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Review Article

Use of Amino Acids in Fish Sperm Cryopreservation: A Review

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Abstract

Sperm is protected against oxidative stress with seminal plasma. Dilution during cryopreservation is reduced the seminal plasma components having cells more sensitive to oxidative stress. Amino acids have antioxidant property and found in seminal plasma at high concentration. Therefore, amino acids have an important biological role for prevention of cell damage during cryopreservation. Thus far, conducted studies in mammalians have demonstrated that supplementation of amino acids (e.g. taurine, hypotaurine, proline, glutamine, glycine, histidin, and methionine) to extenders reduced sperm damage and DNA fragmentation and improved post-thaw motility. Recently, studies about antioxidant property and addition to extenders of amino acids have been performed in different fish species (*Dicentrarchus labrax, Sparus aurata, Oncorhynchus mykiss, Salvelinus fontinalis, Pagrus major, Carassius auratus*). In conducted studies, it has determined that addition of amino acids has reduced DNA fragmentation and protected DNA against strand breaks and also improved some sperm quality parameters post-thaw. In conclusion, amino acids provide better motility and lower DNA damage in fish sperm. However, studies on supplementation of amino acids to extenders in fish sperm cryopreservation are limited. Therefore, future studies in fish having economic and ecologic importance are necessary about effect of supplementation of amino acids in cryopreservation.

Keywords: Sperm; Cryopreservation; Amino acids

Introduction

The cryopreservation of fish sperm is an important technique due to transportation of genetic material among facilities, optimal utilization from aquaculture, reducing the risk of spreading infections, conducting of hybridization studies, biodiversity and gene pool conservation, selective breeding activities, and conservation of endangered species [1-4]. In addition, cryobanks could be provided to store in a genetically stable form of sperm and to maintain the biological functions of sperm cells for long terms [5,6]. On the other hand, even so there are numerous advantages of sperm cryopreservation, a lower physiological activity, structure deformation, DNA fragmentation, impairment of membrane stability and spermatozoa functionality, biochemical and metabolic changes, and a series of alterations could be occurred with cryopreservation process by oxidative stress due to generation of Reactive Oxygen Species (ROS) [7-9].

Sperm is protected against oxidative stress with seminal plasma. Dilution during cryopreservation reduces the seminal plasma components having cells more sensitive to oxidative stress [10]. Amino acids have antioxidant property and found in seminal plasma at high concentration. Therefore, amino acids have an important biological role for prevention of cell damage during cryopreservation. Thus far, conducted studies in mammalians have demonstrated that supplementation of amino acids (e.g. taurine, hypotaurine, proline, glutamine, glycine, histidine, and cysteine) to extenders reduced sperm damage and DNA fragmentation and improved post-thaw motility [11,12]. Recently, studies about benefit from antioxidant property and addition to extenders of amino acids have been performed in different fish species (Dicentrarchus labrax, Sparus aurata, Oncorhynchus mykiss, Salvelinus fontinalis, Pagrus major, Carassius auratus). [4,13-19]. This paper reviews the studies and results about addition of amino acids to extenders in cryopreservation process for fish sperm.

Amino Acids and Antioxidant Effects

Amino acids are the building blocks of peptides and proteins [20]. Particularly, sulfur-containing amino acids are important due to removing of free radicals and protection against oxidative stress. Because, sulphur a fundamental element for amino acids, proteins and other biomolecules. Methionine, cysteine, homocysteine, and taurine are the four common sulfur-containing [21].

Several amino acids (e.g. cysteine, glycine, proline and histidine) are found in seminal plasma. Lahnsteiner [22] stated that in the seminal plasma of *O. mykiss*, the main Free Amino Acids (FAAs) were arginine, glutamic acid, isoleucine, leucine, methionine and proline, in spermatozoa cysteine, arginine and methionine. The main FAAs in the seminal plasma of *C. carpio* were alanine, arginine, cysteine, glutamic acid, histidine, leucine, lysine, methionine and proline. To date, amino acids have been used in sperm cryopreservation as a non-permeating cryoprotectant of many mammalian species to preventing against cold shock [23] and freezing stress [24-29]. Recently, studies about addition of amino acids to extenders have been performed in fish sperm cryopreservation.

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Table 1: The amino acids used for cryopreservation of sperm in various fish species.

Species	Amino acid	Concentration of amino acid (mM)	Thawing temperature (ºC)	Thawing duration (s)	Motility (%)	Researcher
Sparus aurata	Taurine, hypotaurine	1, 10	25	30	60-70	Cabrita et al. [10]
Dicentrarchus labrax	Taurine, hypotaurine	1, 10	25	30	60-70	Cabrita et al. [10], Martínez-Páramo et al. [42]
Salvelinus fontinalis	Methionine	1.5	25	30	19-21	Lahnsteiner et al. [22]
Oncorhynchus mykiss	Methionine	1.5, 3	25	30	17-21	Lahnsteiner et al. [22]
Oncorhynchus mykiss	Taurine	50, 75, 100	35	10	48.8, 34.8, 4.2	Ekici et al. [16]
Cyprinus carpio	L-cysteine	0.5, 1, 1.5, 2			82-92	Kledmanee et al. [46]
Oncorhynchus mykiss	Methionine	1,5	40	5	69	Kutluyer et al. [4]
Pagrus major	Taurine	50, 100			77-78	Liu et al. [15]
Cyprinus carpio	Cysteine	2.5, 5, 10, 20	20	30	50-76	Ogretmen et al. [19]
Carassius auratus	Methionine	1, 1.5, 3, 6	40	5	45	Kutluyer et al. [18]

Use of Amino Acids in Fish Sperm Cryopreservation

Motility, membrane stability and spermatozoa functionality, DNA integrity in fish sperm are affected by oxidative stress due to generation of Reactive Oxygen Species (ROS) during dilution in the extender media, cryoprotectant exposure and cooling process [7,8,10,30]. Especially, DNA integrity is one of indicators of cryopreservation success due to preserving genetic material and can be used in order to select the best treatment for fertilization trials [31]. DNA damage could be a result of free radical-induced damage because of ice crystal formation and recrystallisation during freezing-thawing procedure [17,32,33]. Studies about DNA damage after cryopreservation were performed in several species using the comet assay [34-37]. In some studies, it has been reported that cryopreservation process affected DNA stability by reason of DNA fragmentation [34-36]. Rani et al. [17] suggested that main reason of DNA damage is the toxicity of cryoprotectant. In contrast, Song and colleagues [38] stated that mechanical injury on sperm DNA stability was negligible. Suquet et al. [39] detected that there were not genome alterations in turbot *Psetta maxima* sperm after cryopreservation. Gwo et al. [40] determined that the nucleus of Atlantic croaker *Micropogonias undulatus* sperm was not affected from freeze-thaw process. Additionally, fertilization success depends on selecting the best treatment in cryopreservation process. Due to these reasons, usage of antioxidants in the cryopreservation is important for cryopreservation success. Antioxidants are useful for inhibition of ROS generation [41]. Recently, studies about addition to extenders of amino acids, which have antioxidant property, performed in different fish species (Table 1) [4,14-19,42].

The usage of amino acids in fish sperm cryopreservation and properties of these amino acids are described in the following:

Cysteine

Cysteine is naturally occurring sulphur containing non-essential amino acid. It has antioxidant properties due to being an important precursor in the production of antioxidant glutathione, which protects cells from free radicals [43]. Especially, studies about use of extenders containing cysteine is limited in fish species [44]. Previous studies showed that L-cysteine improved viability of spermatozoa by reducing lipid peroxidation of sperm plasma membrane and preventing DNA damage of spermatozoa from ROS during cryopreservation in fish [44,45]. In studies about supplementation of the extender with amino acids, it has been reported that amino acids reduced both DNA fragmentation parameters and protecting DNA against strand breaks, although they were not significantly affected to the post-thawing motility percentages and motility duration, sperm motility parameters (TM, PM, VCL, VSL and linearity) of sperm. In contrast, Kledmanee and colleagues [46] determined that L-cysteine increased the percentage of sperm motility, duration of sperm motility, the percentage of sperm viability and fertilization capacity. Ogretmen et al. [19] stated that cysteine caused a significant decrease DNA damage of sperm in common carp. In addition, they determined that the fertilization and hatching rate was increased by cysteine.

Methionine

Methionine is one of two sulphur-containing proteinogenic essential amino acids. It has antioxidant properties because of being a glutathione precursor, a tripeptide that reduces Reactive Oxygen Species (ROS) and thus protects cells from oxidative stress. In addition, methionine is required for the synthesis of polyamines (spermine and spermidine), which take part in nucleus and cell division events and the most important methyl group donor for methylation reactions of DNA and other molecules [21]. Due to these properties of methionine, studies about effects of methionine on improvement of post-thaw sperm quality have been performed in fish species. Lahnsteiner [13] suggested that methionine had a positive effect on the sperm viability in rainbow trout *O. mykiss* and carp *C. carpio*. Lahnsteiner et al. determined that methionine only slightly increased post-thaw motility the brook trout (*Salvelinus fontinalis*). Kutluyer et al. [4] found that addition of methionine to extenders increased the post-thaw sperm motility duration in rainbow trout compared to the standard extender. Kutluyer et al. [18] stated that an increase in the concentration of L-methionine caused a significant increase in the motility rate and duration of sperm in goldfish (*Carassius auratus*) and DNA damage reduced compared to control group.

Taurine and Hypotaurine

Taurine (2-aminoethanesulfonic acid) is a sulphonated beta amino acid [47] and has been reported many physiological and pharmacological actions, including membrane stabilization,

antioxidation, osmoregulation, modulation of ion flux, and control of Ca^{2+} homeostasis [48]. It is reduced Reactive Oxygen Species (ROS) and prevents to changes in membrane permeability during cryopreservation [49-53]. Hypotaurine is a sulphuric acid and provides the biosynthesis of taurine [54]. Hypotaurine has a protective effect against oxidants such as the hydroxyl radical, the superoxide radical and hydrogen peroxide and also moves as an endogenous neurotransmitter through transaction on the glycine receptors [54,55]. Cabrita and colleagues [10] reported that DNA fragmentation in gilthead seabream (*S. aurata*) and European sea bass (*D. labrax*) was significantly reduced by taurine and hypotaurine. Martinez-Paramo et al. [14] stated that addition of taurine to extenders (1 mM) improved some parameters of European sea bass sperm quality after thawing. Liu et al. [15] determined that taurine (50 mM) provided the most pronounced protective effect in improving post-thaw quality of red seabream sperm.

Importance of Amino Acid Concentration

Determination of the best concentration and combination of amino acids is important for cryopreservation success. In previous studies, it has been reported that too high amino acid concentration negatively affected to sperm quality due to osmotic toxicity and hyper tonicity [24,26,56-58]. Kledmanee and colleagues [46] they suggested that an increase in the concentration of L-cysteine caused low semen qualities due to its toxic effect. Due to these reasons, the best concentration could be determined for cryopreservation success.

Conclusion

In conclusion, it can also be concluded that amino acids is essential, not only for the increasing of post-thaw sperm quality as observed in different studies but also they are crucial for decreasing DNA damage in fish. Further research could be performed to obtain better information in terms of sperm quality, the DNA integrity and fertilizing capacity and select the best concentration of amino acids.

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