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SPERM CHARACTERISTICS OF WILD AND CULTIVATED COMMON CARP (*Cyprinus carpio* L., 1875)

ABSTRACT

Some sperm characteristics such as volume, motility, motility duration, density, total spermatozoa and pH of wild-caught, intensively and extensively cultured Common Carp were investigated in this study. When the three groups were compared, while lower values in volume, spermatozoa density ($x10^9$ ml), and total spermatozoa were found significant for the third group (extensive) of carp, the wild counterparts showed better values for all parameters. pH values were not different among the groups. A positive correlation between total spermatozoa and volume were found in all groups. However, the weight, length, and volume were positively correlated in just cultured groups. Volume and motility in third group of fish were also positively correlated.

Keywords: Common Carp, *Cyprinus carpio*, Sperm Quality, Sperm Characteristics, Spermatozoa Density

DOĞAL VE KÜLTÜRE ALINMIŞ SAZANLARIN (*Cyprinus carpio* L., 1875) SPERM ÖZELLIKLERI

ÖZET

Bu çalışmada doğal, entansif ve ekstansif koşullarda yetiştirlmiş olan adi sazan bireylerinin hacim, motilite, motilite süresi, yoğunluk, toplam yoğunluk ve pH gibi sperm karakter değerleri ortaya konmuştur. Her üç grup ta karşılaştırıldığında, hacim, spermatozoa yoğunluğu ($x10^9$ ml) ve toplam yoğunluktaki en düşük değerler üçüncü grup (ekstansif) için bulunmuşken, doğadan elde edilmiş olan bireyler tüm parametreler için daha yüksek değerler göstermiştir. pH değerleri bakımından gruplar arasında bir farklılığa rastlanmamıştır. Ayrıca bütün gruplar için toplam yoğunluk ve hacim arasında pozitif bir koralasyona rastlanmıştır. Buna karşın, boy, ağırlık ve hacim arasındaki pozitif korelasyon yalnızca kültür gruplarında vardır. Üçüncü grupta ise hacim ve motilite pozitif korelasyon göstermiştir.

Anahtar Kelimeler: Adi Sazan, *Cyprinus carpio*, Sperm Kalitesi, Sperm Karakteri, Spermatozoa Yoğunluğu

1. INTRODUCTION (GİRİŞ)

The common carp, *Cyprinus carpio* L., is an important fish in global aquaculture, as its annual yield is over 2.22 million tons, which is exceeded only by silver and grass carps [1]. Common carp is by far the most important freshwater fish in Europe, with an annual aquaculture yield of about 147 kton.

The fish farming industry has been more focused on the quality of eggs or larvae rather than that of sperm, even though the quality of both gametes may affect fertilization success and larval survival. In some species, poor sperm quality can be a limiting factor in their culture, however, even when fertilization success is high, differences in sperm quality between males when mixed sperm from multiple males is used may severely reduce the apparent population size and may affect the future genetic integrity of the stock [2].

Mixing of sperm from different males is a very common practice in the culture of many commercial species. So while the fertilization success may seem high, it is possible that not all the males are equally contributing to the gene pool due to sperm competition [3, 4, 5, and 6].

Optimal sperm quality is important for effective broodstock management and should be a criterion in the selection of male broodstock [7]. In fish farms and hatcheries, the biotic and abiotic factors that affect sperm quality are diverse and are dependent on complex interactions between genetic, physiological and environmental factors [2]. Sperm quality can also be influenced by factors such as size or age of individuals [8 and 9].

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

Fish stocking into dam lakes is one of the important activities of State Hydrological Works of Turkey. For this purpose, carp sperm are obtained from the males cultivated in intensive and extensive pond systems and mostly morphological selection are made irrespective of culture systems.

Therefore the aim of this study was to investigate some sperm quality parameters such as sperm volume, spermatozoa concentration, total number of spermatozoa, spermatozoa movement duration, pH, and sperm mortality for common carp obtained from wild, intensive and extensive culture systems in Aquaculture Unit of State Hydraulic Works. Thus, a contribution could be made to form a male common carp broodstock selecting individuals from wild or the two culture systems.

3. MATERIALS AND METHODS (MATERYAL VE METOD)

- **Broodstock and collection of sperm (Anaç ve sperm eldesi):** Sixty three adult males of carp with a mean weight of 1.13±0.62 kg and total length of 39.71±39.71 cm were obtained from Seyhan Dam Lake (Group I), intensive (Group II) and extensive (Group III) culture ponds of Aquaculture Unit of State Hydraulic Works at the reproductive season in 2007.

  The males for first group were found to range from 31.20 to 49.70 cm in total length and total weight was ranged between 0.54 to 1.68 kg. In case of others, the total length and weight were ranged from 30.50 to 54.50 cm and 0.57 to 2.47 kg for group II and 30.80 to 51.71 cm and 0.57 to 2.16 kg for group III.

  Sperm was collected by manual stripping. The stripping method was the same as in most of the sperm quality studies. Fish were not anaesthetized and before sperm collection the urinary bladder was drained by gentle pressure on the abdomen. Sperm was sampled into glass tubes and only used if uncontaminated with water, blood, urine, and feces.

- **Evaluation of sperm quality (Sperm kalitesinin değerlendirilmesi):** The sperm quality parameters including sperm volume (ml), spermatozoa motility (%), motility duration (s), spermatozoa density (X10^9/mL), and sperm pH were determined. Sperm quality were triggered directly in activation medium at ratio 1:1000-2000 and immediately recorded with a 3 CCD video camera (SONY, Japan) mounted on a dark-field microscope (Olympus BX51). These records were analyzed repeatedly with a micro image analyzer (Olympus DP2-BSW application software). Analysis of sperm motility was carried out in triplicate for each sample.
- **Sperm volume (sperm Miktarı):** The sperm was collected in glass tubes graded in millimeters and sperm volume was registered immediately following collection by abdominal massage.
- **Spermatozoa motility (Sperm Motilitesi):** Sperm samples were kept at approximately +4^{0}C throughout the motility tests. Spermatozoa motility was observed under 200x magnification and the percentage of motile spermatozoa were assessed. Only forward movements by the spermatozoa were assessed as motility, whereas simply vibrating sperm were assessed as immobile.
- **Spermatozoa density (Sperm Yoğunluğu):** Due to its rapidity and efficiency, heamocytometric method is widely preferred to determine the sperm density. Therefore, spermatozoa density was determined by the heamacytometric method in this study. Sperm was diluted (1/1000) in Hayem solution and mean sperm count was calculated from three replicate samples for each fish at a magnification of 200x and sperm density was expressed as x10^9/mL.
- **Duration of spermatozoa movement and sperm pH (Spermatozoa hareketlilik süresi ve pH):** The duration of spermatozoa movement was assessed using a sensitive chronometer (1/100s) that was started simultaneously with the addition of activation solution into the sample. Sperm pH was measured with standard pH papers (Merck).

Statistical analysis results are expressed as mean ± standard deviation. 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 α=0.05 confidence level. All statistical analysis was carried out using SPSS 11 for Windows software package.

4. RESULTS (BULGULAR)

Sperm quality parameters were found rather variable (Table 1). When groups were compared, sperm volume, motility, total spermatozoa ($p<0.01$) and density ($p<0.05$) were significantly different but the differences of motility duration and ph were not significant.

Correlation between sperm quality parameters for each group and irrespective of fish groups are shown in Tables 2-5. According to the results of the present study, a positive correlation between total spermatozoa and sperm volume ($p<0.01$), and total spermatozoa and density ($p<0.05$) were determined for the first group of fish.

In group II, a positive correlation between fish size (length and weight) and sperm volume ($p<0.01$) and between total spermatozoa and fish length ($p<0.05$) were found.

Total spermatozoa was positively correlated with fish size (length and weight), sperm volume, and density ($p<0.01$) in the third group. At the same time, there was a positive correlation between sperm volume and density, and motility and total spermatozoa in the same group ($p<0.05$).

In Table 5, the evaluation was made for all individuals irrespective of the groups. While the fish size (length and weight) and sperm volume were positively correlated with density and total spermatozoa at p<0.01 level, a positive correlation between density and total spermatozoa was found at p<0.05 level.

Table 1. Sperm quality parameters for wild and cultured common carp groups
(Tablo 1. Doğal ve kültüre alınmış sazan gruplarına ait sperm kalite parametreleri)

	Fish Lenght (cm) X±sx	Fish Weight (kg) X±sx	Sperm Volume (ml) X±sx	Motility rate (%) X±sx	Motility Duration (s) X±sx	Spz. Density (x10^9/ml) X±sx	Total spz. (x10^9)	Sperm pH
GI	39,32 ±1,18a	0,98 ±0,86a	3,70 ±0,28a	76,26 ±1,01a	68,43 ±1,68a	9,28 ±0,44a	33,80 ±2,38a	7,88 ±0,05a
GII	40,08 ±1,58a	1,23 ±0,13a	3,81 ±0,80a	71,88 ±1,39b	67,38 ±2,48a	9,46 ±0,51a	34,99 ±7,05a	7,88 ±0,05a
GIII	39,74 ±1,30a	1,16 ±0,97a	1,55 ±0,12b	70,18 ±0,64b	62,43 ±1,68a	7,68 ±0,37b	12,34 ±1,27b	7,86 ±0,05a
S.L.	--	--	**	**	-	*	**	-
Means±SX n=63	39,71 ±0,78	1,13 ±0,62	3,02 ±0,31	72,77 ±0,68	66,05 ±1,46	8,81 ±0,27	27,04 ±2,81	7,87 ±0,03
* p<0,05, **p<0,01			S. L.: Significant level					
a,b, : Means with different letters within columns are significantly different								

Table 2. Correlation between fish size (length and weight) and sperm quality parameters for Group I.
(Tablo 2. 1. Grup için balık büyüklüğü (Boy ve Ağırlık) ve sperm kalite parametreleri arasındaki korelasyon)

	Total Spz.	Weight	Length	Sperm Volume	Motility Rate	Motility Duration	Spz. Density
Weight	,078						
Lenght	,085	,953**					
Sperm Volume	,703**	,278	-,369				
Motility Rate	,206	-,328	-,213	,338			
Motility Duration	,183	-,265	-,465	-,026	-,084		
Spz. Density	,542*	-,193	-,023	-,195	-,048	,246	
Sperm pH	-,291	-,022	-,196	-,178	-,041	,091	-,086

* Correlation is significant at 0,05 level.
** Correlation is significant at 0,01 level.

Table 3. Correlation between fish size (length and weight) and sperm quality parameters for Group II.
(Tablo 3. 2. Grup için balık büyüklüğü (Boy ve Ağırlık) ve sperm kalite parametreleri arasındaki korelasyon)

	Total Spz.	Weight	Length	Sperm Volume	Motility Rate	Motility Duration	Spz. Density
Weight	,591**						
Lenght	,527*	,976**					
Sperm Volume	,979**	,681**	,617**				
Motility Rate	-,144	-,055	,042	-,207			
Motility Duration	-,030	,181	,238	,003	,321		
Spz. Density	,066	-,292	-,311	-,114	,377	-,116	
Sperm pH	,040	-,045	-,064	,018	-,055	-,090	,020

* Correlation is significant at 0,05 level.
** Correlation is significant at 0,01 level.

Table 4. Correlation between fish size (length and weight) and sperm quality parameters for Group III.
(Tablo 4. 3. Grup için balık büyüklüğü (Boy ve Ağırlık) ve sperm kalite parametreleri arasındaki korelasyon)

	Total Spz.	Weight	Length	Sperm Volume	Motility Rate	Motility Duration	Spz. Density
Weight	,590**						
Lenght	,593**	,961**					
Sperm Volume	,920**	,612**	,602**				
Motility Rate	,450*	,383	,354	,583**			
MotilityDuration	-,189	,048	,133	-,140	-,011		
Spz. Density	,804**	,338	,330	,530*	,169	-,158	
Sperm pH	-,137	-,326	-,295	-,238	-,263	-,364	-,052

* Correlation is significant at 0,05 level.
** Correlation is significant at 0,01 level.

Table 5. Correlation between fish size (length and weight) and sperm quality parameters for irrespective of fish groups
(Tablo 5. Ayırt etmeksizin bütün gruplar için balık büyüklüğü (Boy ve Ağırlık) ve sperm kalite parametreleri arasındaki korelasyon)

	Total Spz.	Weight	Length	Sperm Volume	Motility Rate	Motility Duration	Spz. Density
Weight	,370**						
Lenght	,349**	,947**					
Sperm Volume	,958**	,455**	,439**				
Motility Rate	,122	-,127	-,013	,091			
Motility Duration	,115	-,026	,039	,084	,177		
Spz. Density	,342**	-,107	-,118	,097	,281*	,107	
Sperm pH	-,015	-,127	-,128	-,017	-,062	-,063	-,014

* Correlation is significant at 0,05 level.
** Correlation is significant at 0,01 level.

5. DISCUSSION (TARTIŞMA)

Pond water was taken directly from the dam lake so that physical conditions of the water were the same for all groups of fish. The water temperature was between 22-24 ^{0}C during the study. There were no statistical difference on the mean length and weight of the studied individuals of all groups. The first group of the sample consisted of wild fish; second and third groups of studied fish were obtained from the intensive and extensive culture systems of the aquaculture unit as it is stated in material and methods. While second group of fish were kept in concrete ponds with flow through system and fed ad libitum with commercial carp feed, third group were kept in fertilized earth ponds and they were not fed with commercial diet.

Volume, motility, motility duration, spermatozoa density, total spermatozoa, and pH of wild and cultured groups of carp were compared in Table 1. There were no statistically significant difference among the mean length and weight of the groups. However, statistically lower level of volume, spermatozoa density (x10^{9} ml), and total spermatozoa were observed for the third group (extensive) of fish. Although we have no clear proof, it can be speculated that the poorer values of this group is probably due to stress as a consequence of insufficient food in the ponds. Stress may act by inducing changes in plasma osmolarity which in turn can affect sperm quality in fish [2]. In sockeye salmon for instance, fish respond to confinement stress with decreased levels of steroids [10]. It is also well accepted that broodstock nutrition can significantly impact reproductive performance [11]. All these information and more stressful character of

aquaculture environment may explain the better values of sperm characteristic of wild carp than that of the culture groups.

Many studies, which have been carried out on different fish species, revealed that motility rate and duration of motility vary throughout the spawning season. Therefore sperm sampling in this study was made in the second half of May when fish reached the peak reproductive activity and it was completed in five days. The mean sperm amount obtained for this study was 3.02±0.31 ml. This result of the present study was confirmed by the reports of Belova (1981)[12] and Bozkurt and Seçer (2006)[13] who reported the values were between 1-9 ml for the same species. However, the value of our study conflicts with the study of [14]. Intraspecific variability in fish sperm quality was determined by many factors such as fish size, age, environmental conditions, and aquaculture manipulations including photoperiod and hormonal applications. Therefore these differences among studies may be attributed to sampling period, size of fish, feeding regime, and hormonal induction.

Although the mean duration of motility (66.05±0.46 s) of the current study was shorter than that reported by [15], similar results in some previous studies [13,16] were observed for the same species.

Feeding is one of the most effective factors on spermatozoa motility as stated by [17,18]. However, spawning time in reproductive season is also another determinative factor on motility. Bozkurt and Secer (2006)[13] found that the motility rates ranged between 76% and 79% in different months. These values are higher than the value (72.77±0.68) of the present study but this motility rate corresponds with the result of [19].

Emri *et al*. (1988)[20] investigated spermatozoa density of carp along the spawning season and they found that the density varied between $0.7\pm0.1x10^{10}$ ml and $1.4\pm2x10^{10}$ ml. Lubzens *et al*., (1997)[21] also determined the spermatozoa density between 5.6 $x10^{9}$ ml and 32.5 $x10^{9}$ ml. The density value ($8.81\pm0.27x10^{9}$ ml) of the present study were in these range and was supported by previous studies.

In regards to total spermatozoa of carp spermatozoa, while the values of our study confirmed the data of [13] who found that the total spermatozoa of carp ranged between $13.06\pm8.06x10^{9}$ and $35.62\pm22.26x10^{9}$ in the period from April to June, our results were clearly lower than that of [15]. The lower dilution rates of fish sperm make counting process difficult due to high level of density and thus, variability among the results of different studies may increase. Therefore, it is assumed that the variability on spermatozoa density data of the same species may be ascribed to the differences in sampling time, dilution rates, and the processes.

The pH values of the studied fish groups were not significantly different and the values (7.86-7.88) observed for this study were similar to the results reported by [15,22].

6. CONCLUSION (SONUÇ)

When correlations between fish size (length and weight) and sperm characteristics were taken into account, positive correlations between total spermatozoa density, weight, length, and volume were found in both cultured groups. Wild males were the better group followed by the second (intensive) group of fish in terms of sperm characteristics. Therefore wild or intensively cultured males of common carp may efficiently be used as broodstock rather than extensively cultured fish.

REFERENCES (KAYNAKLAR)

1. FAO, "Aquaculture Production 2007," Fishstat Plus Software FAO, Roma.
2. Rurangwa, E., Kime, D.E., Ollevier, F., and Nash, J.P., (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture 234,pp:1-28.
3. Bekkevold, D., Hansen, M.M., and Loeschcke, V., (2002). Male reproductive competition in spawning aggregations of cod (Gadus morhua, L.). Mol. Ecol. 11, pp:91– 102.
4. Gage, M.J.G., Stockley, P., and Parker, G.A., (1998). Sperm morphometry in the Atlantic salmon. J. Fish Biol. 53,pp:835-840.
5. Mjolnerod, I.B., Fleming, I.A., Refseth, U.H., and Hindar, K., (1998). Mate and sperm competition during multiplemale spawnings of Atlantic salmon. Can. J. Zool. 76, pp:70– 75.
6. Vladic, T.V., Afzelius, B.A., and Bronnikov, G.E., (2002). Sperm quality as reflected through morphology in salmon alternative life histories. Biol. Reprod. 66, pp:98-105.
7. Tekin,N., Secer, S., Akçay, E., Bozkurt, Y., and Kayam, S., (2003). The effect of age on spermatological properties in rainbow trout (Oncorhynchus mykiss W. 1792). Turk.Vet. Anim. Sci. 27, pp:37-44.
8. Buyukhatipoglu, S. and Holtz, W., (1984). Sperm output in rainbow trout (Salmo gairdneri): effect of age, timing and frequency of stripping and presence of females. Aquaculture 37, pp:63– 71.
9. Chechun, T.Ya, Pantaleeva, N.O., and Samoded, A. Yu., (1994). Seasonal and age-related qualitative and quantitative changes in sperm production in steelhead Oncorhynchus mykiss. J. Ichthyology 34: pp:78-89.
10. Kubokawa, K., Watanabe, T., Yoshioka, M., and Iwata, M., (1999). Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172, 335-349.
11. Izquierdo et al 2001. Izquierdo, M.S., Fernandez-Palacios, H., and Tacon, A.G.J., (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25– 42.
12. Belova, N.V., (1981). The ecological and physiological peculiarities of sperm in pond cyprinids. Journal of Ichthyology 21: pp:90-102.
13. Bozkurt, Y. and Seçer, S., (2006). Aynalı Sazan (Cyprinus carpio) Balıklarında Üreme Mevsimi Boyunca Spermatolojik Özelliklerin Belirlenmesi.E.U. Journal of Fisheries & Aquatic Sciences 23(1/2): pp:195-198.
14. Bozkurt, Y., Öğretmen, F., Erçin, U., and Yıldız, Ü., (2008). Seminal plasma composition and its relationship with physical spermatological parameters of Grass carp (Ctenopharyngodon idella) semen: with emphasis on sperm motility. Aquaculture Research 39: pp:1666-1672.
15. Akçay, E., Bozkurt, Y., and Kayam, S., (2002). Cryopreservation of mirror carp (Cyprinus carpio L. 1758) semen: with emphasis on post-thaw motility. 1st International Congress on Aquaculture, Fisheries Technology and Environmental Management, 8-10 June 2002. Book of Abstracts. p. 5. ECEP, Athens.
16. Tabakoğlu, Ş.S., (2005). Hipofiz Uygulanmış ve Uygulanmamış Farklı Boy Gruplarına Ait Aynalı Sazan (Cyprinus carpio)'ların Sperm Kalitelerinin İncelenmesi.Yayımlanmamış Yüksek Lisans Tezi.Adana: Çukurova Üniversitesi Fen Bilimleri Enstitüsü.
17. Alavi, H.S.M., Rodina, M., Policar, T., and Linhart, O., (2009). Relations between semen characteristics and body size in Barbus

barbus L. (Teleostei:Cyprinidae) and effects of ions and osmolality on sperm motility. Comparative Biochemistry and Physiology, Part A 153: pp:430-437.
18. Billard Billard, R., Cosson, J., Crim, L.W., and Suquet, M., (1995a). Sperm Physiology and Quality. In:Bromage, N., Roberts, R.(Eds), Brood stock Management and Egg and Larval Quality. Blackwell, Oxford, pp:25-52
19. Linhart, O., Rodina, M., and Cosson, J., (2000). Cryopreservation of sperm in common carp Cyprinus carpio: sperm motility and hatching success of embryos. Cryobiology 41: pp:241– 250.
20. Emri, M., Marian, T., Tron L., Balkay, L., and Krasznai, Z., (1998). Temperature adaptation changes ion concentrtions in spermatozoa and seminal plasma of common carp without affecting sperm motility. Aquaculture, 167; pp:85-94.
21. Lubzens, E., Daube, N., Pekarsky, I., Magnus, Y., Cohen A., Yusefovich, F., and Feigin, P., (1997). Carp (Cyprinus carpio L.) spermatozoa cryobanks- strategies in research and application. Aquaculture, 155; pp:13-30.
22. Piironen, J., (1985). Variation in the properties of milt from the Finnish landlocked salmon (Salmo salar Salmo salar m. sebago Girard) during a spawning season. Aquaculture, 48; pp:337-350.