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Lipid Peroxidation in Sperm Cells and the Creation of Reactive Oxygen Species: A Review

Toker MB*

Department of Reproduction and Artificial Insemination, Bursa Uludag University, Turkey

***Corresponding author:** Toker MB, Department of Reproduction and Artificial Insemination, Bursa Uludag University, 16059, Bursa, Turkey, Tel: +90 224 294 1350; Email: berktoker@uludag.edu.tr

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Abstract

The extended storage of spermatozoa represents a pivotal tool for advancing reproductive technologies in the realm of animal medicine. Cryopreservation of sperm stands out as a valuable technique for the preservation of sperm cells, offering the potential for the enhancement of genetic traits and the amplification of selected reproductive characteristics. Over the years, diverse methodologies have been investigated for the cryopreservation of mammalian sperm cells, involving various techniques. However, the cryopreservation of sperm induces substantial biological and functional alterations in spermatozoa. Sperm cell membrane consists high amount of unsaturated fatty acids. Due to the redundancy of fats and cholesterol, reactive oxygen species that were created by lipid peroxidation are prone to impact membranes and compromising their functional capabilities. Prolonged storage of spermatozoa induces consequential biological and functional alterations in sperm cells, thereby compromising their capacity for fertilization. Furthermore, abrupt temperature fluctuations, exemplified by cold shock, and the dynamic processes of ice formation and dissolution during freezing-thawing procedures, exert adverse effects on the structural and functional integrity of key sperm components, including the acrosome, nucleus, mitochondria, axoneme, and plasma membranes. Also, toxic effects of hydroperoxides cause degeneration on cell membranes and altering cell pH. The effects of reactive oxygen species over lipids have one of the strongest impacts on biological systems. This article is a brief review that highlights some of the substances that were created on the way of cryopreservation with the lipid peroxidation fact that called as “Reactive Oxygen Species”.

Keywords: Antioxidants; Cryopreservation; Lipid Peroxidation; Reactive Oxygen Species; Sperm

Abbreviation: ROS: Reactive Oxygen Species; LPO: Lipid Peroxidation; LDL: Low-Density Lipoprotein; PUFAs: Polyunsaturated Fatty Acids.

Introduction

The membrane of sperm cells highly consists of unsaturated fatty acids. Because of the natural structure of the cell, reactive oxygen species (ROS) are created even more than normal by lipid peroxidation (LPO) phenomenon throughout the process of cooling, freezing and thawing to cryopreserve the cell for future generations [1]. The ROS classify as reactive and non-reactive and both class cause physical and chemical stress on cells [2]. The balance between the detoxification ability of cells through ROS under normal conditions is compromised for various reasons, resulting in a decline in motility, vitality, and achievable fertility [3]. Elevated levels of reactive oxygen species (ROS) within the cell contribute to diverse forms of damage and oxidative stress which can be restored to normal by using various antioxidant matters [4].

Long Term Storage of Sperm Cells

Long term storage of sperm cells require an arrest or reducing of the cells to prolong their fertile life for further fertilization purposes. Consequently, researchers investigated the preservation of semen under two conditions: firstly, in a liquid, unfrozen state employing reduced temperatures or alternative methods to mitigate sperm metabolism; and secondly, in a frozen state, entailing conservation at sub-zero temperatures [5]. Extended storage of spermatozoa induces biological and functional alterations in sperm cells, resulting in a detriment to their fertilization capabilities. Furthermore, abrupt temperature fluctuations, such as cold shock, and the formation and dissolution of ice during the freezing-thawing process, exert an impact on the structural and functional integrity of essential sperm components, including the acrosome, nucleus, mitochondria, axoneme, and plasma membrane [6]. The efficacy of cryopreservation hinges on numerous factors, encompassing interactions among cryoprotectants, extenders, cooling rate, thawing rate, packaging, and inherent variability among individual animals [7]. Despite the reversibility inherent in cryopreservation, the process gives rise to detrimental effects, such as cold shock, ice crystallization, and lipid peroxidation. These deleterious consequences can lead to an irreversible decline in the motility, viability, and fertilization capacity of spermatozoa. Consequently, the success of semen cryopreservation relies on the mitigation of these adverse effects and the preservation of post-thaw semen quality [8].

Reactive Oxygen Species

Reactive oxygen species encompass both radical and non-radical oxygen molecules, thus constituting a broad spectrum that includes all oxygen radicals as ROS; however, it is noteworthy that not all ROS are oxygen radicals [9]. The presence of reactive oxygen species in sperm cell is essential, as they play a role in motility, capacitation, acrosome reaction, fertilization, and the fusion of spermatozoon with the oocyte, necessitating a specific level within the cell. The testes present high levels of activity and require oxygen. Consequently, both the process of creating sperm and leydig cells are vulnerable to radical attacks due to their dependence on oxygen [3].

Radical Reactive Oxygen Species

The primary radical reactive oxygen species include; superoxide (O_2-), hydroperoxyl (HO_2), hydroxyl (OH), alkoxyl (RO), and peroxyl (ROO) radicals [3]. The superoxide (O_2-) radical is formed as a result of the one-electron reduction of molecular oxygen, leading to an unstable structure. The superoxide radical, formed through reactions with certain compounds and transition metals, results from enzymatic activities playing a role in sperm hyperactivation, capacitation, and acrosome reaction. Due to its ease of entry into the cell, the superoxide radical exerts a highly potent toxic effect on spermatozoa [10]. The hydroperoxyl (HO_2) radical is a potent oxidant formed through the protonation of the O_2-radical under low pH conditions [11]. "The hydroxyl (OH) radical stands out as a highly potent reactive oxygen species, formed by the three-electron transfer to molecular oxygen [3]. Hydrogen peroxide (H_2O_2) and O_2- react with transition metals possessing one or more unpaired electrons, or through other influences, to generate the OH radical with free radical characteristics. There are several pathways for the formation of this radical [11].

As a result of the Fenton reaction, H_2O_2 is reduced by transition metals such as Fe^{+2} (or similar transition metals such as Cu, Zn, Mn, Cr) to generate the OH radical [10].

$$Fe^{+3} + O- \longrightarrow Fe^{+2} + O_2$$

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH + OH^-$$

As a result of the Haber-Weiss reaction, H_2O_2 reacts with O_2- (catalyzed by Fe^{+2} and Cu^{+2}) to produce hydroxyl radicals [12,13].

$$O_2^- + H_2O_2 \longrightarrow O_2 + OH + OH^-$$

Hydroxyl radicals can also be formed through exposure of water to high-energy ionizing radiation or exposure of H_2O_2 to UV light [11].

$$H_2O \xrightarrow{\text{X or Gama ray}} H + OH$$

$$H_2O_2 \xrightarrow{\text{UltraViole (UV)}} 2OH$$

The formation of alkoxyl (RO) occurs through the reduction of lipid hydroperoxides by transition metals such as Fe^{+2}. It leads to the oxidation of low-density lipoprotein (LDL), resulting in cell death. Peroxyl (ROO), on the other hand, is a radical formed during the degradation of organic substances such as lipids and proteins and has a short half-life [10,11].

Non-Radical Reactive Oxygen Species

Singlet oxygen ($^1O_2$), H_2O_2, peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), and ozone (O_3) can be listed as some non-radical forms of reactive oxygen species. Due to its directional spin, singlet oxygen is a highly reactive form of oxygen. Singlet oxygen, with opposite electron spin directions, is named delta if the electron orbits are on the same path and sigma if they are on different paths. Neutrophils, immune system phagocytic cells, transform H_2O_2 resulting from O_2^- dismutation through enzymes into a potent antibacterial agent, HOCl, by combining it with chloride ions [3,11].

$$2O^- + 2H^+ \longrightarrow 2HO_2$$

The reaction between nitric oxide and O2- in the presence of transition metals results in the formation of the potent oxidant, $ONOO^-$. In the presence of these reactive oxygen species, nitrites and nitrates are produced, leading to protein structural alterations and functional losses [3,11]. H_2O_2, in the absence of ROS but in the presence of Fe^{++} or other transition metals, generates the highly reactive and harmful OH radical through the Fenton reaction, while in the presence of O_2^-, it produces the OH radical through the Haber-Weiss reaction. Hydrogen peroxide, a neutral, membrane-permeable, and lipid-soluble molecule, can impact all cell membranes and is a major secretion product of the phagocytosis mechanism. It can also arise from a one-electron transfer to oxygen (superoxide dismutation) or the addition of two electrons to oxygen (reduction) [3,10,11,14]. Ozone (O_3), although present in the atmosphere in small quantities compared to oxygen, becomes highly unstable when found in high concentrations during reactions. It reacts with nitric oxide to form nitrogen dioxide (NO_2). Additionally, its presence is significant due to its role in the oxidation of unsaturated fatty acids, contributing to ROS formation [3,10].

$$O + O_2 \longrightarrow O_3$$

$$O_3 + NO \longrightarrow O_2 + NO_2$$

Conclusion

Understanding and spot-on precautions against excessive Reactive Oxygen Species throughout the process of cryopreservation stands a great importance for the success of the procedure. Fundamentally, the lipid peroxidation process initiates by highly reactive radicals attacking polyunsaturated fatty acids (PUFAs) in cell membranes, leading to the detachment of a hydrogen atom from the methylene group. Subsequently, a cascade of peroxidation reactions commences, and the effects of the resulting products intensify until intracellular antioxidant substances are depleted. The depletion of antioxidants is concomitant with disruptions in membrane fluidity and permeability [14]. This sequence of events generally unfolds in stages: initiation, propagation, and ultimately, termination. The products resulting from the destruction of the cell and organelle membranes expedite the process leading the cell towards apoptosis. Therefore, using appropriate antioxidant matters with the right strategy and particular effects of them to prevent the Reactive Oxygen Species is still a hot topic in the literature after some elaborative scientific investigations. Since the freezing of sperm cells from males of high genetic merit allows the dissemination of genetic improvement, evaluations of various antioxidants and the effects on cryopreservation will need to continue in the near future.

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