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Relationship Between Spermatozoa Motility, Egg Size, Fecundity and Fertilization Success in Brown Trout (*Salmo trutta fario*)

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Abstract: In this study, the relationship between spermatozoa motility, egg size, fecundity and fertilization rates were investigated. Sperm and eggs were collected without anesthesia by abdominal massage from brown trout (*Salmo trutta fario*). Fertilization rates ranged from 35 to 72% and correlated positively with spermatozoa motility ($r = 0.333$, $p>0.05$) and egg size ($r = 0.749$, $p<0.05$). On the other hand, negative correlation ($r = -0.393$, $p>0.05$) was evaluated between fertilization rates and fecundity. Consequently it was determined that increase in spermatozoa motility and egg sizes effect the fertilization success positively in brown trout.

Key words: Brown trout, *Salmo trutta fario*, motility, egg size, fertilization, fecundity

INTRODUCTION

Salmo trutta forms resident populations in the upper streams of rivers and occurs in North Africa, Europe, West Asia and Anatolia (Geldiay and Balik, 1988) and it is an important potential species for recreational fishery. However, in most parts of these areas, river systems have undergone great changes in their ecology and morphology in recent years and river damming and degradation of spawning habitats have caused a decline in the stocks of *Salmo trutta* (Bozkurt *et al*., 2006).

In commercial fish production the evaluation of semen quality is of interest to increase the efficiency of artificial fertilization. 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; Secer *et al*., 2004). High individual variations of milt quality are frequently reported (Rana, 1995). This may be due to genetic variability but also to sampling location (from testes to the genital pore), contamination of sperm samples by urine and aging of spermatozoa during the spawning season. Because of the close vicinity of sperm ducts and urinary ducts, sperm samples are frequently contaminated with urine. This phenomenon is described in freshwater fish species (Rana, 1995). Intratesticular aging of sperm has been reported for many fish species and it affected sperm quality at the end of the milting period (Rana, 1995).

Techniques for determining semen quality in fish include monitoring spermatozoa motility and fertilization success (Tekin *et al*., 2003). In addition, sperm quality is a measure of the ability of sperm to successfully fertilize an egg. One of the most common parameters employed to study sperm biology is the motility parameters of the spermatozoa (Billard *et al*., 1995). Studies on motility of fish spermatozoa have been restricted to a very limited number of groups, mostly species with commercial value in aquaculture or involved in conservation programs (Billard and Cosson, 1992; Alavi and Cosson, 2002).

Determination of motility has great importance, as sperm must be fully motile to achieve egg penetration (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). 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 parental 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

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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).

From this point of view, the purpose of this study was to find out the relationship between spermatozoa motility, egg size, fecundity and fertilization ratios in brown trout (*Salmo trutta fario*).

MATERIALS AND METHODS

Collection of semen and evaluation of motility: Twenty mature (2+years old) male *Salmo trutta fario* (total weight 1.486±0.15 kg, total length 35.56±2.25 cm) were used as semen donors. This study was carried out at a local trout farm in Bolu province during spawning season (November and December months in 2005) of the *Salmo trutta fario*. 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. Semen from each fish was collected into 25 mL calibrated glass beakers by abdominal massage. A 10 μL sample was taken from each semen 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 and observations were made within 30 min of sperm collection.

Fertilization trials: The fertilization capacity of sperm from each males resulting 60 sperm samples were tested with the same egg pool. All fertilization trials were done as 3 replicates in sterile petri dishes with 10 mL of eggs (100±10 eggs). The dry fertilization technique was used and the insemination dosage was 1×10^5 spz/egg for each fertilization experiment. Sperm obtained from each fish was poured onto the eggs and gently mixed about 20 sec, respectively and one minute later 20 mL fertilization solution (3 g urea, 4 g NaCl and 1 L distilled water) was added. About 30 min later following the fertilization, the eggs were rinsed in hatchery water and incubated in a egg incubator. Fertilization rate was determined as the percent of eyed eggs about fourty five days later following the fertilization.

Statistical analysis: Results are expressed as mean±standard 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 $\alpha = 0.01$ level. All statistical analysis were carried out using SPSS 10 for Windows software package.

RESULTS

The spermatozoa motility was also ranged between 70 and 95% and was found as mean 81.0±10.74%. In addition the fertilization ratio was ranged between 35 and 72% and was found as mean 54.4±11.65%. Statistical analysis shows a significant positive allometry for the relationship between fertilization rates and egg size ($p<0.05$). Also positive allometry was found between fertilization rate and spermatozoa motility ($p>0.05$). On the other hand, a negative allometry for the relationship between fertilization rates and fecundity was determined

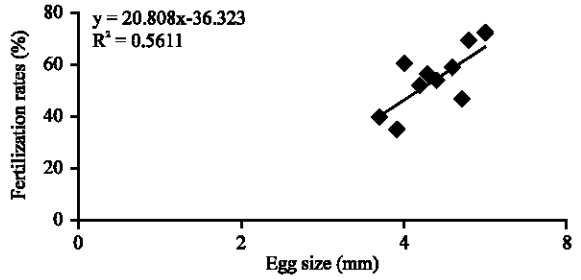

Fig. 1a: Relationship between fertilization rates and egg size

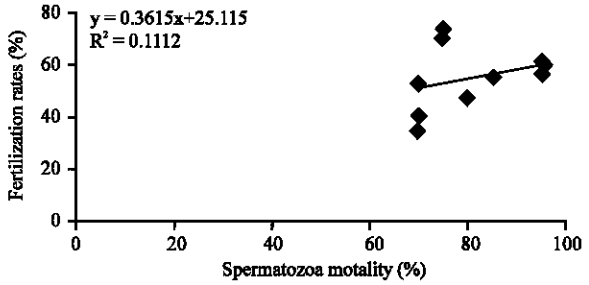

Fig. 1b: Relationship between fertilization rates and spermatozoa motility

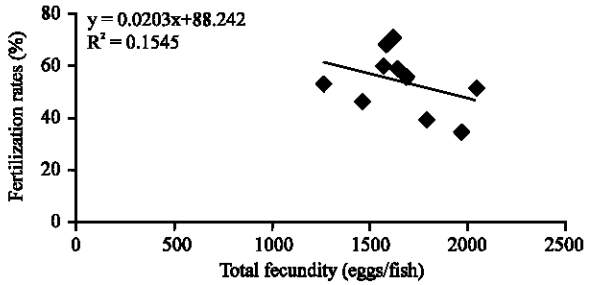

Fig. 1c: Relationship between fertilization rates and fecundity

Table 1: Fecundity, egg size, motility and fertilization rates in brown trout (*Salmo trutta fario*) (n = 20)

	Total Fecundity (eggs/fish)	Egg size (mm)	Motility (%)	Fertilization rates (%)
Mean value (±SD)	1664±225.29	4.36±0.42	81±10.74	54.4±11.65
Range	(1270-2045)	(3.7-5.0)	(70-95)	(35-72)

Table 2: Correlations between spermatozoa motility, egg size, fecundity and fertilization rates in brown trout (*Salmo trutta fario*)

	Spermatozoa motility	Egg size	Fecundity
Egg size	0.121		
Total fecundity	-0.495	-0.469	
Fertilization rates	0.333	0.749*	-0.393

*Correlation is significant at the 0.05 level

(p>0.05). Values related to fecundity, egg size, spermatozoa motility and fertilization rates were shown in Table 1. Correlations and relationships between egg size, spermatozoa motility, fecundity and fertilization rates were shown in Table 2 and Fig. 1a-c.

DISCUSSION

The fish farming industry has been more focused towards the quality of eggs and larvae rather than that of sperm, even though the sperm quality of male broodstock also affects the production of healthy larvae. Nevertheless, in commercial hatcheries, milt is often inadequate both in terms of quantity and quality and does not always give successful fertilization in the artificial insemination procedures commonly used for aquaculture species.

Sperm quality is a very important variable in aquaculture broodstock management (Billard *et al*., 1995; Bromage, 1995), as it can influence the percentage of egg fertilization and thus the total production of viable eggs from a given broodstock.

Any quantifiable physical parameter that directly correlates with the fertilization capacity of sperm could be potentially used as a measure of sperm quality. 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 height of the breeding season (Terner, 1986). Studies on most fish species recorded that motility of spermatozoa may show seasonal variation (Benau and Terner, 1980).

In the case of egg size, this parameter is also very important factor that directly affect the fertilization result. In spite of obtaining of meanly low fertilization result (54.4%), statistically significant correlation (0.749) was determined with the egg size (p<0.05). This result indicates that sperm quality of brown trout broodstock used in this investigation is slightly lower or affected by many factors such as genotype, age and size of broodstock, environmental conditions or handling parameters. 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.

On the other hand, a positive correlation (0.333) was also determined with the fertilization ratios (p>0.05). Similarly 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 semen motility and fertility at 1×10^5-1.5×10^5:1 semen/egg ratio. In addition, Linhart *et al*. (2000) also observed good correlation (r = 0.53) with fresh semen between carp semen motility and fertilization at semen/egg ratios of around 2×10^5:1. Accurate, subjective estimation of motility requires considerable experience. In this study, despite usage of low ratio of spz:egg (100.000 spz:egg), a positive relationship was determined between motility and fertilization rate (p>0.01). The recommended sperm to egg ratio for commercial trout culture is based on the use of stripped milt (Scott and Baynes, 1980).

In conclusion, it can be suggested that egg size, fecundity and spermatozoa motility should be considered together to evaluate fertilization success of the broodstock. The information on gamete physiology obtained from the present study can lead to more efficient gamete management and increased fry yields.

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