Universal Journal of Agricultural Research 1(3): 65-69, 2013
DOI: 10.13189/ujar.2013.010304 http://www.hrpub.org

Effect of Different Extenders and Storage Periods on Motility and Fertilization Rate of Rainbow Trout (*Oncorhynchus Mykiss*) Semen

Temel Şahin[1,*], İlker Zeki Kurtoğlu[2], Fikri Balta[2]

[1]Recep Tayyip Erdoğan University, Maritime Collegue, Rize, Turkey
[2]Recep Tayyip Erdoğan University, Faculty of Fisheries, Rize, Turkey
*Corresponding Author: temel.sahin@erdogan.edu.tr

Abstract The effects of different extenders and storage periods on the motility and fertilization rate of rainbow trout (Oncorhynchus mykiss) semen were evaluated after short-term storage. Semen was collected from anesthetized males by the abdominal massage. After determination of main semen characteristics, the pooled ejaculates were diluted with 4 different extenders at a ratio of 1:3, and stored at 4ºC for 72 h. During preservation, spermatozoa motility (%) were determined every 12 h. Fertilization was carried out using the dry fertilization technique. The sperm-egg ratio was approximately 0.5x106 sperm/egg for optimum fertilization success. The highest motility (64.4±5.27%) and fertilization rate (94.3 ± 0.58 %) were obtained from semen stored with glucose based extender after 72 h storage. These results indicate that glucose based extender is a better preservative than the other solutions used in the study for the short term preservation of rainbow trout semen.

Keywords Rainbow Trout, Extender, Semen, Short-Term Storage, Fertility

1. Introduction

Preservation of fish sperm for short-term duration is generally useful from the commercial point of view and facilitates various hatchery operations. The short-term storage of sperm at low temperature (4ºC) is mostly applied in short-distance transport of gametes collected in different locations, in synchronizing the timing of obtaining good quality of gamete collection from males and females during artificial insemination, in avoiding the aging of sperm, in facilitating hatchery operations, also in experimental programs for genetic studies [1-3].

The fish sperm could be preserved by storage in undiluted and diluted form. Undiluted sperm stored at low temperature has been reported to cause a reduction in fertilization capacity [4]. Storage of diluted sperm with extender provides better control compared to undiluted storage [5]. Extender solutions can be mixed with semen to increase the volume of the semen samples and to prevent it from deteriorating while it is being held or shipped for insemination.

The quality of the stored sperm can be assessed by taking into consideration motility, motility duration, and by its insemination ability [3]. Buyukhatipoglu and Holtz [6] reported that the spermatozoa of rainbow trout were maintained fertilization capacity at 4°C under oxygen and air medium for 15 and 9 days, respectively. Sperm of rainbow trout diluted with ovarian fluid and borax-boric acid buffer and the highest insemination achievement (81.76%) was obtained when ovarian fluid was used as a sperm extender but the spermatozoa demonstrated maximum (34 seconds) forward motility aftcr dilution with borax-boric acid buffer [7]. Bencic et al. [8] suggested that atmospheric air served as a suitable gas for the short-term preservation of salmonid semen. Canyurt et al. [9] indicated that the best results in fertilization and motility were obtained from the sperm samples of rainbow trout diluted with artificial seminal plasma with the rate of 1:1 and stored for 7 days [9]. Hatipoğlu and Akçay [10] found that glucose based extender was a better preservative than Ringer solution for the short term preservation of Abant trout (*Salmo trutta abanticus*) semen. Niksirat et al. [11] reported that semen samples stored in the absence of antibiotics completely lost fertilizing capacity within 19 days of storage. Şahin et al. [12] clarified that *Salmo coruhensis* sperm stored in extenders containing glucose, calcium chloride or magnesium chloride remained motile for 24, 14 and 18 days, respectively.

As seen in previous studies, despite the use of various diluents to preserve fish sperm without reducing fertilizing capacity, results showed that sperm motility, motility duration, viability and fertilizing capacity vary widely. Individual variation, collecting method and storage conditions affect the success in fish sperm dilution [13]. The goal of this study was to identify the effect of cold storage of

sperm from rainbow trout by assessing sperm motility and its insemination ability using different extenders.

2. Materials and Methods

2.1. Broodstock Care and Collection of Sperm and Eggs

The experiment was carried out at the Recep Tayyip Erdoğan University, Iyidere Fisheries Research Centre (IFRC), Rize, Turkey. The broodstock were held in a circular fiberglass tank under a natural illumination and fed with a commercial trout diet at 2% of their body weight per day. The average water temperature was measured as 12.9±0.58 °C (8.0-18.5 °C) during spawning season. A total of 6 mature rainbow trout males (total weight 2536 ±316.3g, total length 56.7 ± 2.82 cm) were randomly selected from broodstock and were used as semen donors in the middle of the spawning season. Fish were fasted for two days prior to collection of semen, anaesthetized in 30 ppm of benzocaine and their abdomens were dried before stripping in order to avoid contamination of semen with urine, mucus and blood cells. The semen was collected into 10 ml graduated glass tubes by gentle abdominal massage. Each male was stripped once only and the total amount of expressible milt was collected individually and the sperm volume was expressed as ml. The semen samples were held on crushed ice (4ºC) before analysis, which was undertaken within 4 h of stripping. Eggs were gathered from 5 mature females without anesthesia.

2.2. Evaluation of Sperm Density and Spermatocrit

Sperm density was determined according to the haemacytometric method [14]. Semen was diluted by pipetting 10 µl semen into 990 µl 0.7% NaCl solution. One droplet of diluted semen was placed on a hemocytometer slide (depth 0.1 mm) with a coverslip, the sperm was allowed to settle for 3-5 min, sperm cells were counted using light microscopy (x 40), and spermatozoa density was expressed as x 10^9cells/ml. Spermatocrit was defined as the ratio of white packed material volume to the total volume of semen multiplied by 100 [15]. Heparinized microhematocrit capillary tubes (75 x 1.1-1.2 mm) were filled with semen and one end was sealed with clay. The capillary tubes were centrifuged at 10,000 rpm for 10 min.

2.3. Evaluation of Sperm Motility, Motility Duration and Ph

The motility of fresh spermatozoa from each male was determined immediately after semen was collected. The percent of spermatozoa exhibiting rapid, vigorous, forward movement was determined subjectively under a microscope (x 400 magnification) by diluting the semen in activation solution (0.3% NaCl) at a ratio of 1:100 (1 µl sperm to 99 µl activation solution). Motility duration was assessed using a sensitive chronometer (1/100) that was started simultaneously with the addition of activation solution into the samples. Sperm motility observations were done using three replicates per sample. All of the experiments were performed at 17-20°C and all of measurements were carried out by the same investigator under the same conditionsfor avoiding subjective errors. pH was measured by using indicator papers (Merck 6.4-8).

2.4. Selection of the Sperm Extenders

Four extenders containing calcium chloride (E1), magnesium chloride (E2), glucose (E3) and calcium+magnesium chloride (E4) [16] were selected for evaluation as potential extenders for rainbow trout sperm. The chemical composition of each extender is shown in Table 1. The semen and extenders were maintained in a refrigerator before dilution. For selecting the most suitable extender, the semen was mixed with extender solution at a ratio of 1:3 and placed into 2 ml small plastic vials that were three replicates per treatment. After a rapid shaking, the vials were stored in a refrigerator at 4°C. The samples were analyzed by 12 hours intervals.

Table 1. The composition of semen extenders.

	Composition of extenders (g/L)					
Extenders	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$	Glucose
E1	8.75	0.20	0.20	-	0.30	-
E2	8.75	0.20	-	0.20	0.40	-
E3	7.25	0.40	-	-	0.80	2.0
E4	8.75	0.20	0.10	0.10	0.40	-

2.5. Fertilization

Eggs were pooled from 5 females. Fertilization took place in dry plastic dishes using dry fertilization technique and a portion of ca. 100 eggs (10 g) was placed into each dish. The egg groups were fertilized with cold preserved semen for 72 h storage. Eggs and sperm cells were gently mixed for 10 s. The proportion of sperm-egg was approximately $0.5x10^6$ sperm/egg for optimum fertilization success [17]. After fertilization, 25 ml of 0.3% sodium chloride was added to the sperm-egg mixture as fertilization solution and left for 45 min for swelling of eggs. Subsequently, the fertilized eggs were rinsed with hatchery water (10ºC) and placed into vertical incubation trays. Unfertilized and dead eggs were counted and removed continuously. The fertilization rate was determined with the percent of eyed-egg 21 days after insemination.

2.6. Statistical Analysis

All measurements were done in triplicate for each sample and the average of three measurements was used in

subsequent statistical analyses. Data were expressed as means±SD. Motility data were normalized through arcsine transformation. Pearson correlation analysis was used to estimation with spermatologic parameters. Differences between parameters were analyzed by one-way analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Tukey HSD) for post-hoc comparisons at a level of $\alpha = 0.05$. All analyses were carried out using SPSS 15.0 for Windows statistical software package.

3. Results

Spermatologic parameters of fresh semen from rainbow trout are shown in Table 2. The volume of semen collected ranged from 10.4 to 21.8 mL. The sperm concentration in the seminal plasma varied between individuals and ranged from 1.6 x 10^9 to 15.4 x 10^9 sperm cells per milliliter of semen. The semen pH ranged from 7.0 to 7.7, and motility ranged from 80 to 100 per cent.

The effects of extenders and storage periods up to 72 h at 4ºC on motility are shown in Table 3.All semen samples were stored in the refrigerator (4ºC) for 72 h and the motility drastically declined during storage period. The mixing of fresh semen with the glucose based extender solution (E3) significantly ($P < 0.05$) increased the motility of spermatozoa. In contrast, the semen mixed with calcium chloride (E1), magnesium chloride (E2), calcium+magnesium chloride (E4) and the semen stored without the extender (control) had a greatly reduced sperm motility.

Table 2. Average spermatologic parameters of rainbow trout semen (n = 6)

Parameters	Min	Max	Means	SD
TL	53.0	61.0	56.7	2.82
BW	2064	2919	2536	316.3
Volume (mL)	10.4	21.8	17.0	4.56
Spermatocrit (%)	25.0	37.6	33.0	4.28
pH	7.0	7.7	7.4	0.28
Motility (%)	80	100	96.7	8.16
Duration of motility (s)	85	206	121	43.9
Density (x 10^9)	1.6	15.4	8.4	4.75

The effect of extenders on fertilization rates are shown in Table 4. The differences between the groups were statistically significant ($p < 0.05$). The highest fertilization rate (94.3±0.58%) was obtained with glucose-based extender.

Table 3. Effect of extenders and storage periods on motility rates.

Storage period (h)	E1	E2	E3	E4	Control
12	79,4±6,82^{a}	82,2±5,65^{a}	93,3±4,33^{b}	82,8±5,65^{a}	87,7±6,18^{ab}
24	68,9±7,41^{a}	74,4±9,17^{a}	86,1±4,17^{b}	67,8±4,41^{a}	82,8±5,65^{cb}
36	56,7±7,50^{a}	59,4±5,27^{a}	83,3±5,59^{b}	53,9±4,86^{a}	77,2±6,18^{cb}
48	53,3±4,33^{a}	55,6±4,64^{a}	73,3±5,00^{b}	44,4±5,83^{a}	56,7±5,59^{a}
60	47,2±9,05^{a}	48,3±7,07^{a}	67,8±7,95^{b}	42,2±3,63^{a}	48,3±6,12^{a}
72	23,9±4,86^{a}	22,8±4,41^{a}	64,4±5,27^{b}	22,2±3,63^{a}	26,7±6,61^{a}

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

Table 4. Effect of extenders on fertilization rates of rainbow trout sperm.

Extenders	Fertilization rates (%)
E-1	66,0±8,54^{a}
E-2	90,3±1,53^{ab}
E-3	94,3±0,58^{b}
E-4	91,0±2,00^{ab}
Control	85,7±2,52^{ab}

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

4. Discussion

In comparison to other salmonid species (Table 5), the sperm volume found in this study was high compared to *S. trutta fario* [18], *O. mykiss* [19], *S. trutta abanticus* [10], *S. trutta macrostigma* [20], *S. coruhensis* [12] but was similar compared to *O. mykiss* [21]. Sperm density recorded for *O. mykiss* was lower than values observed in *S. trutta abanticus* [10] and *S. coruhensis* [12] but higher than values found in *O. mykiss* [19], *S. trutta caspius* [22], *S. trutta macrostigma* [20]. After freshwater activation, 96.7±8.16% of spermatozoa were motile, higher than in *O. mykiss* [19, 22, 23], and *S. trutta magrostigma* [20].

Table 5. Sperm volume**,** density and motility of some salmonid species.

Species	Volume (ml)	Density ($\times 10^9$/ml)	Motility (%)
S. trutta fario [18]	3.9±1.48	-	-
O. mykiss [19]	1.22±0.22	6.06±0.90	73.25±5.15
O. mykiss [21]	18.17±2.74	-	72.29±10.79
O. mykiss [23]	-	-	78.25±3.63
S. trutta abanticus [10]	7.4±0.3	17.9±0.4	-
S. trutta macrostigma [20]	13.93±0.84	6.02±0.46	80.37±2.36
S. coruhensis [12]	1.6±0.64	13.0±4.93	-
S. trutta caspius [22]	-	3.3	-

Spermatozoa that were immotile in the seminal fluid were rapidly activated in contact with fresh water and remained motile for 85-206 s, similar to values reported by Büyükhatipoğlu and Holtz [6], Babiak et al. [24], and Tekin et al. [25], but higher than reported by Bozkurt et al. [21] and Tuset et al. [26] as 78-174 and 22-33 s, respectively. The differences in the properties of sperm may be due to differences in breed, biological characteristics and rearing conditions of brooders, artificial induction of spawning and spawning season.

Short-term storage of sperm using different extenders has been reported in *Oncorhynchus mykiss* [9], *Cyprinus carpio* [27], *Clarias gariepinus* [13], *Salmo trutta abanticus* [10] and *Salmo coruhensis* [12]. In the study, sperm motility was affected during preservation and the proportion of motile cells decreased as the length of storage increased in all groups. The best motility results were obtained with glucose based extender (E3). Similar results for the motility parameters of cold preserved spermatozoa were reported in fish in some experiments [10, 12, 28].

The fertilization results were quite high except for extender E1. Although extenders E2 and E4 were suitable for short-term preservation of rainbow trout sperm, the fertilization rates were all lower than those obtained with extender E3 which gave the best fertilization rates. The reason for the differences among groups in fertilization rate can be explained with decrease in spermatozoa motility. Furthermore, the fertilization can be affected by factors such as dilution ratio of sperm, extender composition, and egg quality [27].

In this investigation, it has been shown that the effect of storage time and extender on motility and fertilization rate was significant ($p < 0.05$).The fertilization success obtained when extender E3 used as a sperm diluent was significantly better ($p < O. 05$) than al1 the other diluents tested. Extender E3 was the most suitable diluent for cold storage of rainbow trout semen at 4°C.

Acknowledgements

This study is based in part on the Scientific Research Project. The authors thank Recep Tayyip Erdoğfan University, Center of Scientific Research Project, Rize, Turkey for the equipment and material support.

REFERENCES

[1] Scott AP, Baynes SM (1980) A review of the biology, handling and storage of salmonid spermatozoa. J Fish Biol 17:707–739.

[2] Stoss J (1983) Fish gamete preservation and spermatozoan physiology. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish Physiology, vol 9, Part B. Academic Press, San Diego, CA, USA, pp 305–350.

[3] Rana K (1995) Preservation of gametes. In: Bromage NR, Roberts R B (eds) Broodstock management and egg and larval quality. Blackwell, Oxford, pp 53-75.

[4] Lahnsteiner F, Weissman T, Patzner RA (1997) Aging processes of rainbow trout semen during storage. Prog

Fish-Cult 59:272–279.

[5] Harvey B, Kelley RN (1984) Chilled storage of Sarotherodon mossambicus semen. Aquaculture 36:85–95.

[6] Büyükhatipoglu Ş, Holtz W (1978) Preservation of trout sperm in liquid or frozen state. Aquaculture 14(1): 49-56.

[7] Heerden E, Vuren JHJ, Steyn GJ (1993) Development and evaluation of sperm diluents for the artificial insemination of rainbow trout (Oncorhynchus mykiss). Aquat Living Resour 6:57-62.

[8] Bencic DC, Krisfalusi M, Cloud JG, Ingermann RL (2000) Short-Term Storage of Salmonid Sperm in Air versus Oxygen. North American Journal of Aquaculture 62:19-25.

[9] Canyurt MA, Akhan S, Takma Ç (2003) A study on short-therm storage of rainbow trout (Oncorhynchus mykiss Walbaum, 1792) milt. EU Journal of Fisheries & Aquatic Sciences 20(3-4):537-542.

[10] Hatipoğlu T, Akçay E (2010) Fertilizing ability of short-term preserved spermatozoa Abant trout (Salmo trutta abanticus T, 1954). Ankara Üniv Vet Fak Derg 57:33-38.

[11] Niksirat H, Sarvi K, Abdoli A, Hajirezaee S (2011) Short-Term Storage of Semen of Rainbow Trout: Interactions of Time, Antibiotic, and Activator. Journal of Applied Aquaculture 23:358-366.

[12] Şahin T, Kurtoğlu İZ, Delihasan Sonay F, Ak A (2013) Quantitative Characteristics and Short-term Storage of Salmo coruhensis Sperm. Isr J Aquacult-Bamidgeh IJA:65.2013.828, 6 pages.

[13] Vuthiphandchai V, Thadsri I, Nimrat S (2009) Chilled storage of walking catfish (Clarias macrocephalus) semen. Aquaculture 296:58–64.

[14] Tvedt HB, Benfey TJ, Martin-Robichaud DJ, Power J (2001) The relationship betweensperm density, spermatocrit, sperm motility and fertilization success in Atlantic halibut, Hippoglossus hippoglossus. Aquaculture 194:191–200.

[15] Rurangwa E, Kime DE, Ollevier F, Nash JP (2004) The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture 234:1-28.

[16] Liu L, Wei Q, Guo F, Zhang J, Zhang T (2006) Cryopreservation of Chinese sturgeon (Acipenser sinensis) sperm. J Appl Ichthyol 22(Suppl. 1):384-388.

[17] Billard, R., 1977. A new technique of artificial insemination for salmonids using a sperm diluent. Fisheries, Vol II, No. 1: 24-25.

[18] Bozkurt Y, Seçer S, Bukan N, Akçay E, Tekin N (2006) Relationship between body condition, physiological and biochemical parameters in brown trout (Salmo trutta fario) sperm. Pak J Biol Sci 9(5):940-944.

[19] Aral F, Şahinöz E, Dogu Z (2007) A study on the milt quality of Oncorhynchus mykiss (Walbaum, 1972) and Carasobarbus luteus (Heckel, 1843) in Atatürk Dam Lake, Southeastern Turkey. Turk. J. Fish. Aquat Sci 7:41-44.

[20] Bozkurt Y, Öğretmen F, Kökçü Ö, Ercin U (2011) Relationships between seminal plasma composition and sperm quality parameters of the Salmo trutta macrostigma (Dumeril, 1858) semen: with emphasis on sperm motility. Czech J Anim Sci 56(8):355-364.

[21] Bozkurt Y, Seçer S, Tekin N, Akçay E (2005) Cryopreservation of rainbow trout (Oncorhynchus mykiss) and mirror carp (Cyprinus carpio) sperm with glucose based extender. Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi 1(1):21-25 (in Turkish).

[22] Hatef A, Niksirat H, Amiri BM, Alavi SMH, Karami M (2007) Sperm density, seminal plasma composition and their physiological relationship in the endangered Caspian brown trout (Salmo trutta caspius). Aquacult Res 38:1175-1181.

[23] Canyurt MA, Akhan S (2008) Effect of ascorbic acid supplementation on sperm quality of rainbow trout (Oncorhynchus mykiss). Turk J Fish Aquat Sci 8:171-175.

[24] Babiak I, Fraser L, Dobosz S, Goryczko K, Kuzminski H, Strzezek J (1999) Computer-controlled freezing of rainbow trout Oncorhynchus mykiss (Walbaum) spermatozoa for routine programmes. Aquacult Res 30:707-710.

[25] Tekin N, Seçer S, Akçay E, Bozkurt Y, Kayam S (2003) Gökkuşağı alabalıklarında (Oncorhynchus mykiss W., 1792) yaşın spermatolojik özellikler üzerine etkisi. Türk J Vet Anim Sci 27:37-44 (in Turkish).

[26] Tuset VM, Dietrich GJ, Wojtczak M, Słowińska M, de Monserrat J, Ciereszko A (2008) Relationships between morphology, motility and fertilization capacity in rainbow trout (Oncorhynchus mykiss) spermatozoa. J Appl Ichthyol 24:393-397.

[27] Bozkurt Y, Secer S (2005) Effect of Short-Term Preservation of Mirror Carp (Cyprinus carpio) Semen on Motility, Fertilization, and Hatching Rates. Isr J of Aquac – Bamidgeh 57(3):207-212.

[28] Bozkurt Y, Öğretmen F, Seçer FS (2009) Effect of Different Extenders and Storage Periods on Motility and Fertilization Success of Grass Carp (Ctenopharyngodon idella) Sperm During Spawning Season. Tarım Bilimleri Dergisi 15 (3):277-284