Sperm quality, egg size, fecundity and their relationships with fertilization rate of grass carp (*Ctenopharyngodon idella*)

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Abstract

Gametes were collected by abdominal massage from 15 anesthesized male and female grass carp. In collected sperm samples the following mean spermatological properties were determined: sperm volume 15.64 ± 5.39 mL, sperm motility 81.20 ± 7.61%, motility duration 62.06 ± 19.40s, density 17.88±5.48 $x10^9$ mL^{-1}, total density 428.90±302.05 $x10^9$, and pH 7.25±0.81. Also egg size (mm) and fecundity (egg/fish) were determined as 1.04±0.028 and 417867 ± 36.274 respectively in female grass carp. The fertilization capacity of sperm was tested with the same egg pool. The insemination dosage was $2x10^5$ spz/egg for each fertilization experiment. Fertilization rate was determined as mean 79.3±2.95% and positively correlated with sperm motility ($r=0.932$, $p<0.01$), egg size ($r=0.513$, $p>0.05$) and fecundity ($r=0.539$, $p>0.05$).

Keywords: Grass carp, Sperm, Motility, Fertilization

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Introduction

Grass carp (*Ctenopharyngodon idella*), also known as White Amur, is a vegetarian fish living in the Amur River in Asia. Because of its herbivorous feeding habits, it has received considerable attention as biological control of aquatic vegetation. Grass carp can reproduces only its own original habitat. Therefore, grass carp culture is an important issue to overcome excess aquatic vegetation. So, gamete quality and artificial insemination is very important to obtain viable larvae for grass carp culture.

The aquaculture industry has been more focused on fish eggs rather than sperm in spite of both gamete may effect fertilization success. However, sperm quality is very important variable in aquaculture broodstock management and it can influence the fertilization of eggs. Parameters to evaluate sperm quality include sperm volume, spermatozoa density, motility and motility period of spermatozoa (Billard et al., 1995). Motility is very important parameter since spermatozoa must be motile to achieve egg penetration (Chauvaud et al., 1995). Spermatozoa motility and duration can vary according to fish species and temperature during spermiation period (Billard, 1986). In addition, spermatozoa density and sperm pH are considered other indicators of sperm quality as they can also effect the fertilization rate (Lahnsteiner et al., 1998). Therefore fecundity, egg size and sperm quality parameters should be considered together to estimate fertilization success.

There is a lack of literature data on sperm quality and its relationship with fertilization in grass carp. From this point of view, the objectives of this research was designed to determine physical sperm quality parameters and as well as to examine the relationships between spermatological parameters, egg size, fecundity and fertilization success in grass carp.

Materials and methods

Broodstock management and collection of gametes

The experiment was carried out at State Hydrolic Works (SHW) Fish Reproduction Station, Adana, Turkey, during spawning season of grass carp in 2010. The parental broodstock were held in sand ponds under a natural photoperiod regime and fasted 48 h before sperm collection. The broodstock were anaesthetized in 100 ppm of MS 222 (Argent Labs., Redmond,WA, USA) before stripping. Water temperature varied between 22-24°C during spawning season.

Sperm and eggs were collected by abdominal massage. Sperm was collected from 15 mature males (TW 4.86±0.44 kg, TL 53.9±1.23 cm) by manual abdominal stripping 12 h after a single injection of 2 mg/kg of carp pituitary extract (CPE) at 24°C water temperature. Sperm was sampled into glass tubes and used in case of uncontaminated with water, blood, urine and faeces. Sperm was stored on ice (4°C) until used for experiment. Eggs were also collected from 15 mature (4-5 years old) females (TW 6.44±0.79 kg, TL 56.7±4.19 cm) that were stripped gently massaging of the abdomen 10-12 h after a double injection of 3.5 mg/kg CPE. The first injection, 10% (0.35 mg/kg) CPE was

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given 10h before the second (3.15 mg/kg). Only transparent, well rounded and unwrinkle eggs were used for fertilization.

Evaluation of semen

Sperm was collected in glass tubes and sperm volume was registered immediately following collection by abdominal massage. Motility was evaluated using a dark-field microscope at x400 magnification and was expressed as percentage. 0.3% NaCl was used as activation solution to estimate motility. For this aim, about 10 µL semen was placed on microscope slide and 100 µL activation solution was added. Each motility determination was performed in triplicate for each semen sample. Same person conducted all the sperm motility observations in order to decrease the degree of variation among observers.

Duration of spermatozoa motility was estimated using a sensitive chronometer (1/100) starting simultaneously with the addition of activation solution into the sample until 5% of the spermatozoa maintained forward swimming activity. Spermatozoa density was determined by the hemacytometric method. For this aim, sperm was diluted (1/1000) with Hayem solution (5 g Na_2SO_4, 1 g NaCl, 0.5 g $HgCl_2$ 200 mL bicine) and a droplet of the diluted sperm was placed on Thoma's hemocytometer slide (depth 0.1 mm) with a coverslip and counted using dark-field microscope. After a few minutes (to allow sperm sedimentation), the number of spermatozoa was counted at x400 magnification and expressed as $x10^9$ mL^{-1}. Semen pH was measured using pH meter (Model GLP 21, Crison, Barcelona) within 30 min of sampling. Semen colour was evaluated visually immediately following collection.

Fecundity, egg size and fertilization

Fecundity was calculated by volumetric method and egg size was determined using a sensitive micrometer. Fertilization experiments were performed in triplicate using an egg pool at 24°C water temperature. Eggs pooled from each 15 females were divided to 45 egg batches in small dishes. Fertilization took place in these dry plastic dishes and 100 g of eggs were placed into each dish. Fertilization capacity of sperm from each males were tested with the same egg pool. The insemination dosage was $2x10^5$ spz/egg for each fertilization experiment. The fertilization solution (3 g of urea, 4 g of NaCl in 1 L of water) and the dry fertilization technique were used. Semen were added over the eggs and gently mixed before activation with 20 mL fertilization solution.

Following fertilization eggs were stirred for 10-15 min and then the eggs were rinsed with hatchery water two or three times to eliminate the stickness of the eggs and placed into the incubation trays. The fertilization ratio was counted in the 4-cell stage under a stereomicroscope at 20-fold magnification.

Statistical analysis

Motility data were normalized through arcsine transformation. Correlations between spermatological parameters, fecundity, egg size and fertilization rates were estimated using Pearson's correlation test. Results are presented as mean±SE. Significance was considered at the level of α=0.05. Statistical analyses were

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performed with SPSS 10 for Windows statistical software package.

Results

Physical characteristics of semen were found to be rather variable and are presented in Table 1. Semen colour was determined as milky white in all samples. The volume of sperm collected for each male was 15.64±5.39 mL (ranged from 4 to 23 mL). Mean spermatozoa motility was 81.2±7.61% (ranged from 70 to 95%) and the mean forward motility duration was 62.06±19.40 s (ranged from 35 to 117s). The spermatozoa density was 17.88±5.48 $x10^9$ spz.mL^{-1} (ranged from 8 to 35 $x10^9$ spz.mL^{-1}). Sperm motility positively correlated with spermatozoa density ($r=0.742$, $p<0.01$) and movement duration ($r=0.855$, $p<0.01$). Mean fertilization rate was 79.3±2.95% (ranged from 60% to 95%) and highly correlated with spermatozoa motility ($r=0.932$, $p<0.01$) and spermatoza density ($r=0.826$, $p<0.01$). Correlations between fertilization rates and sperm quality parameters are shown in Table 2. Mean fecundity of grass carp collected from each female was 417867±36.274 (ranged from 202154 to 715329) and mean egg size was determined as 1.04±0.028 mm (ranged from 0.9 to 1.2 mm). A negative relationship ($r=-0.412$, $p>0.05$) was determined between fecundity and egg size. Statistical analysis shows positive correlation between fertilization rates and fecundity ($r=0.539$, $p>0.05$) and also between fertilization rates and egg size ($r=0.513$, $p>0.05$). Relationships between fertilization rates, sperm quality parameters, fecundity and egg size are shown in Figure 1 (a-h).

Table 1: Sperm quality parameters, fecundity and egg size of grass carp (n=15)

	Minimum	Maximum	Mean	SEM
Volume (mL)	4	23	15.64	5.39
Motility (%)	70	95	81.20	7.61
Movement duration (s)	35	117	62.06	19.40
Density ($x10^9$ mL^{-1})	8	35	17.80	5.48
Total density ($x10^9$)	73	1177	428.90	302.05
pH	6	8.5	7.25	0.81
Egg size (mm)	0.9	1.2	1.04	0.03
Fecundity (egg/fish)	202154	715329	417867	36.274

Table 2: Correlations between sperm quality parameters, and fertilization rates in grass carp

	Sperm Volume	Spermatozoa Motility	Movement Duration	Spermatozoa Density	Total spz Density	Sperm pH
Spermatozoa Motility	0.327					
Movement Duration	0.848*	0.855*				
Spermatozoa Density	0.908*	0.742*	0.934*			
Total spz. Density	0.822*	0.912*	0.935*	0.863*		
Sperm pH	0.244	0.317	0.343	0.250	0.290	
Fertilization Rate	0.871*	0.932*	0.894*	0.826*	0.857*	0.273

*Correlation is significant at the 0.01 level

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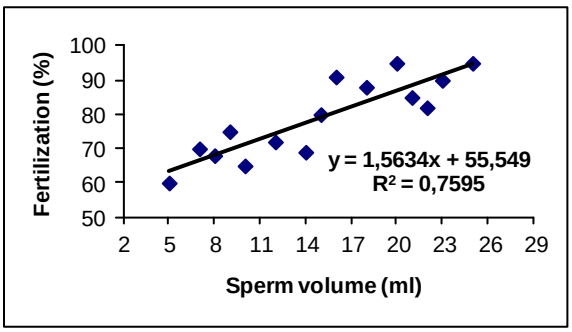

Figure 1a: Relationship between fertilization rates and sperm volume

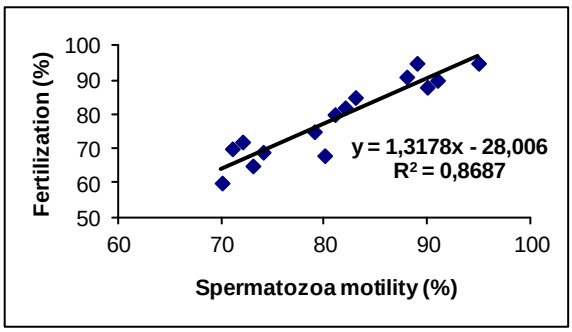

Figure 1b: Relationship between fertilization rates and spermatozoa motility

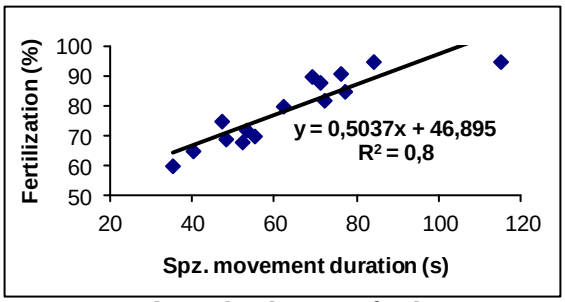

Figure 1c: Relationship between fertilization rates and spermatozoa movement duration

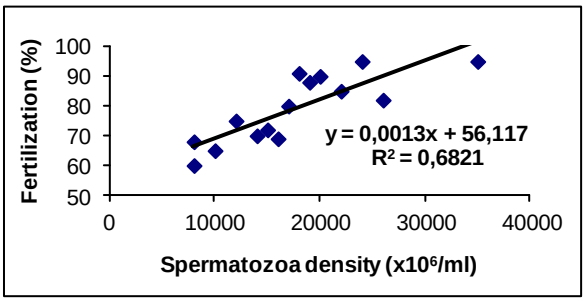

Figure 1d: Relationship between fertilization rates and spermatozoa density

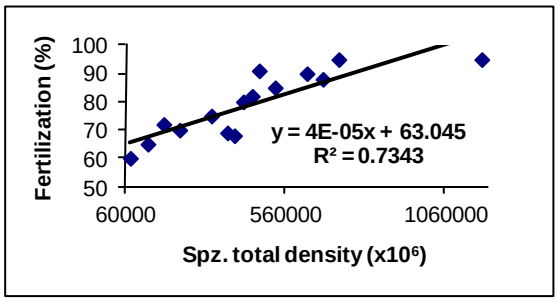

Figure 1e: Relationship between fertilization rates and total spermatozoa density

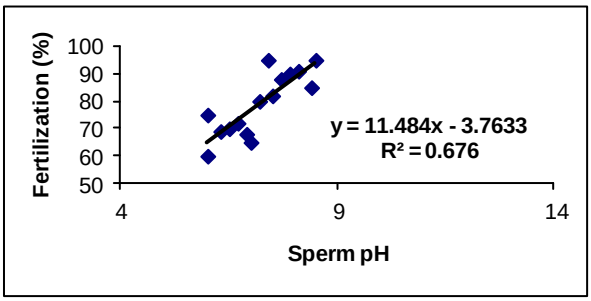

Figure 1f: Relationship between fertilization rates and sperm pH

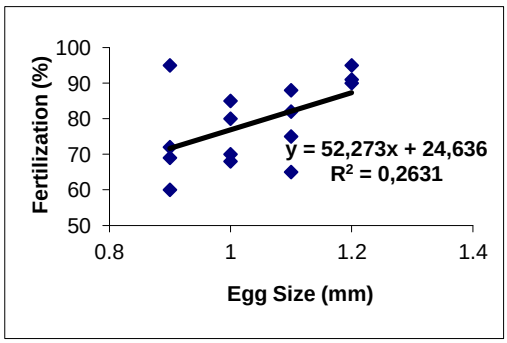

Figure 1g: Relationship between fertilization rates and egg size

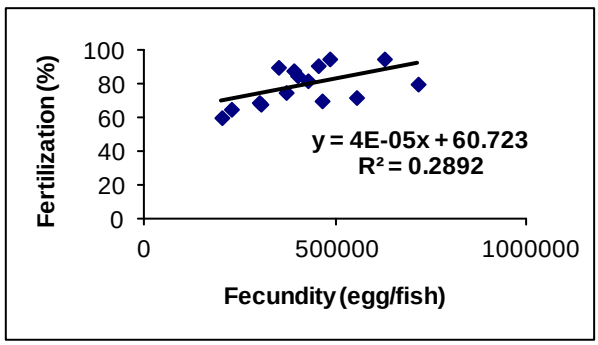

Figure 1h: Relationship between fertilization and fecundity.

Figure1: Variables correlated to fertilization rate: a) sperm volume, b) spermatozoa motility, c) spermatozoa movement duration, d) spermatozoa density, e) total spermatozoa density, f) sperm pH, g) egg size, h) fecundity.

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Discussion

The relationship between sperm quality parameters, fecundity, egg size and fertilization poorly understood in grass carp. For this purpose, the current study was aimed to evaluate semen quality of grass carp determining the spermatological parameters and fertilization rate in order to determine the reproductive capability of this species under captive conditions. Sperm volume is one of the features reflecting the semen yield and spermatozoa density. The mean sperm volume of grass carp (15.64±5.39 mL) was similar to results reported by Bozkurt et al. (2008) for grass carp (14.44±1.16 mL) and Akcay et al. (2004) for mirror carp (13.26±2.51 mL) but different from reported by Bozkurt and Secer (2004) for mirror carp (8.95±12.95 mL). The difference may be due to differences in feeding conditions and regime, environmental factors, or spawning time.

The spermatozoa motility and its duration have great influence on successful fertilization. However, spermatozoa motility varies in vigor and duration not only among males but also within individual males depending on ripeness (Tekin et al., 2003; Bozkurt et al., 2011). It has been shown that sperm motility performances (percentage of motile sperm and period of motility) change at spawning season in some teleost fish (Koldras et al., 1996). The observed changes seem to be related to the changes in seminal fluid composition as well as hormonal changes in the hypothalamous (Bozkurt et al., 2009).

Mean spermatozoa motility of grass carp was determined as 81.2±7.61% in the present study. This value complies with that of Zhukinskiy and Alekseenko (1983) and Bozkurt et al. (2008) for this species. The sperm motility range of samples studied was 70-95% and the fertilization rate range was 60-95%. The motility was well and positively correlated ($r=0.932$, $p<0.01$) with the fertilization yield. The significant positive correlation between sperm motility and fertilization indicates that grass carp eggs can only be fertilized with motile spermatozoa.

Observations on the fertilization process in teleosts demonstrate that spermatozoa must swim actively into the egg micropylar channel since immotile spermatozoa cannot perform the fertilization process (Iwamatsu et al., 1993). On the other hand, mean motility duration (62.06±19.40 s) was lower than that of Zhukinskiy and Alekseenko (1983) for this species. This value also different from reported by Akcay et al. (2004) for mirror carp and Bozkurt (2006) for scaly carp.

Spermatozoa density may also effect the fertilization rate (Aas et al., 1991). Findings on spermatozoa density ($17.88\pm5.48 \times 10^9$ mL^{-1}) in the present study corroborates with the results reported by Bozkurt et al. (2008) for grass carp (15.43±0.72) and Akcay et al. (2004) for mirror carp ($17.33\pm1.22 \times 10^9$ mL^{-1}) but not those reported by Emri et al. (1998). The differences may be due to feeding conditions, age, environmental factors (such as day length and temperature) or

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semen dilution ratio. According to Billard et al. (1995), semen pH can effect spermatozoa motility and maturation. So, determination of variation in sperm pH can provide information on fertilization capacity of spermatozoa. Mean pH determined in this study (7.25±0.81) was generally confirmed by Saad et al. (1988), Lubzens et al. (1997) and Emri et al. (1998).

Fertilization procedure was carried out using $2x10^5$ spermatozoa per egg as stated by Munkittrick and Moccia (1984). Mean fertilization rate (79.3±2.95%) was lower than the findings of Zhukinskiy and Alekseenko (1983) that reported their results in the range of 90.2-97.7%. Dada and Ogunduyile (2011) reported positive relationship between sperm volume and fertilization rates in African catfish (*Clarias gariepinus*). Also, Linhart et al. (2000) indicated good correlation (r=0.53) between motility and fertilization at spermatozoa/egg ratios of around $2x10^5$:1. Similarly, higher positive relationship (r=0.932, p<0.01) was determined between motility and fertilization ratio at $2x10^5$ spermatozoa/egg ratio.

Fecundity and egg size was determined similar to the values reported for *Ctenopharyngodon idella* (Horvath et al., 1984). Most studies on fish reproduction tend to consider fecundity and egg size as separate indicators of reproductive performance. It is generally accepted that there is an inverse relationship between fecundity and egg size that fish produce either more eggs of a smaller size or fewer eggs of a larger size (Bromage et al., 1992). Similarly, negative relationship (r=-0.412, p>0.05) was determined between fecundity and egg size in this research. On the other hand, fertilization rates positively correlated with fecundity (r=0.539, p>0.05) and egg size (0.513, p>0.05). For this reason, it can be concluded that fecundity and egg size should be considered together to evaluate fertilization success in grass carp.

In conclusion the present research showed that mature males releasing sperm with low motility and low density should be culled from the broodstock. Reducing the number of male broodstock maintained for spawning can significantly improve hatchery efficiency and minimize feed costs. This investigation provides useful information for efficient gamete management and increased fry yields in aquaculture operations and can help preperation of gametes for cryopreservation experiments. On the other hand, further experiments are needed to increase the viability, survival and development of larvae used in farming conditions.

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References

Aas, G.H., Refstie, T. and Gjerde, B., 1991. Evaluation of milt quality of Atlantic Salmon. *Aquaculture*, 95, 125–132.

Akcay, E., Bozkurt, Y., Secer, S. and Tekin, N., 2004. Cryopreservation of mirror carp semen. *Turkish Journal of Veterinary and Animal Sciences*, 28(**5**), 837-843.

Billard, R., 1986. Spermatogenesis and spermatology of some teleost fish species. *Reproduction Nutrition Development*, 26, 877-920.

Billard, R., Cosson, J., Crim, L.W. and Suquet, M., 1995. Biology of sperm and artficial reproduction in carp. *Aquaculture*, 129, 95-112.

Bozkurt, Y. and 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, Turkey.

Bozkurt, Y., 2006. Relationship between body condition and spermatological properties in scaly carp (*Cyprinus carpio*) semen. *Journal of Animal and Veterinary Advances*, 5(5), 412-414.

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, 1666-1672.

Bozkurt, Y., Öğretmen, F. and Seçer F. S., 2009. Effect of different extenders and storage periods on motility and fertilization success of grass carp (*Ctenopharyngodon idella*) sperm during spawning season. *Journal of Agricultural Sciences*, 15 (3), 277-284.

Bozkurt, Y., Öğretmen, F., Kökçü, Ö. and Erçin, U. 2011. Relationship between seminal plasma composition and sperm quality parameters of the *Salmo trutta macrostigma* (Dumeril,1858) semen: with emphasis on sperm motility.*Czech Journal of Animal Sciences*, 56 (8), 355-364.

Bromage, N.R., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg

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[DOR: 20.1001.1.15622916.2012.11.4.4.6]

production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 100, 141-166.

Chauvaud, L., Cosson, J., Suquet, M. and Billard, R., 1995. Sperm motility in turbot, *Scopthalmus maximus*: initiation of movement and changes with time of swimming characteristics. *Environmental Biology of Fishes*, 43, 341-349.

Dada, A. A. and Ogunduyile, F.D., 2011. Effects of velvet bean (*Mucuna pruriens*) on sperm quality of African catfish *Clarias gariepinus* (Burchell, 1822) Broodstock. *Journal of Fisheries and Aquatic Sciences*, 6 (6), 655-661.

Emri, M., Marian, T., Tron, L., Balkay, L. and Krasznai, Z., 1998. Temperature adaptation changes ion concentrations in spermatozoa and seminal plasma of common carp without affecting sperm motility. *Aquaculture*, 167, 85-94.

Horvath, L., Tamas, G. and Tolg, I., 1984. Special methods in pond fish husbandry. *Akademiai Kiado*. Budapestsh.148 p.

Iwamatsu, T., Ishijma, S. and Nakashima, S., 1993. Movement of spermatozoa and changes in micropyles during fertilization in medaka eggs. *Journal of Experimental Zoology*, 266, 57-64.

Koldras, M., Loir, M., Maise, G. and Le Gac, F., 1996. Study of the composition of seminal fluid and of sperm motility along the genital tract, during the spawning season, in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources*, 9, 337–45.

Lahnsteiner, F., Berger, B., Weismann, T. and Patzner, R. A., 1998. Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. *Aquaculture*, 163, 163–81.

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, 241–250.

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*, 155,13-30.

Munkittrick, K.R. and Moccia, R.D., 1984. Advances in the cryopreservation of salmonid semen and suitability for a production-scale artificial fertilization program. *Theriogenology*, 21,645-659.

Saad, A., Billard, R., Theron, M.C. and Hollebecq, M.G. 1988. Short-term

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[DOR: 20.1001.1.15622916.2012.11.4.4.6]

preservation of carp (*Cyprinus carpio*) semen. *Aquaculture*, 71,133-150.

Tekin, N., Secer, S., Akcay, E., Bozkurt, Y. and Kayam, S., 2003. The effect of age on spermatological properties in rainbow trout (*Oncorhynchus mykiss* W., 1792). *Turkish Journal of Veterinary and Animal Sciences*, 27, 37-44.

Zhukinskiy, V.N. and Alekseenko, V.R., 1983. Semen quality in common carp, *Cyprinus carpio*,and white amur, *Ctenopharyngodon idella* (Cyprinidae), in different periods of the spawning season and as influenced by extraction methods. *Journal of Icthyology*, 23, 124-133.

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