The Use of Antioxidant in Cryopreservation to Improve the Spermatozoa Quality after Freezing-Thawing: A Review

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Abstract

Cryopreservation is a common procedure used to preserve spermatozoa for a certain period of time, to address the problem of infertility in males. During the cryopreservation process there will be cold shock, osmotic stress and the formation of ice crystals. These three events will cause the quality of spermatozoa to decrease in motility, viability, changes in permeability and changes in lipid components in the membrane. Cryopreservation can modulate mRNA stability, protein and gene expression, and the epigenetic content of spermatozoa as well as the occurrence of apoptosis in spermatozoa. In addition, in cryopreservation there can be excess free radicals that damage spermatozoa.

The addition of antioxidant to cryoprotectant can protect spermatozoa from lethal effects during the freezing process by modifying the ice crystals formed in the medium during freezing to become smaller so that they are able to mechanically inhibit cell membrane damage when the temperature decreases. The use of antioxidants during cryopreservation to protect sperm from damage caused by free radical activity. Antioxidants are nucleophilic compounds that can provide protection to cells from harmful oxidative processes by reducing the activity of free radical. Therefore, it is necessary to add antioxidant in the cryoprotectants in the cryopreservation process to improve the quality of spermatozoa.

Introduction

Cryopreservation of human sperm is important because it is one way to solve the problem of infertility in males. Cancer patients receiving radiotherapy and chemotherapy, patients with oligospermia or genital abnormalities, and practitioners who have been exposed to radiation, electromagnetic radiation, or high temperature for a lengthy period of time are all candidates for sperm cryopreservation. It’s also widely utilized in sperm banks and by those who need to store their sperm for a limited period of time [1, 2].

In recent decades, Assisted Reproductive Technology (ART) has evolved rapidly, and sperm freezing has become an essential element of reproductive medicine. Patients may be able to generate their own offspring via sperm cryopreservation and Assisted Reproductive Technology (ART). Many investigations have discovered that sperm shape and function are harmed during cryopreservation during the freeze-thaw process.

In recent years, research institutes that exist around the world have studied the mechanisms of transition phase damage in human sperm cells, explored ways to improve the freezing process and sperm cryopreservation techniques such as developed new types of freezing phase and creating a carrier, optimizing freezing processes phase, and adding cryoprotectants, as well as creating novel types of freezing carriers phase, improving freezing methods phase, and incorporating cryoprotectants [2-4].

Although sperm cell quality has increased as a result of ongoing cryopreservation technique optimization, changes in sperm cell structure have occurred, epigenetic modification, and semipermanent damage induced by phase freezing injury, as well as accelerator inactivation, still exist, particle alterations and oxidative stress can’t go unnoticed [2]. During cryopreservation, osmotic pressure is triggered by a change in cell volume due to the movement of water and solutes across the plasma membrane of sperm, causing to the formation of ROS [5]. Cold shock occurs due to oxidative stress and the formation of ROS [6]. Antioxidants are a major defense factor against oxidative stress caused by free radicals [7]. For this reason, it is necessary to add antioxidants to cryopreservation media or cryoprotectants. Here, we will discuss about The Use of Antioxidant in Spermatozoa Cryopreservation to Improve the Quality of Post-Thawing Spermatozoa.

Methods

Literature searches were conducted on Embase, PubMed, NCBI, Google Scholar and the Science Direct web. The keywords used in the journal search were, Spermatozoa Cryopreservation, Cryoprotectant, antioxidant, Quality spermatozoa, Pasteurization. The journals used in the literature review were obtained from various international research journals The journal year used is limited to 2018-2022 including: Reproduction and Genetics, Cryobiology, Animal Reproduction Science, Andrology, Reproduction in Domestic Animal, Theriogenology, Cell Tissue Bank and others.

Results and discussions

Spermatozoa Cryopreservation

Cryopreservation of spermatozoa is a technique for storing spermatozoa in liquid nitrogen for a long period. Cryopreservation is the freezing of cells or tissues to sub-zero temperatures, especially 196°C. All biological activity of spermatozoa was interrupted during cryopreservation and was not resumed until they were needed. To preserve spermatozoa from damage during the freeze-thawing process, cryoprotectants such as ethylene glycol, glycol, DMSO (dimethyl sulfoxide), and di-methylformamide are used [8, 9].

The cryopreservation process results in damage to spermatozoa so that it has an impact on fertility. Layek et al. (2016) revealed that the damage from cryopreservation could be up to 50% [10]. Sperm cryopreservation has several destructive effects including loss of sperm motility and viability, mitochondrial membrane depolarization, acrosomal damage, changes in plasma membrane permeability, and even nuclear, and DNA damage [11]. One of the evaluations of male infertility is semen analysis. Normal sperm analysis carried out is descriptive of morphological motility, and sperm concentration, with a threshold level that must be exceeded to be considered as fertile sperm. Assay methods are constantly being developed, therefore newer test methods have been established to investigate sperm physiology and function by monitoring characteristics such as motility, acrosomal reactions, capacitation, sperm DNA damage, reactive oxygen species, chromatin structure, zona pellucida binding, and fusion. sperm-oocyte [12].

Cryoprotectants

Cryoprotectants are components that must be present in the cryopreservation medium that serves to minimize the physical and chemical stress of spermatozoa cells resulting from the cooling, freezing and thawing processes [13]. Cryoprotectants are chemical substances that function to protect cells from negative or lethal effects during the cooling process and freezing process. Cryoprotectants also have the potential to disrupt membrane permeability, alter the expression of spermatozoa cell proteins and reduce motility and fertilization capacity [14]. Cryoprotectants are divided into two groups namely permeable (glycerol, dimethyl acetaldehyde, dimethyl sulfoxide (DMSO), ethylene glycol and propylene glycol [15, 16] passes through the plasma membrane and replaces water in spermatozoa cells) and nonpermeable (raffinose, albumin, sucrose, yolk citrate, polyethylene glycol and polyvinyl pyrrolidone, are an additive that gives protective characteristics but cannot pass through the plasma membrane) [17-19].

The Role of Antioxidants in Improves the Quality of Cryopreservation of Spermatozoa During the cryopreservation process, there is an increase in excessive ROS which can damage motility and fertilization ability, pleated proximation and fatty acid disorders in the plasma membrane of spermatozoa [20]. To prevent such cell damage, antioxidants are needed. Where Antioxidants Serve to Protect Biological Systems to a potentially
damaging effect of a process or reaction that causes oxidation which extends [21]. Antioxidant is a nucleophilic compound or that has ability to reduce, extinguish or compress free radical reactions.

**The addition of Vitamin C**

Vitamin C is known as a powerful antioxidant because it has a strong reducing power that can easily neutralize the resulting free radicals, thereby reducing oxidative damage. The use of Vitamin C as an antioxidant to protect sperm is very useful, while in high doses it can have pro-oxidative effects, especially when there are high levels of transition metals such as copper and iron [22].

It has been tested that Vitamin C as an additive to the extender, its purpose to improves sperm quality after a severe challenge that brought to the cell by cryopreservation. Vitamin C acts as electron donor, to neutralize the resulting free radicals from normal metabolic activity other than the environment challenge. This ability to donate electrons allows to reduction of oxidative stress from Ascorbic Free Radicals (AFR) [23].

**The addition of Vitamin E**

Vitamin E is a very powerful lipophilic chain-breaking antioxidant that is located in the cell membrane and can break the covalent bonds formed by ROS between the membrane lipid fatty acid side chains [26].

Researcher Khaeradmand et al. Beheshti et al. found that the group given vitamin ‘E’ was then stored at 5°C or after freezing-thawing obtained a high percentage of sperm membrane integrity [27,28]. The findings in the study conducted by José Alberto Espina-Ávila et al found that there was an increase in the percentage of sperm with intact membrane permeability during freezing-thawing in diluents to which vitamin “E” was added [29].

**The addition of Trolox (analogue of vitamin E)**

Trolox is a water-soluble analogue of vitamin E. Trolox has strong antioxidant properties, which can reduce ROS production to prevent oxidative stress and apoptotic-mediated processes. Trolox has also been successfully tested in preventing lipid peroxidation of sperm membranes [30-32].

Saeid Nekoonam et al reported that normozoospermia and oligozoospermia patients, the use of Trolox as a frozen extender supplement improves the quality of human sperm in cryopreservation, in terms of early apoptosis and DNA integrity [33].

Research by Marta F. Riesco, et al reported that the addition of 1 mM trolox in cryopreservation extenders is a good approach to improve the quality of thawed semen, reduce cryodamage in ovine sperm and improve reproductive performance in fertility tests. This type of strategy could be a step forward to deploying and implementing AI procedures on ovine species [34].

**The addition of Astaxanthin (AST)**

Astaxanthin (AST), a red xanthophyll carotenoid extracted from the alga Haematococcus pluvialis (H. pluvialis), is a potent antioxidant capacity and can scavenge singlet oxygen and free radicals [35]. Many studies have reported that astaxanthin has various beneficial biological functions, including antioxidant and antiinflammatory agents [36].

Research conducted by Hai-tao Guo et al, evaluation of the effect of astaxanthin on parameters of traditional motility, fatty acid composition, lipid peroxidation, plasma membrane integrity, and boar sperm fertilization capacity at cryopreservation. His research also observed that astaxanthin improves the composition of plasma membranes and has a positive effect on the quality of boar sperm, which cryopreservation performs. Meanwhile, astaxanthin administration has a harmless nontoxic effect on the fertilization capacity of boar sperm carried out cryopreservation [37].

Research conducted by Gizem Dede et al, reported that the addition of astaxanthin to sperm cryopreservation media will positively affect sperm motility and can reduce the number of decondensed sperm [38].

Research conducted by Taha Ghantabpour et al, reported the addition of 1 mM astaxanthin to human semen on cryopreservation media has a protective effect against oxidative stress and may reduce the destructive effect on sperm quality [39].

**The addition of Elamipretide (SS-31, D–Arg–Dmt–Lys–Phe–NH2)**

Elamipretide is a water-soluble peptide that selectively binds to cardiolipine, an acidic lipoprotein exclusively localized in the Deep Mitochondrial Membrane (IMM), necessary for optimal activity of complex I to IV ETC [40]. It is oxidatively sensitive due the presence of conjugated double bonds (C 18 residue: 2). When oxidized occurs then cardiolipin converts cytochrome c into peroxidase, releases cytochrome c and induces apoptosis [41].

Elamipretide binds selectively to cardiolipin and can reduce its oxidative inactivation by cytochrome c [42] protecting the cristase architecture and increases bioenergy activity [43]. During cryopreservation of spermatozoa, cryoprotectants induce an excess of calcium resulting in oxidative damage to cardiolipine, apoptosis and impaired interaction with cytochrome c [44].

Many previous studies have reported that elamipretide is effective against stress, diseases of the heart muscle, nervous system and endocrine system [45-47]. In this study it was also reported that the proper addition of Elamipretide significantly improved the viability and motility of human spermatozoa post thawing, accompanied by a reduction in ROS production and mitochondrial dysfunction.

Hongwei Bai et al reported peptide supplementation targeting mitochondrial Elamipretide to cryopreservation media can significantly improve the quality and function of freeze-thawed sperm. The study provides a new perspective that during cryopreservation. Elamipretide could be promising candidates to eliminate cryodamage on cryopreservation of human spermatozoa [48].
Based on research Alicja Kowalcyk et al reported that Elamipretide supplements to cryopreservation media can significantly improve the quality and function of frozen sperm. This research also shows that Elamipretide can be used as a cryoprotective agent that serves as a cell protector against the negative effects of oxidative stress and improves sperm survival after cryopreservation [49].

The addition of Gltathione (GSH)

Glutathione (GSH) (Lc − glutamyl − L - cysteinyglycine) is the main non protein thiol compound in mammals cell, [50] and is directly involved in neutralizing ROS and keeping exogenous antioxidants such as vitamin C and E in their active form [51].

The results of the study of J. Gadea et al, report that the addition of GSH with concentration of 1.00 mM to the freezing medium significantly improved sperm motility, viability and also resulted in changes in sperm movement patterns with a decrease in the speed of the straight-line and average pathways, and also a decrease in the value of the longitude of the trajectory arch shake and the amplitude of lateral head displacement [52].

Ivan Yáñez-Ortiz et al. report that freeze and thawed donkey sperm can tolerate high concentrations of GSH, this is in contrast to other species that have been observed. This antioxidant capacity suggests that during post-artificial insemination (AI) ROS may be needed, using exogenous antioxidants such as GSH to increase sperm resistance to freezing-thawing. This suggests that the effect of increase is limited to this species [53].

M.S. Ansari et al, reported that the addition of GSH with concentration 0.5 mM in the extender could result in an improvement in sperm structure (i.e. viability, acrosome integrity and plasma membrane integrity), functional integrity (i.e. motility and mitochondrial function) and fertility parameters of Indian red forest birds (Gallus gallus murgha) through enrichment of antioxidant potential and increased oxidative stress [54].

Research conducted by H. Izanloo et al, reported that the addition of 2 mM, 1 mM, 0.5 mM GSH, there was an improvement in plasma membrane integrity, plasma membrane function, morphology and DNA integrity when compared to control groups. The study also showed that GSH has a positive effect on fertility and Mitochondrial Membrane Potential (MMP) [55].

The addition of Melatonin

Melatonin is the main hormone secreted by the pineal gland, has been suggested as a scavenger for free radicals and antioxidants [56]. Melatonin it is one of the most effective antioxidants that protects cells from oxidative stress caused by reactive species [57].

In a study conducted by Gamal M.K. Mehaisen et al, showed that there was a positive effect on the quality of rooster sperm added melatonin on cryopreservation media (cryoprotectant) [58].

Research conducted by K.R. Pool et al showed that in male ram sperm given melatonin does not protect the quality of cryopreservation spermatozoa through scavenging relative oxygen species that are not visible as previously suggested. In contrast, administration of melatonin seems to specifically reduce the production of mitochondrial superoxide, altering sperm function, rather than simply increasing the percentage of live sperm [59].

Research conducted by Wilasinee Inyawilert et al, reported that the addition of 1 mM of melatonin for semen extender in swamp buffalo during cryopreservation may provide the best protection against sperm damage [60].

The addition of Myoinositol

Myoinositol is a vitamin like substance that is widely recognized as part of the vitamin B family and is commonly referred to as vitamin B8 [61]. These biomolecules are involved in number of cellular functions including cell growth, morphogenesis, cell lipid synthesis and cell cytogenesis [62] Mona Abdolsamadi et al report that Myoinositol could be a good supplement in sperm cryopreservation in oligoastenoteratozoosperma, to reduce the detrimental effects of the cryopreservation process especially on DNA Integrity, which is an important factor in the success of Assisted Reproductive Technology (ART). Myoinositol has an affirmative effect on sperm motility and Total Antioxidant Capacities levels. It also prevents increased DNA fragmentation in the cryopreservation process [63].

The study conducted by Verón et al which was the first study to thoroughly evaluate the effect of supplementation with MI (2mg/mL) on frozen-thawed cow semen. As a result, there was a positive impact MI found on a series of quality sperm biomarkers, especially in the kinematics of sperm objective parameters. In addition, sperm motility describes a trend towards a progressive increase in the percentage of total and motile sperm, and in RI. Interestingly Some differences in sperm response were found between breeds, and between replications of individual cows. Important supplementation with MI does not exert a damaging effect on sperm vitality, osmotic plasma membrane competence, acrosome status, nuclear chromatin condensation, or DNA fragmentation [64].

The addition of Resveratrol (RSV)

Resveratrol is a potent non-flavonoid antioxidant that works by scavenging ROS and chelating divalent cations [65]. Resveratrol has been found to be involved in enzymatic pathways, cellular signaling, and apoptosis, mainly by inhibiting the formation of ROS and beneficial in the prevention of vascular disease [66]. It has also been reported that antioxidant resveratrol can protect animal sperm and human sperm during cryopreservation [67].

In a recent study of post-thaw buck sperm, commercial extender supplementation with RSV increased the viability of spermatozoa [68]. Research conducted on buffalo, the addition of resveratrol to the tris citric acid extender can improve quality parameters, post-thawing sperm fertility and antioxidant enzyme levels [69].

Seonggyu BANG et al reports that RSV is one of the best antioxidants for sperm cryopreservation in dogs to maintain sperm quality, the optimal recommended concentration is 200 μM resveratrol. On their findings suggest that there is still room for increased use of antioxidants in reproductive technologies, biology studies and cell pathology. In addition to its use in sperm cryopreservation, RSV can also be used on embryonic cryopreservation, but high doses can have toxic effects so it is necessary to take appropriate precautions [67].
### Figure 1: The Effects of Antioxidant in Spermatozoa Cryopreservation.

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<tr>
<th>Antioxidan</th>
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<th>Result</th>
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<tr>
<td>Vitamin C</td>
<td>S.C.C. Pinto (2020)</td>
<td>Bovine sperm</td>
<td>added groups vitamin C (2.5 mmol/mL) in Tris-egg yolk extender</td>
<td>during cryopreservation of sperm the presence of beneficial effects on sperm motility, acrosome membranes and plasma preservation</td>
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<td>Vitamin E</td>
<td>José Alberto Espina-Ávila (2021)</td>
<td>ram sperm</td>
<td>1. Triladyl group with an additional 10 mg/vitamin E 2. Triladyl group without vitamin E</td>
<td>in the group given vitamin &quot;E&quot; can improve sperm survival.</td>
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<td>Trolox</td>
<td>Saeid Nekoonam et al (2016)</td>
<td>Human sperm (normozoospermia and oligozoospermia)</td>
<td>The sample was 20 normozoospermic and 20 oligozoospermic Addition of Trolox with doses of 0, 20, 40 and 80 mM to the cryoprotectant agent</td>
<td>Improvement of cryopreserved human sperm quality, as measured by early changes in apoptosis and DNA integrity</td>
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<td></td>
<td>Marta F. Riesco et al (2021)</td>
<td>Ram Sperm</td>
<td>Addition of Trolox supplementation of 1.00 mM to the cryopreservation extender</td>
<td>Improves sperm quality, reduces cryodamage to ovine sperm and improves reproducibility in fertility assessment</td>
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<td>Astaxantin(AST)</td>
<td>Hai-tao buo et al (2021)</td>
<td>Boar sperm</td>
<td>Addition of Astaxantin with different concentrations of 0, 0.5, 1, 2 and 5 mM in the clotting medium in boar sperm</td>
<td>Prevents lipid peroxidation, regulates the fatty acid composition of sperm membranes, improves sperm quality after thawing and has no negative effect on the in vitro fertilization capacity (IVF) of pig sperm and the potential forembryo development.</td>
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<td></td>
<td>Gizem Dede et al (2022)</td>
<td>Human sperm</td>
<td>The sample was 30 normozoospermia. Addition of astaxantin with concentrations: Control group: 0 μM, Treatment group: 50, 100, 500 μM in each group</td>
<td>The addition of astaxatin 100 μM affects sperm motility positively and astaxantineffectively reduces chromatin condensation</td>
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<td>Taha Ghantabpour, et al (2022)</td>
<td>Human Spermatozoa</td>
<td>I. 10 samples were addedastaxantin at doses of 0, 0.5, 1 and 2 mM, then evaluation of motility, viability and externalization of phosphatidylserine(PS)</td>
<td>the addition of astaxatin 1 mM to the sperm freezing medium can improve all parameters of sperm motility and viability.</td>
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<td>Il. 25 samples weredivided into 3 groups namely the freshgroup, the controlgroup, and astaxantin added group</td>
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<td>Elamipretide</td>
<td>Hongwei Bai et al.2020</td>
<td>Human sperm</td>
<td>Sperm samples in cryopreservation were added with elamipretide with different doses, namely 0.0, 0.1, 1, and 10 μM</td>
<td>improved parameters of spermatozoa post thawing, including motility, viability, stability of plasma membrane, mitochondria and chromosomes</td>
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<td></td>
<td>Alicja Kowalczyk, et al (2021)</td>
<td>Bull’s sperm</td>
<td>I. control group (not given Elamipretide), II. added Elamipretide TFA (Trifluoroacet ic) at a dose of 0.1; 1; 5; and 10 μM</td>
<td>The result obtained that the most effective concentrations are the addition of elamipretide 5 and 10 μM in terms of the parameters tested from the quality of spermatozoa in cryopreservation</td>
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<td>MS Ansari et al (2021)</td>
<td>Indian red jungle fowl (Gallus gallus murghi)</td>
<td>Semen was diluted with an extender 1: 5 at a temperature of 37 C, Control Group (GSH 0), Treatment Group ( GSH 0.1 , 0.5 and 1.0 mM) then in cryopreservation temperature -196 C in liquid nitrogen</td>
<td>The addition of GSH as much as 0.5 mM in the extender can be: 1. Improve sperm structure, namely sperm viability, plasma membrane and acrosome integrity. 2. Improve functional integrity, namely motility and mitochondrial function</td>
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<td></td>
<td>Iván Yánez-Ortiz (2021)</td>
<td>Donkey Sperm</td>
<td>Control group : GSH 0 treatment group : GSH 2,4, 6, 8 and 10 mM</td>
<td>Administration of GSH 8 and 10 mM (the highest GSH concentration) can affect sperm motility, which suggests that how donkey sperm handles ROS is different from other species. The addition of GSH in the clotting medium is necessary to control intracellular ROS levels, especially H2O2, which is produced during freeze thawing as well as post artificial insemination (AI), and can improve reproductive performance.</td>
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<td></td>
<td>H. Izanloo et al (2022)</td>
<td>Turkey</td>
<td>The addition of GSH 0.5, 1 and 2 mM to semen diluted in a glucose-based extender cryopreservation.</td>
<td>Improves turkey sperm survival/viability after thawing and improves turkey sperm fertility.</td>
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<td>Melatonin</td>
<td>Gamal M.K. Mehaisen (2020)</td>
<td>Rooster sperm</td>
<td>Addition of melatonin with a concentration of 10-3, 10-6 or 10-9 M to the extender, cryopreservation sample</td>
<td>Melatonin supplementation has decreased lipid peroxidation, DNA fragmentation, and changes such as apoptosis after liquefaction.</td>
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<td>Wilasineeinyawilert (2021)</td>
<td>Thai swamp buffalo semen</td>
<td>Addition of melatonin with concentrations of 0.0.1, 0.5, 1.0, 2.0 and 3.0 mM in the sperm of six marsh buffaloes diluted with a tris-citrate egg yolk extender.</td>
<td>The addition of melatonin in semen extenders showed a positive effect on motility, sperm viability and melatonin-added groups of 1.0 mM showed the best results in all parameters.</td>
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<td>K.R. Pool et al (2021)</td>
<td>Ram spermatozoa</td>
<td>Cryopreservation: Control group without melatonin. The treatment group added melatonin with concentrations of 0.1, 1, 10, and 100 mM</td>
<td>In particular, the addition of melatonin can reduce the production of mitochondrial superoxide, which interferes with sperm function, rather than simply increasing the percentage of live sperm.</td>
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<td>Myoinositol</td>
<td>Mona Abdolsamadi et al (2021)</td>
<td>Human sperm (oligoasthenoteratozoospermia)</td>
<td>Sperm samples from 40 patients were divided into two groups, namely the control group and the treatment group to which 2 mg/ml of myoinositol was added.</td>
<td>Myoinositol has an affirmative effect on sperm motility, TAC levels, prevents increased DNA fragmentation. The addition of myoinositol gives good results in cryopreservation of sperm of oligoasthenoteratozoospermic patients.</td>
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<tr>
<td>Verón et al (2021)</td>
<td>Bovine Sperm</td>
<td>Myoinositol supplementation adose of 2 mg/ml in frozen-thawed bovine sperm from 9 bulls</td>
<td>Increase in the percentage of total and progressive motile sperm.</td>
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<tr>
<td>Resveratrol</td>
<td>Seonggyu BANG et al (2021)</td>
<td>dog sperm</td>
<td>Samples from 4 dog sperm and added resveratrol at doses of 0, 100 μM, 200 μM and 400 μM, evaluated post-thawing sperm quality.</td>
<td>Resveratrol is the best antioxidant supplement to maintain the quality of dog sperm on cryopreservation with the recommended dose of optimal concentration 200 μM.</td>
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<td>Hussain Ahmed et al (2020)</td>
<td>buffalo bull (Bubalus bubalis) spermatozoa</td>
<td>Semen from four bulls, divided into five groups namely the control group (T5 = no resveratrol) treatment group with added resveratrol at a dose of T4 = 100 μM, T3 = 50 μM, T2 = 20 μM, T1 = 10 μM, and evaluated post freezing-thawing.</td>
<td>The addition of resveratrol to the extender can improve quality parameters, antioxidant enzyme content, fertilization capacity, and reduce DNA and LDH fragmentation in buffalo sperm during cryopreservation.</td>
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</table>

**Conclusion**

This review shows that the addition of antioxidants is necessary to counteract ROS, prevent cell damage caused during the freezing-thawing process as well as improve spermatozoa quality and functionality parameters. The purpose of this review to improve understanding of the role of antioxidants in cryopreservation. It is necessary to conduct further studies for the clinical application of antioxidants and their effects to determine the optimal antioxidant supplementation during cryopreservation as well as the possibility of adding new antioxidants to cryopreservation reagents to guarantee the quality of spermatozoa.

**References**


