Ankara Üniv Vet Fak Derg, **57**, 33-38, 2010

Fertilizing ability of short-term preserved spermatozoa Abant trout (*Salmo trutta abanticus T,* 1954)*

Taner HATİPOĞLU[1] , Ergun AKÇAY [2]

[1] Ministry of Environment and Forestry, General Directorate of national parks and nature protection, Ankara, Turkey,[2] Department of Animal Reproduction and AI, Faculty of Veterinary Medicine, University of Ankara, Turkey.

Summary: The objective of this experiment was to evaluate spermatological parameters and fertilizing ability of short term stored semen in different extenders from endemic Abant trout (*Salmo trutta abanticus T,*1954). Semen was collected from 30 adult males by the hand stripping method without anesthesia. Having determined the main spermatological properties (volume, motility, movement duration, concentration and pH), the pooled samples were diluted at a 1:2 ratio with two extenders (0.3 M glucose or Ringer solution). The diluted semen was stored for 48 hours at 4°C. Following the cooled storage, motility of the spermatozoa was evaluated after 24 and 48 hours regarding the post-cooled period. Dry fertilization technique was used for the fertilization trials. Eggs were pooled from 30 females by the hand stripping method. Fertilization took place in dry plastic dishes and 600 eggs were used in each fertilization trial. The sperm-egg ratio was approximately $0.25x10^6$ sperm/egg. After swelling, eggs were rinsed with hatchery water (7^oC) and batches were placed into vertical incubation trays. The experimental success was assessed from sperm motility and the percent of eyed-egg 25 days after fertilization. Average fresh semen motility was 81.4 %. According to the results of the experiment, the highest motility (67 % after 24 h, 53 % after 48 h) and movement duration (60 s after 24 h, 42 s after 48 h) were determined by using glucose extender after 24 and 48 hours storage. Fertility rate of fresh semen was 84.4 %. The highest eyed-egg rate (80.4 %) was obtained from semen stored with glucose based extender after 24 h storage. After 48 hour storage fertilization yield for the semen diluted with 0.3 M Glucose decreased to 61.9 % and to 43.8 % for the one diluted with Ringer solution. Our results indicate that glucose based extender is a better preservative than Ringer solution for the short term preservation of Abant trout semen.

Key words: Abant trout, semen, short-term preservation, fertility

Kısa süreli saklanmış Abant alabalığı spermatozoonlarının fertilizasyon yeteneği

Özet: Bu araştırma ile, ülkemize ait endemik bir balık türü olan Abant alabalığının spermatolojik parametrelerinin değerlendirilmesi ve farklı sulandırıcılarda kısa süreli saklanan spermatozoonların fertilizasyon yeteneğinin ortaya konulması amaçlanmıştır. Bu araştırmada, 30 ergin erkek balıktan anestezi yapılmaksızın masaj yoluyla sperma alınmıştır. Birincil spermatolojik parametreler (miktar, motilite, haraket süresi, yoğunluk ve pH) belirlendikten sonra birleştirilen örnekler iki farklı sulandırıcı ile (0,3 M glukoz veya Ringer solusyonu) 1:2 (sperma:sulandırıcı) oranında sulandırılmıştır. Sulandırılan sperma 4°C'de 48 saat saklanmıştır. Saklama işlemi esnasında 24. ve 48. saatlerde motilite değerlendirmesi yapılmıştır. Fertilizasyon denemeleri için kuru fertilizasyon tekniği kullanılmıştır. Yumurtalar 30 ergin dişiden masaj yoluyla alınmıştır. Fertilizasyon kuru plastic kaplarda gerçekleştirilmiş ve her bir fertilizasyon denemesi için 600 yumurta kullanılmıştır. Tohumlama dozu olarak herbir yumurta için $0,25x10^6$ spermatozoa kullanılmıştır. Yumurtaların şişmesinden sonra üzerlerine kuluçka suyu (7^oC) eklenerek dikey inkubasyon tepsilerine yerleştirilmiştir. Deneysel başarının tesbiti için spermatozoon motilitesi ve fertilizasyon sonrası 25. günde gözlü yumurta sayısı dikkate alınmıştır. Araştırmadan elde edilen sonuçlara göre taze sperma motilitesi ortalama % 81,4 olarak bulundu. Saklama sonrası en yüksek motilite (24. saat: % 67; 48. saat: % 53) ve hareket süreleri (24. saat: 60 saniye; 48. saat: 42 saniye) glukoz sulandırıcısı ile elde edildi. Taze spermadan elde edilen fertilizasyon oranı % 84,4 olarak bulundu. En yüksek gözlü yumurta oranı (% 80,4) 24 saat glukoz sulandırıcısında saklanan spermadan elde edildi. Saklamanın 48. saatinde bu oran glukoz sulandırıcısı için % 61,9'a, ringer sulandırıcısı için % 43,8'e düştü. Sonuçlara göre, Abant alabalığı spermasının kısa süreli saklanmasında glukoz tabanlı sulandırıcı Ringer sulandırıcısına oranla daha iyi koruyucu özellliklere sahip olduğu tesbit edilmiştir.

Anahtar sözcükler: Abant alabalığı, fertilite, kısa süreli saklama, sperma

Introduction

Salmo trutta abanticus is native to lake Abant and the nearest rivers and creeks. And it is known to be endemic to Lake Abant, Turkey (17) being reared in natural conditions as well. Moreover, *Salmo trutta abanticus* is important as a biological gene source for restocking in Turkey. Nowadays, *Salmo trutta abanticus* populations are badly affected by natural phenomena

* This research summarized from PhD thesis entitled "Determination of Some Reproductive Properties of Abant Trout (*Salmo Trutta Abanticus*), Short Term Storage of Semen and Fertilitization Yield"

(e.g., droughts and climate change) or human activities (e.g., overfishing, tourism, pollution and industry).

Short-term preservation of fish semen has many applications. It can be used in longterm genetic studies aimed at overcoming low fecundity (32). 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 (10). 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 (9), Lahnsteiner et al. (23), and Tekin et al. (39).

The objective of this study was to determine the main spermatological parameters of endemic Abant trout (*Salmo trutta abanticus T,*1954), to study fertilitization yield of insemination made by native and short term stored semen in different extenders and to use these results in feasible mass production of this subspecies.

Materials and Methods

Collection of semen and eggs: In this experiment, adult males and females of Abant trout aging between 2 and 5 years old were obtained from Fish Production Station in Yedigöller, Bolu, Turkey. In the spawning period, adult fishes were kept seperately in small ponds under constant environmental condition and were fed with a pelleted diet (50 % protein). Water temperature varied between 7 and 12°C during the spawning period (december-january) and adult fishes were fasted 48 hour prior to semen collection. The semen was collected into the 20 ml calibrated glass beakers by abdominal massage from 30 mature male Abant trout without anesthesia and samples contaminated with faecal material or urine were discarded. Eggs were collected from 30 mature females which were stripped by gently massaging the abdomen without anesthesia. The eggs used for the experiments were well-rounded and transparent.

Evaluation of semen volume, motility, movement duration, concentration and pH: Semen was collected into calibrated glass beakers by abdominal massage and the volume was measured with ml. Motility was evaluated using a light microscope at x400 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 used for cooled storage. Motility and movement duration were evaluated based on following criteria: 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. The semen concentration was estimated by using the hemocytometric method and expressed as spermatozoa $x10^9$/ml. pH was measured by using indicator papers.

Dilution, short-term preservation, and activation of semen: Collected semen from 30 males was pooled in equal amounts to eliminate the effect of individual variability of gamete donors. The pooled semen was diluted at a ratio of 1:2 (semen/extender) with two different extenders. Extender I, contained 0.3 M glucose (glucose-based solution); extender II contained 0.11 M NaCl, 0.04M KCl, 0.0024 M $NaHCO_3$ and 0.002 M $CaCl_2$ (Ringer Solution). The semen and extenders were kept at 4ºC prior to dilution. For the cooling storage diluted spermatozoa were stored at 4°C for 48 h in 10ml glass tubes in a refrigerator. Motility was estimated after 24 h and 48 h during storage. For the activation, saline solution (0.3 % NaCl) was used.

Fertilization: For the fertilization, dry fertilization technique was used. Eggs were pooled from 30 females. Fertilization took place in dry plastic dishes and 600 eggs (about 60 g) was placed into each dish. Batches of eggs were inseminated with cold stored semen for 24 h or 48 h storage or fresh semen for control. Eggs and sperm cells were gently mixed for 10 s. The sperm-egg ratio was approximately $0.25x10^6$ sp/egg. After insemination, 25 ml fertilization solution (0.3 % NaCl) was added on sperm-egg mixture and left for 45 min to allow eggs to swell. After swelling, eggs were rinsed with hatchery water (7^oC) and batches were placed into vertical incubation trays. The experimental success was determined with the percent of eyed-egg 25 days after fertilization.

Statistical analysis: Data were presented as mean ± standard error of mean (X±Sx). Differences between motility and fertility averages regarding hours were analyzed by Wilcoxon test. Differences between hours regarding motility and fertility were analyzed by Mann-Whitney U test. All analyses were carried out using SPSS 11 for Windows statistical software package.

Results

Spermatological parameters of fresh semen from Abant trout are shown in Table 1.

Motility values of fresh and short-term stored semen for 24 - 48 h are presented in Table 2.

Table 1: Average spermatological parameters of Abant trout semen
Tablo 1: Abant alabalığında ortalama spermatolojik parametreler

	Volume (ml)	Motility (%)	Movement Duration (s)	Concentration ($x10^9$/ml)	pH
X± Sx (n=30)	7.4±0.3	81.8±1.7	72.1±1.7	17.9±0.4	7.5±0.1

Table 2. Motility values of fresh and short-term stored semen
Tablo 2: Taze ve kısa süreli saklanmış spermada motilite değerleri

		Fresh semen		Short-term stored semen			
	No	Mot. (%)	Motility (control)	Motility 24 h (glucose)	Motility 24 h (Ringer)	Motility 48 h (glucose)	Motility 48 h (Ringer)
1st trial	1	90					
	2	75	81,6	70	50	60	40
	3	80					
2nd trial	4	70					
	5	90	75.0	50	40	35	30
	6	70					
	7	70					
3rd trial	8	90					
	9	70	78,3	70	40	60	30
	10	75					
4th trial	11	80					
	12	90	86,6	65	40	50	30
	13	90					
5th trial	14	90					
	15	80	88,3	70	50	60	40
	16	95					
X ± Sx			81.5±6.4	65.0±3.9	44.0±4.9	53.0±2.4	34.0±2.4

Table 3: Differences in motility between extenders and storage periods
Tablo 3: Sulandırıcılar ve saklama süreleri arasındaki motilite farklılıkları

Extender	24 h X ± Sx	48 h X ± Sx	p
Glucose	65.0 ± 3.9	53.0 ± 4.9	*
Ringer	44.0 ± 2.4	34.0 ± 2.4	*
p	**	*	

* : $p < 0.05$
** : $p < 0.01$

The effects of extenders and storage periods up to 48 h at 4ºC on the postactivation motility are shown in Table 3.

Fertility results obtained by fresh or stored semen are shown in Table 4. Dry fertilization was applied and approximately 600 eggs per batch were inseminated (five replicates per treatment) with frozen or fresh (control) semen. Fertility changes in fertilization trials with different extenders are shown in Figure 1. The effects of extenders and storage periods up to 48 h at 4ºC on the fertility are shown in Table 5.

Table 4: Fertility data obtained from fertilization trials
Tablo 4: Fertilizasyon denemelerinden elde edilen fertilite bulguları

Trils	Glucose 24 h	Ringer 24 h	Glucose 48 h	Ringer 48 h	Control
	n (fertilized egg/600)				
1st trial	426	391	346	264	438
2nd trial	384	318	277	179	413
3rd trial	397	342	306	217	420
4th trial	390	329	287	202	415
5th trial	414	354	332	233	426
X±Sx	402.2±7.8	346.8±12.6	309.6±13.1	219.0±14.3	422.4±10.1
%	80.4	69.4	61.9	43.9	84.5

Table 5: Differences in fertility between extenders and storage periods
Tablo 5: Sulandırıcılar ve saklama süreleri arasındaki fertilite farkları

Extender	24 h X ± Sx	48 h X ± Sx	p
Glucose	402.2 ± 7.8	309.6 ± 13.1	*
Ringer	346.8 ± 12.6	219.0 ± 14.3	*
p	**	*	

* : $p < 0.05$
** : $p < 0.01$

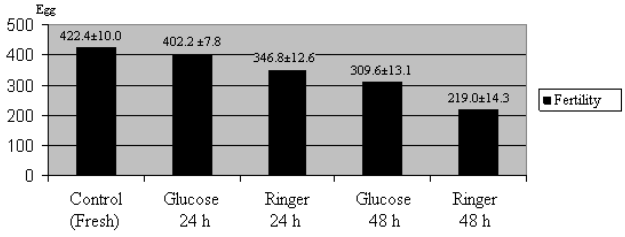

Figure 1: Fertility results after cooled storage
Şekil 1: Kısa süreli saklama sonrası fertilite sonuçları

Discussion and Conclusion

There is no report is the first one on this subspecies in the literature. Because of this, our results were compared with salmonidae family. Mean semen volume was similar to the results reported by Gjerde (18),

Munkittrick and Moccia (29) but different from those reported by Erdahl et al. (16), Büyükhatipoğlu and Holtz (13), Tekin et al. (38). The difference may be due to differences in breed, feeding conditions and regime, environmental factors, or spawning time. The mean spermatozoa motility observed in this study was similar to the findings of Bozkurt et al. (11), Çevik (15) but different from those of Schmidt-Baulain and Holtz (34), Levanduski and Cloud (24).

The most reliable indicator of the sperm quality is spermatozoa motility. Subjective estimation of motility requires considerable experience and it is practically used in the selection of sperm for insemination and preservation. However, motility varies in vigor and duration not only among males but also within an individual male depending on its ripeness (2). Studies on most fish species recorded that motility of spermatozoa may show seasonal variation (3, 5). Results reported here regarding post-activation motility and duration of movement are in agreement with other studies (27, 39). Both properties decreased by time but the proportion of motile cells decreased faster in activated semen samples than in fresh ones. Spermatozoon density corroborates results reported by Büyükhatipoğlu ve Holtz (13), Baynes and Scott (4), Billard (8), Tekin et al. (38). The mean pH for trout semen was generally confirmed by Piironen (30) and Billard (8).

Our results confirmed the data of Munkittrick and Moccia (29) and Ciereszko and Dabrowski (14) who found a correlation between motility rate and fertilization capacity for the trout sperm using subjective estimation methods for motility determination. Magyary et al. (26) concluded a strong, positive correlation of frozen-thawed carp spermatozoa motility and fertility at 1-1.5x10^5:1 spermatozoa/egg ratio. In addition, Linhart et al. (25) also observed good correlation with fresh sperm between carp spermatozoa motility and fertilization at 2x10^5:1 sperm/egg ratio. The recommended sperm to egg ratio for trout culture is based on the use of stripped milt (33). In this study, high fertility rates were achieved by the semen with a high motility rate at 250.000 sperm:egg ratio.

Under the suitable conditions, salmonid milt can be stored for a few weeks unfrozen. This methodology is important because it is not always practical or even possible to harvest milt and use it to fertilize eggs immediately. Previous investigations determined that successful short-term storage of salmonid milt depends on numerous factors, including temperature, fluid volume, and gaseous environment (20, 22, 31, 37). The most commonly used method of short-term storage has been under an atmosphere of 100 % Oz at low temperatures (7, 12, 31, 37).

In this experiment, average fertility (hatching rate %) of fresh semen was found as 84.5 %. We observed significant differences in motility and fertility of sperm stored in different extenders (Table 1). Sperm stored in Ringer solution showed lower motility and fertility values than sperm stored in glucose extender ($p<0.05$). The best motility and fertility rates were obtained by glucose extender ($p<0.05$). The average results were lower than the results of Uysal and Alpbaz (41). This difference seems to be depend on genetic factors and farm conditions. Likewise, it may be attributed to 76 and 72 % fertility rates in trout species differences in previous studies.

In this study, the highest fertilization rate by glucose extender was obtained 65 % after 24 h storage and the sperm-egg ratio was approximately 2.5x10^5 sperm/egg. Hatching rates were considerably higher in this study than that of Erdahl et al. (16) who reported 20 %. On the other hand, Hatipoğlu and Akcay (20), Billard (7) and Stoss and Holtz (36) reported similar hatchability rates. The low fertilization results mostly correlated with the low spermatozoa/egg ratio of 1 x 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.

In salmonids, the viability of chilled, stored gametes is almost negatively correlated with the temperature (22). 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 (31). In the present study, collected semen from 30 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 (19, 31). 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 methods of storage (1, 31, 33, 35).

The possible precautions that could prolong the sperm viability may include: (i) the prevention of desiccation (especially important in the case of storage in capped containers) either by using a moisture-securing system (36) or by dilution with immobilizing diluents (28, 40); (ii) lowering the storage temperature to below 1°C, which prevents bacterial growth and stops metabolic processes in spermatozoa (36, 40); (iii) optimizing gaseous atmosphere: an oxygen atmosphere can be sub-optimal, as demonstrated by Bencic et al. (6); (iv) securing sterility by using sterile dilution media (21) or antibiotics (6, 37) to prevent excessive bacterial growth, which was observed in some samples in the present study after 2 weeks of storage; (v) avoid urine contamination (19).

The present study indicates that Abant trout 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.

References

1. **Akçay E, Tekin N, Seçer S** (1995): *Balık spermasının prezervasyonu*. Ege University J Fish Aquat Sci, **12,** 367-373.
2. **Akçay E, Bozkurt Y, Kayam S** (2002): *Cryopreservation of mirror carp (Cyprinus carpio L. 1758) semen: with emphasis on post-thaw motility.* pp. 5. In: 1st Int Cong Aquacult-Fish Technol Environ Management., June 8-10, Athens.
3. **Akçay E., Bozkurt Y., Tekin N., Seçer S** (2004): *Cryopreservation of mirror carp semen.* Turk J Vet Anim Sci, **28,** 837-345
4. **Baynes SM, Scott AP** (1985): *Seasonal variations in parameters of milt production and in plasma concentration of sex steroids of male Rainbovv trout (Salmo gairdneri).* Gen Comp Endocrinol, **57,** 150-160.
5. **Benau D, Terner C** (1980): *Initiation, prolongation and reactivation of the motility of salmonid spermatozoa.* Gamete Res, **3**,247- 257.
6. **Bencic DC, Cloud JG, Ingermann RL** (2000): *Short-term storage of salmonid sperm in air versus oxygen*. N Am J Aquaculture, **62**, 19-25.
7. **Billard R** (1981): *Short-term preservation of sperm under oxygen atmosphere in rainbow trout (Salmo Gairdneri).* Aquaculture, **23**, 287-293.
8. **Billard R** (1992): *Reproduction in Rainbow trout: sex differentiation, dynamics of gametogenesis, biology and preservation of gametes*. Aquaculture*,* **100**, 263-298.
9. **Billard R, Cosson MP** (1992): *Some problems related to the assesment of sperm motility in freshwater fishes.* J Exp Zool, **261,** 122-131.
10. **Bozkurt Y, Secer S** (2004): *Determination of some spermatological properties of mirror carp (Cyprinus carpio L. 1758) semen and evaluation of short-term preservation using different extenders.* In: 3th Nat. Cong. Reprod. Art. Insem., September 30-October 2. Antalya.
11. **Bozkurt Y, Secer S, Bukan N, Akcay E, Tekin N** (2006): *Relationship between body condition, physiological and biochemical parameters in brown trout (Salmo trutta fario) sperm.* Pakistan J Biol Sci, **9**, 940-944.
12. **Büyükhatipoglu Ş, Holtz W** (1978): *Preservation of trout sperm in liquid or frozen state.* Aquaculture, **14**, 49-56.
13. **Büyükhatipoglu Ş, Holtz W** (1984): *Sperm output in Rainbow trout (Salmo gairdneri) effect of age, timing and frequency of stripping and presence of females.* Aquaculture, **37**, 63-71.
14. **Ciereszko A, Dabrowski K** (1994): *Relationship between biochemical constituents of fish semen and fertility: The effect of short-term storage.* Fish Physiol and Biochem, **12**, 357–357.
15. **Çevik M** (2000): *Alabalık (Gökkuşağı Alabalığı) Spermasının Dondurulması ve Değerlendirilmesi.* Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora Tezi, Ankara
16. **Erdahl AW, Erdahl DA, Graham EF** (1984): *Some factors affecting the preservation of Salmonid spermatozoa.* Aquaculture**, 43,** 341-350.
17. **Geldiay R, Kocataş A** (1988): *Deniz Biyolojisine Giriş.* Ege Üniversitesi Fen Fakültesi Yayınları.
18. **Gjerde B** (1984): *Variation in semen production of farmed Atlantic salmon and Rainbow trout*. Aquaculture*,* **40,** 109-114.
19. **Glogowski J, Kwasnik M, Piros B, Dabrowski K, Goryczko K, Dobosz S, Kuzminski H, Ciereszko A** (2000): *Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success.* Aquacult Res, **31,** 289–296.
20. **Hatipoğlu T, Akçay E** (2008): *Short-term storage of Abant trout (Salmo trutta abanticus Tontonese, 1954) semen and fertility trials.* 16th International Congress on Animal Reproduction, 13-17 July Budapest, Rep Dom Anim, **43**, 141.
21. **Jenkins JA, Tiersch TR** (1997): *A preliminary bacteriological study of refrigerated channel catfish sperm*. J World Aquaculture Soc, **28**, 282-288.
22. **Jensen JOT, Aiderdice DF** (1984): *Effect of temperature on short-term storage of eggs and sperm of chum salmon (Oncorhynchus keta).* Aquaculture, **37**, 251-265.
23. **Lahnsteiner F, Berger B, Weismann T, Patzner RA** (1996): *Motility of spermatozoa of Alburnus alburnus (Cyprinidae) and its relationship to seminal plasma composition and sperm metabolism*. Fish Physiol Biochem, **15,** 167-179.
24. **Levanduski MJ, Cloud JG** (1988): *Rainbow trout (Salmo gairdneri) semen: effect of non-motile sperm on fertility*. Aquaculture*,* **75,** 171-179
25. **Linhart O, Rodino M, Cosson J** (2000): *Cryopreservation of sperm in common carp Cyprinus carpio: sperm motility and hatching success of embryos.* Cryobiology, **41,** 241-250.
26. **Magyary I, Urbanyi B, Horvath L** (1996): *Cryopreservation of common carp (Cyprinus carpio L.) sperm: II. Optimal conditions for fertilization.* J App Ichthyol, **12**, 117-119.
27. **Mansour N, Lahnsteiner F, Berger B** (2004): *Characterization of the testicular semen of the African catfish, Clarias gariepinus (Burchell, 1822) and its short-term storage.* Aquacult Res, **35,** 232-244.

28. **Mc Niven MA, Gallant RK, Richardson GF** (1993): *Fresh storage of Rainbow trout (Oncorhynchus mykiss) semen using a non-aqueous medium.* Aquaculture, **109,** 71-82.
29. **Munkittrick KR, Moccia RD** (1987): *Seasonal changes in the quality of Rainbow trout (Salmo gairdneri) semen: effect of a delay in stripping on spermatocrit, motility, volume and seminal plasma contituents.* Aquaculture, **64,** 147-156.
30. **Piironen J** (1985): *Variation in the properties of milt from the Finnish landlocked salmon (Salmo salar Salmo salar m. sebago Girard) during a spavvning season.* Aquaculture, **48,** 337-350.
31. **Rana K** (1995): *Preservation of gametes*. 53-75. In: NR Bromage, RJ Roberts (Eds), Broodstock Management and Egg and Larval Quality. Oxford Blackwell Science, England.
32. **Saad A, Billard R, Theron MC, Hollebecq MG** (1988): *Short-term preservation of carp (Cyprinus carpio) semen.* Aquaculture , **71,** 133-150.
33. **Scott AP, Baynes SM** (1980): *A review of the biology, handling and storage of salmonid spermatozoa.* J Fish Biol, **17,** 707-739.
34. **Schmidt-Baulain R, Holtz W** (1989): *Deep freezing of rainbow trout (Salmogairdneri) sperm at varying intervals after collection.* Theriogenology, **32,** 439-443.
35. **Stoss J** (1983): *Fish gamete preservation and spermatozoan physiology*. 305–350. In: W Hoar, D Randall, E Donaldson (Eds), Fish Physiology. Academic Pres, New York.
36. **Stoss J, Holtz W** (1983): *Successfull storage of chilled rainbow trout (Salmo gairdneri)*. Aquaculture, **31,** 269-274.
37. **Stoss J, Refstie T** (1983): *Short-term storage and cryopreservation of milt from Atlantic salmon and sea trout.* Aquaculture, **30**, 229-236.
38. **Tekin N, Seçer S, Akçay E, Bozkurt Y, Kayam S** (2003): *Gökkuşağı alabalıklarında (Oncorhynchus mykiss W., 1792) yaşın spermatolojik özellikler üzerine etkisi.* Turk J Vet Anim Sci, **27,** 37-44.
39. **Tekin N, Seçer S, Akçay E, Bozkurt Y** (2003): *Cryopreservation of rainbow trout (Oncorhynchus mykiss) semen.* Isr J Aquacult-Bamid, **55,** 208-212.
40. **Truscott B, Idler DR, Hoyle RJ, Freeman HC** (1968): *Sub-zero preservation of Atlantic salmon sperm.* J Fish Res Bd Can, **25**, 363–72.
41. **Uysal İ, Alpbaz A** (2003): *Abant alabalığı (Salmo trutta abanticus T., 1954) ile gökkuşağı alabalığı (Oncorhynchus mykiss w., 1792) yumurtalarının döllenme, gözlenme, larva çıkışı ve yaşama oranlarının karşılaştırılması.* Ege University J Fish Aquat Sci, **20,** 95-101.

Geliş tarihi: 26.12.2008 / Kabul tarihi: 15.05.2009

Address for correspondence
Assoc. Prof. Ergun Akçay
Department of Animal Reproduction and
Artificial Insemination
Faculty of Veterinary Medicine
University of Ankara
06110 Dışkapı, Ankara, Turkey
E-mail: akcay@veterinary.ankara.edu.tr