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The Relationship Between Body Condition, Sperm Quality Parameters and Fertilization Success in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Rainbow trout (*Oncorhynchus mykiss*) sperm was collected without anesthesia by abdominal massage. In collected sperm, volume, motility, movement duration, density, total density and pH were recorded. Furthermore, body weight and body length were measured and correlations beetween spermatological properties and these parameters were assessed. Fertilization was carried out using the dry fertilization technique at 1x105 spz/egg ratio. The highest fertilization and hatching rates were found as 93 and 100%, respectively. There were significant positive correlations between body weight and length ($r= 0.800$, $p<0.01$), body weight and movement duration ($r= 0.556$, $p<0.05$), body length and pH ($r= 0.613$, $p<0.05$) and density and total density ($r= 0.864$, $p<0.01$). Significant negative correlations were found between body length and density ($r= -0.609$, $p<0.05$) and body length and total density ($r= -0.570$, $p<0.05$). In addition, it was observed that there was a positive relation between motility and fertilization rate (0.944, $p<0.01$) and also between motility and hatching rate (0.689, $p<0.01$). Furthermore, fertilization and hatching rates significantly correlated ($r= 0.742$, $p<0.01$) with each other.

Key words: *Oncorhynchus mykiss*, sperm quality, fertilization, hatching

INTRODUCTION

The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of viable larvae[1]. Techniques for determining sperm quality in fish include monitoring sperm density, motility and fertilization success[2].

Sperm quality data are required for successful artificial insemination and semen handling techniques. In addition, sperm quality is a measure of the ability of sperm to successfully fertilise 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.

Nevertheless, in commercial hatcheries, sperm is often inadequate both in terms of quantity and quality and does not always give successful fertilisation in the artificial insemination procedures commonly used for aquaculture species. 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[2-4] .

Sperm quality can also be influenced by factors such as size or age of individuals[4,5]. Often, older, more experienced males produce higher milt volumes with higher sperm density and greater fertilization capacity as compared with younger, less mature fish[5]. Considered together, available data indicate that there are individual differences in sperm quantity and quality that must be identified to select the most valuable fish for broodstock. In addition, a relationship between motility and fertilizing capacity has been assumed by several authors[6,7]. From this point of view, it seems that sperm quality data are required for successful artificial insemination and semen handling techniques and also sperm quality is a measure of the ability of sperm to successfully fertilise an egg.

The objectives of this study were to examine the relationships between body condition and quality parameters of rainbow trout (*Oncorhynchus mykiss*) sperm as well as influence on fertilization ratio of eggs.

MATERIALS AND METHODS

Broodstock and collection of gametes: 15 adult (4-5 years old) males of rainbow trout with a mean weight of 2.06±0.31 kg (Range:1.58-2.55 kg) and a total length of 52.27±4.21 cm (Range:45-57 cm) were obtained from a local freshwater fish hatchery at the early reproductive season. In the pre-spawning period the parental brood fish were kept seperately in small ponds and fasted 48 h prior to sperm collection. Sperm and eggs were collected by manual stripping. Before collection of gametes, 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 faeces. Eggs were collected from 5 mature (3-4 years old) females (mean weight 2.35±0.56 kg, mean total length 46.30±0.24 cm) which were stripped by gently massaging the abdomen. Only transparent, well-rounded and unwrinkle eggs were used for fertilization experiments.

Evaluation of sperm quality: The sperm quality parameters including sperm volume (mL), spermatozoa motility (%), spermatozoa density (x10^9/mL), spermatozoa movement duration (s) and sperm pH were evaluated.

Sperm volume: The sperm was collected in glass tubes graded in millimetres and sperm volume was registered immediately following collection by abdominal massage.

Spermatozoa motility: The motility of sperm in each sample was evaluated within five minutes following sperm collection. Sperm samples were kept at approximately +4°C throughout the motility tests. About 10 μL sperm was placed on a glass microscope slide (1.0-1.2 mm depth) and 100 μL activation solution (0.3% NaCl) was added. Spermatozoa motility was observed under 200x magnification and the percentage of motil spermatozoa were assessed. Only forward movements by the spermatozoa were assessed as motility, whereas simply vibrating sperm were assessed as immobile.

Spermatozoa density: Spermatozoa density were determined by the haemacytometric method. Sperm was diluted (1/1000) in Hayem solution (5 g Na_2SO_4, 1g NaCl, 0.5 g $HgCl_2$, 200 mL bicine) 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. Counting chambers were always kept in a moist atmosphere for at least 10 min before cell counting.

Duration of spermatozoa movement and sperm 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 standart pH electrodes within five minutes of sampling.

Fertilization: For the aim of fertiliation, five egg pools were used each consisting of eggs from two to three females. 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 sterile petri dishes with 12.5 mL of eggs (200±10 eggs). The dry fertilization technique was used and the insemination dosage was 1x10^5 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 min later following the fertilization, the eggs were rinsed in hatchery water and incubated in a vertical egg incubator. Fertilization rate was determined as the percent of eyed eggs about fifteen days later following the fertilization. Hatching occurred between 320 and 330 day-degree (mean water temperature about 10°C).

Statistical analysis: Results are expressed as mean ± standart deviation. Differences between parameters were analysed by repeated analysis of variance (ANOVA). The arcsine transformation was used to the fertilization percentages before analysis. Significant means were subjected to a multiple comparison test (Duncan) for post-hoc comparisons at α =0.05 level. All statistical analysis were carried out using SPSS 10 for windows software package.

RESULTS AND DISCUSSION

Sperm quality parameters were found rather variable and presented in (Table 1). Correlations between body weight-length, sperm quality parameters, fertility and hatching of eggs are shown in (Table 2). The fertilization and hatching rates for individual samples ranged from 45 to 93% and from 64 to 100%, respectively. Statistical analysis shows negative allometry for the relationships between fertilization rates and spermatozoa movement duration, sperm pH, body weight, body length and positive allometry for the relationships between fertilization rates and other sperm quality parameters. Relationships between fertilization rates and sperm quality and body condition parameters are shown in (Fig. 1).

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 sucessful animal production operation and the success of reproductive process is dependent on a supply of high quality gametes[8]. In this study, we evaluated sperm quality parameters and their relationships with the fertilization rates.

Mean sperm volume was similar to results reported by[9] but differed from results reported by[10,11]. The differences may be due to the feeding conditions and regime, water quality, environmental factors or spawning time.

Table 1: Sperm quality parameters, fertilization and hatching data of rainbow trout (*Oncorhynchus mykiss*)

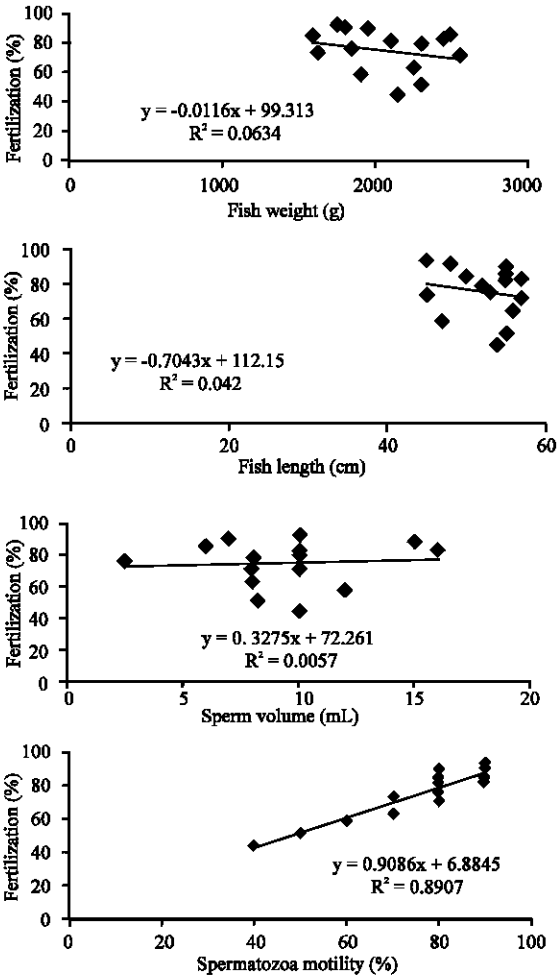
	Sperm volume (mL)	Spermatozoa motility (%)	Movement duration (s)	Spermatozoa density (x10^9/mL)	Total spz. density (x10^9)	Sperm pH	Fertilization Rate (%)	Hatching rate (%)
Means ± SD	9.3±3.33	75.3±15.05	72.4±26.98	7.7±4.31	70.0±42.53	7.3±0.30	75.6±14.49	84.0±11.67
Range	2.5-16	40-90	43-143	3.250-19.100	16.12-190.10	7-8	45-93	64-100

Table 2: Correlations between body weight-length, sperm quality parameters, fertility and hatching of rainbow trout (*Oncorhynchus mykiss*) eggs

	Weight	Length	Volume	Movement motility	duration	Total density	density	pH	Fertilization
Llength	0.800**								
Volume	-0.003	0.033							
Motility	-0.032	-0.058	-0.028						
Mov. duration	0.556*	0.447	-0.319	0.059					
Density	-0.490	-0.609*	-0.179	0.289	-0.074				
TotTotal density	-0.457	-0.570*	0.310	0.215	-0.234	0.864**			
pH	0.412	0.613*	-0.080	0.051	0.232	-0.375	-0.385		
Fertilization	-0.252	-0.205	0.075	0.944**	-0.081	0.328	0.290	-0.083	
Hatching	-0.361	-0.357	0.020	0.689**	-0.420	0.301	0.275	-0.007	0.742**

*Correlation is significant at 0.05 level
**Correlation is significant at 0.01 level

In the present study, the mean spermatozoa motility agreed with the findings of[12,11] but not with[13]. In the case of movement duration; the finding was rather similar with that of[14] but not with[9,13]. Spermatozoa motility varies in vigor and duration not only among males but



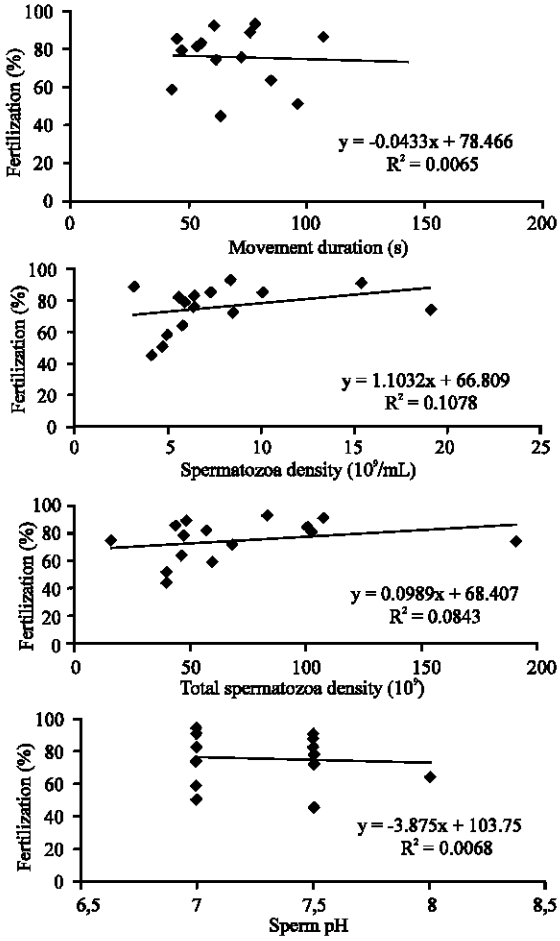


Fig. 1: Variables correlated to fertilization rate: a) Fish weight, b) Fish length. c) Sperm volume, d) Spermatozoa motility, e) Movement duration, f) Spermatozoa density, g) Total spermatozoa density, h) Sperm pH. All correlations were significant at $p<0.05$, except for (d) which was significant at $p<0.01$.

also within an individual male depending on ripeness[15]. Most studies on fish species have shown that the duration and motility of sperm may vary seasonally[16]. The findings on spermatozoa density in the present study agrees with the findings of[14,11] but different from[9,17-19]. The differences may be due to feeding conditions, age, environmental factors or dilution ratios. Sperm pH was found in the range of generally confirmed[9,20].

Sperm production and quality can be affected by both fish age, size and physiological status. Relationships found in this experiment between fish size and sperm quality indices indicate that physical condition of mature fish has a influence on sperm quality. In addition to these; poor sperm quality may result from the effects of genetics, diet, environmental stres (toxicants, water quality, fish density) or disease. According to[21], fish held in captivity experience conditions that often increase stress and lead to reduced gamete quantity. Similarly, low sperm volumes and spermatozoa densities were obtained from 4-5 years old mature rainbow trouts in this study.

Our results confirmed the data of Munkittrick and Moccia[9] and Ciereszko and Dabrowski[22] who found a correlation between motility rate and fertilization capacity for the rainbow trout sperm using subjective estimation methods for motility determination. 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. The recommended sperm to egg ratio for commercial trout culture is based on the use of stripped milt[23]. On the other hand, when excess spermatozoa were present, neither density nor motility of the spermatozoa were major factors in determining the percentage of eggs that reached the stage of eye-up. These results suggest that when low numbers of spermatozoa:egg are present early in the reproductive season, samples showing high motility are capable of fertilizing more eggs than samples with lower motility. This also indicates that subjective motility estimation techniques give a reliable indication of the unidentified properties of spermatozoa which are important in the fertilization of eggs.

CONCLUSION

In this study indicated that sperm quality parameters have been affected by the age, size and spawning season. Also it can be concluded 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. Our data can be used to select high quality mature males for fertilizing eggs in a commercial aquaculture operation. In addition to these, the information on sperm physiology obtained from the present study can lead to more efficient gamete management and increased fry yields and aid suitability of sperm for frozen or liquid preservation. On the other hand, further studies are needed for adjusting the sperm/egg ratio to increase the efficiency of sperm to fertilize the larger egg batches used in aquaculture.

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