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Review Article

An overview of lipids, lipid peroxidation and antioxidants, and their impact on sperm

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Abstract

Fatty acids are essential nutrients that improve male reproductive efficiency by altering the fatty acid composition and protecting the integrity of the sperm membrane. The performance of sperm motility, acrosomal response and sperm-oocyte fusion are all influenced by the fatty acid profile. Lipid metabolism is helped to maintain cellular functions such as sperm motility, capacitation, acrosome response or fusion. However, oxidative degradation of lipids causes lipid peroxidation and oxidative stress. Furthermore, the imbalance between reactive oxygen species and antioxidants in the body causes oxidative stress, which can lead to sperm destruction, malformation and subsequently male infertility. Antioxidants are used to treat male infertility by reducing oxidative stress and increasing sperm characteristics.

Key words: Antioxidant, Lipid, Lipid peroxidation, Sperm plasma membrane

Highlights

- Lipids found in the sperm plasma membrane are essential not only for the health and integrity of mammalian spermatozoa, but also for regulating critical phases such as sperm motility, capacitation, acrosome response or fusion.
- Reactive oxygen species (ROS) have a significant role in both the positive and negative modulation of sperm function and excess concentrations of reactive oxygen species cause sperm pathologies such as ATP depletion leading to insufficient axonemal phosphorylation, lipid peroxidation and loss of sperm motility.
- Lipid peroxidation is a process where oxidants such as free radicals damage lipids typically polyunsaturated fatty acids.
- Antioxidant supplementations improve sperm function and survivability, which have a positive impact on fertilization success.

INTRODUCTION

Fatty acids (FAs) are carbon chains with one end having a methyl group (CH_3) and the other having a carboxyl group (COOH). In mono- and polyunsaturated fatty acids (PUFA), the carbon chain may be saturated or include one or more double bonds. In the omega system, the first carbon of the methyl group is termed omega, while the other carbons are named omega-3 (n-3), omega-6 (n-6) and omega-9, depending on their distance from omega. Several fatty acids may be found in plants and animals; olive and canola oils have the greatest concentrations of omega-9 fatty acids such as oleic acid (C18:1 n-9). Safflower, sunflower, cottonseed and soyabean oils are high in omega-6 fatty acids (linoleic acid: C18:2 n-6). There are three major dietary n-3 fatty acids: a-linolenic acid (C18:3 n-3) from linseed oil, eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) from fish oil. DHA (C22:6 n-3) for ruminants (Esmaeili *et al.*, 2014; Fair *et al.*, 2014), DPA (C22:5 n-6) for boars, rats and rabbits, and

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docosatetraenoic acid (C22:4 n-6) for domestic birds have been identified as important constituents in spermatozoa phospholipids (Alizadeh *et al.*, 2014). Animals are unable to generate certain n-6 and n-3 fatty acids, which are required to control fluidity and acrosome responsiveness, due to a lack of appropriate fatty acid desaturase enzymes. FAs are a source of energy and as precursors of bioactive lipid mediators, have a significant impact on cellular responses and activities (Innes and Calder, 2018). The fatty acid composition influences not only sperm motility but also acrosomal response and sperm-oocyte fusion. The FA content of phospholipids heavily influences sperm membrane fluidity, flexibility and sperm-oocyte. In this review, the role of FA composition in sperm quality is reviewed.

Role of fatty acids in spermatozoon function

Fatty acids are part of the phospholipid layer of cell membranes and may have an impact on the characteristics of the membrane and interactions between the membrane's constituent parts. The hypothalamic-pituitary-testicular axis, which has a direct relationship with PUFA or their derived eicosanoids, is involved in the reproductive system's function in males which controls hormones involving testicular development and spermatogenesis. Sperm are stored in the epididymis, where they undergo the process of maturation and plasma membrane modification also takes place. Remodeling involves the ingestion of produced epididymal glycoproteins, consumption of phospholipids from the membrane bilayer, translocation of protein and lipid contents during maturation, and the development of sperm's increasing motility and capacity to fertilize an egg (Gatti *et al*., 2004). Spermatozoa's viability and motility are dependent on the integrity of the mitochondrial sheath, of which phospholipids constitute a key component. There was a similar distribution of saturated (47.8%) and unsaturated (49.8%) fatty acids in buffalo spermatozoa (Jain and Anand, 1976). Furthermore, the amount of unsaturated fatty acids was relatively high (71%) in ram spermatozoa. Goat spermatozoa have a very high proportion (67%) of saturated fatty acid chains linked to the phospholipid fractions (Rana *et al*., 1991).

Fatty acids accumulate in testicular cells via two different mechanisms i.e. passive diffusion through the lipid bilayer and/or protein-mediated transport mediated by the CD36 glycoprotein, which is abundant in Sertoli cells. Sphingomyelin (SM) and phosphatidylcholine (PC) are one of the few membrane phospholipids mostly found in the outer layer of the sperm plasma membrane, whereas phosphatidylethanolamine (PE) and phosphatidylserine (PS) are found in the inner membrane. Rival *et al*. (2019) demonstrated that PS on sperm and PS receptors on eggs are required for fertilization. PS is required for the fusion of sperm and eggs (Rival *et al.*, 2019). Abnormal PC and PE levels can be used to predict sperm fertilization capacity. PC is frequently employed as a "protective agent," for example, to reduce sperm ultrastructural damage induced by cold shock (Mafolo *et al*., 2020).

Role of cholesterol in spermatozoon function

The fusion of the genomes from two gametes during fertilization is a critical stage in life that results in the creation of a new organism. This process, which is dependent on a complex spatial reorganization of sperm membrane components, is greatly facilitated by cholesterol depletion. Cholesterol regulates reproductive activities in male animals including spermatogenesis, secondary sexual characteristics, behaviour effects and metabolic effects. Cholesterol also influences spermatozoa plasma membrane permeability/fluidity, which might impair capacitation and fertilization. Cholesterol efflux from the sperm membrane produces changes in its architecture, increased bilayer permeability and fluidity, which results in the sperm cells becoming capacitated (Puga Molina *et al.*, 2018). Due to the hydrophobic nature of cholesterol, which makes it insoluble in aqueous solutions, diffusible serum carrier proteins (such as lipoproteins) are needed to

bind the substance to move cholesterol throughout the body (Uehara and Saku, 2014). The control of capacitation-related sperm cholesterol efflux is most likely mediated by a cholesterol transporter such as ATP binding cassette transporters and scavenger receptors. Loss of cholesterol from the plasma membrane is one of the first stages of sperm capacitation. This cholesterol efflux causes lipid rearrangement in the plasma membrane, which eventually makes the membrane more permeable to Ca^{2+}, HCO_3 and K^+. A spermatozoon must have high intracellular concentrations of these ions in order to conduct the acrosome reaction and fuse with the egg (Bhakta *et al.*, 2019). When enough cholesterol is eliminated from the sperm plasma membrane during capacitation, the membrane becomes unstable and is better able to fuse with the outer acrosomal membrane, which causes the acrosome response. Variations in the cholesterol/phospholipid ratio have been associated with capacitation duration and cryopreservation survival across mammalian species because of the stabilizing abilities of cholesterol.

How ROS generate and their impact on sperm?

All of the essential physiological processes of sperm, including maturation, motility, activation, capacitation and acrosome responses, are mediated by ROS, which acts as intracellular signaling molecules. The action of ROS is controlled by the redox state of cysteine residues. Enzymatic activity is determined by the redox state of thiol groups, which activates adenylate cyclase (AC) by raising the level of intracellular cyclic AMP (cAMP). Protein kinase A (PKA) is then activated by cAMP, resulting in the activation of several downstream cell signalling pathways that are unique to particular stages of spermatozoa maturation (Thompson *et al.*, 2013). The capacitation and hyperactivation mechanism is initiated by induction of Ca^{2+} and HCO_3^- influx through inactivation of plasma membrane Ca^{2+}-ATPase and cytosolalkalization. The activation of the AC and increased synthesis of cAMP, which stimulates PKA, are mediated by calcium ions and ROS (mainly O_2^-). PKA causes serine (Ser) and tyrosine (Tyr) residues to be phosphorylated, which activates protein tyrosine kinase (PTK). The sperm flagellum cytoskeleton and the axoneme fibrous sheath following tyrosine residues are phosphorylated by PTK. ROS, particularly superoxide, are thought to constitute the penultimate step in sperm hyperactivation by promoting phosphorylation of tyrosine residues (Thompson *et al.*, 2013; Agarwal *et al.*, 2014). Furthermore, ROS may enhance the capacitation process by activating cell signalling pathways that entail an increase in intracellular cAMP levels, activation of PKA, and phosphorylation of mitogen-activated protein kinase (MEK) and downstream regulatory proteins. The cumulative effect of ROS-mediated activation of fibrous sheath proteins orchestrates the physiological process of sperm capacitation and male germ cell acquisition of the necessary potency to accomplish acrosome reaction. When sperm and oocytes fuse, ROS substantially contributes to increased plasma membrane fluidity. ROS prevent phospholipase A2 (PLA2) deactivation via inhibiting protein tyrosine phosphatase activity. Thus, PLA2 activation may cleave secondary fatty acids from membrane phospholipid triglycerides which results in increased plasma membrane fluidity (Cannarella *et al.*, 2020).

If the spermatozoa do not recognize and fertilize an egg, the prolonged production of ROS will result in a build-up of oxysterols in both the mitochondrial and plasma membranes. The inclusion of oxysterols in mitochondrial membranes may therefore result in increased mitochondrial ROS production and activation of the intrinsic apoptotic cascade. H_2O_2 diffuses across sperm membranes and interacts with transition metals to generate a highly reactive hydroxyl radical, which destroys the structural and functional characteristics of the sperm. ROS and H_2O_2 are produced by defective or dead sperm cells and phagocytic cells in an ejaculate or phagocytic cells in the female reproductive

tract (Lapointe *et al*., 2000). The predominant leukocytes in semen are granulocytes (50–60%), followed by macrophages (20–30%) and by T lymphocytes (2–5%). Granulocytes are the major generators of ROS, followed by macrophages. Furthermore, seminal plasma contains significant levels of several cytokines which are normally present in the male genital tract. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are the most important interleukins that arise during inflammatory illnesses of the genital tract, and they have a definite association with leukocytospermia. At physiological proportions, some pro-inflammatory cytokines enhance the degree of lipid peroxidation in sperm membranes, which may be significant for the sperm fecundation process. However, infection–inflammation concentrations of various cytokines, such as IL-8 and TNF-α, either alone or in the presence of leukocytes, have been shown to increase lipid peroxidation of the spermatozoa plasma membrane to levels that can decrease sperm reproductive ability (Shokri *et al*., 2014).

Because of extreme reactivity, free radicals cause damage at the cell, tissue, and organ levels by altering the structure of biomolecules such as lipids, proteins and nucleic acids. During spermiogenesis sperm DNA breaks apart to facilitate chromatin remodeling. In this context, sperm damage owing to DNA immaturity is the degree of chromatin compactness. McPherson and Longo (1992) illustrated that damaged DNA strands were found, and it was suggested that the breaks may be caused by an inaccurate replacement of histones with protamine. In addition to increased oxidative stress also causes chromatin, protein, and membrane lipid damage (Fig. 1). Histones containing oxidative modification sites such as

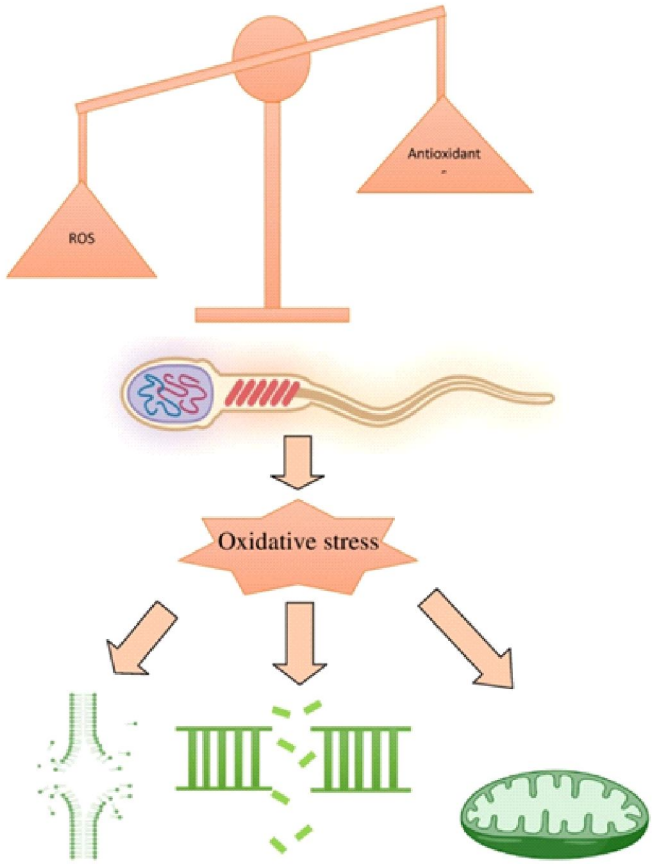

Fig. 1. Schematic representation of pathological effects of ROS on sperm causing lipid peroxidation, DNA damage and mitochondrial dysfunction

arginine and lysine may be responsible for disrupting paternal epigenomic regulation during the early phases of embryonic development, resulting in abortion.

Bacterial impact on ROS generation

In the male genital tract, gram-positive bacteria such as Staphylococci and Streptococci are the most common microorganisms. Persistent exposure of spermatozoa to bacteria results in peroxidative sperm damage, because it disturbs sperm lipid membranes during gamete maturation or transportation. Bacteria are known to draw white blood cells to the inflammatory site and activate the host's defence against infection. Activated macrophages and neutrophils are thought to be the principal sources of reactive oxygen radicals, and released inflammatory cytokines may be detrimental to spermatozoa, potentially affecting fertility. Fraczek *et al*. (2007) illustrated that *B. ureolyticus*, *S. Haemolyticus* and *E. coli* caused the greatest damage to sperm membrane lipids. Urogenital tract infection caused by *U. urealyticum* is thought to impair sperm quality and fertilizing capacity by forming H_2O_2 and hydroxide anion (OH), both of which are very toxic to sperm cells. Infection with *U. urealyticum* in the sperm results in a reduction in microelements such as zinc or selenium in the sperm fluid, which are crucial for the preservation of the sperm's antioxidative defence.

Influence of antioxidants to overcome ROS production

Antioxidants are substances that can neutralize or eliminate free radicals, keeping them from causing harm to cells. The typical shallow cytoplasm of sperm cells makes them extremely sensitive to oxidative stress because they lack the cytoplasmic enzyme repair mechanisms required for antioxidant action. Hence, antioxidants are used to protect them from harmful effects. Among all accessible antioxidants (enzymatic such as superoxide dismutase, catalase and glutathione peroxidase, and the nonenzymatic antioxidant system which includes ascorbic acid, urate, vitamin E, pyruvate, glutathione, lycopene, β-carotene, taurine and hypotaurine) vitamins C and E, carnitine, N-acetyl cysteine, selenium and zinc are the most commonly used.

Superoxide dismutase (SOD) is found in all eukaryotic cells. It lowers the steady-state level of O^{2-}. SOD is highly active in semen plasma. Similarly, catalase is found in intracellular organelles and peroxisomes. It lowers the level of H_2O_2. Catalase decomposes hydrogen peroxide into molecular oxygen and water. Catalase is involved in the activation of sperm capacitation induced by nitric oxide. Glutathione peroxidase is found in the cytoplasm and mitochondrial matrix. It also lowers the level of H_2O_2 (Pfeifer *et al*., 2001). In the epididymis and testes, the glutathione peroxidase is the primary endogenous antioxidant defense against lipid peroxidation (Mora-Esteves and Shin, 2013).

Vitamin E (α–tocopherol) is mostly present in membranes and detoxifies products of membrane lipid peroxidation (LPO) (Keskes-Ammar *et al*., 2003). It neutralizes hydroxyl free radicals and superoxide anions. vitamin E exhibits some anti-inflammatory activity, and therefore, may reduce leukocyte-initiated sperm oxidative stress. Vitamin C (ascorbic acid) is found in cytosol, chloroplast, mitochondria, peroxisome and vacuole, and detoxifies H_2O_2 (Ilić *et al*., 2018). It is active against endogenous oxidative damage such as hydroxyl, superoxide and peroxide radicals. It has been demonstrated that adding vitamin C (and vitamin E) to sperm lowers the degree of DNA breakage induced by ROS. Glutathione is located on non-protein thiol in cells and deactivates free radicals. Combined usage of vitamins C and E resulted in a considerable reduction in sperm DNA fragmentation (SDF) and a significant increase in pregnancy and implantation rates (Greco *et al*., 2005). By boosting protamine 2 binding to sperm DNA, zinc promotes sperm DNA integrity, making spermatozoa less vulnerable to oxidative

damage (Brewer *et al.*, 2002). Zinc also inhibits NADPH oxidase (an enzyme complex contained in the cell membrane and mitochondria) which is one of the important sources of ROS generation. Selenium (Se) is a trace element that is important for proper testicular growth and spermatogenesis, as well as for preserving sperm DNA from oxidative stress damage. Selenium is mainly used for the enhancement of enzymatic antioxidant activity. In a nutshell, in the context of oxidative stress, antioxidant treatment may restore normal male reproductive function.

Conclusion

The sperm plasma membrane consists of PUFA which has a fundamental role in the normal homeostasis of the male gamete such as the function and integrity of mammalian spermatozoa. The present focus of nutrition and male reproductive research is on sperm FA profiles, and the positive and negative effects of fatty acids. Understanding sperm plasma membrane mobility and the sensitivity to oxidative damage might be enhanced by having a thorough understanding of how lipid influences the composition of the sperm's lipid membrane, which is important for sperm functioning. Such data will aid in the development of specialized nutritional therapies to enhance male reproductive efficiency. It is also advantageous for the sperm to withstand the biochemical and physical changes and obstacles encountered during cryopreservation and results in a higher post-thaw quality accessible for artificial insemination.

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