DETERMINATION OF THE EFFECT OF METHIONINE, TOCOPHEROL AND URIC ACID 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 4 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): 1.5 mmol/l L- methionine, 0.25 mmol/l α-tocopherol and 2.0 mmol/l uric acid. After dilution the straws were placed on the tray, frozen for 10 min, and plunged into liquid nitrogen. The present study indicates that uric acid and carnitine are the important antioxidant in sperm of rainbow trout for increase the quality of sperm and fertility. Additionally, supplementation of the extender with uric acid, L-methionine and α-tocopherol increased the post-thaw motility rate and duration. There were significantly differences

among the treatments in sperm motion characteristics (VAP, VCL, VSL, LIN, STR and WOB) of frozen-thawed sperm ($p > 0.05$).

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). As concerns O. mykiss, the knowledge about the use of extenders containing antioxidants is limited. Therefore, to obtain more information about effect of supplementation of extender with antioxidants (L-methionine, α-tocopherol and uric acid) on motility and fertility of sperm in rainbow trout. In present study, it was examined whether addition of antioxidants (1.5 mmol/l L- methionine, 0.25 mmol/l α-tocopherol and 2.0 mmol/l uric acid) 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, coworking 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

Methionine, α-tocopherol and uric acid 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 ($p < 0.05$). In table 1; there were significantly differences among the treatments in sperm motion characteristics (VAP, VCL, VSL, LIN, STR and WOB) of frozen-thawed sperm ($p > 0.05$). Especially, α –tocopherol has showed lower ratio than methionine and uric acid in VAP and VCL ($p < 0.05$).

Our results indicated that the post-thaw motility rate increased in extender supplemented with L-methionine, α-tocopherol and uric acid ($p < 0.05$). A post-thaw fertility of 86.71±2.11% was obtained with the standard extender. In table 2, fertilization rate and hatching rate of frozen-thawed semen was not affected by the tested antioxidants ($p > 0.05$).

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

Extenders	Standard extender	Methionine	α -tocopherol	Uric acid
VAP	83.77±10.78^{b}	79.62±17.78^{b}	59.49±11.61^{a}	86.68±28.41^{b}
VCL	110.99±15.74^{ab}	120.53±26.00^{a}	107.07±20.39^{bc}	125.46±26.15^{a}
VSL	71.51±13.50^{b}	51.59±23.24^{a}	55.43±14.07^{a}	51.94±37.15^{a}
LIN	66.54±18.35^{c}	43.34±19.02^{a}	55.86±29.01^{b}	46.38±7.35^{a}
STR	85.27±11.45^{b}	63.25±21.97^{a}	92.48±4.73^{c}	62.18±35.61^{a}
WOB	77.08±14.79^{c}	66.96±12.25^{ab}	59.37±26.98^{a}	70.57±27.21^{bc}

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

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
Methionine	89.56±4.21	79.54±4.05
α -tocopherol	88.72±3.36	78.27±5.16
Uric acid	88.01±4.25	78.11±4.65
P	-	-

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

DISCUSSION

Cabrita et al. (2011) reported that addition of α-tocopherol did not significantly increase the post-thaw motility parameters of motility in gilthead seabream (Sparus aurata) and European seabass (Dicentrarchus labrax). Martinez-Paramo et al. (2012) demonstrated that supplementation of freezing media with α-tocopherol improved sea bass sperm motility, resulting in higher percentages of motile spermatozoa with higher curvilinear velocity. Sperm velocities play a key role in success of fertilization (Kutluyer et Al., 2014). Uric acid has positive effect on sperm motility parameters due to its stability and to be strong reducing agent, which is formed from xanthine and hypoxanthine (Berg et al., 2006). Additionally, uric acid can be use in sperm cryopreservation extenders of aquatic animals due to inexpensive and efficacy (Lahnsteiner and Mansour, 2010). Lahnsteiner and Mansour (2010) stated that uric acid was the major antioxidant of sperm and it improved the motility rate (%) and the rate of sperm membrane integrity in Alburnus alburnus, Lota lota, Perca fluviatilis, S. trutta and the sperm velocity in P. fluviatilis. Lahnsteiner et al. (Lahnsteiner et al., 2010) found that uric acid had highest concentrations in spermatozoa of brown trout and play a major role in antioxidative protection of spermatozoa under in vivo conditions. The present results agree with these reports. Uric acid increased the post-thaw sperm motility rate in comparison to the standard extender. 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.

In conclusion, the present study indicates that uric acid and carnitine are the important antioxidant in sperm of rainbow trout for increase the quality of sperm and fertility. 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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