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Effect of extenders and storage periods on the motility performance of common carp, *Cyprinus carpio* var. *communis* sperms in Kashmir Himalaya

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Abstract
The effect of short term storage on the motility rate and motility duration was assessed at different storage periods. The sperms were diluted with3 different extenders viz. Extender I contained 300 mM glucose solution. Extender II contained 1% NaCl solution. Extender III contained 75 mM NaCl, 70 mM KCl, 2 mM $CaCl_2$, 1 mM MgSO4, 20 mM Tris (Modified ionic solution) at ratio of 1:3. Glucose containing extender showed the best results. The highest motility rate and duration was found with the glucose based extender at 0 hour storage period. Therefore it is found that glucose based extender is the best extenders than other extenders used in the study and motility performance of sperm of *Cyprinus carpio* var. *communis* decreases with the storage time.

Keywords: Motility rate, extender, *Cyprinus carpio* var. *communis*, sperms

1. Introduction
Common carp is the most commercially important species. Major production of the species comes from the aquaculture operation. However, the quality of seed gets deteriorated in hatchery due to inbreeding depression, negative selection etc. So, the preservation of genetic diversity is a great challenge. Short term preservation of sperms can be considered as an important tool for reducing the genetic erosion that invariably results from inbreeding and that can allow the preservation of the various male donors from all the possible strains [1].
For the successful hatchery operations, short-term preservation of fish sperm is very important. The short-term storage of sperm at low temperature (4ºC) is mostly used to obtain the good quality gamete of both male and female sex at the same time during artificial insemination, also in research programs for genetic studies. Maturation of gametes and the time of spawning between the sexes at different times has been documented in number of species. To overcome these problems, researchers have examined procedures such as cryopreservation, short-term, refrigerated storage of milt. Short-term, refrigerated storage of milt have been developed for several teleosts, such as walleye [2] Red drum *Sciaenops ocellatus* [3], Atlantic sturgeon *Acipenser oxyrinchus* [4] Mozambique tilapia *Tilapia mossambica* [5], and Paddlefish *Polyodon spathula* [6].The fish sperm could be preserved by storage in undiluted and diluted form. Sperm stored at low temperature in undiluted form has been reported to cause a reduction in fertilization capacity [7]. Undiluted storage provides less control compared to the diluted storage of sperm with extender [5]. The dilution of the sperm in a suitable medium helps in the preservation for short time periods, while transporting from the place of collection to the place of employment, as it helps to keep the physicochemical properties of semen stable [8,9]. These solutions, however, helps in keeping the sperms inactive, and thus prevent the exhausting energy reserves of the spermatozoa [10].

2. Materials and methods
2.1 Milt collection
Mature male brooders were collected from the hatchery of Faculty of fisheries SKUAST K during the breeding season. Each male was stripped once only and the total amount of expressible milt was collected individually by gently pressing the abdomen. The semen was collected directly into clean 15 ml graduated centrifuge tubes. Care was taken to avoid the contamination of semen with water, urine, blood or faecal matter. The tubes were covered and immediately transported on ice (4°C) to the laboratory for analyses.

2.2 Estimation of Sperm motility

Motility was evaluated using a light microscope (Olympus CX31) at 40x magnification and was expressed as percentage of motile spermatozoa. An activating solution of 0.3% NaCl was used to estimate motility. For the evaluation of motility, about 10 µl of semen was placed on a glass microscope slide and 100 µl of activation solution was added [11].

2.3Short-term storage of sperm

Three extenders were selected for evaluating potential extender for fishes as shown in table 1. The semen was diluted at ratio of 1:3 with one of three extenders. Extender I contained 300 mM glucose solution as described by Tekin *et al.* (2003). Extender II contained 1% NaCl solution. Extender III contained 75 mM NaCl, 70 mM KCl, 2 mM $CaCl_2$, 1 mM MgSO4, 20 mM Tris (Modified ionic solution) as described by Lahnsteiner *et al.* (1998). The diluted sperm was packaged in small laboratory tubes. Undiluted semen samples were used as control. Experimental and control samples were stored at 4°C for 4 hours in a refrigerator. During refrigerated preservation, motility (%), motility durations (s) of stored sperm were evaluated at 1 hour intervals.

Table-1: Composition of semen extenders

Extenders	Composition
Extender I	300 mM of glucose solution [12]
Extender II	1% NaCl solution
Extender III	75 mM NaCl, 70 mM KCl, 2 mM $CaCl_2$, 1 mM MgSO4, 20 mM Tris (Modified ionic solution.[13]

3. Results

3.1 Short term preservation of Common carp sperm

3.1.1 Post-activation motility rate

Data on the effect of extenders and storage periods on motility rate are given in Table 2. Spermatozoa motility percentage decreased significantly with increasing storage period in diluted and undiluted sperm during spawning season. When the effect of extenders were tested, the best results (74% motility) were achieved with extender I containing glucose solution at the e 0 hours storage periods. On the contrary, the lowest motility (4.66%) was obtained with control group at 4 hour storage periods. Table 2, shows that statistically there was a significant difference in motility rate in extenders, extender 1, extender 2, extender 3 and no extender at storage time 0, 1, 2, 3 and 4 hr. The maximum value of extender 1 was at 0 hr and minimum value was at 4 hr storage period indicating significant decrease in motility percentage from 0 hr storage to 4 hr storage. The decrease in motility (%) was also found in extender 2, extender 3 and no extender from 0 hr storage to 4 hr storage. At 0 hr storage the maximum value of motility rate (74%) was of extender 1 and minimum value (53.66%) was of extender 3. At 1 hr storage, the maximum value (56.33%) was of extender 1 and minimum value (42.66%) was of extender 2. The maximum value (40%) at 2 hr storage was of extender 1 and minimum value (31%) was that of control (undiluted milt). The maximum value (30%) at 3 hr storage was of extender 1 and minimum value (15%) was of extender control (undiluted milt). The maximum value (14.33%) at 4 hr was of extender 1 and minimum value (4.66%) was of control (undiluted milt). The Extender 1 showed better performance of motility rate at all storage periods with a mean value of 42.9% followed by Extender 2 with a mean value of 35.66%.

Table 2: Effect of extenders and storage periods on motility rates of Scale carp sperm during spawning season

Storage period (hour)	Extender (%)			Control (sec)	Mean (sec)
	1	2	3		
0	74.00	66.33	53.66	69.66	65.91
1	56.33	42.66	44.666	50.33	48.50
2	40.00	35.00	38.66	31.00	36.16
3	30.00	25.33	29.66	15.00	25.00
4	14.33	9.00	10.00	4.66	9.50
Mean	42.90	35.66	35.33	34.133	

CD at 5% level of significance; Storage = 2.7435; Extender =2.4538; Storage × Extender = 5.487

3.1.2 Post-activation motility durations

Data on the effect of extenders and storage periods on motility durations are given in Table 3. In case of testing extenders, the highest motility duration (54 sec) was achieved with extender 1 at 0 hours storage periods during spawning season. On the contrary, the lowest motility duration of 5 sec was observed with extender 3 at 4 hour storage period. Decrease in motility duration is significantly important with increasing storage period in diluted and undiluted sperm during spawning season, differences among storage hours and extenders were statistically significant during experiment. The maximum value (54 sec) of extender 1 was at 0 hr and minimum value at 4 hr storage (16.33 sec) indicated significant decrease in motility duration with storage. The decrease in motility duration was also observed in extender 2, extender 3 and control (undiluted milt) from 0 to 4 hr storage. The maximum value (56 sec) of motility duration at 0 hr was that of extender 2 and minimum value (42 s) was that of extender 3. The maximum value at 1 hr was that of extender 1 (48 sec) and minimum value (33 sec) was that of extender 3. The maximum value (31 sec) at 2 hr was that of extender 1 and minimum value (24 sec) was that of extender 3. The maximum value (21 sec) at 3 hr was that of extender 1 and minimum value (11.66 sec) was that of control (undiluted milt). The maximum value (16.33 s) at 4 hr was that of extender 1 and minimum value (5 sec) was that of extender 3. The extender 1 shows better performance of motility duration at all storage periods with a mean value of 34.067 sec of followed by extender 2 with a mean value 29.22 sec, control (undiluted milt) with a mean value of 26.133 sec and extender 3 with a mean value 24.33 sec.

Table 3: Effect of extenders and storage periods on motility duration of scale carp carp sperm during spawning **season**

Storage period (hour)	Extender (sec)			Control (sec)	Mean (sec)
	1	2	3		
0	54.00	56.00	42.00	46.00	49.50
1	48.00	42.00	33.00	39.00	40.50
2	31.00	27.00	24.00	25.00	26.75
3	21.00	13.00	19.66	11.66	16.33
4	16.33	8.00	5.00	9.00	9.588
Mean	34.067	29.22	24.733	26.133	

CD at 5% level of significance; Storage = 3.3828; Extender =3.0257; Storage X Extender = 6.766

4. Discussion

The most important indicator for the success of a preservation protocol is the post-activation motility. The present work reports that the spermatozoa motility get affected during preservation. Glucose containing extender was found with the best motility results. The motility percentage decreased faster with time in undiluted sperm samples than diluted ones. The motility of diluted sperm was always significantly greater than that of undiluted sperm, Similar results for the motility parameters of spermatozoa were reported by different worker [14,15, 16] also reported that no significant changes in the quality were found when the spermatozoa are kept in appropriate extender at low temperature (4°C), [5] also observed that diluted sperm with extender provides better control compared to undiluted storage. [17]Also reported that the best results in fertilization and motility are obtained from the sperm samples of rainbow trout diluted with artificial seminal plasma with the rate of 1:1 stored for 7 days. It is possible that by using suitable activating mediums, duration of motility will be increased that will enhance fertilizing capacity of the fish.

Sperm dilution is a major factor in the induction of sperm motility [18, 19,20] reported that the duration of sperm motility and intensity of spermatozoa in *A. baeri* increased when the dilution rate increased from 1:6 to 1:100. In the present study, the maximum duration of motility has been determined with extender I containing 300 mM glucose. Therefore the extender I provided longer duration of motility since glucose served as an energy resource for spermatozoa. Similar findings were reported by [21] Hatipoglu and Akçay (2010) who found that glucose based extender is a better preservative than Ringer solution for the short term preservation of Abant trout (*Salmo trutta abanticus*) semen.

5. Conclusion

It is concluded from the present study that glucose based extender is best at the dilution ratio of 1:3. But the development of suitable protocol for short term preservation of milt more research in future.

6. References

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