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EFFECTS OF DIFFERENT DOSES OF TAURINE IN THE GLUCOSE-BASED EXTENDER DURING CRYOPRESERVATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) SEMEN

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ABSTRACT

This study aimed to investigate the effects of different doses of taurine added to glucose extender. Semen was collected from rainbow trout (Oncorhynchus mykiss) by abdominal massage and diluted (1:2) with 300 mM glucose (G) extender containing different taurine doses (50, 75 and 100 mM). The control group extender did not contain taurine. After dilution and dosing, samples were filled to 0.5 ml straws, frozen in nitrogen vapour and stored in liquid nitrogen. The comparison of the results obtained for the 50 mM taurine group and the control group showed no significant difference between the post-thawing motility percentages and motility duration of sperm, and only a very low statistically difference for eyed-embryo percentages ($P < 0.05$).

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Keywords: Rainbow trout, *Onchorhynchus mykiss*, taurine, sperm, cryopreservation

Introduction

Cryopreservation of sperm was first used in the development of reproductive technologies for agricultural animals and then transferred to aquaculture (7). This technology is used in hatcheries to store semen for the purpose of breeding all year round (7, 17). Cryopreservation is an effective strategy for saving endangered fish species by facilitating the storage of their semen in a gene bank (17, 23) and the transport of sperm between fish farms (13). Frozen fish semen can also be used in hybridization (23), sex reversal and gene transfer programmes (14, 22).

Spermatozoa are affected by many factors during cryopreservation, such as temperature change in the environment, osmotic stress, toxic effect of cryoprotectants and forming of ice crystals (19, 20, 29). Oxidative stress is another important stress factor which the spermatozoa are exposed to (18, 29). Oxidative stress is generated as a result of imbalance between free radical formation and the antioxidant defense, thus causing tissue damage. Therefore it has been confirmed that adding antioxidants to the diluent provides protective effect over mammal and human sperm (1, 15).

Antioxidants (taurine, hypotaurine, vitamin E, and vitamin C) can also be added to the diet and the extender to increase the resistance of the cells during cryopreservation. This operation provides resistance to spermatozoa against oxidative stress (13). It balances the lipid peroxidation with the help of the antioxidants existing in the semen and prevents the excessive production of free radicals (1, 2, 4, 10). Taurine is a ubiquitous sulphur-containing amino acid that has been proposed to be an antioxidant (2) and which is present in the reproductive tract. It can traverse the sperm plasma membrane and inhibit lipid peroxidation and can protect the sperm cells against reactive oxygen species (ROS) (3, 15, 21). Therefore, in studies using taurine as an antioxidant, enhancement of the liveliness, membrane integrity and motility of rabbit, ram, buffalo, bull and cat sperm has been observed (3, 6, 15, 27).

So far, to the best of our knowledge, the effect of taurine on the cryopreserved rainbow trout sperm quality has not been reported. Therefore, the present study was conducted to determine the effect of taurine on sperm quality parameters like post-thaw motility, and fertilization rate during cryopreservation of rainbow trout spermatozoa.

Materials and Methods

Gamete collection

Semen and eggs were collected by abdominal massage from 2- to 3-year-old males and 3- to 4-year-old female rainbow trout. The obtained semen samples were pooled and stored at 4 °C during the experiment.

Motility observations

One microliter of fresh semen was mixed with 20 µl of spermatozoa activating solution (0.3% NaCl) and placed onto glass slides. Observations were performed at 4 °C. Motile and non-motile sperm were counted at 4 °C under a light microscope at ×40 magnification. Sperm density was counted by the hemocytometric method. pH was measured with indicator papers (Merck).

Extender composition

Ten percent DMSO solution extended with 300 mM of glucose (300 mMG) solution. This glucose-based extender containing different taurine (T) doses (50, 75 and 100 mM). In this experiment, control group (not containing taurine) (300 mMG), 50 mMT, 75 mMT and 100 mMT solution were used as the cryopreservation solution.

Cryopreservation

One part semen was mixed with two parts cryopreservation solution. The experiments were conducted within 10 min following dilution. Straws were cooled to 4 °C before loading with semen. Then, diluted semen was sucked into the straws, and the sealed straws (by PVA) were placed on the tray. Rainbow trout sperm was cryopreserved in 0.5 mL straws. Freezing was performed in an insulated box on a tray in the vapour of liquid nitrogen. Straws were frozen at 2.5 cm above the level of liquid nitrogen, with a holding time of 10 min. After that the straws were stored in liquid nitrogen.

Thawing and Fertilization

The straws were instantly transferred to a water bath at 35 °C for 10 seconds. Fertlization technique was used at a dosage of 3×10^6 sperm per egg. Eggs were pooled from 3 females. For the fertilization, eggs of 25 ml (approximately 200 eggs per batch) were transferred into petri dishes (90 mm in diameter, 17 mm in height). Egg batches were fertilized with frozen and fresh semen. Eggs and sperm were gently swirled for 10 s and then 25 ml of fertilization solution (3 g urea, 4 g NaCl, 1 liter water) was added to the sperm-egg mixture. The fertilization rate was determined by the number of eyed-stage embryos in relation to the total number of eggs.

Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) ($P < 0.05$).

Results and Discussion

Antioxidants are substances which restrain oxidation. Concordantly antioxidants prevent the production of free radicals as a result of oxidative stress. Production of ROS increases during cryopreservation and as a result, the sensitivity against lipid peroxidation rises (5, 11). It is generally accepted that an increase in ROS production causes a decrease in the level of antioxidants. In this context, the cryopreservation procedure causes antioxidants in the semen to decrease (5, 8, 10, 15).

The antioxidant effect of natural components of the seminal plasma (taurine, hypotaurine, catalase, desferal, ascorbic acid) has been used recently (2). One direction of studies is towards adding substances that have antioxidant characteristics (hypotaurine, inositol, proline, ascorbic acid, alpha-tocopherol, despheral, taurine) into the diluent for freezing sperm of different mammals (12, 15). It is considered that antioxidants added into the diluent may have positive effects on the spermatological parameters after thawing (15). The cryoprotective characteristics of antioxidants give better results than those for sperm frozen without these substances (16).

Lower fertilization rates are achieved in the fertilization process when frozen sperm is used. This condition arises from factors such as cold shock, extender content, osmolality, oxidative damage and membrane instability (29). Owing to the freezing/thawing processes, antioxidant enzyme levels in the spermatozoa and the seminal plasma decrease, making them more sensitive to spermatozoal lipid peroxidation. As a result permanent damages occur on spermatozoa membranes and all functions of the spermatozoa are negatively affected (19, 29).

The antioxidant mechanisms in human and mammalian animal spermatozoa have been studied (3), however there is not enough information about these mechanisms in fish spermatozoa.

The study of Bucak et al. (10) confirmed that antioxidants (taurine, trehalose, cysteamine) added into the extender could increase the ram spermatozoa motility rates and show a cryoprotective effect. Yıldız and Daşkın (28) reported that adding several antioxidants together into the extender is more effective (highest motility and the lowest dead count) than using them individually.

In a study in which taurine (50 mM) was used as the antioxidant, the bull sperm morphology was preserved during the freezing-thawing process but there was no increase in the sperm motility after thawing (27). In another study, taurine did not increase the survival rate of ram spermatozoa (25). Taurine (25 or 50 mM) has been found to exert protective effect on ram spermatazoa (increased post-thaw motility) but when sperm samples were diluted with 100 mM taurine, their post-thaw motility decreased (24). Another study showed that the effect of 50 mM taurine in skimmed milk-glucose extender was successfully employed on cat spermatozoa motility at 5 °C (6).

TABLE 1

Mean sperm and fertilization rate parameters in rainbow trout semen supplemented with different taurine concentrations

Taurine concentration	Post-thawing motility [%]	Motility duration [sec]	Eyed embryos [%]
50 mM	63.3 ± 9	48.8 ± 12.3	63.7 ± 2.75
75 mM	63.3 ± 4.7	34.8 ± 2.3	29.0 ± 3.27
100 mM	3.3 ± 4.7	4.2 ± 6.1	9.0 ± 4.4
Control (not containing taurine)	61.7 ± 2.4	47.3 ± 2.6	69.5 ± 1.8
Fresh semen	90.0 ± 2.9	48.5 ± 1.9	87.6 ± 2.6

In our study, extender containing glucose (300 mM), which is also used by many researchers (9, 26), was applied in the freezing of the rainbow trout sperm. Taurine was used as an antioxidant. When the motility and the eyed embryo percentages of the 50 mMT, 75 mMT, 100 mMT, control and the fresh semen groups were compared after thawing, statistically significant differences ($P < 0.05$) were obtained (**Table 1**).

The differences between the eyed embryo percentages of the 50 mMT and 75 mMT groups were statistically significant ($P < 0.05$), but the difference in their motility percentages after thawing were non-significant (**Table 1**). This difference in the eyed embryo percentages could be related to the survival period of the spermatozoa, since the approximate motility duration of the 50 mMT group was 49 seconds, and that of the 75 mMT group, about 35 seconds. Concordantly, the difference in motility periods is thought to affect the fertilization success. When the eyed embryo percentages between the 50 mMT and control groups were compared, very low difference was found ($P < 0.05$) but no significant difference was observed regarding the spermatozoa motility percentages and spermatozoa motility duration (**Table 1**). However, in this study it was determined that the fertilization rate was not increased by using taurine as an antioxidant.

Conclusions

In our study, taurine was used as an antioxidant in the diluent during freezing of rainbow trout spermatozoa. Depending on the taurine concentration used, it was determined that the motility periods and percentages of the rainbow trout after thawing differed. It was also observed that the spermatozoa motility decreased in the case of increased taurine concentration and the fertilization success decreased accordingly. It was determined that 50 mM taurine in the diluent was not effective in increasing the fertilization success compared to the control group (300 mM glucose).

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