MEDDOCS Open Access Publisher

Annals of Reproductive Medicine

Open Access | Research Article

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

Zuraida[1]*; Silvia Werdhy Lestari[2]; Andon Hestiantoro[3]; Mulyoto Pangestu[4]

[1]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Jakarta 10430 Indonesia.

[2]Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia.

[3]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia.

[4]Education Program in Reproduction and Development, Department of Obstetrics and Gynecology, School of Clinical Sciences, Monash University, Victoria, Australia.

***Corresponding Author(s): Zuraida**

Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Jakarta 10430 Indonesia.

Email: zuraidazuraida455@gmail.com

Received: Jan 09, 2023

Accepted: Feb 02, 2023

Published Online: Feb 06, 2023

Journal: Annals of Reproductive Medicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

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 apaptosis 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.

Cite this article: Zuraida, Silvia Werdhy L, Andon H, Mulyoto P. The Use of Antioxidant in Cryopreservation to Improve the Spermatozoa Quality After Freezing-Thawing: A review. Ann Reprod Med. 2023; 2(1): 1005.

To this purpose, a computerized search of EMBASE, PubMed, Scopus and Google Scholar databases from 2010 to 2022 were performed on the general term such as “sperm cryopreservation”, “cryoprotectant”, “antioxidant”. The purpose of this review is to improve understanding of the role of antioxidants in cryopreservation of spermatozoa. 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. It is necessary to conduct further studies for clinical application of antioxidants and their effects to determine the optimal antioxidant supplementation during cryopreservation.

Introduction

Cryopreservation of human sperm is important because it is one way to solve the problem 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 medicine2. 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 cryopresevation 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 oxidetive stress can’t go unnoticed2. 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 Scolar and the Science Direct web. The keywords used in the journal search were, Spermatozoa Cryopreservation, Cryoprotectant, antioxidant, Quality spermatozoa, Pascathawing. 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 along 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 glycerol, 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 permeabilityand 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 propile 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 ions 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].

Seren Çelik et al reported that vitamin C supplementation to prepare spermatozoa produce recovery rates better sperm parameters and DNA integrity after vitrification [24].

S.C.C. Pinto et al, reported Extender supplementation combined with reduced vitamin C and glutathione showed beneficial effects on sperm motility, acrosome membranes and plasma preservation during sperm cryoprepreservation, which is also a group that demonstrates the value of reactive oxygen species and may be a potential strategy for increasing bovine spermatogenesis in artificial insemination and embryogenesis in vitro [25].

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 freeze-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 oligoozoospermia 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 anticancer 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 cardiolypine, an acidic lipoprotein exclusively localized in the Deep Mitichondrial 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 cardiolypine, 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 a promising candidate to eliminate cryodamage on cryopreservation of human spermatozoa [48].

Based on research Alicja Kowalczyk 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 Gluthatione (GSH)

Glutathione (GSH) (Lc – glutamyl – L - cysteinylglycine) 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 Yánez-Ortiz et al. report that freez 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 oligoastenoteratozoospermia, 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 Capacity 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.

Antioxidan	Referensi	Sampel	Study design	Result
Vitamin C	S.C.C. Pinto **(2020)**	Bovine sperm	added groups vitamin C (2.5 mmol/mL) in Tris-eggyolk extender	during cryopreservation of sperm thepresence of beneficial effects on sperm motility, acrosome membranes and plasma preservation
Vitamin E	José Alberto Espina-Ávila (2021)	ram sperm	1. Triladyl group with anadditional 10 mg / mlof vitamin E 2. Triladyl group withoutvitamin E	in the group given vitamin "E" canimprove sperm survival.
Trolox	Saeid Nekoonam et al (2016)	Human sperma (normozospermia and oligozospermia)	The sample was 20normozoospermic and 20 oligoozoospermia Addition of Trolox with doses of 0, 20, 40 and 80mM to the cryoprotectant agent	improvement of cryopreserved human sperm quality, as measured by early changes in apoptosis and DNA integrity
	Marta F.Riesco et al (2021)	Ram Sperm	Addition of trolox supplementation of 1.00 mM to the cryopreservation extender	Improves sperm quality, reduces cryodamage to ovine sperm andimproves reproducibility in fertility assessment
Astaxantin(AST)	Hai-tao buoet al (2021)	Boar sperm	Addition of Astaxantin with different concentrations of 0, 0.5, 1, 2 and 5 mM in theclotting medium in boar sperm	Prevents lipid peroxidation, regulates the fatty acid composition of sperm membranes, improves sperm quality after thawing and has no negative effecton the in vitro fertilization capacity (IVF) of pig sperm and the potential forembryo development.
	Gizem Dedeet al (2022)	Human sperm	The sample was 30normozoospermia. Addition of astaxantinwith concentrations: Control group: 0 µM, Treatment group: 50, 100, and 500 µM in eachgroup	The addition of astaxatin 100 µM affectssperm motility positively and astaxantineffectively reduces chromatin condensation
	Taha Ghantabpour, et al (2022)	Human Spermatozoa	I. 10 samples were addedastaxantin at doses of 0, 0.5, 1 and 2 mM,then evaluation of motility, viability andexternalization of phosphatidylserine(PS)	the addition of astaxantin 1 mM to the sperm freezing medium can improve allparameters of sperm motility and viability.
			II. 25 samples weredivided into 3 groups namely the freshgroup, the controlgroup, and astaxantin added group	
Elamipretide	Hongwei Baiet al.2020	Human sperm	Sperm samples in cryopreservation were added with elamipretidewith different doses, namely 0.0, 0.1, 1, and 10 µM	improved parameters of spermatozoa post thawing, including motility, viability, stability of plasma membrane,mitochondria and chromosomes
	Alicja Kowalczyk, et al (2021)	Bull's sperm	I. control group (not given Elamipretide), II. added ElamipretideTFA (Trifluoroacetic)at a dose of 0.1; 1; 5; and 10 µM	The result obtained that the most effective concentrations are the additionof elamipretide 5 and 10 µM in terms ofthe parameters tested from the quality ofspermatozoa in cryopreservation
Glutathione	**MS** Ansari etal (2021)	Indian red jungle fowl (Gallus gallus murghi)	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	The addition of GSH as much as 0.5 mM in the extender can be: 1. Improve sperm structure, namely sperm viability, plasma membraneand acrosome integrity. 2. Improve functional integrity, namely motility and mitochondrialfunction
	Iván Yánez-Ortiz (2021)	Donkey Sperm	Control group : GSH 0 treatment group : GSH 2,4 6, 8 and 10 mM	Administration of GSH 8 and 10 mM (the highest GSH concentration) can affect sperm motility, which suggests that how donkey sperm handles ROS isdifferent from other species. The addition of GSH in the clotting mediumis necessary to control intracellular ROSlevels, especially H2O2, which is produced during freeze thawing as wellas post artificial insemination (AI), and can improve reproductive performance.
	H. Izanloo etal (2022)	Turkey	The addition of GSH 0.5, 1 and 2 mM to semendiluted in a glucose-based extendercryopreservation.	Improves turkey sperm survival(viability) after thawing and improves turkey sperm fertility

Melatonin	Gamal M.K. Mehaisen (2020)	Rooster sperm	Addition of melatoninwith a concentration of10-3, 10-6 or 10-9 M to the extender, cryopreservation sample	Melatonin supplementation has decreased lipid peroxidation, DNA fragmentation, and changes such as apoptosis after liquefaction.
	WilasineeInyawilert (2021)	Thai swamp buffalo semen	Addition of melatoninwith concentrations of 0,0.1, 0.5, 1.0, 2.0 and 3.0 mM in the sperm of six marsh buffaloes dilutedwith a tris-citrate egg yolk extender.	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
	K.R. Pool etal (2021)	Ram spermatozoa	Cryopreservation : Control group without melatonin The treatment group added melatonin with concentrations of 0.1, 1, 10, and 100 mM	In particular the addition of melatonin can reduce the production of mitochondrial superoxide, which interferes with sperm function, ratherthan simply increasing the percentage oflive sperm.
Myoinositol	Mona Abdolsamadi et al (2021)	Human sperm (oligoasthenoteratozoospermia)	Sperm samples from 40 patients were dividedinto two groups, namely the control group and thetreatment group to which2 mg/ml of myoinositol was added.	Myoinositol has an affirmative effect onsperm motility, TAC levels, preventsincreased DNA fragmentation. The addition of myoinositol gives goodresults in cryopreservation of sperm of oligoastenoteratozoospermic patients
	Verón et al(2021)	Bovine Sperm	Myoinositol supplementation atadose of 2 mg/ml infrozen-thawed bovine sperm from 9 bulls	increase in the percentage of total andprogressive motile sperm
Resverator	Seonggyu BANG et al(2021)	dog sperm	Samples from 4 dog sperm and added resverator at doses of 0, 100 µM, 200 µM and 400 µM, evaluated post-thawing sperm quality	Resveratrol as the best antioxidantsupplement to maintain the quality of dog sperm on cryopreservation with therecommended dose of optimal concentration 200 µM
	Hussain Ahmed, et al (2020)	buffalo bull (Bubalus bubalis) spermatozoa	Semen from four bulls, divided into five groups namely the control group(T5 = no resveratrol) treatment group withadded resveratrol at a dose of T4 = 100µM, T3 = 50µM, T2 = 20µM, T1 = 10µM, and evaluatedpost freezing-thawing	the addition of resveratrol to the extender can improve quality parameters, antioxidant enzyme content, fertilization capacity, andreduce DNA and LPO fragmentation inbuffalo sperm during cryopreservation.

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

1. Chey G. Dearing CNJ, Kevin S. Lindsay. Human sperm cryopreservation in cancer patients: Links with deprivation and mortality. Cryobiology. 2017: 5.
2. Maryam Hezavehei MS, Homa Mohseni Kouchesfahani,, Ralf Henkel AA, Vahid Esmaeili, Abdolhossein Shahverdi,. Sperm cryopreservation: A review on currentmolecular cryobiology and advanced approaches. RBMO. 2018; 3: 13.
3. Lusignan XL, Herrero G . Delbes, Chan PTK. Effects of different cryopreservation methods on DNA integrity and sperm chromatin quality in men. Andrology. 2018; 7.
4. Pamela Uribea B, Christian Rojasa, Juan Meriñoa B, Fabiola Zambranoa, Juana V. Villegas, et al. Effect of incubation temperature after devitrification on quality parametersin human sperm cell. Cryobiology. 2017; 4.
5. Ball BA. Oxidative stress,osmotic stress and apoptosis: Impacts on sperm function and preservation in the horse. Animal Reproduction Science. 2008; 107: 257-267.
6. Gadea J, Molla M, Selles E, Marco MA, Garcia-Vazquez FA, et al. Reduced glutathione content in human sperm is decreased after cryopreservation: Effect of the addition of reduced glutathione to the freezing and thawing extenders. Cryobiology. 2011; 62.
7. Silva S, Soares A, Batista A, Almeida F, Nunes J, et al. In Vitro and In Vivo Evaluation of Ram Sperm Frozen in Tris Egg-yolk and Supplemented with Superoxide Dismutase and Reduced Glutathione. Reproduction in Domestic Animal. 2011; 46: 8.
8. Phiwayinkosi V. Dludlaa BJ, Amsha Viraragavana, Carmen Pheiffera, Rabia Johnsona, et al. A dose-dependent effect of dimethyl sulfoxide on lipid content, cell viabilityand oxidative stress in 3T3-L1 adipocytes. Toxicology Reports. 2018; 7.
9. Serap Erdal BJM, Natalia Gurulen, Marianne Berwick, Emily Gonzales, Johanna Byrdd, et al. Application of mutagen sensitivity assay in a glioma case-control study. Toxicology Reports. 2018; 6.
10. Layeka SS, Kumaresana TKM, Parks JE. Cryopreservation of bull semen : Evolution from egg yolk based to soybean based extenders. Animal Reproduction Science. 2016; 9.
11. Maryam Ezzati DS, Kobra Hamdi, Sara Rahbar, Maryam Pashaiasl. Influence of cryopreservation on structure and functionof mammalian spermatozoa: an overview. Cell Tissue Bank. 2019; 16.
12. Amena Khatun MSR, Myung-Geol Pang. Clinical assessment of the male fertility. Obstetrics & Gynecology Science. 2018; 13.
13. OS A, TL Y, D S, RI A. Effect of glycerol and dimethylformamide (DMF) cryoprotectants on buck Etawah Crossbreed frozen semen using modified tris diluents. JITV. 2013; 18: 12.
14. Bogle OA, Kumar K, Attardo-Parrinello C, Lewis SEM, Estanyol JM, et al. Identification Of Protein Changes In Human Spermatozoa Throughout The Cryopreservation Process. Andrology. 2016; 13.

15. Hossen S, Sukhan ZP, Cho Y, Kho KH. Effects of Cryopreservation on Gene Expression and Post Thaw Sperm Quality of Pacific Abalone, Haliotis discus hannai. Frontiers in Marine Science. 2021; 8: 16.

16. Parmegiani L, Minasi MG, Arnone A, Casciani V, Cognigni GE, et al. "Universal Warming" Protocol For Vitrified Oocytes To Streamline Cell Exchange For Transnational Donation Programs: A Multi-Center Study. Journal of Assisted Reproduction and Genetics. 2020; 37: 7.

17. Fernandez-Gonzalez L, Jewgenow K. Cryopreservation Of Feline Oocytes By Vitrification Using Commercial Kits And Slush Nitrogen Technique. Wiley Reproduction Domestic Animal. 2016; 51: 1.

18. Li K, Chen X, Song X, Wu X, Xian Y. Cryopreservation Of Luciola Praeusta Kiesenwetter (Coleoptera: Lampyridae) Embryos By Vitrification. Cryobiology. 78; 6.

19. Bergstein-Galan TG, Bicudo LC, Rodello L, Weiss RR, Bicudo SD. Sperm Membrane Integrity And Stability After Selection Of Cryopreserved Ovine Semen On Colloidal Solutions. Andrologia. 2017; 6.

20. Landeras J, Spermatozoa JG. Seminal Plasmafatty Acids As Predictors Ofcryopreservation Success. Andrology. 2012; 1: 11.

21. Lenzia A, Gandinia L, Lombardoa F, Boitanid MPC, Marescab V, et al. Polyunsaturated Fatty Acids Of Germ Cell Membranes, Glutathione And Blutathione-Dependent Enzyme-Phgpx: From Basic To Clinic. Contraception. 2002; 65: 5.

22. Hininger I, Waters R, Osman M, Garrel C, Fernholz K, et al. Acute Prooxidant Effects Of Vitamin C In Edta Chelation Therapy And Long-Term Antioxidant Benefits Of Therapy. Free Radical Biology & Medicine. 2005; 38: 6.

23. Sebastian J. Padayatty MRCP P, MD AK, MD YW, PhD PE, PhD OK, PhD J-HL, et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. Journal of the American College of Nutrition. 2013; 22: 1.

24. Çelik S, Özekici Ü, Çakıl D. Effects Of Antioxidants On Motility And DNA Integrity In Frozen- Thawed Sperm. Maltepe Medical Journal. 2020.

25. Pinto SCC, Almeida DS, Alves MBR, Florez-Rodriguez SA, Júnior GSA, Alves NBe, et al. Does Supplementation Of Vitamin C, Reduced Glutathione Or Their Association In Semen Extender Reduce Oxidative Stress In Bovine Frozen Semen? Arq Bras Med Vet Zootec. 2020; 72: 19.

26. Jeong Y-J, Kim M-K, Song H-J, Kang E-J, Ock S-A, Kumar BM, et al. Effect of a-tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes. Cryobiology. 2009; 58: 9.

27. A K, H B, Abshenas J. Comparative Evaluation Of The Effect Of Antioxidant In The Chilled- Stored Ram Semen. Itanian Journal of Veterinary Research, University of Shiraz. 2006; 7.

28. Beheshti R, Asadi A, Maheri-Sis N. The Effect Of Vitamin E On Post-Thawed Buffalo Bull Sperm Parameters. Journal of American Science. 2011; 7: 6.

29. Espina-Ávila JA, Magaña-Monforte JG, Aké-Villanueva JR, Aké-López JR. Effect Of The Use Of Vitamin "E" In The Diluent On The Viability Of Ram Sperm. Tropical Animal Health and Production. 2021; 53: 330.

30. Anel-Lópeza L, Álvarez-Rodríguez M, García-Álvareza O, Álvarez M, Maroto-Moralesa A, Anel L, et al. Reduced glutathione and Trolox (vitamin E) as extender supplements in cryopreservation of red deer epididymal spermatozoa. Animal Reproduction Science. 2012; 135: 10.

31. Domínguez-Rebolledo ÁE, Fernández-Santos MR, Bisbal A, Ros-Santaella JL, Ramón M, Carmona M, et al. Improving The Effect Of Incubation And Oxidative Stress On Thawed Spermatozoa From Red Deer By Using Different Antioxidant Treatments. Reproduction. Fertility and Development. 2010; 22: 14.

32. Maiaa MdS, Bicudo SD, Sicherle CC, Rodello L, Gallego ICS. Lipid peroxidation and generation of hydrogen peroxide in frozen-thawed ram semen cryopreserved in extenders with antioxidants. Animal Reproduction Science. 2010; 1226.

33. Nekoonam S, Nashtaei MS, naji M, Zangi BM, Amidi F. Effect Of Trolox On Sperm Quality In Normozospermia And Oligozospermia During Cryopreservation. Cryobiology. 2016; 72: 6.

34. Riesco MF, Alvarez M, Anel-Lopez L, Neila-Montero M, Palacin-Martinez C, Montes-Garrido R, et al. Multiparametric Study of Antioxidant Effect on Ram Sperm Cryopreservation—From Field Trials to Research Bench. Animals. 2021; 11.

35. Bahmanzadeh M, Vahidinia A, Mehdinejadiani S, Shokri S, Alizadeh Z. Dietary Supplementation With Astaxanthin May Ameliorate Sperm Parameters And DNA Integrity In Streptozotocin- Induced Diabetic Rats. Cerm (Clin Exp Reprod Med). 2016; 43.

36. Najafi D, Taheri RA, Najafi A, Shamsollahi M, Alvarez-Rodriguez M. Effect Of Astaxanthin Nanoparticles In Protecting The Post-Thawing Quality Of Rooster Sperm Challenged By Cadmium Administration. Poultry Science. 2020; 99.

37. Guo H-t, Wang J-r, Sun L-z, Jin X-h, Shi X-y, Lin J-y, et al. Effects Of Astaxanthin On Plasma Membrane Function And Fertility Of Boar Sperm During Cryopreservation. Theriogenology. 2021; 164.

38. Dede G, Saylan A. The Effect Of Astaxanthin On Human Sperm Parameters After Cryopreservation. Can Urol Assoc J. 2022; 14.

39. Ghantabpour T, Nashtaei MS, Nekoonam S, Rezaei H, Amidi F. The Effect of Astaxanthin on Motility, Viability, Reactive Oxygen Species, Apoptosis, and Lipid Peroxidation of Human Spermatozoa During the Freezing–Thawing Process. BIOPRESERVATION AND BIOBANKING. 2022; 20.

40. Lesnefsky EJ, Hoppel CL. Oxidative Phosphorylation And Aging. Ageing Research Reviews. 2006; 5: 32.

41. Kagan VE, Bayır HA, Belikova NA, Kapralov O, Tyurina YY, Tyurin VA, et al. Cytochrome C/Cardiolipin Relations In Mitochondria: A Kiss Of Death. Free Radical Biology & Medicine. 2009; 46: 15.

42. Szeto HH. REVIEW : First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. British Journal of Pharmacology 2014; 171: 22.

43. Allen ME, Pennington ER, Perry JB, Dadoo S, Makrecka-Kuka M, Dambrova M, et al. The Cardiolipin-Binding Peptide Elamipretide Mitigates Fragmentation Of Cristae Networks Following Cardiac Ischemia Reperfusion In Rats. COMMUNICATIONS BIOLOGY. 2020; 3: 12.

44. Handy DE, Loscalzo J. Redox Regulation of Mitochondrial Function. ANTIOXIDANTS & REDOX SIGNALING. 2012; 16.

45. Dao-Fu Dai M, PHD, Tony Chen B, Hazel Szeto M, PHD, Madeline Nieves-Cintro´n P, Vassily Kutyavin B, Luis F. Santana P, et al. Mitochondrial Targeted Antioxidant Peptide Ameliorates Hypertensive Cardiomyopathy. Journal of the American College of Cardiology. 2011; 58.

46. Li J, Chen X, Xiao W, Ma W, Li T, et al. Mitochondria-Targeted Antioxidant Peptide Ss31 Attenuates High Glucose-Induced Injury On Human Retinal Endothelial Cells. Biochemical and Biophysi-

cal Research Communications. 2011; 404: 8.

47. Zhao X, Xia J, Liu Y. Contrast of Real-Time Fluorescent PCR Methods for Detection of Escherichia coli O157:H7 and of Introducing an Internal Amplification Control. microorganisms. 2019; 7: 14.

48. Bai H, Zhang Y, Tian S, Hu R, Liang Y, Gao J, et al. Elamipretide As A Potential Candidate For Relieving Cryodamage To Human Spermatozoa During Cryopreservation. Cryobiology. 2020; 95: 5.

49. Kowalczyk A, Piątkowska EC. Antioxidant Effect Of Elamipretide On Bull's Sperm Cells During Freezing/Thawing Process. Andrology. 2021; 9: 7.

50. Atmaca G. Antioxidant Effect of Sulfur-Containing Amino Acids. Yonsei Medical Jurnal. 2004; 45.

51. Gadea Jn, Selle´s E, Marco MA, Coy P, Mata´s C, Romar R, et al. Decrease in glutathione content in boar sperm after cryopreservation Effect of the addition of reduced glutathione. Theriogenology. 2004; 62: 690-701.

52. Gadea J, Molla M, Selles E, Marco MA, Garcia-Vazquez FA, Gardon JC. Reduced Glutathione Content In Human Sperm Is Decreased After Cryopreservation: Effect Of The Addition Of Reduced Glutathione To The Freezing And Thawing Extenders. Cryobiology. 2011; 62.

53. Yánez-Ortiz I, Catalán J, Delgado-Bermúdez A, Carluccio A, Miró J, Yeste M. Addition of Reduced Glutathione (GSH) to Freezing Medium Reduces Intracellular ROS Levels in Donkey Sperm. Veterinary Sciences. 2021; 8.

54. Ansari MS, Rakha BA, Akhter S, Akhter A, Blesbois E, Santiago-Moreno J. Effect Of Glutathione On Pre And Post-Freezing Sperm Quality Of Indian Red Jungle Fowl (Gallus Gallus Murghi). Theriogenology. 2021; 172.

55. Izanloo H, Soleimanzadeh A, Bucak MN, Imani M, Zhandi M. The Effects Of Glutathione Supplementation On Post-Thawed Turkey Semen Quality And Oxidative Stress Parameters And Fertilization, And Hatching Potential. Theriogenology. 2022; 179: 6.

56. Meng X, Li Y, Li S, Zhou Y, Gan R-Y, Xu D-P, et al. Dietary Sources and Bioactivities of Melatonin. nutrients. 2017; 9: 64.

57. Hardeland Rd, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin-A pleiotropic, orchestrating regulator molecule. Progress in Neurobiology. 2011; 93.

58. Mehaisen GMK, Partyka A, Ligocka Z, Niżański W. Cryoprotective Effect Of Melatonin Supplementation On Postthawed Rooster Sperm Quality. Animal Reproduction Science. 2020; 212: 7.

59. Pool KR, Rickard JP, Graaf SPd. Melatonin Improves The Motility And DNA Integrity Of Frozen-Thawed Ram Spermatozoa Likely Via Suppression Of Mitochondrial Superoxide Production. Domestic Animal Endocrinology. 2021; 74: 8.

60. Inyawilert W, Rungruangsak J, Liao Y-J, Tang P-C, Paungsukpaibool V. Melatonin Supplementation Improved Cryopreserved Thai Swamp Buffalo Semen. Reproduction in Domestic Animals. 2021; 56: 6.

61. Oliva M, Lippa EM, Iaconianni P, Vaiarelli A. Effect of Myoinositol and Antioxidants on Sperm Quality in Men with Metabolic Syndrome. International Journal of Endocrinology. 2016; 5.

62. CONDORELLI RA, VIGNERA SL, MONGIOÌ LM, LAGANÀ2 AS, CIMINO L, CALOGERO AE. Myo-inositol as a male fertility molecule: speed them up. European Review for Medical and Pharmacological Sciences. 2017; 21.

63. Abdolsamadi M, Mohammadi F, Nashtaei MS, Teimouri M, Sardar R, Dayani M, et al. Does Myoinositol Supplement Improve Sperm Parameters And DNA Integrity In Patients With Oligoasthenoteratozoospermia After The Freezing– Thawing Process? Cell Tissue Bank. 2020; 21: 8.

64. Verón GL, Paladea R, Bello R, Manjon, Antonella A, Monti JI, et al. Evaluation of Myo-inositol Effects upon Frozen-Thawed Bovine Sperm Quality. International Journal of Animal Breeding and Genetics. 2021; 10: 15.

65. Stojanovic S, Sprinz H, Brede O. Efficiency and Mechanism of the Antioxidant Action of trans- Resveratrol and Its Analogues in the Radical Liposome Oxidation. Archives of Biochemistry and Biophysics. 2001; 391: 11.

66. Delmas D, Jannin B, Latruffe N. Resveratrol: Preventing Properties Against Vascular Alterations And Ageing. Mol Nutr Food Res. 2005; 49.

67. BANG S, QAMAR AY, TANGA BM, FANG X, CHO J. Resveratrol Supplementation Into Extender Protects Against Cryodamage In Dog Post-Thaw Sperm. The JUrnal of Veterinary Medical Science. 2021; 83.

68. Lv C, Larbi A, Wu G, Hong Q, Quan G. Improving The Quality Of Cryopreserved Goat Semen With A Commercial Bull Extender Supplemented With Resveratrol. Animal Reproduction Science 2019; 208: 10.

69. Ahmed H, Jahan S, Ullah H, Ullah F, Salman MM. The Addition Of Resveratrol In Extender Ameliorates Post-Thaw Quality Parameters, Antioxidant Enzymes Levels And Fertilizing Capability Of Buffalo Bull (Bubalus Bubalis) Spermatozoa. Theriogenology. 2020; 34.