.0
10	-do-		71.0
11	Ringer's solution		41.2
12	cow serum		20.6
13	-do-		43.2
C.	Control (Fresh, no dilution)		
14	artificial propagation in hatchery tanks		40.0
15	-do-		11.1

Table 4. The post-thawing motility of cryopreserved milkfish sperm in various extenders and concentrations.

Extender	Concentration (M)	Motility Scores					
		% motile sperm			Vitality		
		1	2	3 Run	1	2	3 Run
NaCl	0.10	1	1	–	2	1	–
	0.15	3	1	–	3	1	–
	0.20	3	1	–	2	1	–
	0.25	1	1	–	2	1	–
	0.30	1	1	–	1	1	–
Glucose	0.10	1	1	–	1	1	–
	0.20	1	1	–	2	1	–
	0.30	1	1	–	2	1	–
	0.40	3	1	–	4	2	–
	0.50	0	0	–	–	–	–
	0.70	0	–	–	–	–	–
	1.00	0	–	–	–	–	–
Ringer's solution		3	1	1	3	1	1
Cow serum		3	3	4	3	3	4
Milkfish serum		4	2	4	4	3	4

The suitability of milkfish serum as extender for milkfish sperm preservation both at 0-4°C and -196°C storage conditions, may be attributed to suitable osmotic potential and/or presence of proteins which may have directly or indirectly influenced sperm viability. This finding leads to the necessity of studying the blood serum itself and the techniques of collection and preservation of the serum. It becomes necessary, for example, to investigate the effects of milkfish serum on the motility and fertilizing capacity of sperm at different durations of storage.

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