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Relationship Between Spermatozoa Motility, Egg Size, Fecundity and Fertilization Success in *Salmo trutta abanticus*

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Abstract: Sperm and eggs were collected without anesthesia by abdominal massage from *Salmo trutta abanticus*. The relationship between spermatozoa motility, egg size, fecundity and fertilization rates were investigated. Fertilization rates ranged from 72% to 80% and correlated positively with spermatozoa motility (r= 0.111, p>0.01) and egg size (r=0.459, p>0.01). On the other hand, negative correlation (r=-0.535, p>0.01) was evaluated between fertiliation rates and fecundity. Consequently it was determined that increase in spermatozoa motility and egg sizes effect the fertilization success positively in *Salmo trutta abanticus*.

Key Words: *Salmo trutta abanticus*, fertilization, motility, egg size, fecundity

Salmo trutta abanticus'larda Fertilizasyon Başarısı ile Spermatozoa Motilitesi, Yumurta Büyüklüğü ve Yumurta Verimi Arasındaki İlişki

Öz: Abant alabalıklarından (*Salmo trutta abanticus*) sperma ve yumurta abdominal masaj yoluyla alınmıştır. Fertilizasyon oranları ile spermatozoa motilitesi, yumurta büyüklüğü ve yumurta verimi arasındaki ilişki araştırıldı. Fertilizasyon oranı ile spermatozoa motilitesi (r=0.111, p>0.01) ve fertilizasyon oranı ile yumurta büyüklüğü (r=0.459, p>0.01) arasında pozitif yönde korelasyon belirlenirken fertilizasyon oranı ile yumurta verimi (r=-0.535, p>0.01) arasında ise negatif yönde bir korelasyon belirlenmiştir. Sonuç olarak, spermatozoa motilitesi ve yumurta büyüklüğündeki artışların Abant alabalık (*Salmo trutta abanticus*)'larında fertilizasyon başarısını olumlu yönde etkilediği belirlenmiştir.

Anahtar Kelimeler: *Salmo trutta abanticus*, fertilizasyon, motilite, yumurta büyüklüğü, yumurta verimi

Introduction

Salmo trutta abanticus is native to lake Abant and the nearest rivers and creeks and is known to be endemic to Lake Abant, Turkey (Geldiay and Balik 1988) being reared in natural conditions as well. Moreover, *Salmo trutta abanticus* is important as a biological gene source for restocking for Turkey. Nowadays, *Salmo trutta abanticus* populations are badly affected by natural phenomena (e.g., droughts and climate change) or human activities (e.g., overfishing, tourism, pollution and industry).

The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of viable larvae (Kjorsvik et al. 1990). 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 (Bozkurt et al. 2006). It is well known that the size of eggs of fish shows considerable intra- and inter- specific variation. Even parental fish of the same strain, weight and length have eggs that in different size (Bagenal 1971). Egg size is primarily determined by the genotype of parents of fish and it is also known to be affected by other factors including age and size of the female parent. Gall (1974) have shown in studies of hatchery-reared trout that older and heavier females produce larger eggs than younger and smaller fish. The availability of food also affects egg size (Springate et al. 1985). Alterations in egg size also occur in batch-spawning fish as the season progresses (Bagenal 1971) and in synchronous spawners as a result of photoperiodic modifications of maturation (Bromage et al. 1984).

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On the other hand, sperm quality data are required for successful fertilization. In addition, sperm quality is a measure of the ability of sperm to successfully fertilize an egg. Any quantifiable physical parameter that directly correlates with the fertilization capacity of sperm could be potentially used as a measure of sperm quality. Optimal sperm quality is important for effective broodstock management and should be a criterion in the selection of male broodstock (Bozkurt et al. 2006).

Nevertheless, in commercial hatcheries, sperm is often inadequate both in terms of quantity and quality and it does not always give successful fertilization in the artificial insemination procedures commonly used for aquaculture species. Spermatozoa motility is the most commonly used criterion to evaluate semen quality (Bozkurt, 2006). However, investigations of the relationship between spermatozoa motility and fertility have given conflicting results or were of little value in predicting fertilization potential (Graham et al. 1978).

From this point of view, it seems that fecundity, egg size and spermatozoa motility should be considered together to estimate fertilization success. Therefore the aim of this study was to determine the relationship between motility, egg size, fecundity and fertilization rates in *Salmo trutta abanticus*.

Materials and Methods

Broodstock care and collection of sperm: Fifteen mature *Salmo trutta abanticus* males (total weight 1.452±1.86 kg, total length 45.39±4.25 cm) and females (total weight 1.310±480.83 kg, total length 44.5±3.53 cm) were used as sperm and egg donors respectively. The broodstock were held in raceways under a natural photoperiod regime and fed with a commercial trout diet at 2% of their body weight per day. Water temperature varied between 7-8 °C during spawning season. Sperm from each fish was collected into 25 ml glass beakers by abdominal massage.

Evaluation of spermatozoa motility: A 10 µl sample was taken from each sperm batch and placed on a microscope slide and 100 µl activation solution (0.3% NaCl) was added to determine spermatozoa motility. The percentage of motility was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as actively swimming in a forward motion. Sperm cells that vibrated in place were not considered to be motile and observations were made within thirty minutes of sperm collection.

Fecundity and egg size: Fecundity and egg size was evaluated from fifteen females. Fecundity was calculated by volumetric method and egg size was determined by using a sensitive micrometer (at 0.01 mm sensitivity).

Fertilization: The fertilization capacity of sperm from each males resulting 45 sperm samples were tested with the same egg pool. All fertilization trials were done as 3 replicates in dishes with 10 ml of eggs (90±10 eggs). The dry fertilization technique was used and the insemination dosage was $2x10^5$ spz/egg for each fertilization experiment. Sperm obtained from each fish was poured onto the eggs and gently mixed about 20 s respectively and one minute later 20 ml fertilization solution (3 g urea, 4 g NaCl and 1 l distilled water) was added. About 30 minutes later following the fertilization, the eggs were rinsed in hatchery water and incubated in a egg incubator. Fertilization rate was determined as the percentage of eyed eggs about fourty days later following the fertilization.

Statistical Analysis: Results are expressed as mean±standart deviation. The arcsine transformation was used to the fertilization percentages before statistical analysis. Significant means were subjected to a multiple comparison test (Duncan) for post-hoc comparisons at α=0.01 level. All statistical analysis were carried out using SPSS 10 for Windows software package.

Results and Discussion

Fertilization ratio ranged between 72% and 80% and was found as mean 76.0±4.0%. Statistical analysis shows positive allometry for the relationships between fertilization rates and spermatozoa motility and negative allometry for the relationship between fertilization rates and fecundity. Values related to fecundity, egg size, spermatozoa motility and fertilization rates were shown in Table 1. Correlations and relationships between spermatozoa motility, egg size, fecundity and fertilization rates are shown in Table 2 and Figure 1 (a-c).

Table 1. Fecundity, egg size, motility and fertilization rates in *Salmo trutta abanticus* (n=15).

	Total Fecundity (eggs/fish)	Egg size (mm)	Motility (%)	Fertilization rates (%)
Mean value (±sd)	1468±180.21	5.42±0.25	75.2±3.24	76±2.58
Range	1125-1800	5.0-5.8	60-85	72-80

Table 2. Correlations between spermatozoa motility, egg size, fecundity and fertilization rates in *Salmo trutta abanticus*

	Spermatozoa motility	Egg size	Fecundity
Egg size	-0.100		
Total Fecundity	0.055	-0.156	
Fertilization rates	0.111	0.459	-0.535

* Correlation is not significant at the 0.01 level.

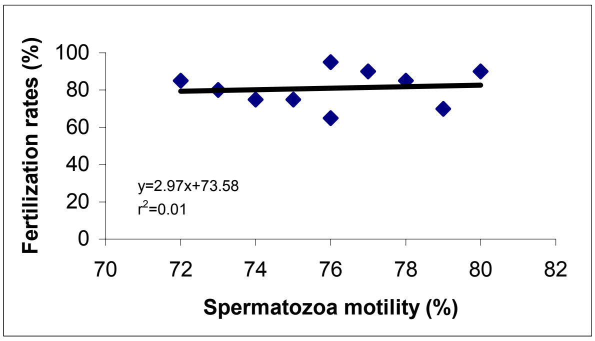

Figure 1a. Relationship between fertilization rates and spermatozoa motility.

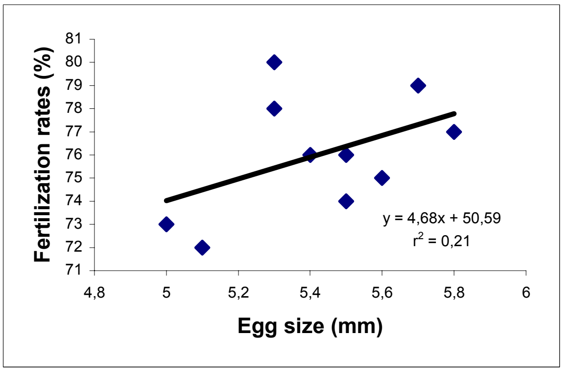

Figure 1b. Relationship between fertilization rates and egg size.

The global growth of intensive aquaculture has increased the need for efficient and effective means of conserving fish gametes. Viable sperm is an essential component in any successful animal production operation and the success of reproductive process is dependent on a supply of high quality gametes (Cruz-Casallas et al., 2005).

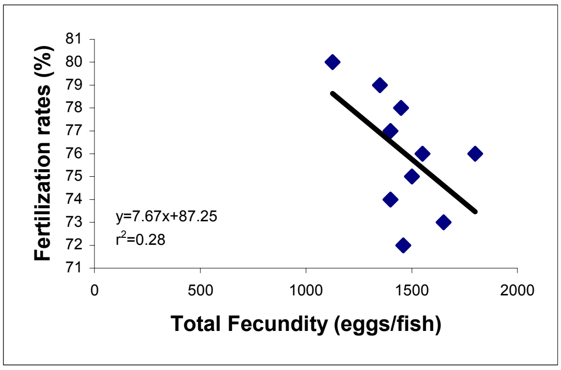

Figure 1c. Relationship between fertilization rates and fecundity.

The most reliable indicator of the sperm quality is spermatozoa motility. Subjective estimation of motility requires considerable experience and this indicator is being used in the selection of sperm for insemination and preservation. However, spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on its ripeness. The highest motility of the spermatozoa is observed at the peak of the breeding season (Terner 1986). Studies on most fish species recorded that motility of spermatozoa may show seasonal variation (Benau and Terner 1980).

Our results confirmed the data of Munkittrick and Moccia (1987) and of Ciereszko and Dabrowski (1994) who found a correlation between motility rate and fertilization capacity for the rainbow trout sperm using subjective estimation methods for motility determination. Magyary et al. (1996) concluded a strong, positive correlation (r=0.85) of frozen/thawed carp spermatozoa motility and fertility at $1x10^5$-$1.5x10^5$:1 spermatozoa/egg ratio. In addition, Linhart et al. (2000) also observed good correlation (r=0.53) with fresh sperm between carp spermatozoa motility and fertilization at spermatozoa/egg ratios of around $2x10^5$:1. The recommended sperm to egg ratio for commercial trout culture is based on the use of stripped milt (Scott and Baynes 1980). In this study, a positive relationship was determined between motility and fertilization rate (p>0.01) at 200.000 spz:egg ratio.

In the case of fecundity, this parameter was found lower than values reported for rainbow trout (Bromage and Cumaranatunge 1988, Estay et al. 1994). However values related to the egg size were found higher than that of rainbow trout (Springate et al. 1984, Estay et al. 1994). Most studies of 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 in which fish produce either more eggs of a smaller size or fewer eggs of a larger size (Springate et al. 1985, Bromage et al. 1992). Similarly, a negative allometry was obtained between fecundity and egg size in this study. Also a negative relationship was determined between fecundity and fertilization rates. For this reason, it can be concluded that egg size is more important than fecundity for the fertilization success.

In conclusion, the information obtained from the present study can lead to more efficient gamete management and increased fry yields. On the other hand, further studies are needed to increase the viability, survival and development of larvae used in farming conditions.

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