SHORT-TERM PRESERVATİON OF BROWN TROUT (*SALMO TRUTTA MACROSTİGMA*) SPERM: EFFECT OF EXTENDERS ON MOTİLİTY

İ. YAVAŞ[1], Y. BOZKURT[2], F. KARACA[1]
[1] *Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Hatay, Turkey*
[2] *Mustafa KemalUniversity, Faculty of Fisheries, Department of Aquaculture, Hatay, Turkey*

KRATKOTRAJNO OČUVANJE SPERME POTOČNE PASTRMKE (*SALMO TRUTTA MACROSTİGMA*): DELOVANJE EKSTENDERA NA POKRETLJİVOST

Abstrakt

Cilj eksperimenta je bila procena spermatoloških parametara kratkotrajno čuvane sperme potočne pastrmke (*Salmo trutta macrostigma*) korišćenjem različitih ekstendera. Mleč je uzorkovan od odraslih mužjaka istiskanjem rukom, bez anestezije. Po određivanju najvažnijih karakteristika sperme (volumen, pokretljivost, trajanje pokretljivosti, gustina, pH) uzorci sperme koji su pokazali >80 pokretljivosti su prikupljeni i razblaženi u odnosu 1:3 sa tri različita ekstendera.

Razblažena sperma je čuvana 72 sata na 4°C. Tokom čuvanja na svaka 24 h je procenjivan motilitet spermatozoida (%). U zaključku, rezultati studije su pokazali da se ekstender I pokazao boljim od druga 2 za kratkotrajno očuvanje sperme potočne pastrmke.

Ključne reči: *Salmo trutta macrostigma, kratkotrajno očuvanje, pokretljivost, mleč.*

INTRODUCTION

In combination with illegal methods and heavy fishing pressure, spawning success was reduced caused by pollution of streams, degradation of spawning habitats, river damming and interspecific competition with introduced rainbow trout has caused a decline in the stocks or extinction of native trout populations in Turkey. Brown trout (*Salmo trutta macrostigma*) is endangered by illegal catching methods, human pressures and

degradation of spawning habitats (Alp et al., 2005). Consequently, there is need for good management practices for the conservation of brown trout stocks in Turkey. To fulfill this task, it is necessary to achieve a good knowledge of the reproduction of this subspecies.

Short-term preservation of fish sperm have many applications. Collection, evaluation, and storage of sperm 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 Seçer, 2005). The basic objective of sperm preservation 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, 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 best quality semen prior to freezing and to determine fertility of the stored semen following thawing.

The objective of this experiment was to determine the main spermatological parameters of brown trout (*Salmo trutta macrostigma*), short term preservation of semen in different extenders and to use these results in feasible mass production of this subspecies.

MATERIALS AND METHODS

Broodstock management and collection of sperm

This experiment was carried out at the Çamlıyayla Fish Production Station during the spawning season of brown trout (*Salmo trutta macrostigma*). In the spawning period, adult males were kept seperately in small ponds under constant environmental condition. Water temperature varied between 7 and 11°C during the spawning period (December-January) and adult males were fasted 48 hour prior to semen collection.

Semen was collected into the 20 ml calibrated glass beakers by abdominal massage from 10 adult males (aging between 2 and 5 years old) by the hand stripping method without anesthesia and samples contaminated with faecal material or urine were discarded.

Evaluation of sperm

Semen volume was measured with ml in calibrated glass beakers. Motility was evaluated using a light microscope at x40 magnification and was expressed as percentage of motile spermatozoa before cooling. 0.3 % NaCl was used to estimate motility as activating solution. For the evaluation of motility, about 5 µl semen was placed on a cold glass microscope slide and 100 µl activation solution was added, mixed and covered with a coverslip. For each sample, at least five microscopic fields were observed. The sperm motility was observed by two observers. Only samples showing high motility (>70 %) were pooled and used for storage at 4ºC.

Motility and movement duration were evaluated based on following criterias: 1) Mass progressive motility when most of the spermatozoa were still actively swimming with progressive movement 2) Total duration of movement until most spermatozoa stopped swimming. Movement duration of spermatozoa was estimated using a sensitive chronometer. Semen density was estimated by using the hemocytometric method and expressed as spermatozoa $x10^9$/ml. pH was measured by using indicator papers.

Dilution of sperm and short-term preservation

The pooled samples were diluted at a 1:3 ratio with three different extenders. Extender I contained 600 mg NaCl, 315 mg KCl, 15 mg $CaCl_2.2H_2O$, 20 mg $MgSO_4.7H_2O$, 100 ml distilled water, 1.5 g BSA, 0.5 g sucrose, 7% egg yolk, 1% methanole (modified Lahnsteiner et al., 2000). Extender II contained 4.68 g/lt NaCl, 2.98 g/lt KCl, 0.11 g/lt $CaCl_2$, 3.15 g/lt Tris, 1% DMSO (modified Billard and Cosson, 1992) and extender III contained 600 mg NaCl, 315 mg KCl, 15 mg $CaCl_2.2H_2O$, 20 mg $MgSO_4.7H_2O$, 100 ml distilled water, 1.5 g BSA, 0.5 g sucrose, 7% egg yolk, 10% methanole (Lahnsteiner et al., 2000). Only samples with good initial motility (total motility >70 %) were chosen for experiment. The diluted semen was stored for 72 hours at 4°C. During the cool storage, motility of the spermatozoa was evaluated every 24 hours.

Statistictical analysis

All data were expressed as mean±standard deviation. Relative quantities were transformed by angular transformation and metric data were tested for normality. Data were analyzed one-way multifactorial variance (ANOVA) with subsequent Tukey's-b test.

RESULTS

Average fresh semen motility was 80.4%. According to the results of the experiment, the highest motility (58% after 24 h, 46% after 48 h, 24% after 72 h) and movement duration (60 s after 24 h, 42 s after 48 h, 21 s after 72 h) were determined by using first extender after 24, 48 and 72 hours storage. Spermatological parameters of fresh semen from brown trout are shown in Table 1. Motility values of fresh and short-term stored semen for 72 h are presented in Table 2.

Table 1. Spermatological parameters of brown trout (*Salmo trutta macrostigma*) semen.

	Volume (ml)	Motility (%)	Movement Duration (s)	Density ($x10^9$/ml)	Total Density	pH
X± Sx (n=30)	8.4±0.4	84.7±1.4	68.1±1.3	21.9±0.3	657±..5	7.0±0.5

Table 2. Effects of short-term preservation on sperm motility of brown trout (*Salmo trutta macrostigma*).

Extender Type	Motility (0 h) X ± Sx	Motility (24 h) X ± Sx	Motility (48 h) X ± Sx	Motility (72 h) X ± Sx
I	79.5±5.4 [a]	61,4±5.7 [a]	49,4±3.1 [a]	21.5±3.1 [a]
II	20.3±6.3 [b]	5.1±3.1 [b]	0 [b]	0 [b]
III	61.4±3.1 [c]	21.4±2.4 [c]	0 [b]	0 [b]
Control	81.5±2.4 [a]	0 [d]	0 [b]	0 [b]
p	**	**	**	**

Data expressed as the mean ± SD (n=30)
a, b, c, d : Different superscripts within lines denote significant differences.
** : p< 0,01 : Difference between among groups were significant.

DISCUSSION

Limited amounts of data are available on short-term preservation of brown trout sperm. Most of experiments in this field focused on rainbow trout. The present paper describes the first attempt in Turkey to preserve the brown trout sperm for short periods.

Mean semen volume was similar to the results reported by Gjerde (1984), Munkittrick and Moccia (1987) but different from those reported by Erdahl et al. (1984) and Tekin et al. (2003b). The difference may be due to differences in breeding, feeding conditions and regime, environmental factors, or spawning time. The mean spermatozoa motility observed in this study was similar to the findings of Bozkurt and Secer (2006) but different from those of Schmidt-Baulain and Holtz, (1989). Spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on ripeness (Akçay et al., 2002). Most studies on fish species have shown that the duration and motility of semen can vary seasonally (Akçay et al., 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 Lubzens et al. (1997), and Tekin et al. (2003a). Post-activation motility is one of the most important indicators of the success of a preservation protocol. Spermatozoa motility was affected during preservation in this research. The proportion of motile cells decreased faster with time in undiluted sperm samples than diluted ones. Similar results for the motility parameters of chilled stored spermatozoa were reported in fish in some experiments (Stoss and Holtz 1983, Bozkurt and Secer 2005). It is possible to enhance the fertilizing capacity of fish by using suitable activating mediums that increase motility duration.

Knowledge of the factors influencing spermatozoa motility has tremendous importance in fish breeding and aquaculture. In this research spermatozoa stored without oxygen supply at 4°C showed a rapid decrease in motility (Table 2). It can be concluded from the results of this research that, aerobic conditions are necessary for maintaining the viability of spermatozoa during in vitro storage. Similarly, previous studies have shown that short-term fish sperm preservation can be improved with the addition of oxygen. According to Billard (1981), rainbow trout sperm survival was improved after storage in an oxygen atmosphere in comparison with storage in air. Under suitable conditions, salmonid sperm can be stored for a few weeks in unfrozen form. This methodology is important because it is not always practical or even possible to harvest sperm and use it to fertilize eggs immediately (Bencic et al. 2000). Previous investigations showed that successful short-term storage of salmonid sperm depends on numerous factors such as temperature, fluid volume, and gaseous environment (Rana, 1995). The most commonly used method of short-term storage has been under an atmosphere of 100 % O_2 at low temperatures (Billard, 1981, Rana, 1995).

Storage temperature is also important factor affecting viability of fish gametes in vitro experiments. Viability can be prolonged by maintaining gametes and embryos close

to 0°C to reduce metabolic rate. However, the ability to tolerate low temperature may vary between temperate and tropical species (Leung and Jamieson 1991). According to Carpentier and Billard (1978) sperm stored in vitro conditions could be survived for one to several days at 1–4°C. (Buyukhatipoglu and Holtz (1978) also reported that the spermatozoa of rainbow trout were fertile for 21 days at 4°C under oxygen or air. However, the motility percentage and beat frequency of the flagellum continued to decline with an increase in storage period and the fertilizing capacity of sperm was completely lost after 6 days of storage. Harvey and Kelley (1984) found that post-activation motility of undiluted Sarotherodon mossambicus sperm stored at 5°C declined to zero in 60–120 h.

The efficiency of spermatozoa storage was affected by individual sample variability, but not by the genetic source of donors. This individual variability of samples, usually named ''individual male variability'' results from biological variation among individuals, as well as from collection techniques (Piironen, 1985). In the present study, collected semen from 10 males were pooled in equal amounts to eliminate the effect of individual variability of gamete donors. Contamination with urine is considered as an important interference factor, randomly affecting quantitative characteristics of spermatozoa (Glogowski et al, 2000). This may play an important role in short-term storage of spermatozoa. The method of storage showed a highly significant effect on fertilization ability, as well as on motility and concentration of refrigerated spermatozoa. In addition different storage methods (bags, foils, glass and plastic tubes) may affect motility and fertility in cooled semen samples. Also the changes in spermatozoa concentrations during storage indicate that a desiccation process has likely been the reason for differences among different storage methods (Bozkurt et al. 2005).

CONCLUSIONS

In this experiment, significant differences in motility of sperm stored were observed in different extenders. The best motility rates were obtained by modified Lahnsteiner solution. In conclusion, the present study indicates that brown trout (*Salmo trutta macrostigma*) semen can be successfully preserved for 48 h at 4ºC prior to fertilization. However, further investigations are 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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