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## Research Article

# In Vitro Effect of Zinc: Evaluation of the Sperm Quality of Endangered Trout *Salmo Coruhensis* and Rainbow Trout *Oncorhynchus Mykiss* and Fertilizing Capacity

## Abstract

This study was intended to reveal the usefulness of Zinc in endangered trout *Salmo coruhensis* and rainbow trout *Oncorhynchus mykiss* sperm. Spermatozoa were activated in sperm motility-activation solutions (NaCl, 0.3%; NaHCO<sub>3</sub>, 1%) containing the Zinc [Control (0), 0.5, 1, 2, 3, 4 and 5 mM]. The percentage and duration of motility, fertility and hatching rate were determined in sperm samples. Our results indicated that the percentage and duration of motility, fertility and hatching rate increased when activation solution containing NaHCO<sub>3</sub> was supplemented with 1 mM Zinc in rainbow trout (*O. mykiss*). On the contrary, motility rate in endangered trout (*S. coruhensis*) was increased by Zinc compared to control group. The percentage and duration of motility, fertility and hatching rate were affected by increasing concentrations of Zinc in endangered trout (*S. coruhensis*) and rainbow trout (*O. mykiss*) ( $p < 0.05$ ). In conclusion, sperm quality was affected by quantitative changes different concentrations of Zinc and the best results were obtained from a concentration of 1 mM for rainbow trout.

## Introduction

*Oncorhynchus mykiss* and *Salmo trutta* are the most important Salmonid fish species owing to its aquaculture potential, economic value and wide consumer demand [1,2]. *Salmo trutta* forms inhabit naturally in the upper streams of rivers and distributed especially in North Africa, Europe, West Asia and [2-4]. In addition, it is an important potential species for recreational fishery. Recently, *S. t. labrax* ecotype has been described by Turan et al. [5] as *S. coruhensis* [6,7]. In addition, *S. coruhensis* is an endemic anadromus fish and only distributed in the rivers of Eastern Black Sea Region [8]. 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 [9-12]. Sperm motility is the essential functional parameter for successful fertilization in fish [13,14]. 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 [14,15]. Therefore, characteristics of activation solutions are crucial in terms of initiation and progression of sperm motility [14].

Trace elements have a crucial role for the male reproductive process owing to their high activity at the molecular level and Zinc is an essential trace mineral for the normal functioning of the male reproductive system [16], and biological and psychological processes including growth, development, enzyme systems [16-19]. In addition, Zinc is important in terms of help to stabilize the cell membrane and nuclear chromatin of spermatozoa and it is an indispensable microelement in spermatogenesis [19,20-23]. Moreover, it affects activation, production, maturity, and capacitation of sperm [19-23]. Several studies on Zinc and its compounds have been published over the past decade the latest available literature about the embryo development on equine [24], antioxidant and anti-inflammatory effects on testicular damage in rats [25], effects of dietary Zinc on growth performance, antioxidant responses and reproductive performance in different fish species (*Oreochromis niloticus*, *Megalobrama amblycephala*, *Morone chrysops* × *Morone saxatilis*, *Oreochromis niloticus* × *Oreochromis aureus*) [19,26-32], protection of sperm against reactive oxygen species in men [33], male sex hormones and semen quality in rats [23], toxic effect on male reproduction system of different species (e.g. *Gyrodactylus turnbulli*, *Danio rerio*, *Poecilia reticulata*) [34-36].

As far as the authors of this work are aware, no attempt has been made to use of Zinc in sperm quality of fish species *in vitro*. Within this context, the present study was conducted to obtain more information about effect of Zinc (0.5 mM; 1 mM; 2 mM; 3 mM; 4 mM; 5 mM) in sperm quality and fertilization of endangered trout *S. coruhensis* and rainbow trout *O. mykiss*.

## Materials and Methods

Six mature endangered trout males (1652.71±0.52 g, 45.61±2.62 cm as mean±SD) and rainbow trout (1388.00±0.55 g, 44.52±2.62 cm as mean±SD) were randomly selected from a broodstock at natural photoperiod and temperature in Fish Production Station Meryemana Stream, Trabzon, Turkey for sperm collection. Water temperature and dissolved oxygen were 5.0±1°C and 8.6±0.4 mg l<sup>-1</sup>, respectively. Males were anesthetized (Benzoacaine, 50 mg/L) before stripping. All procedures were approved by ethical of animal usage committee of Karadeniz Technical University. Caution was exercised to prevent contamination of the semen with urine, feces, blood, mucus or water. The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice (2–4 °C) until use.

Zinc chloride (ZnCl<sub>2</sub>) was separately added to the activation solutions (NaCl, 0.3%; NaHCO<sub>3</sub>, 1%) (one per experimental group): Control (o) (C), (a) 0.5 mM, (b) 1 mM, (c) 2 mM, (d) 3 mM, (e) 4 mM and (f) 5 mM. Each sample was evaluated for the motility parameters using a light microscope with a digital image processing software connected to the computer (Eclipse E50; Nikon Corporation, Tokyo, Japan) to evaluate the percentage of spermatozoa motility and duration. 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% [14,37–40].

The pH of sperm samples was measured with a pH meter (Thermo Scientific Orion 5-Star Plus pH meter, USA). The sperm density was diluted at a ratio of 1:1000 with Hayem's solution (5 g Na<sub>2</sub>SO<sub>4</sub>, 1 g NaCl, 0.5 g HgCl<sub>2</sub> and 200 ml bidistilled water). The spermatocrit is defined as the ratio of white packed material 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.

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<sup>5</sup>: 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 Deviation. 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.

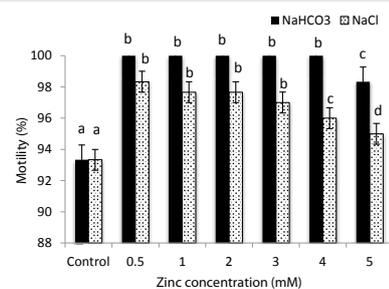
## Results

Sperm parameters (mean ± SD) are presented in table 1. Effect of Zinc on the percentage and duration of sperm motility in *S. coruhensis* are shown in figures 1,2. Highest motility (98.33%) was in 0.5 mM Zinc in activation solution containing NaCl whilst highest duration of motility (64 s) was obtained from control group. In activation solution containing NaHCO<sub>3</sub>, highest motility (100%) was in the concentrations of 0.5, 1, 2, 3 and 4 mM while highest duration of motility (64 s) was obtained from control group. Zinc concentrations of 0.5–5.0 mM had a positive effect on sperm motility compared to control group. In contrast, motility duration was negatively influenced by supplementation of Zinc.

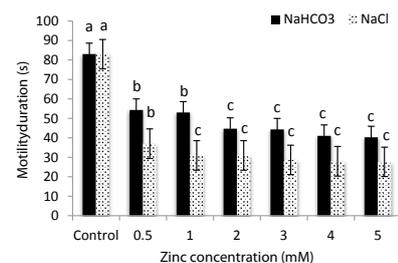
Effect of Zinc on motility and duration of *O. mykiss* sperm is presented in figures 3,4. The trials indicated that highest motility (95.00%) and duration of motility (82.50 s) were obtained from control group in activation solution containing

**Table 1:** Sperm parameters (Mean±SD) of *S. coruhensis* and *O. mykiss*.

Species	Sperm volume (ml)	pH	Spermatocrit (%)	Sperm density (×10 <sup>9</sup> )
<i>Salmo coruhensis</i>	6.67±0.53	7.71±0.14	50.00±0.35	6.18±0.52
<i>Oncorhynchus mykiss</i>	7.33±0.18	7.17±0.34	40.00±0.18	3.81±0.24



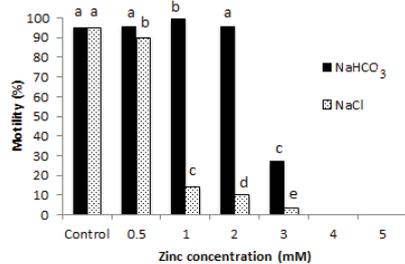
**Figure 1:** Effect of supplementation of zinc to different activation solutions on the motility rate of *S. coruhensis* sperm (n=6). Different letters show differences between treatments (p<0.05).



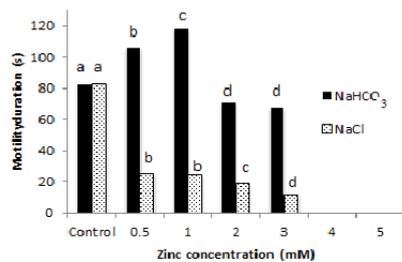
**Figure 2:** Effect of supplementation of zinc to different activation solutions on the motility duration of *S. coruhensis* sperm (n=6). Different letters show differences between treatments (p<0.05).

NaCl ( $p < 0.05$ ). For activation solution containing  $\text{NaHCO}_3$ , highest motility (100%) and duration of motility (118 s) were in a concentration of 1 mM ( $p < 0.05$ ). Zinc concentrations of 2.0–5.0 mM had a negative effect on sperm motility duration compared to control group. Sperm motility was not detected in the Zinc concentrations of 4.0–5.0 mM.

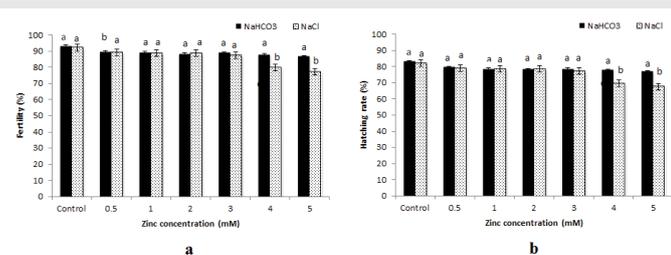
Effects of Zinc on fertility and hatching rate of *S. coruhensis* and *O. mykiss* are presented in figures 5,6. Highest fertility and hatching rate were in control group for two activation solutions



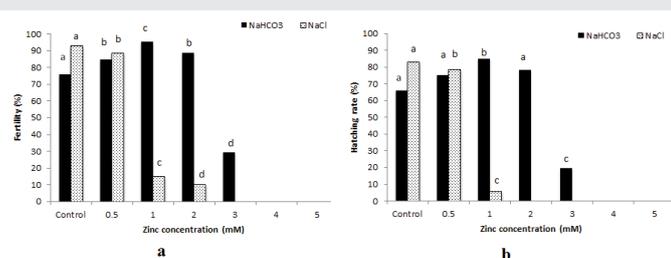
**Figure 3:** Effect of supplementation of zinc to activation solutions on the motility rate of *O. mykiss* sperm ( $n=6$ ). Different letters show differences between treatments ( $p < 0.05$ ).



**Figure 4:** Effect of supplementation of zinc to activation solutions on the motility duration of *O. mykiss* sperm ( $n=6$ ). Different letters show differences between treatments ( $p < 0.05$ ).



**Figure 5:** Effect of supplementation of zinc to different activation solutions on the fertility (a) and hatching rate (b) of *S. coruhensis* sperm ( $n=6$ ). Different letters show differences between treatments ( $p < 0.05$ ).



**Figure 6:** Effect of supplementation of zinc to activation solutions on the fertility (a) and hatching rate (b) of *O. mykiss* sperm ( $n=6$ ). Different letters show differences between treatments ( $p < 0.05$ ).

(NaCl;  $\text{NaHCO}_3$ ) ( $p < 0.05$ ). For activation solution containing  $\text{NaHCO}_3$ , highest fertility (93.05%) and hatching rate (83.07%) were in a concentration of 1 mM. In contrast, highest fertility (92.98%) and hatching rate (82.89%) were in control group for activation solution containing NaCl ( $p < 0.05$ ). Fertility and hatching rate were not detected in the Zinc concentrations of 4.0–5.0 mM. In *O. mykiss*, the Zinc concentrations range of 1.0–5.0 mM had a negative effect on fertility and hatching rate compared to control group.

## Discussion

To the best of our knowledge, this is apparently the first report about *in vitro* effect of Zinc in *S. coruhensis* and *O. mykiss* sperm, although studies on Zinc and its compounds have been conducted about the embryo development [24], antioxidant and anti-inflammatory effects on testicular damage [25], growth performance, antioxidant responses and reproductive performance [19,26–32], protection of sperm against reactive oxygen species [33], male sex hormones and semen quality [23] and toxic effect of Zinc on male reproduction system [34–36]. There is little information about effect of Zinc on reproduction system in the scientific literature. Thus far, a study has been conducted to investigate the effect of dietary Zinc on sperm motility parameters (VCL, LIN, VSL, VAP, ALH, and MAD) by Jiang et al. [19].

In this study, we demonstrated the usefulness of Zinc in different activation solutions for in all investigated species. Using 0.5 mM Zinc in activation solution containing NaCl provided high sperm motility rate (98.33%) in *S. coruhensis*. In contrast, highest percentage and duration of sperm motility, fertility and hatching rate in *O. mykiss* were obtained from activation solution containing  $\text{NaHCO}_3$  in a concentration 1 mM as 100.00%, 118 s, 93.05% and 83.07%, respectively. This may be due to the fact that Zinc is involved in a number of metabolic processes and interact with critical biological substances, including pyridoxine, polysaccharides, dehydroascorbic acid, riboflavin, and the pyridine nucleotides [41–44]. In addition, the percentage and duration of sperm motility, fertility and hatching rate decreased with increasing concentration of Zinc. This may be inhibition of motility due to toxic effect of Zinc. Additionally, motility in *O. mykiss* was not detected in the Zinc concentrations of 4.0–5.0 mM. The lack of motility, independently of cell rupture, may be explained by the fact that Zinc can lead to mitochondrial disruption that are necessary for sperm motility.

In conclusion, based on our results, Zinc had species-specific effects. Zinc was efficiently used for *O. mykiss* sperm. The effective concentrations were 0.5–1 mM. Zinc can be recommended to increase the quality of sperm. Our study provides new insights related to use of Zinc on fish sperm quality. The knowledge of effects of Zinc and its mechanism of action might be helpful for both research and commercial use. Further studies would be needed to understand the precise mechanisms.

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