

The Impact of Adding Melatonin and Other Antioxidants on Post-Thaw Human Sperm Quality During Cryopreservation

Riyam Hussein¹, Lina Hasan Abbas², Suhaila Rayhaan³, Hawraa Abbas Fadhil⁴, Zainab R. AL-Mousawi⁵

¹High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, ²Clinical Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq, ³Department of Biochemistry, College of Medicine, University of Al-Ameed, Karbala, ⁴Department of Medical Laboratory Technology, Al-Zahrawi University College, Kerbala, Iraq, ⁵Department of Anatomy and Histology, College of Medicine, University of Al-Ameed, Karbala, Iraq

Abstract

Sperm cryopreservation is the process of storing sperm for an extended period of time in order to maintain male fertility. Cryopreservation involves exposing germ cells to substances that prevent freezing, cooling them to below-freezing temperatures, storing, melting, and then removing the anti-freezing material when it has been used. It is helpful in cancer patients before chemotherapy and radiotherapy. Spermatozoa can be adversely impacted by reactive oxygen species by its detrimental effects on sperm membrane lipids, which cause ice crystal formation and the induction of oxidative stress (OS) during cryopreservation. Owing to the tight relationship between OS induction and cryopreservation, several recent researches have concentrated on the function of antioxidants in preserving male fertility. A variety of antioxidants have been developed for *in vitro* supplementation in an attempt to prevent the cellular harm brought on by cryopreservation. Examples of antioxidants include melatonin, catalase, superoxide dismutase, tocopherol, ascorbic acid, and carotenoids. When added to sperm extenders, melatonin, a natural hormone that plays a role in a number of sperm physiological processes, has frequently increased sperm viability and fertility. In order to determine whether it can protect human sperm from the damaging effects of cryopreservation, it was added to the sperm cryopreservation solution. Melatonin was added to freezing extenders in recent studies on mammals, and this increased the post-thaw activities of human sperm. Therefore, this study was aimed to review the background documents on the state-of-the-art scientific literature in this area of work. Also, this study reviewed the feasibility of employed melatonin in cryopreservation because it has antioxidant ability.

Keywords: Antioxidant, melatonin, oxidative stress, reactive oxygen species, cryopreservation

INTRODUCTION

The study of cryobiology has advanced significantly in recent years. Cryobiology is the study of life in cold environments. Cryobiology is a relatively new study field. According to a literature search, the term “cryobiology” was first used to refer to the relatively new field of low temperature biology in the early 1950s. In order to maintain the existence of life, creatures must be able to adapt to the altering surface environment of the earth.^[1] Due to their greater cryoresistance, quantity, and straightforward methods, sperm cryopreservation has the longest history in the context of the cryobiology of reproductive cells and tissues and is the most frequently used in human reproductive medicine.^[2] Cryopreservation involves exposing gametes to anti-freezing substances, cooling them to below-freezing temperatures, storing

them, melting them, and then removing the anti-freezing material when it has been used.^[3] It is helpful in cancer patients before chemotherapy and radiotherapy.^[4] Additionally, it can be used in a number of assisted reproductive procedures, including *in vitro* fertilization, intracytoplasmic sperm injection, and intrauterine insemination in the cases of testicular sperm biopsy and aspiration in people with oligoasthenoteratospermia, oligospermia, and oligoasthenospermia,^[5] as well as for

Address for correspondence: Ms. Riyam Hussein, MSc, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq.
E-mail: reyam.hussain@ierit.nahrainuniv.edu.iq

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the prevention of the spread of sexually transmitted diseases or when the male partner is unable to provide a sufficient sample on the day of ovum pickup.^[6]

Despite the drawbacks of sperm cryopreservation, it also damages sperm cells by resulting in thermal shock, intracellular ice crystal formation, an increase in salt content, and oxidative stress (OS).^[7] The most widely used method for sperm cryopreservation since the first report of human sperm freezing, slow freezing, involves a technique of gradual cooling over a period of 2–4 h and involves the use of permeable cryoprotectants (CPAs). This method allows relatively large volumes of ejaculate sperm preparations to be preserved.^[8] The post-thawed sperm and, subsequently, the outcomes of its usage in treatment programs may depend on a number of variables, including the thawing technique, sample amount, the type of container, the type of CPA, and freezing procedure.^[9]

METHODS OF CRYOPRESERVATION

There are two methods for performing cryopreservation: (a) slow freezing and (b) verification or ultra-rapid freezing. The most traditional and frequently used cryopreservation technique for many years has been the slow freezing of tissue and sperm. Slow freezing is frequently replaced with vitrification as a technique.^[10] Two quick and economical techniques for sperm cryopreservation are rapid freezing and vitrification,^[11] and they can typically be carried out by workers with less training and with fewer equipment than slow-freezing.^[12] Vitrification is a process that results in the solidification of living cells in a manner similar to glass without the creation of ice crystals during cooling.^[13] By comparing their method of sperm vitrification to traditional slow freezing, Isachenko and colleagues^[14] found that it produced equal rates of sperm motility and deoxyribonucleic acid (DNA) integrity. The most significant part is that vitrified spermatozoa have already produced a number of live births in humans.^[15] Sherman first suggested rapid freezing, and for this technique, the straws must be in direct contact with nitrogen vapors for 8–10 min although submerged in liquid nitrogen at -196°C .

First, an equal amount of CPA is blended dropwise with the sample. To finish the incubation process, the mixture is then put into the straws and kept at 20°C for 10 min. The liquid nitrogen is applied to the straws after 15 min although they are held 15–20 cm above the liquid nitrogen level (about -80°C). In order to lessen the difference in temperature between the two ends of the straws as they cool, they should be laid out horizontally.^[8]

SPERM PARAMETERS AFFECTED BY CRYOPRESERVATION

Sperm parameters and chromatin quality are negatively impacted by the freezing process.^[16] In comparison to their

pre-freeze condition, after freezing, spermatozoa typically lose part of their vitality, velocity, plasma membrane functioning, and acrosome integrity.^[17] The proportion of motile spermatozoa dropped from (50.6 to 30.3) following “cryopreservation”. However, the exact process underlying the loss of motility is still not known. When spermatozoa are thawed, mitochondrial abnormalities and the percentage of immotile spermatozoa are strongly correlated.^[17] Increased reactive oxygen species production and reduced antioxidant enzyme activity in spermatozoa can both lead to lower sperm viability.^[8] The properties of the mitochondrial membrane are readily changed by cryopreservation, and the generation of reactive oxygen species (ROS) rises. This might lead to DNA oxidation and the high rates of single-strand and double-strand DNA breakage.^[18] Furthermore, it has been shown that deficiencies in DNA repair enzymes are another factor contributing to DNA damage following freezing.^[19]

Morphology is another variable that may be impacted by freezing, as an uncontrolled liquid input into “spermatozoa” can change cellular osmolality and disrupt the membrane structure. Therefore, following spermatozoa freezing, loose head and tail abnormalities, such as coiled and looped tails, are common.^[17]

In spermatozoa after cryopreservation, OS and apoptosis-inducing compounds are assumed to be the culprits for the disintegration of nucleoprotein structure, disulfide linkages, and the DNA-protamine complex.^[20] Cryopreserved spermatozoa from infertile males had a greater rate of single-strand breaks than spermatozoa from fertile males.^[8]

CRYOPRESERVATION AND OS

There are two ways of producing ROS by spermatozoa, the nicotinamide adenine dinucleotide phosphate oxidase system, which is present in the sperm plasma membrane, or the numerous mitochondria, which may be the main source of ROS generation through the nicotinamide adenine dinucleotide-dependent oxido-reductase reaction. As a result, the more defective spermatozoa there are in the semen, the more ROS are produced, which negatively affects mitochondrial activity and decreases sperm function and motility.^[21] There are several methods for measuring OS as shown in Figure 1.

By damaging sperm membrane lipids, which result in the generation of ice crystals and the induction of OS during cryopreservation, ROS can negatively affect spermatozoa.^[23] Reduced oxygen and the byproducts of their interactions with other molecules are included in reactive oxygen stress. Some, but not all, are free radicals.^[24] Free radicals are reactive chemical intermediates with a brief lifetime that include one or more unpaired-spin electrons. The pro- and anti-oxidant components of human ejaculate interact in a dynamic way. Every

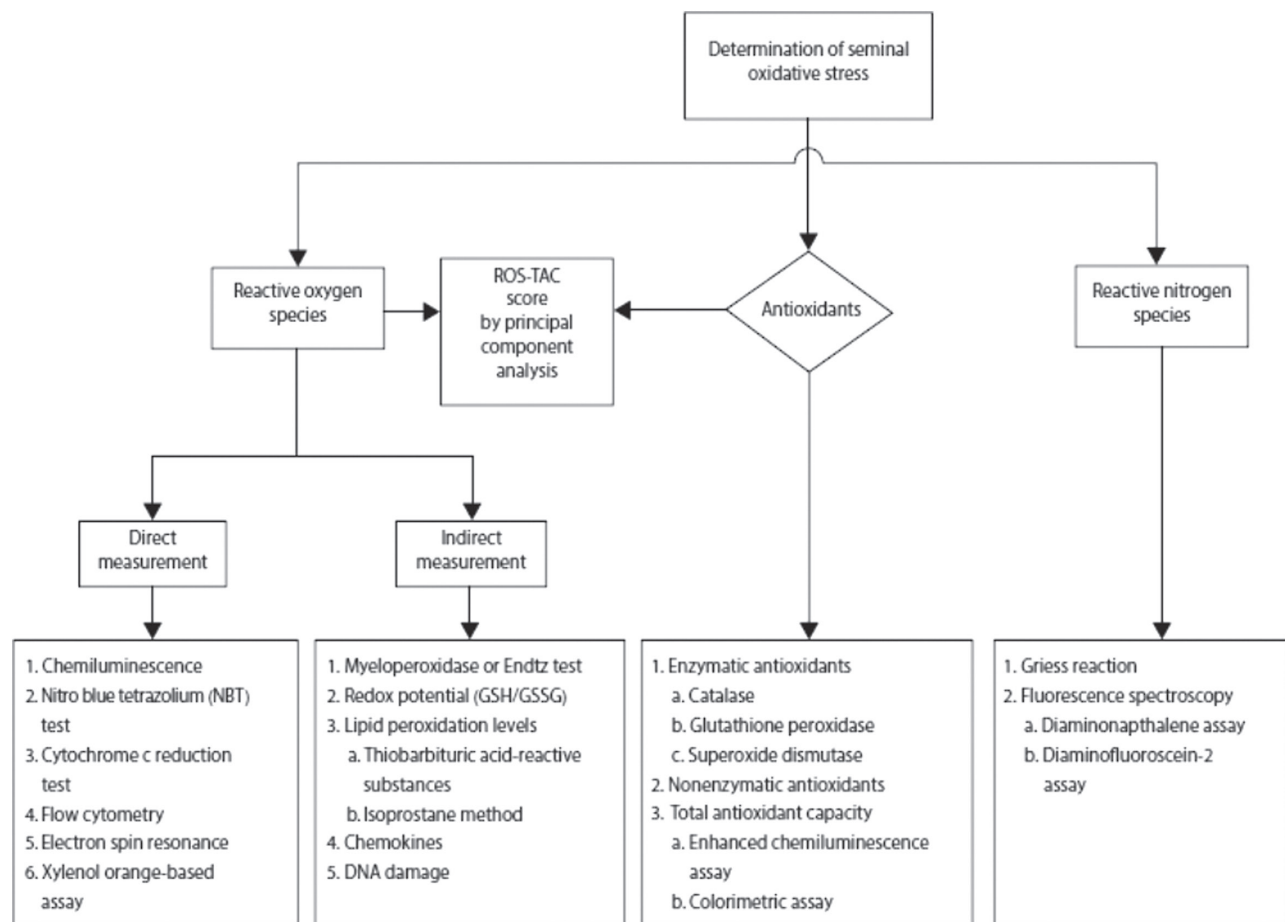


Figure 1: Flowchart showing various methods for measuring OS^[22]

human ejaculate contains potential ROS secretors such as active leukocytes, precursor germ cells, or sperm cells with abnormal morphology. A human ejaculate, on the other hand, contains intracellular and extracellular antioxidants of enzymatic and non-enzymatic systems. Human semen contains enzyme-based and low-molecular weight antioxidants that act as self-defense mechanisms to scavenge free radicals.^[25]

In general, it is understood that ROS can influence cellular death at supraphysiological levels and survival at low quantities. The resulting cellular consequences, including cellular senescence, apoptosis, and altered cellular signaling, are controlled by OS, which is the modification of redox homeostasis to encourage the production of ROS.^[26] It is also crucial to note that “ROS” can serve as signaling molecules for cellular signaling processes at a physiological level.^[27]

Understanding the numerous characteristics of ROS generated in cells, the intracellular origins of these ROS, and how cells detoxify these dangerous species are essential to understand the influence of ROS on cryopreservation.^[28] The idea that persistent OS is related to “senescence,” a response to cellular stress, and that the relative quantities of the reactive species within the cell

determine the specific effects of each, is supported by a number of lines of evidence. Senescence is a reaction to cellular stress, and it has been linked to sustained OS.^[29] Figure 2 summarizes the biological effects of these species at various stages.^[30]

ANTIOXIDANTS IN HUMAN SEMEN

Vitamins E and C, beta-carotene, superoxide dismutase, catalase, and other substances referred to be antioxidants protect cells against harm from free radicals that are produced as a consequence of OS.^[32] There are few options for medical therapy in situations of male infertility. Antioxidants appear to be viable alternatives in this aspect for treating male infertility.^[33]

The majority of the antioxidants that shield sperm from oxidizing elements come from seminal plasma.^[34] Table 1 shows the most antioxidants in human semen. The epididymis and prostate release the most of these enzymes into the seminal plasma.^[35] It is crucial to keep in mind that antioxidants must accumulate in the reproductive tract at high levels in order to enhance spermatogenesis.^[36] Gonadal cells and mature spermatozoa are protected from oxidative damage by antioxidant defense systems in spermatozoa, although the effectiveness of these defense

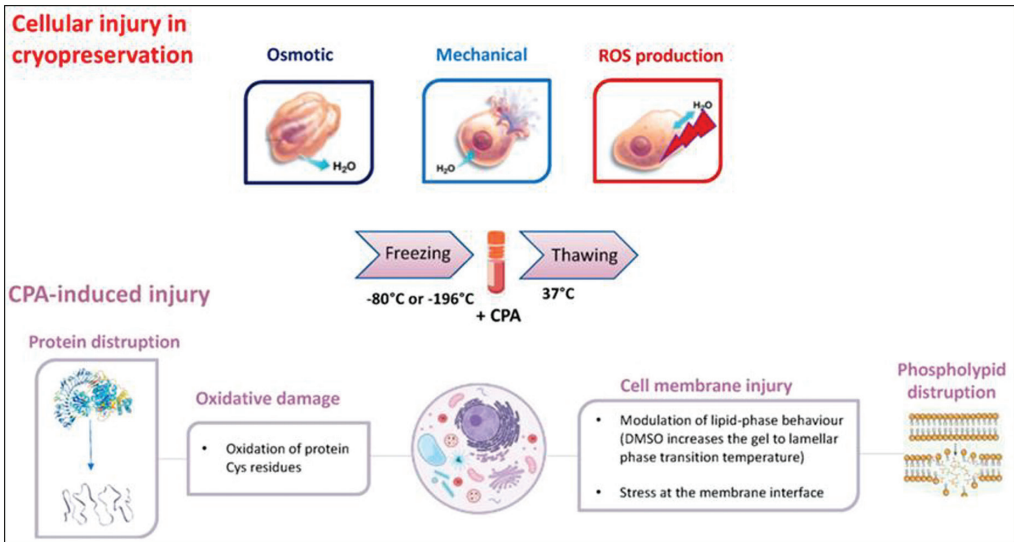


Figure 2: Cell damage caused by cryopreservation and CPA^[31]

Table 1: The most important antioxidants in human ^[25]	
A: Enzymic antioxidants	B: Low molecular weight antioxidants
1. Superoxide dismutase	1. α-Tocopherol
2. Catalase	2. β-Carotene
3. Glutathione peroxidase	3. Ascorbate
	4. Urate
	5. Transition-metal chelators
	6. Transferrin
	7. Lactoferrin
	8. Caeruloplasmin

mechanisms is limited.^[28] By lowering free radical and OS levels and enhancing fertility potential in assisted reproductive technology methods, antioxidants appear to be of major therapeutic significance.^[37] The semen and male reproductive system have a powerful antioxidant mechanism that balances the quantity and the rate of ROS formation in the physiological environment.^[38]

MELATONIN

In many cells, melatonin functions as an efficient antioxidant and free radical scavenger.^[39] Based on its ability to contain and combat free radicals, melatonin serves an antioxidant role.^[40] Due to its lipophilic nature, melatonin may function well in a variety of cellular compartments. Melatonin should be present around “free radicals” at the precise instant they are formed. When melatonin interacts with free radicals, some of the metabolites that are created have significant antioxidant capacities.^[41] The term “antioxidant cascade” refers to the interaction between melatonin’s antioxidant capacity and that of its metabolites.^[42] Due to melatonin’s great efficiency as a hydroxyl radical (•OH) scavenger, several studies have supported its beneficial properties as an antioxidant.^[43] Melatonin, which is regarded to be the pineal gland’s

main hormone of production, is crucial in the regulation of various physiological processes. Melatonin has been found to decrease the “OS” brought on by ROS in the mitochondria of sperm.^[44] Melatonin can detoxify singlet oxygen (O₂), “nitrogen oxide”, the Peroxynitrite anion, as well as its byproducts “peroxynitrous acid” and “hydrogen peroxide”.^[45]

Melatonin’s capacity to combat free radicals and stop OS-related tissue damage was nearly a coincidental discovery. One of the most well studied antioxidants in this respect is melatonin, which has both direct and indirect antioxidant effects. In addition to directly scavenging ROS, melatonin can also activate other enzymatic antioxidants.^[46] Melatonin also has indirect mechanisms to decrease the harmful effects of reactive species in addition to these functions, which would lessen tissue damage brought on by free radical production. Melatonin supports the preservation of cellular processes and mitochondrial integrity. It counteracts ROS and reactive nitrogen species’ harmful effects.^[47]

MELATONIN IN CRYOPRESERVATION

Many ROS are produced during the freezing and thawing of “spermatozoa”.^[48] The lipid peroxidation chain of chemical reactions is started by ROS and results in the production of reactive metabolites such “malondialdehyde”. “Lipid peroxidation reduces membrane fluidity and the activity of membrane enzymes and ion channels”; both of which are important for sperm motility and fertility.^[49] Numerous substances and various techniques for adding them to cooling/freezing fluid have been investigated in order to reduce harm to the sperm induced by ROS during cryopreservation. Examples of antioxidants include melatonin, “catalase”, “superoxide dismutase”, tocopherol, “ascorbic acid”, and carotenoids.^[50] Melatonin, a naturally occurring

hormone that affects a variety of sperm physiological processes, has regularly been shown to boost sperm viability and fertility.^[51] when used with sperm extenders. It was added to the sperm cryopreservation solution to see if it might shield human sperm from the negative effects of freezing.^[52]

PERSPECTIVES AND CONCLUSIONS

Adding antioxidants before freezing increases post-thaw sperm normal morphology and DNA integrity. This review was included in a larger proposal that suggested the likelihood of using melatonin as a suitable cryoprotective for semen during cryopreservation. Melatonin was added as an antioxidant to the semen freezing media to enhance the quality of the cryopreserved spermatozoa in terms of mobility and sperm morphology.

Ethical approval

Not applicable.

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Conflicts of interest

There are no conflicts of interest.

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