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Review Article

Physio-pathological effects of reactive oxygen species on sperm

S. P. Pannu[1*], R. K. Baithalu[2], M. Pandey[2], T. kumar[3], D. Sarkar[1] and N. Bhatia[1]

[1]Animal Reproduction, Gynaecology and Obstetrics, ICAR- National Dairy Research Institute, Karnal- 132 001, Haryana, India; [2]Molecular Reproduction Laboratory, Animal Biotechnology Center, ICAR-National Dairy Research Institute, Karnal- 132 001, Haryana, India; [3]Department of Veterinary Gynaecology and Obstetrics, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

Abstract

Reactive oxygen species are molecular messengers which play a crucial role in sperm physiological processes such as capacitation, acrosome reaction, and sperm-oocyte interaction. ROS are necessary for good sperm activity at normal levels; but, when present in excessive amounts, ROS can have negative impact on the quality and function of sperm. High levels of ROS induce oxidative stress. Oxidative stress is seen as a health risk and causes infertility via sperm damage and deformity. To improve sperm quality, antioxidant therapy can be used which helps in reducing oxidative stresses.

Key words: Antioxidant, Cryopreservation, Fertility, Reactive oxygen species, Sperm

Highlights

- ROS are unavoidable by-products of normal cell metabolism.
- ROS plays a dual role as both toxic compounds and regulators of many biochemical events.
- Detoxification of free radicals is paramount to the resilience of all aerobic life forms.

INTRODUCTION

Free radical generators have been demonstrated in mammalian spermatozoa for the first time in 1943. The reactive oxygen species are reactive molecules that have short lives (10^{-9} s). A vast spectrum of bio-logical substances can react with free radicals in which most prominently substances are unsaturated fatty acids, proteins, and nucleic acids. Sperm plasma membranes with a high concentration of polyunsaturated fatty acids are sensitized to free radicals disruption (Barik *et al*., 2019). An excessive amount of free radical production affects sperm plasma membrane lipids through lipid peroxidation (LPO) and also oxidized proteins and DNA associated with poor sperm function and subfertility. As a consequence of reactive oxygen species belching into a biological system's environ-ment and its inability to rapidly detoxify the resulting reactive intermediates or repair the resulting damage, oxidative stress occurs, damaging sperm (Agarwal *et al*., 2003). The most significant loss caused by free radicals is LPO. LPO causes loss of membrane fluidity and impairs the sperm's capacity to undergo the acrosome response leading to decreased sperm motility. The fertilization process, which involves capacitation, hyperactivation, acrosome formation, and sperm-oocyte attachment, requires a little amount of ROS (Du Plessis *et al*., 2015). Furthermore, similar effects were observed on cryopreserved semen due to ROS production. Sperm viability is usually reduced by 50%, and fertilization potential is impaired seven-fold after cryopreservation (Khumran *et al*., 2020). Therefore, understanding the etiologies and free radical's influence on sperm, such as assessing oxidative stress and treating it according to the etiologies, is believed to be critical in improving male fertility.

*Corresponding Author, E Mail: pannusp85@gmail.com

Classification of ROS

Reactive oxygen species are categorized into three forms (a) oxygen-free radicals which include superoxide anion (O_2^-), hydroxyl radical (OH^-) and hyperoxyl radical (HOO), etc. (b) non-radical species, for example hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2), etc. (c) reactive nitrogen species and free nitrogen radicals, such as nitroxyl ion, nitrous oxide and peroxynitrite, etc. (Talebi, 2011).

Sources of free radicle generation

The primary source of free radicals is mitochondria which generate energy in the shape of adenosine triphosphate (ATP). Free radicals are overexpressed by multiple non-mitochondrial processes, including the peroxisomal-oxidation, cytochrome P450 enzymes, and NADPH oxidase; these processes are all non-mitochondrial in nature (Brochier-Armane *et al*., 2009). ROS is produced mostly by nascent spermatozoa and immune cells (Akbari and Jelodar, 2013). During spermiogenesis, spermatids undergo membrane modification and a 70% reduction in cytoplasmic volume, whereas, the leftover body is released and phagocytosed by Sertoli cells during spermiation. The cytosolic enzyme glucose-6-phosphate dehydrogenase (G6PDH) has increased, and it controls the flow of glucose through the HMP shunt and increases NADPH availability. Furthermore, excessive ROS emission is caused by NADPH oxidase. NADPH oxidase acts as an electron source, resulting in excessive ROS production (Aitken, 2017). As a consequence, spermiogenesis or spermiation dysregulation leads to the discharge of aberrant spermatozoa containing cytoplasmic droplets, which are a significant source of ROS.

Pathway of free radicles generation

It's claimed that free radicals react to biological substances like lipids, DNA, and proteins in a non-specifically and indiscriminate manner, inhibiting biological events (Halliwell and Gutteridge, 2015). Reactive oxygen species are produced when molecular oxygen (O_2) undergoes sequential electron reduction. Superoxide anion radical (O2-) is formed when one electron (e-) is reduced, hydrogen peroxide (H_2O_2) is formed when two electrons are reduced and hydroxyl radical (HO-) is formed when three electrons are reduced. Hydroxyl radicals are extremely powerful initiators of the lipid peroxidation (LPO) cascade, which can result in sperm function loss due to membrane fluidity disruption (Chen *et al*., 2013; Agarwal *et al.*, 2019). By reducing molecular oxygen to one electron, enzymes including xanthine oxidase and NAD(P)H oxidase emit superoxide. The dismutation reaction generates hydrogen peroxide when two molecules of superoxide interact to allocate or receive ions, either naturally or by superoxide dismutase catalysis.

Physio-pathological effects of reactive oxygen species

The production of free radicals speeds up the interaction between sperm and oocytes (Esmaeili *et al*., 2015). However, low and regulated levels of free radicals are linked to various physiological processes including capacitation, hyperactivation, acrosome reaction, and sperm-oocyte interaction, all of which are mandatory for proper fertilization (Bisht *et al.*, 2017). Sperm capacitation is a redox-dependent process that necessitates careful control of ROS levels in order to prevent the harmful consequences produced by oxidative stress (O'Flaherty, 2014, 2015). During capacitation of sperm, ROS initiate an array of phosphorylation reactions (O'Flaherty *et al*., 2006 a,b). Capacitation causes the phosphorylation of proteins in the flagellar fibrous sheath, such as calcium-binding and tyrosine phosphorylation-regulated protein (CABYR) and protein A-kinase anchoring proteins (AKAPs), implying that the cAMP-PKA pathway is involved in hyperactivation regulation (Young *et al*., 2016). Enhanced protein phosphorylation rates and increased levels of the signaling molecules (cAMP and

Ca^{2+}) have been related to ROS generation during the capacitation phase (Aitken, 2017). These phosphorylations and subsequent ROS generation need protein kinase C (PKC), mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinases 1 and 2 (ERK1 and 2) (Dewas *et al*., 2000), and protein tyrosine kinase (PTK). Capacitation and hyperactivation are the final stages of spermatozoa maturation which result in successful ovum fertilization (Choudhary *et al*., 2010). Pathological defects arise when the equilibrium between free radicals and antioxidants is disrupted because the high level of ROS overcomes the antioxidant defense mechanisms. An oxidative impact on sperm membrane lipids triggers ROS-induced spermatozoa destruction. Sperm plasma membrane is highly susceptible to free radical attack, leading to a cascade of chemical reactions known as lipid peroxidation (LPO) (Agarwal *et al*., 2020). The LPO cascade removes over 60% of the fatty acid from the sperm plasma membrane as a result of which alteration in membrane fluidity, ion gradients, receptor transduction, and transport mechanisms, and these changes have negative consequences for sperm function, such as the potential to fuse with oocytes (Gonzalez-Fernandez *et al*., 2012). According to Zribi *et al*. (2011), the sperm nucleus chromatin is vulnerable to oxidative stress, which causes base changes and DNA damage, all of which lead to sperm mortality.

Measurement of ROS

Various methods are used for the measurement of ROS like the chemiluminescence method, nitro blue tetrazolium (NBT) or cytochrome c reduction method, and flow cytometry. Electron spin resonance method and aromatic traps method can also be used for measurement of ROS.

1) Chemiluminescence method- This assay measures the quantity of free radicals present at a given time, not the degree of sperm-damaging ROS and most widely used method for measuring the ROS emitted by spermatozoa. Luminal is a probe that responds to various forms of free radicals. This approach is used to assess both intracellular and extracellular free radical production.

2) Nitro blue tetrazolium (NBT) or cytochrome c reduction method- In this method, nitro blue tetrazolium and ferricytochrome-c (Cyt) are the two most widely used reagents for detecting superoxide anion radicals. The decrease in cytochrome c or NBT can both reliably predict whether ROS were formed by leucocytes or abnormal spermatozoa (Agarwal *et al*., 2014; Gosálvez *et al*., 2017). When the enzyme superoxide dismutase produces a decrease in diformazan production from NBT or no ferricytochrome-c production from cytochrome-c, the presence of superoxide is verified. Nitro blue tetrazolium can be diminished by extracellular matrix superoxide and other chemicals, whereas cytochrome-c reduction only tests extracellular superoxide (Esfandiari *et al*., 2003). The chemiluminescence technique can only show the total quantity of free radicals in the semen and not the origin of free radical generation.

3) Flow cytometry- In this method, the fluorescent strength of ROS-oxidized compounds is determined. The fluorescence intensity of the substances oxidized by ROS is assessed in flow cytometry. The chemicals that may permeate into cells are dihydrorhodamine 123 and 2, 7 dichlorofluorescein diacetate (DCFH-DA). They are then deacetylated, and their fluorescence is lost. Furthermore, when ROS, which is created within the cell, oxidizes them, they become highly fluorescent. It is possible to quantify the fluorescence, which indicates the rate and amount of ROS generated.

Strategies to reduce sperm damage

Antioxidants contained in seminal plasma and spermatozoa help to preserve the sperm from oxidative damage. Seminal antioxidants include β-mercaptoethanol, protein, alpha-tocopherol, ascorbic acid, β-mercaptoethylamine, cysteine, taurin, and 2-aminoethane-

sulfinic acid limit the amount of seminal ROS production (Pahune *et al*., 2013; Fanaei *et al*., 2014). Endogenous antioxidants in seminal plasma protect spermatozoa from oxidative damage (Dorostghoal *et al*., 2017). The antioxidants are categorized into two forms (i) enzymatic which includes superoxide dismutase (SOD), catalase and peroxidase, glutathione peroxidases (GPXs), etc. that catalytically extract free radicals from biological systems. Peroxiredoxins (PRDXs) are enzymes with a molecular weight of 20–31 kDa. They have one (PRDX6) or two (PRDX1-5) cysteine (Cys) residues that are required for their action and engage in the redox cycle (Rhee, 2016). By combining with either the thioredoxin/ thioredoxin reductase system (PRDX1-5) (Perkins *et al*., 2015) or the glutathione/ glutathione reductase system mediated by glutathione S-transferase (PRDX6) (Manevich *et al*., 2004), they may decrease both organic and inorganic hydroperoxides and peroxynitrite (Dubuisson *et al*., 2004). PRDX6, like glutathione peroxidase 4 (GPX4), eliminates phospholipid hydroperoxides, inhibiting lipid peroxidation chain reactions or mending peroxidized cell membranes (Arevalo and Vázquez-Medina, 2018). Hydrogen peroxide, single-chain organic peroxides, and peroxynitrite can all be reduced by PRDXs (O'Flaherty and Matsushita-Fournier, 2017). PRDX6 contains calcium-independent phospholipase A2 (Ca2+-iPLA2) and lysophospholipid acyltransferase activities, and having ability to eliminate phospholipid hydroperoxides (using glutathione). The inadequate level of PRDX6 had a detrimental influence on spermatozoa quality such as lower motility, elevated DNA fragmentation and oxidation of protein, and aberrant sperm chromatin structure, resulting in poor fertility. In neutrophils, SOD and catalase also remove O^{-}_{2} produced by NADPH oxidase and play a vital role in lowering LPO and preserving spermatozoa from oxidative damage (Purandhar and Seshadri, 2013). Bucak *et al.* (2007) reported that catalase is present in ram and cow sperm, and it might have a role in the aging process as well as the control of oxidative stress in cells, which is predominantly caused by H_2O_2. The sperm contains metal-binding proteins such as albumin, ferritin, myoglobin, and non-enzymatic antioxidants such as ascorbic acid, alpha-tocopherol, and other phytonutrients (Panesar and Marwaha, 2013). Antioxidant supplementation prior to cryopreservation is suggested to aid in the establishment of sperm cryopreservation process.

Conclusion

Under normal conditions, the male genital system strikes equilibrium between free radical production and antioxidant activity; however, excessive free radical generation can impair sperm or seminal plasma antioxidant defense mechanisms in sperm, resulting in oxidative damage. Furthermore, oxidative stress is one of the major contributory factors to male infertility. The proportion of reactive oxygen species (ROS) in the body must be kept low enough to cause acceptable physio-pathological damage. Antioxidant therapy may increase semen production and male fertility by reducing oxidative stresses.

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Author's contribution: All authors are equally participated in designing this article.

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