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Research Article

Dose Dependent Treatment with Boric Acid Induces More Changes in the Sperm Cells of Endangered Anatolian Trout Salmo Rizeensis

Abstract

The aim of this study was to test the usefulness of boric acid for endangered Anatolian trout *Salmo rizeensis* sperm. Activation media was supplemented with boric acid (0.5, 1, 2, 3, 4 and 5 mM). Sperm motility and duration were determined in sperm samples. In addition, fertility and hatching rate were examined. Our data indicated that addition of boric acid (3 mM) to activation media was increased the percentage and duration of motile sperm, fertility and hatching rate in endangered Anatolian trout (*S. rizeensis*). On the other hand, the motility rate decreased with increasing the concentration of boric acid ($p<0.05$). In particular, a remarkable increase was observed after concentration 3 mM. Consequently, sperm quality was affected by quantitative changes different concentrations of boric acid and the best results were obtained at concentration 3 mM.

Introduction

Salmo trutta are the most important Salmonid fish species owing to its aquaculture potential, economic value and wide consumer demand [1]. *Salmo trutta* forms inhabit in the upper streams of rivers and occur in North Africa, Europe, West Asia and Anatolia [2,3]. In addition, it is an important potential species for recreational fishery. Recently, *S. t. macrostigma* ecotype has been described by Turan et al. [4], as *S. rizeensis* [5]. In particular, populations of the species are affected by natural hybridization, the local devastation in water sources through habitat fragmentation and modification, water eutrophication and contaminations, environmental instability and global warming [6-10]. Sperm motility is the essential functional parameter for successful fertilization in fish [11,12]. Sperm cells in most fish species immotile in seminal fluid and require to release into the water in order to trigger motility and become metabolically active [12,13]. Therefore, characteristics of activation media are crucial in terms of initiation and progression of sperm motility [12].

Trace elements have a crucial role for the male reproductive process. Boric acid (H_3BO_3) is a bioactive beneficial element [14], and widely used in glass, ceramic, detergent, plastic, agricultural, textile, metallurgy, nuclear and medicine industries owing to its excellent characteristics and it is produced from boron ore and salt lake brine [15-17]. The greatest majority of boric acid is produced by Eti Maden Works (Turkey) in the world [18]. Increasing production does not meet the demand due to using as extensive [19,20]. Several studies about the nutritional benefits [14,19,21,22], metabolic functions [23-26], U shaped dose responses on growth of embryonic fish and frogs [27,28], and therapeutic applications [29-31], toxic effect on male reproduction system of different species (e.g. rat, rodent, dog, human) [32-35], of boron and its compounds have been published in the latest available literature. As far as the authors of this work are aware, no attempt has been made to use of boric acid in sperm activation medium of fish species. In this context, the aim of this study was to examine effect of activation media supplemented with boric acid (0.5 mM; 1 mM; 2 mM; 3 mM; 4 mM; 5 mM) on sperm quality and fertility of endangered trout *S. rizeensis*.

Materials and Methods

Six mature endangered Anatolian trout males (1388.00±0.55 g, 44.52±2.62 cm as mean±SD) were captured from Uzungöl Stream, Trabzon, Turkey for sperm collection. F spermatocrit ish were anesthetized in 50 mg/L Benzoacaine, sperm samples were collected through abdominal massage and special care was taken to prevent contamination (e.g. blood, feces or urine).

The pH of sperm samples was measured with a pH meter (Thermo Scientific Orion 5-Star Plus pH meter, USA). The spermatocrit is defined as the ratio of white packed material

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volume to the total volume of semen × 100. Microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) were used for spermatocrit measurement. Microhaematocrit capillary tubes filled with sperm were centrifuged at 3000 rpm for 10 min in a LD5-2B centrifuge (Beijing Shiningsun Technology, Japan) and then spermatocrit was calculated on the basis of the ratio of spermatozoa volume (white part) to total volume of sperm × 100.

Boric acid was separately added to the activation media (NaCl, 0.3%) (one per experimental group): (a) 0.5 mM, (b) 1 mM, (c) 2 mM, (d) 3 mM, (e) 4 mM, (f) 5 mM for pure water [The concentrations were chosen because of that in preliminary studies, concentrations (<0.5 mM) did not significantly influenced sperm quality, concentrations (>5 mM) caused to death of sperm cells]. Control groups were not supplemented with boric acid. Motility parameters were measured using an automated system, SCA (Sperm Class Analyzer v. 4.0.0. by Micro tic S.L., Barcelona, Spain). The spermatozoa movement was monitored using a camera (Basler A312fc, with sensor type CCD) at 50 Hz mounted on a Nikon Eclipse 50i microscope, co-working with SCA. The percentage of sperm motility was estimated as the cell performing progressive forward movement while the duration of motility was determined as the time until forward movement stops. Determining the percentage of sperm motility was assessed using an arbitrary scale with 10% interval increments in which non motile represents 0% [36].

Fertilization experiments were conducted at 8–10°C. One homogenous egg pool was used for the fertilization experiments. From the eggs the ovarian fluid was drained off and the eggs were placed in fertilization solution a ratio of 1:2 (eggs: solution), then the semen was added and the components were mixed with each other. 100 ± 5 eggs were fertilized with 100 µl sperm (sperm to egg ratio: X10^5: 1). Three to 5 minutes after fertilization the eggs were rinsed in hatchery water and incubated in flow incubators at water temperature of 9 ± 0.5 °C. The experimental success was determined as the percentage of eyed embryos in relation to the total number of eggs 28 to 30 d after fertilization [37].

Statistical analysis was performed using the software package SPSS 14.0 for Windows and results were expressed as means ± Standard error. Differences among the treatments were tested by one-way ANOVA. The Duncan test was used for all *post-hoc* comparisons. Significance was set at $p<0.05$.

Results

Sperm parameters (mean ± SD) are presented in table 1. Effect of boric acid on the percentage and duration of motile sperm for *S. rizeensis* is shown in figure 1. The results of the present study indicated that differences in the percentage and duration of motile sperm of *S. rizeensis* sperm were significant among the treatments ($p<0.05$). Highest motility rate (80.00%) and duration of motility (60 s) were at concentration 3 mM. After concentration 3 mM, a remarkable decrease was observed in motility rate and duration. Effect of supplementation of boric acid on fertility and hatching rate for *S. rizeensis* is presented in figure 2. The trials in this study indicated that differences in the fertility and hatching rate were significant among the treatments ($p<0.05$). Highest fertility (92.17%) and hatching rate (82.09%) were at concentration 3 mM. In particular, a significant decrease was observed in fertility and hatching rate after concentration 3 mM.

Discussion

To the best of our knowledge, this is apparently the first report on effect of activation medium supplemented with boric acid on *S. rizeensis* sperm, although studies have been conducted about the nutritional benefits [14,19,21,22], metabolic functions [23-26], U shaped dose responses on growth of embryonic fish and frogs [27,28], and therapeutic applications [29-31], toxic effect on male reproduction system of different species (e.g. rat, rodent, dog, human) [32-35], of boron and its compounds. In this study, we demonstrated the usefulness of boric acid in different activation mediums for *S. rizeensis* sperm. Using 3 mM boric acid in activation media resulted in high sperm motility rate and duration, fertility and hatching rate of *S. rizeensis*. This may be due to the fact that boric acid is involved in a number of metabolic processes and interact with critical biological substances, including pyridoxine, polysaccharides, dehydroascorbic acid, riboflavin, and the pyridine nucleotides [38-40,23]. However, motility rate and duration decreased with increasing concentration of boric acid. In particular, a

Table 1: Sperm parameters (Mean±SD) of *Salmo rizeensis*.

Species	Sperm volume (ml)	pH	Spermatocrit (%)	Sperm density (×10^9)
Salmo rizeensis	7.00±0.25	7.76±0.22	55.33±0.24	9.27±0.56

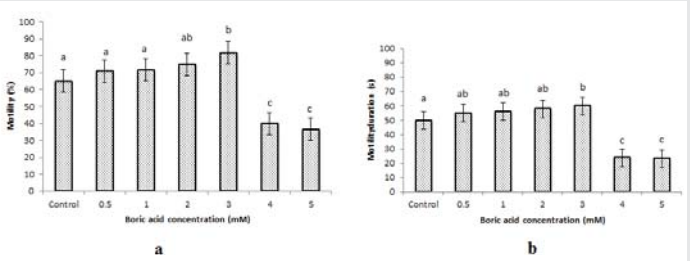

Figure 1: Effect of supplementation of boric acid on a) the motility rate and b) motility duration of S. rizeensis sperm (n=6). Different letters show differences between treatments ($p<0.05$).

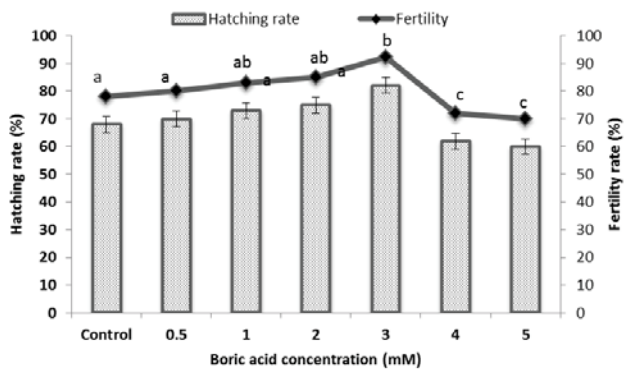

Figure 2: Effect of supplementation of boric acid on the fertility and hatching rate of S. rizeensis sperm (n=6). Different letters show differences between treatments ($p<0.05$).

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significant decrease after concentration 3 mM was observed in motility rate and duration. This may be due to toxic effect of boric acid and, inhibition of motility and fertility might be realized as a result of toxic effect. In addition, the results showed a U-shaped response for boric acid. This finding is in agreement with results from previous studies: [27,28].

Bozkurt et al. [41], determined that motility rate and duration of *S. t. macrostigma* were 80.37% and 81.47 for 0.3% NaCl. Bozkurt et al. [42], reported that motility duration of *S. t. macrostigma* was 32-57 s and 24-53 s for 1% $NaHCO_3$ and 0.3% NaCl. Iaffaldano et al. [43], motility rate and duration of *S. t. macrostigma* were 72.85% and 64.57 for 0.3% NaCl. In present study, highest motility rate and duration of motility were 80.00% and 60 s, respectively.

In conclusion, based on these results, boric acid was used efficiently for activation media in *S. rizeensis* sperm. Our study provides new insights related to use of boric acid on fish sperm quality. The knowledge of effects of boric acid and its mechanism of action might be helpful for both research and commercial use. Further studies would be needed to evaluation the precise mechanisms of boric acid response.

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