DETERMINATION OF THE EFFECT OF SUPEROXIDE DISMUTASE AND REDUCED GLUTATHIONE ON THE QUALITY OF CRYOPRESERVED SEMEN IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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ABSTRACT

In present study, it was examined whether addition of different antioxidants to the cryopreservation extenders had an effect on semen post-thaw fertility and motility in rainbow trout (Oncorhynchus mykiss) and also it was investigated the sperm characteristics post-thaw sperm characteristics and fertility. The collected semen was pooled to minimize individual variation. Each pooled ejaculate was split into 3 equal aliquots and diluted with base extenders supplemented with the antioxidants, and a base extender with no additives (control). The pooled semen samples diluted at the ratio of 1:10 by the extenders were subjected to cryopreservation. Antioxidants were separately added to the extenders (one per experimental group): 250 U/l superoxide dismutase and 1.5 mmol/l reduced glutathione. After dilution the straws were placed on the tray, frozen for 10 min, and plunged into liquid nitrogen. There were not significantly differences between standard extender and reduced glutathione in sperm motion characteristics except WOB (VAP, VCL, VSL, LIN and STR) of frozen-thawed sperm ($p>0.05$). SOD increased sperm kinematic parameters such as VAP, VCL and VSL, while decreased LIN, STR and WOB ($p < 0.05$). Our results indicated that antioxidants and oxidative defensive enzymes on the fertility and hatching of frozen-thawed rainbow trout

sperm for SOD and reduced glutathione respectively (89.23 ± 3.49, 87.26 ± 2.05; 79.25 ± 3.51, 77.62 ± 2.45). ($p>0.05$).

INTRODUCTION

Cryopreservation of sperm is common procedures in aquaculture, particularly used for routine in artificial insemination. However, these application cause damages and adversely affected sperm motility, viability and consequently lower hatching rates. Rainbow trout, O. mykiss, is one of the most important fish species in world due to its aquaculture potential, economic value and wide consumer demand (Kutluyer et al., 2014). Lipid peroxidation of sperm cell membranes, damage of midpiece, axonemal structure, and DNA, malfunctions of capacitation and acrosomal reaction, loss of motility, and infertility may carry out when there is a high production of reactive oxygen species (ROS) in gametes, which are aerobic cells. Moreover, unsaturated fatty acids in plasma membranes of spermatozoa are very sensitive to free radical attack. Antioxidants are molecules protecting against free radical damage and inhibited oxidation (Kutluyer et al., 2014). As concerns O. mykiss, the knowledge about the use of extenders containing antioxidants is limited. In present study, it was examined whether addition of antioxidants (250 U/l superoxide dismutase and 1.5 mmol/l reduced glutathione) to the cryopreservation extenders had an effect on semen post-thaw fertility and motility in rainbow trout and also the sperm kinematic characteristics post-thaw.

MATERIALS AND METHODS

Experiments were conducted with gametes of +2 and +3-year-old rainbow trout were obtained from the fish farm Keban Trout Production Facility (Elazığ, Turkey). The sperm was collected by a gentle abdominal massage, collected into glass vials and stored on ice until use. Semen samples with a motility rate ≥90% were excluded from the experiment. Antioxidants were separately added to the extenders. After dilution the semen (1:10) with 3 extenders was aspirated into 0.25 ml straws, the straws were placed on the tray, frozen for 10 min, and plunged into liquid nitrogen. The straws were thawed in 40°C water for 5 sec. Motility parameters were measured using an automated system, SCA (Sperm Class Analyzer v. 4.0.0. by Microptic 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, at room temperature (20°C). 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:fertilization solution), then the semen was added and the components were mixed with each other. 100±5 eggs were fertilized with 100 µl cryopreserved semen or 25 µl untreated semen (sperm to egg ratio: $x10^5$:1). Three to 5 min 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 percentages of eyed embryos in relation to the total number of eggs 28–30 d after fertilization. Statistical analysis was performed using the software package SPSS 14.0 for Windows and significance was set at $p < 0.05$.

RESULTS

SOD and reduced glutathione increased the post-thaw sperm motility rate in comparison to the standard extender. Differences in the motility rate of frozen-thawed semen were significant among the treatments ($p < 0.05$). SOD and reduced glutathione increased the post-thaw sperm motility duration in comparison to the standard extender ($p < 0.05$). There were not significantly differences between standard extender and reduced glutathione in sperm motion characteristics except WOB (VAP, VCL, VSL, LIN and STR) of frozen-thawed sperm ($p>0.05$). SOD increased sperm kinematic parameters such as VAP, VCL and VSL, while decreased LIN, STR and WOB ($p < 0.05$).

Table 1. Effect of antioxidants and oxidative defensive enzymes on the motility parameters of frozen-thawed rainbow trout sperm.

Extenders	Standard extender	Reduced glutathione	SOD (superoxide dismutase)
VAP	83.77±10.78[a]	88.75±11.91[ab]	136.67±49.64[c]
VCL	110.99±15.74[ab]	102.16±8.75[a]	191.92±46.69[c]
VSL	71.51±13.50[a]	71.06±25.05[a]	107.95±60.64[b]
LIN	66.54±18.35[b]	69.36±23.98[b]	55.78±635[a]
STR	85.27±11.45[ab]	79.11±24.28[a]	74.82±23.25[a]
WOB	77.08±14.79[ab]	86.89±9.18[c]	70.98±17.13[a]

a, b, c: Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

Our results indicated that antioxidants and oxidative defensive enzymes on the fertility and hatching of frozen-thawed rainbow trout sperm for SOD and reduced glutathione respectively (89.23 ± 3.49, 87.26 ± 2.05; 79.25 ± 3.51, 77.62 ± 2.45). ($p>0.05$).

Table 2. Effect of antioxidants and oxidative defensive enzymes on the fertility and hatching of frozen-thawed rainbow trout sperm.

Extenders	Fertility (%)	Hatching (%)
Standard extender	86.71±2.11	76.75±2.16
Reduced glutathione	87.26±2.05	77.62±2.45
SOD (superoxide dismutase)	89.23±3.49	79.25±3.51
P	-	-

–: The same column shows no significant differences among proportions ($p > 0.05$).

DISCUSSION

SOD had significantly higher VCL, VSL and VAP. In contrast, the best results in fertilization were obtained from extender supplemented with carnitine. BCF, LIN, STR and WOB were higher in extender including carnitine. The present results may be due to male and female gamete interactions and maternal genetic and non-genetic constituents, egg quality and female donor that it is important on a male's fertilization ability for fish (Butts et al., 2010; Saleh and Agarwal, 2002).

In conclusion, the present study indicates that SOD, reduced glutathione are the important antioxidant in sperm of rainbow trout for increase the quality of sperm. Additionally, supplementation of the extender with uric acid, L-methionine and α-tocopherol increased the post-thaw motility rate and duration. Fertilization process is not affected by antioxidants significantly. Further research is required in order to select the best concentration and combination of antioxidants.

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