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EFFECT OF SHORT-TERM PRESERVATION OF MIRROR CARP *(CYPRINUS CARPIO)* SEMEN ON MOTILITY, FERTILIZATION, AND HATCHING RATES

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Abstract

Mirror carp (*Cyprinus carpio*) semen was collected by hand stripping for short-term preservation. Two groups of pooled semen were diluted with one of two extenders; a control group was undiluted. The three groups were stored at 4ºC for 72 h. During preservation, spermatozoa motility (%) and duration of spermatozoa movement (s) were determined every 12 h. Following preservation, fertilization was carried out using the dry fertilization technique at 1 x 10^5 spermatozoa/egg. The highest fertilization rates were 21±3.60% in May and 16.67±2.08% in June, both significantly higher than in the control. The highest hatching rates, 69.60±11.86% in May and 89.13±10.75% in June, did not significantly differ from the control.

Introduction

Controlled breeding in captivity is an integral component of aquaculture and allows the production of juveniles throughout the year. However, breeding may be impossible due to a lack of spermiating males or asynchronization of maturity in male and female broodstock.

Artificial insemination requires a large quantity of good quality semen. Collection and storage of good quality semen can improve artificial insemination by reducing the stress to male broodstock caused by repeated semen sampling that reduces semen quality (Ritar, 1999). Short-term storage of semen at 4ºC is

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mostly applied to overcome asynchrony in maturation and difficulties in gamete transportation.

Short-term preservation of fish semen has many applications. It can be useful in artificial propagation and complement cryopreservation (Saad et al., 1988). It can be used in long-term genetic studies aimed at overcoming low fecundity. Collection, evaluation, and storage of semen for several days enable choosing the highest quality semen for desired pair matings. Freshly collected and stored semen can be shipped to other locations for fertilization or cryopreservation (Bozkurt and Secer, 2004).

The basic objective of preservation of spermatozoa is to reduce spermatozoa motility during storage. Spermatozoa motility is the most commonly used criterion to evaluate semen quality. However, in numerous fish species with external fertilization, the duration of sperm motility is very short. Also, studies on most fish species show that the duration and motility of semen may vary seasonally. Therefore determining semen motility is an important component of a preservation program to prevent choosing poor quality semen prior to freezing and to determine fertility of the stored semen after thawing. The relationship between motility and fertilizing capacity has been discussed by Billard and Cosson (1992), Lahnsteiner et al. (1996), and Tekin et al. (2003).

The main purpose of this study was to investigate the effects of short-term preservation of mirror carp (*Cyprinus carpio*) semen on fertilization and hatching rates during the spawning season.

Materials and Methods

Collection of semen and eggs. In the prespawning period, males were kept separately in small ponds and fasted 48 h prior to semen collection. Males were injected with a single dose (2 mg/kg) of carp pituitary extract (CPE), anesthetized with MS 222 (0.1 g/l), and their abdomen and urogenital papillae were dried prior to hand-stripping. Females received a double injection of 3 mg/kg of CPE, the first 10% (0.5 mg/kg CPE) was given 10 h before the second (2.5 mg/kg). Ten to twelve hours after the second injection, their abdomens and urogenital papillae were dried and eggs were hand-stripped. Samples contaminated with fecal material or urine were discarded.

Evaluation of semen volume, motility, movement duration, and pH. Semen was collected into calibrated glass beakers by abdominal massage and the volume was determined in ml. Motility was evaluated by placing about 10 µl semen on a glass microscope slide, adding 100 µl activation solution (0.3% NaCl), and examining the semen under a light microscope at x40 magnification. Motility was expressed as the percentage of spermatozoa that were motile. Only samples having at least 70% motility were used for short-term preservation. The duration of the spermatozoa movement was measured with a sensitive chronometer. During preservation, motility and movement duration were evaluated at 12 h intervals until most of the spermatozoa stopped swimming.

Dilution, short-term preservation, and activation of semen. Samples with at least 70% motility were selected and pooled. The pooled semen was diluted at a ratio of 1:3 with one of two extenders. Extender I contained 440 mg NaCl, 620 mg KCl, 22 mg $CaCl_2$, 20 mg $NaHCO_3$, and 8 mg $MgCl_2$ as described by Kurokura et al. (1984). Extender II contained 350 mM glucose and 30 mM Tris as described by Horvath and Urbanyi (2001). The semen and extenders were kept at 4ºC prior to dilution.

Fertilization. Fertilization was performed by mixing semen with 200 eggs for about 20 s in a petri dish and adding 25 ml of a fertilization solution (3 g urea, 4 g NaCl, and 1 l water) one minute later. About 45 minutes after fertilization, the eggs were rinsed in hatchery water and incubated in a vertical egg incubator. The success of the fertilization was determined 24 h following fertilization as the percent of eyed eggs. The percent of hatched larvae was determined about 72 h after fertilization.

Statistical analysis. Results are presented as means±SD. Differences between parameters were analyzed by repeated analysis of variance (ANOVA). Significant means were

subjected to a multiple comparison test (Duncan) for post-hoc comparisons at a level of $\alpha = 0.05$. All analyses were carried out using SPSS 11 for Windows statistical software package.

Results

Properties of the semen before preservation are shown in Table 1. The effects of short-term storage up to 72 h at 4ºC on the post-activation motility and movement duration, and fertilization and hatching rates are shown in Table 2.

Discussion

Mean semen volume was similar to results reported by Akcay et al. (2002, 2004) but different from those reported by Bozkurt and Secer (2004). The difference may be due to differences in feeding conditions and regime, environmental factors, or spawning time.

The mean spermatozoa motility was similar to the findings of Bozkurt and Secer (2004) but differed from those of Akcay et al. (2002, 2004). Spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on ripeness (Akcay et al., 2002). Most studies on fish species have shown that the duration and motility of semen can vary seasonally (Benau and Terner, 1980; Akcay et al., 2004).

Spermatozoon density corroborates results reported by Akcay et al. (2002, 2004) but not those reported by Emri et al. (1998). The difference may be due to differences in feeding conditions, age, environmental factors, time of spawning, or dilution ratio. The mean pH for mirror carp was generally confirmed by Saad et al. (1988), Lubzens et al. (1997), and Bozkurt and Secer (2004).

Results regarding post-activation motility and duration of movement are supported by similar studies (Kumar, 1988; Jayaprakas and Lal, 1996; Mansour et al., 2004). Both properties decreased with time but the proportion of motile cells decreased faster in fresh semen samples than in activated ones. Movement duration was similarly affected. Similar results were reported by Lahnsteiner et al. (1996), Lubzens et al. (1997), and Bozkurt and Secer (2004).

The highest fertilization rate was 21%, obtained with extender I and similar to the findings of Harvey and Kelley (1984) who reported around 30% fertility and Hulata and Rothbard (1979). Fertilization results were quite low and depend on factors such as dilution ratio, extender type, and egg quality. The low fertilization results mostly correlate with the low spermatozoa/egg ratio of 1×10^5 activated spermatozoa per egg. It can be concluded that a higher concentration of spermatozoa should have been used to obtain a greater number of viable spermatozoa and possibly increase the percentage of spermatozoa surviving the short-term storage. Evaluation of the optimal semen/egg ratio is critical in determining the fertilization capacity of activated semen which can vary among individuals and according to the final concentration of diluted semen and breeding season.

Hatching rates were considerably higher in this study than in that of Munoz and Ojanguren (2002) who reported rates around

Table 1. Spermatological properties of mirror carp *(Cyprinus carpio)* semen (n = 10).

	Sperm volume (ml)	*Spermatozoa motility (%)*	*Movement duration (s)*	*Spermatozoa density (x 10^9/ml)*	*Total spermatozoa (x 10^9)*	*pH*
Range	0.1-55.3	40-95	56-1635	10.73-24.44	1.11-684.3	6.5-8.0
Means±SD	8.95±12.95	77.67±13.87	360.16±177.0	14.53±3.99	152.51±324.35	7.15±0.35

Table 2. Effects of short-term storage preservation on spermatozoa of mirror carp *(Cyprinus carpio)*.

Period of Storage (h)	*May*			*June*		
	Extender I	*Extender II*	*Undiluted semen*	*Extender I*	*Extender II*	*Undiluted semen*
Post-activation motility (%)						
0	81.67±7.63^{a}	80.00±5.00^{a}	80.00±5.00^{a}	80.00±10.00^{a}	76.67±5.77^{a}	85.00±5.00^{a}
12	65.00±13.22^{a}	70.00±10.00^{ab}	71.67±5.77^{a}	60.00±0.00^{a}	66.67±5.77^{a}	68.33±7.63^{a}
24	45.00±13.22^{a}	53.33±15.27^{b}	46.67±32.14^{a}	40.00±10.00^{b}	63.33±5.77^{a}	63.33±5.77^{a}
36	41.67±7.63^{a}	38.33±10.40^{a}	36.67±28.86^{a}	30.00±10.00^{a}	36.67±5.77^{a}	36.67±5.77^{a}
48	33.33±5.77^{a}	30.00±10.00^{a}	16.67±11.54^{b}	26.67±5.77^{a}	20.00±0.00^{a}	23.33±5.77^{a}
60	25.00±5.00^{a}	13.33±5.77^{ab}	6.67±2.88^{b}	23.33±5.77^{a}	13.33±5.77^{ab}	8.33±2.88^{b}
72	18.33±2.88^{a}	11.67±7.63^{a}	5.00±0.00^{a}	18.33±2.88^{a}	6.67±2.88^{b}	5.00±0.00^{b}
Post-activation movement duration (s)						
0	697.33±398.45^{a}	182.00±33.64^{c}	421.33±380.11^{b}	423.33±86.21^{ab}	476.67±124.13^{a}	285.00±47.69^{b}
12	603.33±183.87^{a}	132.67±23.02^{c}	345.33±246.54^{b}	333.33±118.46^{a}	380.00±55.67^{a}	257.00±50.26^{a}
24	458.33±98.78^{a}	115.33±25.54^{b}	232.00±128.42^{b}	221.67±42.52^{a}	303.33±24.67^{a}	216.33±81.24^{a}
36	206.67±67.88^{a}	97.00±33.86^{ab}	168.33±68.98^{b}	143.33±15.27^{a}	233.33±35.11^{a}	141.67±16.07^{a}
48	118.67±19.55^{a}	65.33±20.42^{a}	74.33±11.01^{a}	103.33±5.77^{a}	76.67±27.53^{a}	101.00±15.09^{a}
60	99.67±18.23^{a}	69.67±16.50^{a}	40.00±7.81^{a}	58.33±10.40^{a}	51.67±16.07^{a}	54.00±14.42^{a}
72	46.33±7.76^{a}	36.67±11.50^{a}	7.67±2.51^{a}	35.67±6.02^{a}	25.33±11.67^{a}	11.67±5.68^{a}
Fertilization rate						
	21.00±3.60^{a}	15.33±1.15^{a}	9.00±1.41^{b}	16.67±2.08^{a}	14.33±0.70^{a}	7.00±1.41^{b}
Hatching rate						
	69.60±11.86^{a}	58.90±7.75^{a}	62.50±17.67^{a}	88.05±6.20^{b}	89.13±10.75^{b}	70.85±5.86^{b}

Different superscripts in a row indicate significant differences at $p<0.05$.

20%. On the other hand, Linhart et al. (2000) and Warnecke et al. (2003) reported 35-80% hatchability, rather similar to this study.

The present study indicates that mirror carp (*Cyprinus carpio*) semen can be successfully preserved for 72 h at 4ºC prior to artificial insemination. However, further investigation is needed to determine the optimal semen/egg ratio and to evaluate the viability, survival, and development of larvae produced from short-term stored semen.

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