Chapter

Male Infertility, Oxidative Stress and Antioxidants

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Abstract

Within the male reproductive system, oxidative stress (OS) has been identified as prevailing etiology of male infertility. The effects of reactive oxygen species (ROS) on male fertility depend on the dimensions, "modus operandi" of the ROS and the oxido-reduction potential (ORP) of the male reproductive tract. Hereupon, for an adequate response to OS, the cells of our body are endowed with a well-sophisticated system of defense in order to be protected. Various antioxidant enzymes and small molecular free radical scavengers, maintain the delicate balance between oxidants and reductants (antioxidants), crucial to cellular function and fertility. Therapeutic use of antioxidants is an optimal and coherent option in terms of mitigating OS and improving semen parameters. Therefore, recognizing and managing OS through either decreasing ROS levels or by increasing antioxidant force, appear to be a requesting approach in the management of male infertility. However, a clear defined attitude of the experts about the clinical efficacy of antioxidant therapy is still deprived. Prominently, antioxidant such as coenzyme Q10, vitamin C and E, lycopene, carnitine, zinc and selenium have been found useful in controlling the balance between ROS production and scavenging activities. In spite of that, healthy lifestyle, without smoke and alcohol, everyday exercise, reduction of psychological stress and quality well-designed meals, are habits that can overturn male infertility.

Keywords: Male infertility, reactive oxygen species, oxidative stress, antioxidants, sperm parameters

1. Introduction

The World Health Organization (WHO) defines infertility as the inability (failure) to attain clinical pregnancy after one year or more of regular unprotected sexual intercourse [1]. Since infertility presents a certain disability (impaired reproductive function), medical assessment and treatment falls under the umbrella of the United Nations Convention on the Rights of Persons with Disabilities – UNCRPD, which is formally accepted by many countries. The article 1 of this Convention summarizes the overall objective as: "to promote, protect and ensure the full and equal enjoyment of all human rights and fundamental freedoms by all persons with disabilities, and to promote respect for their inherent dignity" [2]. Due to its health, cultural and socio-economic impact, infertility is a major globally underestimated public health concern [3, 4]. Therefore, proper evaluation of male

infertility is a substantial stride in qualifying, quantifying and configuring necessary laboratory assessment, credential treatment strategies as well as policies to diminish the burden of this global sensitive health issue.

There are approximately 186 million infertile people [5] or 15% of couples globally, 50% due to male factor infertility which experience problems in conceiving [6, 7]. In male dominated societies, generally, the female partner is blamed for barrenness, even though ancient Greeks were aware that male factor is a contributor to the reproductive success [8].

In fertile couples, spontaneous conception is most likely to occur in 30% of cases during the first month, 75% after 6 months, 90% after 12 months and 95% between 18 to 24 months [9]. Also, there are studies which consider that 80% of couples having unprotected sexual intercourse will achieve pregnancy in the 6-month [10] or 12-month interval [11].

In addition, male fertility reaches its maximum potential at ages of about 25 to 30 years and declines sharply in the beginning of fifties [12], however, there are men reported to give life to offspring into their eighties [13]. Paternal age of >40 years is associated with more than 20% higher chance of congenital defects in the offspring [14]. Over the past decades, an age-related decline in semen quality resulting in declined fertility was observed [15].

Oxidative stress (OS) has been identified as one of the major contributors affecting male fertility potential [16] and has thus been extensively studied in the last three decades. Although cells of the human body have efficient mechanisms to cope with factors disturbing the normal cell homeostasis, OS may arise due to an imbalance between generation of oxidants and antioxidants mechanisms, resulting in cell damage.

Reactive oxygen species (ROS) are important mediators of OS status, because of their capacity to oxidize proteins, lipids, and DNA, resulting in cellular dysfunction [17]. ROS are oxygen-based molecules that have unpaired electrons on their most outlier spin-orbit, derived from the reaction of carbon-centered radical with oxygen (except hydrogen peroxide), which makes them highly reactive [18]. The most common ROS are hydroxyl radical (OH•), hydrogen peroxide (H_2O_2) and the superoxide anion (O_2•-). ROS are generated not only by leukocytes (neutrophils and macrophages mostly) [19], but also by any aerobe living cell including spermatozoa [20]. Moreover, another subclass of free radicals deriving from nitrogen-based molecules are called reactive nitrogen species (RNS) [21, 22]. At physiologic amount, RNS are important for various functions within the male reproductive tract such as: (1) signal transduction, (2) regulation and assembly of tight junction within the blood-testis barrier, (3) mediation of cytotoxic and pathological events, (4) production of hormones, (5) inflammation and (6) other important physiological changes of spermatozoa [23].

Some of the most common ROS and RNS are listed in **Table 1**. Effects, consequences, mode of formation and action of these molecules are presented in details in **Table 2**.

Under physiological conditions, high levels of ROS are counterbalanced by antioxidants, which competently maintain a delicate redox balance by donating their electrons to the ROS and thus interrupting further intake of electrons from surrounding compartments [24]. The seminal antioxidant system comprises a network of enzymatic and non-enzymatic molecules, dispersed mostly within seminal plasma and spermatozoa [25]. The three major antioxidant enzymes are glutathione peroxidase (GPx), catalase (CAT) and the superoxide dismutase (SOD) [26].

With an increasing knowledge on the role of OS in the clinical manifestation of male infertility, antioxidant prescription and its implementation in treating male infertility may be helpful. Several antioxidant compounds are currently prescribed

Reactive oxygen species				**Reactive nitrogen Species**	
Radicals		**Non-radicals**			
Lipid peroxyl	**$LOO^{\bullet}$**	*Lipid hydroperoxide*	**LOOH**	*Nitryl chloride*	**NO_2Cl**
Thyl	**$RS^{\bullet}$**	*Ozone*	**O_3**	*Nitrous acid*	**HNO_2**
Peroxyl	**$RO_2^{\bullet}$**	*Singlet oxygen*	**$^{-1}O_2$**	*Nitrogen dioxide*	**NO_2**
Nitric oxide	**$NO^{\bullet}$**	*Hydrogen peroxide*	**H_2O_2**	*Dinitrogen trioxide*	**N_2O_3**
Superoxide	**$O_2^{\bullet-}$**	*Hypochloric acid*	**HOCl**	*Nitroxyl anion*	**NO^{-}**
Hydroxyl	**$OH^{\bullet}$**	*Peroxynitrite*	**$ONOO^{-}$**	*Nitroxyl cation*	**NO^{+}**

Table 1.
Most common ROS and RNS.

Hydrogen peroxide (H_2O_2)	**Ref.**
Hydrogen peroxide is not a free radical, because it does not contain an unpaired electron, but it is classified as ROS because it participates in the generation of highly reactive hydroxyl free radicals through interactions with iron and copper, based on the Fenton reaction.	[22, 27–29].
Superoxide ($O_2\bullet^{-}$)	Ref.
It is generated by electron transport leaks from several reaction in cytosol. It does not spread easily and faraway its origin. It is responsible for cell injury, by deconstructing iron–sulphur clusters in proteins through the inactivation of iron regulatory protein-1. Superoxide is insoluble for the cell membrane.	[30–32]
Hydroxyl (•OH)	Ref.
This represents the neutral form of the hydroxide ion, deriving from the reaction between Fe^{2+} and H_2O_2 (Fenton reaction). It is the most reactive free radical. The hydroxyl radicals and hydroxide ions can be generated also by the reaction of H_2O_2 and $O_2\bullet$- catalyzed by iron (Haber-Weiss reaction). The hydroxyl radical has the potential of reacting fast and nonspecifically.	[33, 34]
Peroxynitrite ($ONOO^{-}$)	Ref.
It is generated during reaction of nitric oxide (NO) with O_2^{-}, it can react with thio groups of structural proteins, resulting in the formation of nitrosotioles, which can disunite metal-protein interactions and result in the formation of metal-derived free radicals.	[35]
Peroxyl radical ($ROO^{\bullet}$)	Ref.
Peroxyl radicals remove electrons from lipids during the process of lipid peroxidation. During this process, intermediates are generated that participate in further reactions with oxygen to form lipid peroxyl (LOO•) and lipid hydroperoxide (LOOH) which are responsible for sperm DNA and protein damage.	[36–38].
Hypochloric acid (HOCl)	Ref.
Hypochloric acid is produced by macrophages and neutrophils during respiratory burning that accompanies phagocytosis. This radical is generated in the reaction between H_2O_2 and chloride ion (Cl^{-}).	[39]

Table 2.
The mode of formation of the biologically active ROS responsible for the major consequences of oxidative stress.

without any scientific rationale, ensuing neither semen parameters improvement, nor fertilization outcomes. Contrary, some other studies even showed a worsening of semen parameters [40–42], because an excess intake of antioxidants can contribute in the establishment of reductive stress (RS), a condition which has been reported being as harmful as OS [43]. Therefore, there still lack of conclusive consensus regarding the clinical advantages of antioxidants - based therapy in male infertility.

2. Oxidative stress and male infertility

OS is a condition characterized by an elevated generation of ROS and a reduced response of biological mechanisms to promptly neutralize the reactive intermediates or to repair the damage [44]. An increased quantity of ROS and RNS has now been established with strict evidence to be a prominent attribute of many acute and chronic pathologies [45].

Nearly eight decades after the Macleods discovery in 1943, highlighting ROS as key players in cell physiology and sperm motility [46], scientists all over the world turned their attention toward the association between free radicals and the male infertility.

2.1 Sources of ROS

Semen comprises a variety of cells including spermatozoa, germ cells, leukocytes and epithelial cells [47], whereby leukocytes produce about 1000-times more ROS than immature sperm cells [48].

ROS originate from a different countless endogenous and exogenous sources.

Endogenous sources of ROS can be generated extracellularly and intracellularly. Intracellular ROS include O_2^{-}, H_2O_2 and OH^{-}, generated mainly in the mitochondria [49]. In the mitochondria, about 5% of the consumed oxygen is physiologically converted into ROS. The ROS production is increased when the electron transporting chain (ETC) derails as a result of mitochondrial dysfunction [50].

Exogenous sources of ROS include smoking, alcohol and drugs abuse, environmental pollutants, heavy metals, ionizing radiation, diets rich in energy-yielding nutrients like carbohydrates, saturated fats and proteins [51].

2.2 Mechanism of ROS production within human sperm

ROS are generated in two pathways: the extrinsic and the intrinsic pathway, described in **Figure 1**.

Leukocytes are responsible for the extrinsic pathway of generating ROS, while spermatozoa for the intrinsic pathway of ROS generation [52]. Granulocytes are the white blood cells (WBC) in seminal fluid which are predominantly responsible for demolishing pathogens by ROS production [53, 54].

An association between OS and the elevated leukocyte numbers has been found [19]. On the other hand, the relationship between the seminal leukocyte concentration and male infertility is not clear. In fact, leukocytospermia, i.e. the presence of more than 1×10^6 WBC/mL, is not predictive of male infertility [55, 56]. However, the significance of WBC activation in ROS generation and its impact on elevated OS levels cannot be left unnoticed. Various studies reported high levels of proinflammatory chemokines in human semen along with high ROS quantity [57, 58]. Recently, in the seminal plasma of oligozoospermic and azoospermic men it was observed a negative correlation between levels of interleukin-6 (IL-6), interferon alpha (IFN-α) and interferon gamma (IFN-γ) and sperm parameters such as concentration, motility and morphology [59, 60].

Among spermatozoa, it has been shown that morphologically abnormal spermatozoa are the main source of ROS generation [61]. Excess residual cytoplasm (ERC) around the mid-piece of spermatozoa (observed in teratozoospermic sperm) contains high levels of cytoplasmic enzymes responsible for generating ROS [62].

ERC has a considerable amount of enzymes to regulate glucose metabolism, specifically glucose-6-phosphate dehydrogenase (G6PD) [63], which induces

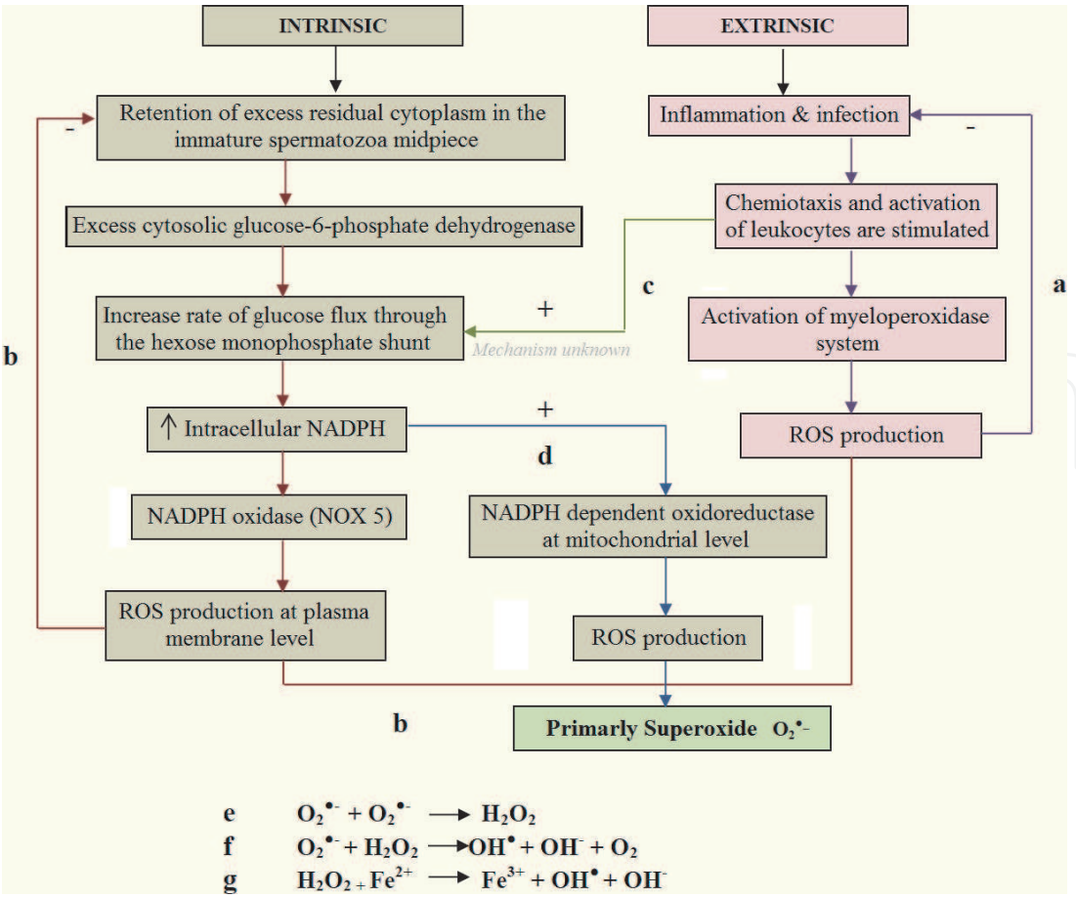

Figure 1.
Mechanism of free radical production within semen. (a) The intrinsic and extrinsic pathway contribute in the formation of $O_2^{\bullet-}$. (b) Superoxide is transformed directly and indirectly to secondary (e, f, g) ROS. Adapted from reference [31]. (mathematical symbols + and - stand for positive and negative feedback).

increased ROS levels by activating (1) the NADPH - nicotinamide adenine dinucleotide phosphate located in the plasma membrane of spermatozoa, and (2) NADPH – dependent oxide-reductase, known as diphorase, detected in the middle piece of mitochondrial level [64–66]. In a study by Sabeur et al., calcium-dependent NADPH oxidase 5 (NOX5) of spermatozoa plays a considerable role in ROS generation [67]. However, there is a difference between NOX5 found in spermatozoa, which does not require protein kinase C for expressing its activity, and in leukocytes, where protein kinase C is essential [68].

2.3 Physiological role of ROS

ROS are very important molecules as they act as cellular mediators essential for (1) normal spermatogenesis, (2) activation of steroidogenic pathway, (3) modulation of mitochondrial and death receptor-apoptotic pathways. These fundamental cascades are required for the process of: maturation, hyperactivation, capacitation, acrosome reaction as well as sperm-oocyte fusion, crucial for the fertilization process, all presented in **Figure 2**.

2.3.1 Maturation

After spermiation, spermatozoa are transported into the epididymis where they undergo a maturation process, leading to significant chemical and physiological modifications including recombination of cell-surface proteins, and enzymatic and

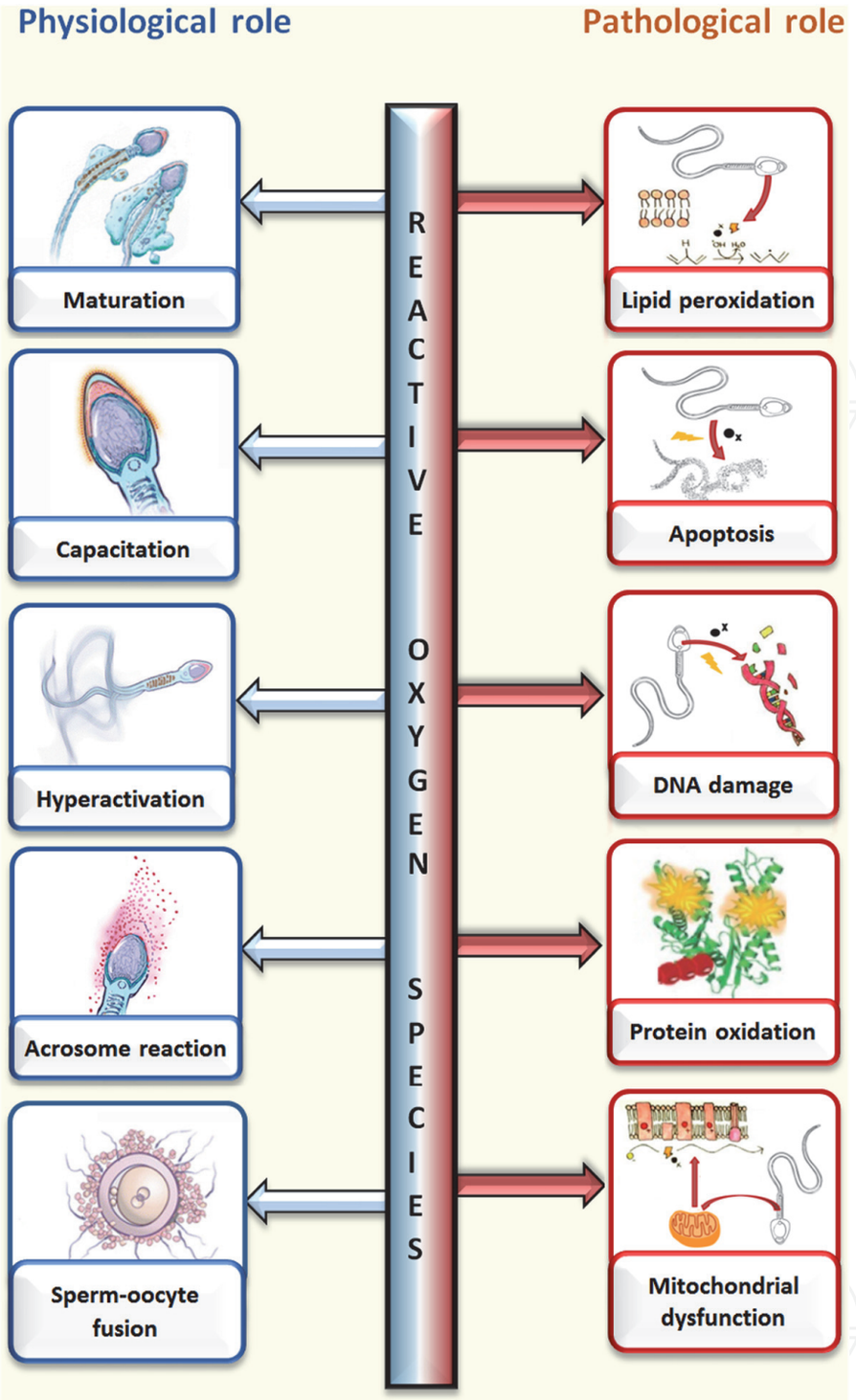

Figure 2.
Physiological and pathological consequences of ROS. ROS dose is a critical parameter in determining the ultimate cellular response, low (necessary) dose for physiological processes and high (toxicity) dose expressing their pathological effects.

nuclear modifications [69, 70]. These result in the assembly of the signal transduction machinery that is crucial for the sperm capacity to undergo hyperactivation and capacitation [69, 71]. The nuclear DNA of mammalian spermatozoa is densely packed, as histones are substituted by smaller-sized (arginine-rich) protamine [72]. Protamines substitute histones during spermiogenesis [73] and compact DNA tightly through inter/intramolecular disulphide bonds between cysteine residues [74]. The oxidizing process of thiol groups on protamines and the formation of disulfide bonds increase chromatin stability and DNA protection from any physical or chemical damage [75], which is fundamental because human spermatozoa have

limited capability to repair DNA damage [76]. Protamination occurs when spermatozoa pass through the caput and caudal part of the epididymis [77].

Another important event is the formation of "mitochondrial capsule" made by a complex protein material, which is necessary to abolish proteolytic degradation [78].

2.3.2 Hyperactivation

Hyperactivation is a particular state of sperm motility characterized by vigorous, large asymmetric flagellar (whiplash-type) beat and head sperm shifting (large lateral head displacement) [79]. Hyperactivation is reported to facilitate the capacitation process and is indispensable for successful accomplishment of acrosomal reaction, sperm-egg fusion, and fecundation [74].

Undoubtedly, ROS play an inclusive role in the regulation of these processes, by triggering hyperactivation and capacitation. This occurs by induction of Ca^{2+} and HCO_3^- influx, probably through the deactivation of the enzyme Ca^{2+}-ATPase and further basification of the cytosol [80]. ROS (especially O_2•-) upregulate the Ca^{2+} mediate adenylate cyclase (AC) enzymatic activity, increasing cAMP (cyclic adenosine monophosphate) generation by activating protein kinase A (PKA). Further, this triggers NADPH oxidase activation and thereby promotes the upregulation of ROS production [81]. PKA-mediated phosphorylation leads to protein tyrosine kinase (PTK) activation, phosphorylating consecutive tyrosine residues in the axonemal fibrous sheath and the cytoskeleton of sperm tail [69, 82].

2.3.3 Capacitation

Capacitation has been documented in 1951 by Austin and Chang [83, 84]. Capacitation involves cholesterol outflow from the sperm membrane and a global intensification of tyrosine phosphorylation [85]. The signal transduction pathway is guided by the cAMP and modulated by the oxido-reductive state [86]. During capacitation, spermatozoa undergo molecular modifications such as alkalization of inner cell pH, activation of cAMP-dependent pathways, cholesterol efflux from cell-membrane and phosphorylation of surface proteins by cAMP-dependent kinase [87]. Researchers have emphasized the impact of free radicals in modulating the cAMP pathway, which involves PKA activation and phosphorylation of its substrates [88]. A correlation between elevated protein phosphorylation rate, increased presence of the second messengers and ROS synthesis have been observed during capacitation [69]. The cholesterol oxidation and its consequent discharge from the sperm membrane is necessary in tuning-up spermatozoa for the next step, resulting in greater bicarbonate and Ca^{2+} ion permeability via activation of sodium/bicarbonate cotransporter (NBC) and ion channels [89].

2.3.4 Acrosome reaction (AR)

The hyperactivated spermatozoon tends to penetrate over the cumulus-oocyte-complex and attach to the zona pellucida of the egg, whereas acrosome reaction (AR) is a well-regulated exocytotic reaction in response to coordinated stimuli [90]. These changes are triggered by tyrosine phosphorylation of sperm-membrane proteins regulated by ROS signaling [63, 88]. NO is implicated in AR by activating the second messenger cyclic guanosine-mono-phosphate (cGMP), PKC and protein kinase G (PKG) [91]. Physiological levels of H_2O_2, O_2^- and NO are needed for AR [88]. In the oocyte, the release of Ca^{2+} is followed by cleavage of phosphatydilinositol-4,5-bisphosphonate (PIP_2) into inositol tri-phosphate (IP_3) and

diacylglycerol (DAG), which are responsible for acrosomal exocytosis and activation of PKC. This further results in Ca^{2+} inflow and activation of PLA2 (phospholipase A2), which play a key role in the cleavage of secondary fatty and consequently increasing the membrane fluidity, necessary sperm-oocyte fusion [92].

2.3.5 Sperm-oocyte fusion

ROS are also necessary in the finalization of the fertilization process. This final step is due to enhanced membrane fluidity, which is controlled and directed by ROS in inhibiting the protein tyrosine phosphatase activity, which prevents deactivation of PLA_2, a necessary step for accomplishing sperm-oocyte fusion [93]. When the spermatozoon penetrates the zona pellucida and the corona radiata, the oocyte changes the composition of the vitelline layer [27]. This envelope is catalyzed by ovoperoxidase making o,o-dityrosine crosslinks to prevent polyspermy [94].

2.4 Pathological repercussions of oxidative stress

High levels of ROS have the potential to damage cellular components by mediating lipid peroxidation, apoptosis, DNA damage, mitochondrial dysfunction and protein oxidation.

2.4.1 Lipid peroxidation (LPO)

Sperm membranes are mostly constituted by poly-unsaturated fatty acid (PUFAs), which represents a disadvantage in terms of OS susceptibility [95].

Lipid peroxidation (LPO) is as a chemical reaction by which oxidants assault carbon double bond(s) in lipid compounds, especially PUFAs, by detaching hydrogen and adding oxygen to carbon, by generating LOO• and LOOH [96]. *In vitro* research highlighted a negative correlation between malondialdehyde (MDA - end product of LPO) concentration, and sperm morphology and motility [97–99]. LPO is a self-propagating process passing through three phases: (1) initiation; (2) propagation; (3) termination. Through all three phases free radicals enter in a radical-chain reaction [35].

The propagation of the oxidative wave can also result in DNA fragmentation and protein damage, affecting particularly sperm motility, morphology and fertilizing capacity.

2.4.2 Apoptosis

The programmed cell death, known as apoptosis, is a physiological phenomenon. In the male reproductive tract, apoptosis is responsible for supervising the excess production of male gametes, a process being regulated by extrinsic and intrinsic stimuli [80]. The intrinsic stimuli include apoptosis-including genes like p53, Bax and Fas, but also Bcl-2 and c-kit genes which act as apoptosis suppressors [100], while extrinsic stimuli consist of varicocele, infection, heat stress, environmental toxins, advanced male age lifestyle factors, ionizing and nonionizing radiations, defective protamination and idiopathic causes [101, 102] . During the process of spermatogenesis, spontaneous germ cell apoptosis in all developing stages of spermatozoa has been seen in the testis of normozoospermic and non-obstructive azoospermic men [20]. This guarantees that only functionally and genetically competent germ cells become mature spermatozoa [103]. Prolactin and insulin are considered as pro-survival hormones which bind to specific receptors on sperm membrane [104]. The inhibition of this cascade will result in increased ROS

generation by mitochondria, followed by the release of cytochrome C, which in turn activates the apoptotic caspases, triggering the apoptosis [74, 82, 105]. High levels of cytochrome C have been found in seminal plasma of infertile men [82, 106].

2.4.3 DNA damage

It is reported that infertile males with high seminal OS levels present high fragmentation of sperm DNA [107]. Numerous contributors can include lifestyle factors, radiation, advanced male age, varicocele, infection and idiopathic causes [108, 109]. Guanine base (G) is the most common DNA's organic base exposed to OS assault and converts into 8-hydroxy-deoxyguanosine (8-OHdG) by free radicals [110]. Mechanisms by which OS cause DNA damage involve warping single and double-stranded DNA crosslinks, direct oxidation of DNA bases and DNA mutations [111]. Comparing to nuclear DNA, mitochondrial DNA is more susceptible to DNA damage, due to the lack of histones and protamines, and nucleotide excision repair mechanisms [112].

In addition, mitochondrial damage affectsthe interior mitochondrial membrane, causing electron outflow from the transporting chain, inducing a further increase of OS status [113].

2.4.4 Mitochondrial dysfunction

Mitochondria represent the most important place in generating ATPs, which serves as a fuel for sperm to move. This is why its proper function represents a fundamental key point in the mosaic of male infertility problems. Defects in the pathway for ATP production correlate with low sperm motility, known as asthenozoospermia [114]. There is an inactivation of genes which encode constituting proteins of the electron transport chain, mainly those that are involved in ATP formation [115]. When the extent of such injury overwhelms DNA repair capacity mechanisms, the subsequent alterations in mitochondrial biology stimulate the activation of the genes responsible for stress–response, hereby inducing apoptosis [116].

2.4.5 Protein oxidation

Formation of radical amino acids is of the result of protein oxidation (PO), especially of the alpha-central carbon, causing disintegration of peptide skeletons [117]. Moreover, the SH-rich lateral chains of methionine and cysteine are inclined to be oxidised with propagation of methionine sulphoxide and disulphides, respectively [87]. Similarly, arginine, proline, threonine and lysine are oxidised, resulting in the formation of carbonylated proteins (aldehyde and ketones), markers of PO status [117]. These alterations impact the protein morphology and physiology, with a wide impact on spermatogenesis and fertility potential.

3. Antioxidants in male infertility treatment

Antioxidants are defined as chemicals compounds with the ability to donate electrons and thereby neutralize an excessive production of ROS [118]. Humans possess a well-sophisticated antioxidant system to shelter the body's cells and tissues against oxidation [119].

As a physiological response to OS, seminal plasma is endowed with various scavengers acting enzymes indexed as total antioxidant capacity (TAC) measured to be 10x higher comparing to blood plasma [120].

The anti-oxidant defense system implicates a co-action of different endo/exogenous players to scavenge the potential oxidative damage of ROS [121]. These consist of CAT, SOD, glutathione peroxidase (GPx), peroxiredoxins and glutathione-S-transferase [122], and water-soluble and fat-soluble vitamins [123]. The role and effect of endogenous and exogenous antioxidants are discussed below.

3.1 Endogenous antioxidants

The major endogenous antioxidant enzymes are: (1) CAT, (2) SOD and (3) GPx. Studies about their efficacy in clinical trials are presented in **Table 3**.

3.1.1 Catalase

Activity of catalase (tetrameric protein) is consisted in dissolving hydrogen peroxide into water and oxygen, through the oxidation of hydrogen ion donors, such as methanol (CH_3OH), ethanol (CH_3CH_2OH), with the consumption of 1 mol of H_2O_2 [128]. In addition, CAT has an important role in terms of physiological effects during sperm capacitation, inducing NO activity and the removal of ROS [129].

3.1.2 Superoxide dismutase (SOD)

SOD is known as metallo-enzyme, as it has the catalytic metal in the active site [130]. The SOD enzyme consists of three different classes existing in both extra- and intracellular compartments. SOD-1 or CuZnSOD is the first intracellular enzyme, with Cu and Zn in the active center; it is usually localized in the cytosol [131]. SOD-2 or MnSOD is the second intracellular isoform, localizing in mitochondria and showing Mn in the active center [132]. The extracellular form of SOD (EC-SOD or SOD-3) is a glycosylated homotetramer mainly secreted into the extracellular area. It is upregulated by cytokines, downregulated by TNF-α, and anchored to the extracellular matrix [133]. CuZnSOD is highly active (75%) in comparison with SOD-3 (25%) [119, 130].

3.1.3 Glutathione peroxidase (GPX)

GPx is a cytosolic antioxidant seleno-enzyme mainly expressed in the epididymis and testis [134]. GPx catalyzes the reduction of detrimental hydroperoxides with thiol cofactors [119]. A “catalytic triad” is formed by the selenocysteine in the active site with tryptophan and glutamine: this activates the selenium portion and

Enzyme	Study findings	Ref.
CAT	• ↑ CAT activity in the group that received antioxidant therapy, comparing to control samples that received placebo. • Positive correlation between levels of CAT and fertilization rates. • Studies are limited in this field.	[124, 125]
SOD	• Its levels are positively associated with sperm concentration ($p<0.001$) and motility ($p=0.008$). • Negative relationship was found with DNA fragmentation ($p=0.014$).	[126]
GPx	• 10x greater GPx activity in the fertile group comparing with the GPx activity in infertile men. • Statistically significant ($p<0.001$).	[127]

Table 3.
The role of endogenous antioxidants enzymes.

neutralizes peroxides [135]. It is mainly expressed in the mitochondrial sperm matrix, while nuclear isoform of GPx has been correlated with sperm DNA preservation from oxidative detrimental impact and chromatin condensation [136]. GPx reduces fat hydroperoxides into alcohols and free H_2O_2 to H_2O, it is fundamental for protecting lipid integrity and maintaining sperm viability and membrane integrity [134].

3.2 Exogenous antioxidants

Most common exogenous antioxidants refer to carnitines, α-tocopherol, ascorbic acid, carotenoids, zinc and selenium. Spermatozoa carry with them minimal endogenous antioxidant amounts, thus during the entire process of spermatogenesis, sperm rely on exogenous antioxidants [137]. Studies about their efficacy in clinical trials are presented in **Table 4**.

3.2.1 Carnitines

L-carnitine (LC) and L-acetyl carnitine (LAC), a water-soluble antioxidant, are implicated in sperm metabolism, motility and viability [147]. It helps in preventing lipid peroxidation, sperm DNA protection and apoptosis [148]. The highest concentration of carnitine is found in the epididymis and spermatozoa [132]. Studies of the semen samples of infertile men, especially oligoasthenoteratozoospermic (OAT) men, have shown lower carnitine levels compared to fertile men [133].

3.2.2 Vitamin C (L-ascorbic acid)

This is a water-soluble vitamin. Humans and other vertebrates lack the enzyme L-glucono-gamma lactone oxidase (LGGLO), which is essential for *in vivo* synthesis. Hence, its intake with diet or as a supplement is fundamental. Vitamin C concentration is 10-times higher in seminal plasma comparing to serum [149]. It nullifies the activity of •OH, O_2•- and H_2O_2 radicals, thereby protecting against oxidative damage [150].

3.2.3 Carotenoids

Carotenoids can be found naturally in fruits and vegetables. Carotenoid cannot be synthesized by humans, by introduced by the diet. Lycopene, a fat-soluble aromatic carotenoid, is reported to be strong neutralizer of $^{-1}O_2$, but a combination of carotenoids seem to be more effective [151]. It can alter the levels of antioxidant enzymes by modification of the levels of ROS, making great contribution to the human antioxidant system [43, 119]. There are studies on fertile men that show high concentration of Lycopene, and reduced levels in seminal plasma of infertile men [152].

3.2.4 Coenzyme Q-10 (CoQ10)

CoQ10 is an intermediate of the mitochondrial electron transport chain [153, 154]. Low seminal plasma/sperm concentrations of CoQ10 have been associated with reduced sperm motility [155].

3.2.5 Zinc (Zn)

Zn is one of the most abundant elements in human [156]. It acts as metalloprotein cofactor in the metabolism of nucleic acids transcription, signal transduction, protein synthesis and cell death regulation [157]. Moreover, Zn is fundamental

Antioxidants	Study findings	Ref.
LC & LAC	• Analyzed in certain systematic reviews and meta-analysis. • Intake (two times daily, not more than 30 weeks) is associated with a remarkable increase in sperm motility and morphology.	[138, 139]
Vit. C	• Studies suggest positive association between levels of ascorbic acid in seminal plasma and sperm morphology and viability. • Very effective in controlling sperm agglutination. • Kobori et al. treated 169 males for 6 months with vitamin C, E and CoQ10, and reported a noteworthy improvement of sperm concentration and sperm motility.	[140, 141]
Carotenoids	• In a randomized clinical trial, Nouri et al. included 44 patients with oligozoospermia. • Tretament with 25 mg lycopene resulted in increased sperm count, concentration, total motility and TAC.	[142]
CoQ10	• Alahmar et al., study treated 65 oligoasthenozoospermic men and 40 fertile control groupwith 200 mg/day CoQ10 for 3 months. • Authors observed a significant improvement in total sperm motility, sperm concentration, TAC, and GPx levels as well as reduced SDF.	[143]
Zn	• Randomized cross-sectional study and case study, combined antioxidant formula. • Significantly correlated with sperm density ($r = 0.341$, $p < 0.0001$), motility ($r = 0.253$, $p < 0.0001$) and viability ($r = 0.286$, $p < 0.0001$) • Decrease levels of MDA, enhancing sperm motility and concentration ($p < 0.001$). • No significant change of Protein Carbonyl (PC) ($p=0.554$).	[144, 145]
Se	• Longitudinal study by Mossa et al. • Included 12 males, treated twice daily with 50 microgram in 3 months period. • Significantly increase in sperm count (39.24 ± 27.4–58.1 ± 21.6; $p<0.01$), motility (22.14 ± 12.9–50.7 ± 17.6; $p<0.01$) and morphology (68 ± 5.7–82.1 ± 6.4; $p<0.01$).	[146]

Table 4.
The role and effect of exogenous antioxidants enzymes.

for optimal sustain of spermatogenesis and adequate function of the male reproductive organs [158]. It also plays a key role in preventing LPO and preserves sperm structure, by reducing generation of H_2O_2 and •OH, through separating active redox transition metals, such as Fe and Cu [144].

3.2.6 Selenium (Se)

Se is an important trace mineral, implicated in many biological processes. Se is the constituent of enzymes such as GPx and seleno-proteins, it shows a major impact in redox defense system, spermatogenesis and increased fertility capacity in both males and females [159]. It protects sperm DNA against OS damage, although the mechanism is still unclear [160].

3.2.7 Role and effect of vitamin E in male reproduction

Vitamin E is the major lipophilic antioxidant [156] and it has been recognized as an essential nutrient for reproduction since its discovery in 1922 [161]. It neutralizes •OH and O_2•- by lessening lipid per-oxidation commenced by ROS, thus protecting cell membranes from oxidation [160]. Vitamin E ameliorates other scavenging oxidants manners and helps maintaining sperm morphology and motility (which

depends on the integrity of the mitochondrial sheath) [162]. Effects and the roles of vitamin E are presented in **Figure 3**.

Various vitamin e isoforms have been found, but their role and importance remains enigmatic, and of the eight naturally occurring forms, only α-tocopherol is maintained in the plasma [163]. Therefore, vitamin E is crucial in maintaining all the necessary functions of healthy sperm and protecting it from detrimental effects of OS. Studies show lower levels of vitamin E in infertile men compared to fertile men [135], allowing somehow to increase concentration of the peroxidation by-product MDA in the seminal fluid [164]. It is mainly used in combination with other vitamins and minerals. In vitro and in vivo studies which show improvements exclusively in the sperm motility and other semen parameters, successful pregnancies and mitigation of oxidative stress markers, presented in **Table 5**.

Vitamin E intake and its dosage should exclusively be determined by a healthcare professional because of adverse events due to vitamin toxicity. The recommended daily dose of vitamin E is 15 mg (30 IU) for adults [173], a dose of 200–800 mg/day may cause gastrointestinal distress, while a daily dose greater than 1000 mg (1500 IU) is associated with increased risk of hemorrhage (antiplatelet effects), thrombophlebitis,, elevated creatinine, gonadal dysfunction and death [163, 174].

Infertile patients which want to increase concentrations vitamin E, its sources can be found in nuts, seeds, vegetable oils, leafy vegetables and fortified cereals.

It needs proper and critical analysis for establishing the correct dosage and duration of antioxidants administration. In case of raised OS status, remedy must be administered at least for 12 weeks, according to the proper minimal period for spermatogonia (72 ± 3 days), or for three to six months [175, 176].

Referring to the studies analyzed above, vitamin E consumption has its obvious beneficial effects. But, the question here is whether vitamin E is more effective solely or in a combination? If used solely, is the efficacy more accentuated in *in vivo*

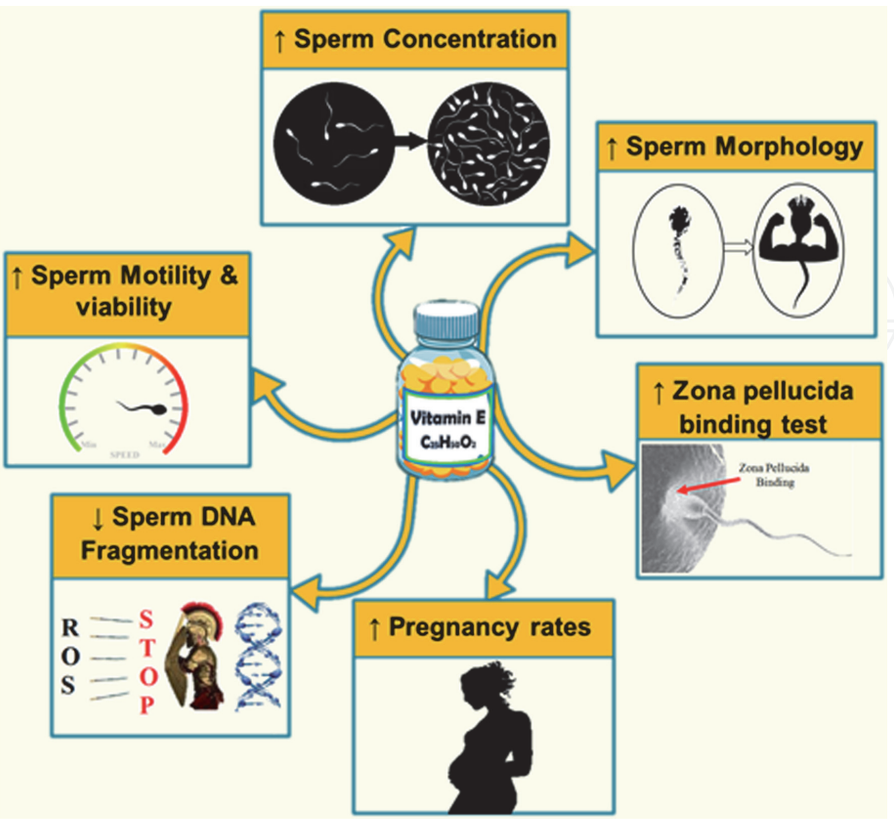

Figure 3.
Effects of vitamin E in male reproduction physiology.

Study design	Number of study subjects/abnormality	Dose/duration	Results	Ref.
Vitamin E *in vivo* studies				
Double-blind, placebo-controlled, randomized study	101 couples (50 in the vitamin E group and 51 in the placebo group)	400 mg/daily p.o	↑motility in the vitamin E group; Morphology was better in the placebo group; Statistically significant higher live-birth rate per transfer in the vitamin e group.	[165]
Randomized placebo-controlled double-blind trial	87 asthenospermic men (52 treated with vitamin E; 35 placebo treatment)	100 mg s.3x1 p.o./ 6 months	↑motility in the vitamin E group, comparing to placebo group ($p<0.001$); ↑Pregnancy (81% with a live birth); ↓ MDA levels (sperm LPO).	[162]
Randomized controlled study	45 infertile men after varicocelectomy, n=22 receiving vitamin E and n=23 control group without supplementation.	300 mg s.2x1 p.o./ 12 months	No significant differences were found in terms of sperm count, sperm motility and pregnancy rates comparing to control group.	[166]
Vitamin E *in vitro* studies				
Double-blind randomized placebo cross-over controlled trial	30 healthy men with high levels of ROS in semen.	300 mg s.2x1 p.o./ 3 months	Improvement of the performance of the spermatozoa in the zona pellucida binding test ($p=0.004$); No significant effect was demonstrated in the conventional semen parameters and levels of ROS;	[167]
Evaluation study	43 subjects, normal (n=23) and abnormal (n=20).	100 or 200 μmol Vitamin E to cryopres-ervation medium	↑ post-thaw motility ($p=0.041$); No improvements in sperm vitality and the degree of DNA fragmentation.	[168]
Experimental study	50 asthenoterato-zoospermic men	2 mM (milli-molar) vitamin E.	Significantly higher total sperm motility ($p<0.001$), progressive motility ($p<0.001$) and viability ($p<0.001$) compared with control group after 2, 4 and 6 hours of incubation; MDA levels were decreased significantly after 6 hours ($p<0.001$).	[169]
Vitamin E in combination with one or more vitamins				
Randomized controlled trial	54 voluntary infertile men	Vit. E 100 mg s.2x2/3 months Selenium 35 μg s.3x2/3 months	Significant improve in sperm motility ($p<0.05$), without significant effects on other parameters; Significant decrease in the MDA concentration.	[170]
Comparative prospective randomized trial	90 idiopathic oligoastheno-zoospermic men	Vit. E 400 mg s.1x1/6 months Clomiphene	Significant increase in sperm concentration ($p=0.001$); Improvement in the mean	[171]

Study design	Number of study subjects/abnormality	Dose/duration	Results	Ref.
		citrate 25 mg s.1x1/6 months	total sperm motility ($p<0.001$).	
Randomized controlled trial	60 asthenozoospermic men	Vit. E 400 mg s.1x1/2 months Vit. C 1000 mg s.1x1/2 months	Increased sperm total motility ($p\leq0.05$); No significant effect on other parameters.	[172]

Table 5.
The role and effect of vitamin E solely and in combination.

or *in vitro* studies? Data presented above from different studies demonstrate the complexity and the unpredictability of vitamin E or antioxidant supplementation, even though there are studies that suggest improvements in sperm parameters, decrease of oxidative stress status, improvements in zona pellucida binding test and higher pregnancy rates.

Vitamin E doesn't work only as an antioxidant, but it is also involved in the modulation of cellular responses by modulating enzymes or by regulating the activity of specific transcription factors [173, 177].

4. Conclusion

ROS are very important in certain physiological processes; however they can be very dangerous for male fertility potential if the levels overcome a physiological threshold.

Therefore, normal fine redox equilibrium between ROS and antioxidants is extremely important. The understanding of this fine balance will facilitate steps towards proper diagnosis and treatment in ideal dosages of antioxidant treatment.

The most widely utilized antioxidants either as single therapy or combined are: vitamin C, E, NAC, carnitines, CoQ10, zinc, selenium, and lycopene.

According to current literature we can conclude that vitamin E used alone is more effective when used for *in vitro* procedures, and very effective used in a dual, triple or more combinations in terms of sperm parameters and oxidative stress status.

Further augmentative clinical trials are needful to ascertain the right and effective antioxidant combination, for reliable and appropriate guiding of this sensitive medical issue.

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References

[1] Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, Vanderpoel S; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009;92(5):1520–4. DOI: 10.1016/j.fertnstert.2009.09.009.

[2] Convention on the Rights of Persons with Disabilities. 2007. Accessed on 30th December, 2020. URL: https://www.un.org/esa/socdev/enable/rights/convtexte.htm

[3] McDonald Evens E. A global perspective on infertility: An under recognized public health issue. Carolina Papers International Health.2004; 18: 1–42 http://cgi.unc.edu/research/pdf/Evens.pdf

[4] Mumtaz Z, Shahid U, Levay A. Understanding the impact of gendered roles on the experiences of infertility amongst men and women in Punjab. Reprod Health. 2013;10: 3. DOI: 10.1186/1742-4755-10-3

[5] Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015; 21: 411–426. DOI: 10.1093/humupd/dmv016

[6] Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, Schlegel PN, Howards SS, Nehra A, Damewood MD, Overstreet JW, Sadovsky R. Best practice policies for male infertility. Fertil Steril. 2002;77(5):873–82. DOI: 10.1016/s0015-0282(02)03105-9.

[7] Hamada AJ, Montgomery B, Agarwal A. Male infertility: a critical review of pharmacologic management. Expert Opin Pharmacother. 2012; 13: 2511–2531. DOI: 10.1517/14656566.2012.740011.

[8] Zhaku V, Beadini Sh, Beadini N, Xhaferi V, Golaboska J. The role of semen analysis in the expression of male infertility in southwestern part of North Macedonia (Experiences from 7 municipalities). UNIVERSI–International Journal of Education Science Technology Innovation Health and Enviroment. 2019;5(2):96–104.

[9] Taylor A. ABC of subfertility: extent of the problem. BMJ. 2003;327(7412): 434–436. DOI:10.1136/bmj.327.7412.434

[10] Gnoth C, Godehardt D, Godehardt E, Frank-Herrmann P, Freundl G. Time to pregnancy: results of the German prospective study and impact on the management of infertility. Hum Reprod. 2003;18(9): 1959–66. DOI: 10.1093/humrep/deg366.

[11] Zhao Y., Kolp L., Yates M., Zacur H. Clinical Evaluation of Female Factor Infertility. In: Carrell D., Peterson C. (eds) Reproductive Endocrinology and Infertility. Springer, New York, 2010. DOI:10.1007/978-1-4419-1436-1_10

[12] Samal S, Dhadwe K, Gupta U. Epidemiological Study of Male Infertility. Indian Medical Gazette, 2012;5:174–80. DOI: 10.18203/2320-1770.ijrcog20161710

[13] Chandra A, Mosher WD. The demography of infertility and the use of medical care for infertility. Infertility and Reproductive Medicine Clinics of North America 1993; 52(2): 283–296. DOI: 10.1016/j.fertnstert.2015.10.007

[14] Lian Z, Zack M. M, Erickson J.D. Paternal age and the occurrence of birth defects. Am J Hum Genet. 1986; 39: 648–660 PMCID: 1684057.

[15] Sengupta P, Borges E Jr, Dutta S, Krajewska-Kulak E. Decline in sperm count in European men during the past 50 years. Hum Exp Toxicol. 2018; 37(3): 247–255. DOI: 10.1177/ 0960327117703690.

[16] Aggarwal R, Puri M, Dada R, Saurabh G. Correlation between leukocytospermia and oxidative stress in male partners of infertile couples with leukocytospermia. Int J Reprod Contracept Obstet Gynecol 2015;4:168–72. DOI: 10.5455/2320-1770.ijrcog 20150230.

[17] Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. Circ Res. 2018 Mar 16;122(6):877–902. DOI: 10.1161/CIRCRESAHA.117.311401.

[18] Papa S, Skulachev VP. Reactive oxygen species, mitochondria, apoptosis and aging. Mol Cell Biochem. 1997; 174 (1–2):305–19. PMID: 9309704.

[19] Sharma RK, Pasqualotto AE, Nelson DR, Thomas AJ Jr, Agarwal A. Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. J Androl. 2001; 22(4):575–583.PMID: 11451354.

[20] Agarwal A, Saleh RA, and Bedaiwy MA: Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003; 79(4): 829–843. DOI: 10.1016/s0015-0282(02) 04948-8.

[21] Sikka SC: Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001; 8(7): 851–62. DOI: 10.2174/ 0929867013373039.

[22] Agarwal A, Prabakaran S, Allamaneni S. What an andrologist/ urologist should know about free radicals and why. Urology.2006;67:2–8. DOI:10.1016/j.urology.2005.07.012

[23] Doshi S. B, Khullar K., Sharma R. K., Agarwal A. Role of reactive nitrogen species in male infertility. Reproductive Biology and Endocrinology. 2012; 10, 109. DOI:10. 1186/1477–7827–10-109

[24] Alahmar AT. The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia. Clin Exp Reprod Med. 2018; 45(2):57–66. DOI:10.5653/cerm.2018.45.2.57.

[25] Huang C, Cao X, Pang D, et al. Is male infertility associated with increased oxidative stress in seminal plasma? A-meta analysis. Oncotarget. 2018;9(36):24494–24513. Published 2018 May 11. DOI:10.18632/ oncotarget.25075

[26] Nakamura BN, Lawson G, Chan JY, Banuelos J, Cortés MM, Hoang YD, Ortiz L, Rau BA, Luderer U. Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. Free Radic Biol Med. 2010; 49(9):1368–1379. DOI:10. 1016/j.freeradbiomed.2010.07.019

[27] Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJ. Free radical biology and medicine: it's a gas, man! Am J Physiol Regul Integr Comp Physiol. 2006;291 (3):R491–511. DOI: 10.1152/ ajpregu.00614.2005.

[28] Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem J. 1991;273 (Pt 3):601–604. DOI:10.1042/bj2730601

[29] Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev. 1995;7(4):659–68. DOI: 10.1071/rd9950659.

[30] Tvrdá E, Massanyi P, Lukáč N. Physiological and Pathological Roles of Free Rad-icals in Male Reproduction In: Rosaria Meccariello, Rosanna Chianese

(Eds.), Sperma-tozoa - Facts and Perspectives. 2018. DOI:10.5772/intechopen.70793

[31] Lampiao F, Opperman CJ, Agarwal A, du Plessis SS. Oxidative stress. In: S.J. Parekattil and A. Agarwal (eds.), Male Infertility: Contemporary Clinical Approaches, Andrology, ART & Antioxidants, 1st edn. Springer, New York, 2012; 225–235. DOI: 10.1007/978-1-4614-3335-4_22.

[32] Schrader M, Fahimi HD. Mammalian peroxisomes and reactive oxygen species. Histochem Cell Biol. 2004;122(4):383–93. DOI: 10.1007/s00418-004-0673-1.

[33] Aprioku J.S. Pharamacology of free radicals and the impact of reactive oxygen species on the testis. Journal of reproduction & infertility. 2013; 14(4), 158–172. PMCID: 3911811.

[34] Mesa-Garcia M. D, Plaza-Diaz,J, Gomez-Llorente C. Molecular Basis of Oxidative Stress and Inflammation. In: Del Moral A.M, Garcia C.M.A. Obesity: Oxidative stress and dietary antioxidants. Elsevier. 2018; 41–62. DOI: 10.1016/b978-0-12-812504-5.00003-9.

[35] Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of Aprioku JS therapeutics. Nat Rev Drug Discov. 2007;6(8):662–80. DOI: 10.1038/nrd2222

[36] Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxid Med Cell Longev. 2019 Oct 13; 2019:5080843. DOI: 10.1155/2019/5080843.

[37] Desai N, Sabanegh Jr, Kim E t, Agarwal A. Free radical theory of aging: Impli-cations in male infertility. Urology. 2010; 75:14–19. DOI:10.1016/j.urology.2009.05.025

[38] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation. In: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014; 1–31. DOI: 10.1155/2014/360438.

[39] Kothari S, Thompson A, Agarwal A, du Plessis SS. Free radicals: their beneficial and detrimental effect on sperm function. Indian J EXP Biol. 2010; 48(5):425–35. PMID: 20795359.

[40] Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. Hum Reprod.1999; 14: 1028–1033. DOI: 10.1093/humrep/14.4.1028

[41] Silver EW, Eskenazi B, Evenson DP, Block G, Young S, Wyrobek AJ. Effect of antioxidant intake on sperm chromatin stability in healthy nonsmoking men. J Androl. 2005; 26: 550–556. DOI: 10.2164/jandrol.04165

[42] Menezo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, Chapuis F, Clement P, Benkhalifa M. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. Reprod Biomed Online. 2007; 14: 418–421. DOI: 10.1016/s1472-6483(10)60887-5

[43] Castagne V, Lefevre K, Natero R, Clarke PG, Bedker DA. An optimal redox status for the survival of axotomized ganglion cells in the developing retina. Neuroscience. 1999; 93: 313–320. DOI: 10.1016/s0306-4522(99)00138-4

[44] Madkour LH. Nanoparticles induce oxidative and endoplasmic reticulum stresses. Springer, Cham, Switzerland.2020; 329–401. DOI: 10.1007/978-3-030-37297-2

[45] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. New York: Oxford University Press; 1999. DOI:10.1093/acprof:oso/9780198717478.001.0001

[46] MacLeod J. The role of oxygen in the metabolism and motility of human spermatozoa. Am J Physiol.1943;138: 512–518. DOI:10.1152/ajplegacy.1943.138.3.512

[47] Aitken RJ, West KM. Analysis of the relationship between reactive oxygen species production and leucocyte infiltrationin fractions of human semen separated on Percoll gradients, Int J Androl. 1990; 13(6): 433–51. DOI: 10.1111/j.1365-2605.1990.tb01051.x.

[48] Plante M, de Lamirande E, Gagnon C. Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. Fertil Steril. 1994; 62: 387–393. DOI:10.1016/s0015-0282(16)56895-2.

[49] Ritchie C, Ko EY. Oxidative stress in the pathophysiology of male infertility. Andrologia.2020;00:e13581. DOI: 10.1111/and.13581.

[50] Hayyan M, Hashim M. A, Al Nashef I. M. Superoxide ion: Generation and chemical implications. Chemical Reviews. 2016; 116(5), 3029–3085. DOI:10.1021/acs. chemr ev.5b00407

[51] Barazani Y, Katz BF, Nagler HM, Stember DS. Lifestyle, environment, and male reproductive health. Urol Clin North Am. 2014;41(1):55–66. DOI: 10.1016/j.ucl. 2013.08.017.

[52] Garrido N, Meseguer M, Simon C, Pellicer A, Remohi J. Prooxidative and anti-oxidative imbalance in human semen and its relation with male fertility. Asian J Androl. 2004;6(1): 59–65. PMID: 15064836.

[53] Wolff H. The biologic significance of white blood cells in semen. Fertil Steril. 1995; 63: 1143–1157. DOI: 10.1016/s0015-0282(16)57588-8

[54] Depuydt CE, Bosmans E, Zalata A, Schoonjans F, Comhaire FH. The relation between reactive oxygen species and cytokines in andrological patients with or without male accessory gland infection. J Androl. 1996; 17: 699–707.PMID: 9016401

[55] Rodin DM, Larone D, Goldstein M. Relationship between semen cultures, leukospermia, and semen analysis in men undergoing fertility evaluation. Fertil Steril. 2003;79 Suppl 3:1555–8. DOI: 10.1016/s0015-0282(03)00340-6.

[56] World Health Organization. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. New York: Cambridge University Press, 1999

[57] Martinez P, Proverbio F, Camejo MI. Sperm lipid peroxidation and proinflammatory cytokines. Asian J Androl. 2007;9(1):102–7. DOI: 10.1111/j.1745-7262.2007.00238.x.

[58] Nandipati KC, Pasqualotto FF, Thomas Jr AJ, Agarwal A. Relationship of interleukin-6 with semen characteristics and oxidative stress in vasectomy reversal patients. Andrologia. 2005;37(4):131–4. DOI: 10.1111/j.1439-0272.2005.00668.x.

[59] Paradisi R, Mancini R, Bellavia E, Beltrandi E, Pession A, Venturoli S, Flamigni C. T-helper 2 type cytokine and soluble interleukin-2 receptor levels in seminal plasma of infertile men. Am J Reprod Immunol. 1997;38(2):94–9. DOI: 10.1111/j.1600-0897.1997.tb00282.x.

[60] Fujisawa M, Fujioka H, Tatsumi N, Inaba Y, Okada H, Arakawa S, Kamidono S. Levels of interferon alpha and gamma in seminal plasma of normozoospermic, oligozoo-spermic,

and azoospermic men. Arch Androl. 1998;40(3):211–4.DOI: 10.3109/014850 19808987944.

[61] Tomlinson M, White A, Barratt C, Bolton A, Cooke I. The removal of morphologically abnormal sperm forms by phagocytes: a positive role for seminal leukocytes? Hum Reprod. 1992; 7:517–522. DOI: 10.1093/oxfordjournals.humrep.a 137682.

[62] Gomez E, Buckingham DW, Brindle J, Lanzafame F, Irvine DS, Aitken RJ. Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: correlation with biochemical markers of the cytoplasmic space, oxidative stress, and sperm function. J Androl. 1996; 17(3):276–87. DOI: 10.1002/j.1939-4640.1996.tb01783.x.

[63] Agarwal A., Sengupta P. Oxidative Stress and Its Association with Male Infertility. In: Parekattil S, Esteves S, Agarwal A. (Eds) Male Infertility. Springer, Cham, 2020. DOI:/10.1007/978-3-030-32300-4_6.

[64] Park J, Rho HK, Kim KH, Choe SS, Lee YS, Kim JB. Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. Mol Cell Biol. 2005;25(12):5146–57. DOI: 10.1128/MCB.25.12.5146-5157.2005.

[65] Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. Etiologies of sperm oxidative stress. Int J Reprod Biomed. 2016;14(4):231–40. PMID: 27351024;

[66] Siegel D, Gibson NW, Preusch PC, Ross D. Metabolism of diaziquone by NAD(P)H: (quinone acceptor) oxidoreductase (DT-diaphorase): role in diaziquone-induced DNA damage and cytotoxicity in human colon carcinoma cells. Cancer Res. 1990; 50(22):7293–300. PMID: 2121335.

[67] Sabeur K, Ball BA. Characterization of NADPH oxidase 5 in equine testis and spermatozoa. Reproduction. 2007; 134 (2):263–70. DOI: 10.1530/REP-06-0120.

[68] Ghanbari H, Keshtgar S, Kazeroni M. Inhibition of the CatSper channel and NOX5 enzyme activity affects the functions of the progesterone-stimulated human sperm. Iran J Med Sci. 2018;43(1): 18–25. PMID: 29398748.

[69] Thompson A, Agarwal A, Du Plessis SS. Physiological role of reactive oxygen species in sperm function: a review. In: Parekattil SJ, Agarwal A, editors. Antioxidants in male infertility: a guide for clinicians and researchers. New York, USA: Springer Science and Business Media; 2013. p. 69–89.

[70] Gil-Guzman E, Ollero M, Lopez MC, Sharma R.K, Alvarez J,G, Thomas Jr A.J, Agarwal A. Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. Hum Reprod Update. 2001;16(9):1922–39. DOI: 10.1093/humrep/16.9.1922

[71] Aitken RJ, Ryan AL, Baker MA, McLaughlin EA. Redox acitivity associated with the maturation and capacitation of mammalian spermatozoa. Free Radic Biol Med. 2004;36(8):994–1010. DOI: 10.1016/j.freeradbiomed.2004.01.017

[72] Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi P, Bianchi U. Origin of DNA damage in ejaculated human spermatozoa. Rev Reprod. 1999;4:31–7. DOI: 10. 1530/ror.0.0040031.

[73] Bao, J, Bedford, M. Epigenetic regulation of the histone-to-protamine transition during spermiogenesis, *Reproduction.*2016; *151(*5):55–70. DOI: 10.1530/REP-15-0562

[74] Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and

management, Arab Journal of Urology. 2019; 17:2, 87–97. DOI:/10.1080/2090598X.2019.1599624.

[75] Rousseaux J, Rousseaux-Prevost R. Molecular localization of free thiols in human sperm chromatin. Biol Reprod. 1995;52(5):1066–72. DOI: 10.1095/biolreprod52.5.1066.

[76] De Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa - I. Effects on the motility of intact spermatozoa and on sperm axonemes. J Androl. 1992; 13(5):368–78. PMID: 1331006.

[77] Saowaros W, Panyim S. The formation of disulfi de bonds in human protamines during sperm maturation. Experientia. 1978;35(2):191–2. DOI: 10.1007/BF01920608.

[78] Roveri A, Ursini F, Flohe L, Maiorino M. PHGPx and spermatogenesis. Biofactors. 2001; 14: 213–22. DOI:/10.1002/biof.5520140127.

[79] Suarez SS. Control of hyperactivation in sperm. Hum Reprod Update. 2008;14(6): 647–57. DOI: 10.1093/humupd/dmn029

[80] Parekattil S, Esteves C, Agarwal A, editors. Male Infertility Contemporary Clinical Approaches, Andrology, ART and Antioxidants: Contemporary Clinical Approaches, Andrology, ART and Antioxidants. 2 ed. Springer; 2020. 61p. DOI: 10.1007/978-3-030-32300-4.

[81] Dutta, S., Henkel, R., Sengupta, P., & Agarwal, A. Physiological role of ROS in sperm function. In S. J. Parekattil, S. C. Esteves, & A. Agarwal (Eds.), Male infertility: Contemporary clinical approaches, Andrology, ART and antioxidants. 2020; 2:337–345. Cham, Switzerland:Springer. DOI: 10.1007/978-3-030-32300-4_27].

[82] Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32(1):1–17. DOI: 10.5534/wjmh.2014.32.1.1

[83] Austin, C. R. Observations of the penetration of sperm into the mammalian egg. Australian Journal of Scientific Research. 1951; Series B, 4, 581–596. DOI: 10.1071/bi9510581.

[84] Chang M.C. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature. 1951;168, 697–698. DOI: 10.1038/168697b0.

[85] Visconti P. E, Bailey J. L, Moore G. D, Pan D, Olds-Clarke P, Kopf G. S. Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. Development. 1995; 121, 1129–1137. PMID: 7743926.

[86] Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Dev. 2017;84:1039–1052. DOI:10.1002/mrd.22871

[87] Baskaran S, Finelli R, Agarwal A, Henkel R. Reactive oxygen species in male reproduction: A boon or a bane? Andrologia. 2020;00:e13577. DOI: 10.1111/and.13577.

[88] Du Plessis SS, Agarwal A, Halabi J, Tvrda E. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. J Assist Reprod Genet. 2015;32(4): 509–20. DOI: 10.1007/s10815-014-0425-7.

[89] Suzuki F, Yanagimachi R. Changes in the distribution of intramembranous particles and filipin-reactive membrane sterols during in vitro capacitation of golden hamster spermatozoa. Gamete Research. 1989; 23(3), 335–347. DOI: 10.1002/mrd. 1120230310.

[90] Fujii J, Tsunoda S. Redox regulation of fertilisation and the spermatogenic

process. Asian J Androl. 2011;13(3):420–3. DOI: 10.1038/aja.2011.10.

[91] Revelli A, Costamagna C, Moffa F, Aldieri E, Ochetti S, Bosia A, ... Ghigo D. Signaling pathway of nitric oxide-induced acrosome reaction in human spermatozoa 1. Biology of Reproduction. 2001; 64(6), 1708–1712. DOI: 10.1095/biolreprod64.6.1708.

[92] O'Flaherty C, Breininger E, Beorlegui N, Beconi M. T. Acrosome reaction in bovine spermatozoa: Role of reactive oxygen species and lactate dehydro-genase C4. Biochimica et Biophysica Acta. 2005;1726:96–101. DOI: 10.1016/j.bbagen.2005.07.012

[93] Khosrowbeygi A, Zarghami N. Fatty acid composition of human spermatozoa and seminal plasma levels of oxidative stress biomarkers in subfertile males. Prostaglandins Leukot Essent Fatty Acids. 2007;77(2):117–21. DOI: 10.1016/j.plefa.2007.08.003.

[94] Min L. The biology and dynamics of mammalian cortical granules. Reproductive Biology and Endocrinology. 2011;9:149. DOI: 10.1186/1477-7827-9-149.

[95] Bal R, Türk G, Tuzcu M, Yılmaz Ö, Kuloğlu T, Baydaş G, Naziroğlu M, Yener Z, Etem E, Tuzcu Z. Effects of the neonicotinoid insecticide, clothianidin, on the reproduct-ive organ system in adult male rats. Drug Chem Toxicol. 2013;36(4):421–9. DOI: 10.3109/01480545.2013.776575.

[96] H Yin, L Xu, N. A. Porter. "Free radical lipid peroxidation: mechanisms and analysis," Chemical Reviews. 2011; 111(10):5944–5972. DOI: 10.1021/cr200084z.

[97] Jedrzejczak P, Fraczek M, Szumała-Kakol A, Taszarek-Hauke G, Pawelczyk L, Kurpisz M. Consequences of semen inflammation and lipid peroxidation on fertilization capacity of spermatozoa in in vitro conditions. Int J Androl. 2005; 28(5):275–83. DOI: 10.1111/j.1365-2605.2005.00547.x.

[98] Colagar A. H., Pouramir M., Marzony E. T., Jorsaraei S. G. A. Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. Braz arch biol technol. 2009; 52(6):1387–1392. DOI:10.1590/S1516-89132009000600010.

[99] Hsieh Y. Y, Chang C. C, Lin C. S. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. International Journal of Biological Sciences. 2006; 2(1), 23–29. DOI: 10.7150/ijbs.2.23.

[100] Sinha HAP, Swerdloff RS. Hormonal and genetic control of germ cell apoptosis in the testis. Rev Reprod. 1999;4:38–47. DOI:10.1530/ror.0.0040038.

[101] Gunes S, Al-Sadaan M, Agarwal A. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. Reprod Biomed Online. 2015; 31(3):309–19. DOI: 10.1016/j.rbmo.2015.06.010.

[102] Ahmad G, Agarwal A. Ionizing radiation and male fertility. In: Male infertility: New York, USA: Springer; 2017. p. 185–96. DOI: 10.1007/978-3-030-32300-4.

[103] Henkel R, Samanta L, Agarwal A. Oxidants, antioxidants, and impact of the oxidative status in male reproduction. London, UK: Elsevier. 2018.

[104] Carpino A, Rago V, Guido C, Casaburi I, Aquila S. Insulin and IR-beta in pig spermatozoa: A role of the hormone in the acquisition of fertilizing ability. International Journal of Andrology. 2010; 33:554–562. DOI: 10.1111/j.1365-2605.2009.00971.x

[105] Aitken RJ, Baker MA, Nixon B. Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress? Asian J Androl. 2015;17 (4):633–9. DOI: 10.4103/ 1008-682X.153850.

[106] Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev. 2016; 28(1–2):1–10. DOI: 10.1071/RD15325.

[107] Erenpreiss J, Elzanaty S, Giwercman A. Sperm DNA damage in men from infertile couples. Asian J Androl. 2008;10(5):786–90. DOI: 10.1111/j.1745-7262.2008.00417.x.

[108] Gunes S, Al-Sadaan M, Agarwal A. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. Reprod Biomed Online. 2015; 31(3):309–19. DOI: 10.1016/j. rbmo.2015.06.010.

[109] Ahmad G, Agarwal A. Ionizing radiation and male fertility. In: Male infertility: New York, USA: Springer; 2017. p. 185–96. DOI: 10.1007/978-3-030-32300-4.

[110] Butkowski E. Oxidative stress markers in diabetes. In: Preedy VR (ed) Diabetes (2nd edition). Academic Press, 2020:3–11. DOI:10.1016/B978-0-12-815776-3.00001-2.

[111] Sharma R. K, Said T, Agarwal A. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian Journal of Andrology. 2004; 6(2), 139–148. PMID: 15154089

[112] Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA. 1988;85(17):6465–7. DOI: 10.1073/pnas. 85.17.6465.

[113] Bui A, Sharma R, Henkel R, Agarwal A. Reactive oxygen species impact on sperm DNA and its role in male infertility. Andrologia. 2018; 50 (8):e13012. DOI: 10.1111/and. 13012.

[114] Folgerø T, Bertheussen K, Lindal S, Torbergsen T, Oian P. Mitochondrial disease and reduced sperm motility. Hum Reprod. 1993;8(11):1863–8. DOI: 10.1093/oxfordjournals.humrep. a137950.

[115] Nowicka-Bauer K., Lepczynski A, Ozgo M, Kamieniczna M, Fraczek M, Stanski L, ... Kurpisz M. K. Sperm mitochondrial dysfunction and oxidative stress as possible reasons for isolated asthenozoospermia. Journal of Physiology and Pharmacology. 2018; 69 (3), 403–417. DOI: 10.26402/ jpp.2018.3.05.

[116] Kumar D. P, Sangeetha N. Mitochondrial DNA mutations and male infertility. Indian Journal of Human Genetics. 2009; 15(3), 93–97. DOI: 10.4103/0971-6866.60183.

[117] Gao S, Li C, Chen L, Zhou X. Actions and mechanisms of reactive oxygen species and antioxidative system in semen. Molecular and Cellular Toxicology. 2017; 13(2), 143–154. DOI: 10.1007/s13273-017-0015-8.

[118] Halliwell B. How to characterize an antioxidant: an update. Biochem Soc Symp. 1995;61:73–101. DOI: 10.1042/ bss0610073.

[119] Adewoyin M, Ibrahim M, Roszaman R, Isa M.L.M, Alewi N.A.M, Rafa A.A.A, Anuar M.N.N. Male Infertility: The Effect of Natural Antioxidants and Phytocompounds on Seminal Oxidative Stress. *Diseases*. 2017; 5, 9. DOI: 10.3390/diseases5010009.

[120] Rhemrev J.P, van Overveld F.W, Haenen G.R, Teerlink T, Bast A, Vermeiden J.P. Quantification of the nonenzymatic fast and slow TRAP in a post-addition assay in human seminal plasma and the antioxidant

contributions of various seminal compounds. J. Androl. 2000;21: 913–920. PMID: 11105918.

[121] Sheweita S.A, Tilmisany A.M, Al-Sawaf H. Mechanisms of male infertility: Role of antioxidants. Curr Drug Metabol. 2016; 6:495–501. DOI: 10.2174/1389200057743305 94

[122] Lazzarino G, Listorti I, Bilotta G, Capozzolo T, Amorini A.M, Longo S, Caruso G, Lazzarino G, Tavazzi B, Bilotta P. Water- and fat-soluble antioxidants in human seminal plasma and serum of fertile males. Antioxidants. 2019; 8(4), 96. DOI: 10.3390/antiox 8040096

[123] O'Flaherty C. Peroxiredoxins: Hidden players in the antioxidant defence of human spermatozoa. Basic Clin. Androl. 2014; 24, 4.DOI: 10.1186/2051-4190-24-4.

[124] Ali M, Martinez M, Parekh N. Are antioxidants a viable treatment option for male infertility? Andrologia. 2020; 00:e13644. DOI: 10.1111/and.13644

[125] Rubio-Riquelme N, Huerta-Retamal N, Gómez-Torres MJ, Martínez-Espinosa RM. Catalase as a Molecular Target for Male Infertility Diagnosis and Monitoring: An Overview. Antioxidants (Basel). 2020;9 (1):78. DOI:10.3390/antiox9010078

[126] Yan L, Liu J, Wu S, Zhang S, Ji G, Gu A. Seminal superoxide dismutase activity and its relationship with semen quality and SOD gene polymorphism. J Assist Reprod Genet. 2014;31(5):549–554. DOI:10.1007/s10815-014-0215-2

[127] Giannattasio A, De Rosa M, Smeraglia R, Zarrilli S, Cimmino A, Di Rosario B, Ruggiero R, Colao A, Lombardi G. Glutathione peroxidase (GPX) activity in seminal plasma of healthy and infertile males. J Endocrinol Invest. 2002;25(11):983–6. DOI: 10.1007/BF03344072.

[128] Aebi H. [13] Catalase *in vitro*, Methods in enzymology. 1984; 105:121–126. DOI:10.1016/S0076-6879(84)05016-3

[129] De Lamirande E, Leclerc P, Gagnon C. Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. Molecular Human Reproduction. 1997; 3 (3):175–194. DOI: 10.1093/molehr/3.3.175

[130] Griveau J. F, Le Lannou D. Reactive oxygen species and human spermatozoa: Physiology and pathology. International Journal of Andrology.1997; 20(2), 61–69. DOI: 10.1046/j.1365-2605.1997.00044.x.

[131] R Peeker, L Abramsson, S L Marklund, Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. *Molecular Human Reproduction.* 1997; 3(12): 1061–1066. DOI: 10.1093/molehr/3.12.1061

[132] Agarwal A, Leisegang K, Majzoub A, Henkel R, Finelli R, … , Shah R. Utility of Antioxidants in the Treatment of Male Infertility: Clinical Guidelines Based on a Syste-matic Review and Analysis of Evidence. World J Mens Health. 2021;39:e2. DOI: 10.5534/wjmh.200196

[133] Ahmed SD, Karira KA, Jagdesh, Ahsan S. Role of L-carnitine in male infertility. J Pak Med Assoc 2011;61:732–6. PMID: 22355991

[134] Bhabak K. P, Mugesh G. Functional mimics of glutathione peroxidase: Bioinspired synthetic antioxidants. Accounts of Chemical Research. 2010; 43(11):1408–1419. DOI: 10.1021/ar100059g.

[135] Mora-Esteves C, Shin D. Nutrient supplementation: Improving male fertility fourfold. Seminars in Reproductive Medicine. 2013; 31(4), 293–300. DOI: 10.1055/s-0033-1345277.

[136] Lanzafame F. M, La Vignera S, Vicari E, Calogero A. E. Oxidative stress and medical antioxidant treatment in male infertility. Reproductive BioMedicine Online. 2009; 19:638–659. DOI: 10.1016/j.rbmo.2009.09.014.

[137] O WS, Chen H, Chow PH. Male genital tract antioxidant enzymes–their ability to preserve sperm DNA integrity. Mol Cell Endocrinol. 2006; 250(1–2): 80–3. DOI:10.1016/j.mce.2005.12.029.

[138] Zhang X, Cui Y, Dong L, Sun M, Zhang Y. The efficacy of combined l-carnitine and l-acetyl carnitine in men with idiopathic oligoasthenoteratozoospermia: a systematic review and meta-analysis. Andrologia 2020;52:e13470. DOI: 10.1111/and.13470

[139] Zhaku V, Beadini Sh, Beadini N, Murtezani B. Combination of Maca, Korean ginseng extract and antioxidant therapy for male with oligoasthenozoospermia: case study. Journal of Hygienic Engineering and Design. 2019; 26(1):28–35.

[140] Dawson EB, Harris WA, Rankin WE, Charpentier LA, McGanity WJ. Effect of ascorbic acid on male fertility. Ann N Y Acad Sci. 1987; 498:312–23. DOI:10.1111/j. 1749–6632.1987.tb23770.x.

[141] Kobori Y, Ota S, Sato R, Yagi H, Soh S, Arai G, Okada H. Antioxidant cosupplem-entation therapy with vitamin C, vitamin E, and coenzyme Q10 in patients with oligo-asthenozoospermia. Arch Ital Urol Androl. 2014; 86(1):1–4. DOI: 10.4081/aiua.2014.1.1.

[142] Nouri M, Amani R, Nasr-Esfahani M, Tarrahi MJ. The effects of lycopene supplement on the spermatogram and seminal oxidative stress in infertile men: A rand-omized, double-blind, placebo-controlled clinical trial. Phytotherapy Research : PTR. 2019;33(12):3203–3211. DOI: 10.1002/ptr.6493.

[143] Alahmar AT, Calogero AE, Sengupta P, Dutta S. Coenzyme Q10 Improves Sperm Parameters, Oxidative Stress Markers and Sperm DNA Fragmentation in Infertile Patients with Idiopathic Oligoasthenozoospermia. World J Mens Health. 2020 Jan 20. DOI: 10.5534/wjmh.190145.

[144] Chia SE, Ong CN, Chua LH, Ho LM, Tay SK. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. J Androl. 2000;21(1):53–7. PMID: 10670519.

[145] Zhaku V, Beadini Sh, Beadini N, Xhaferi V. Role of antioxidants in the diminution of oxidative stress and amelioration of semen parameters in idiopathic male infertility. Acta Medica Balkanica. 2020; 5(9–10):69–80.

[146] Mossa, M, Azzawi M, Dekhel H. Effect of Selenium in Treatment of Male Infertility. Exp. Tech. Urol. Nephrol. 2018, 1, ETUN.000521. DOI: 10.31031/ ETUN. 2018.01.000521

[147] Agarwal A, Said T.M. Carnitines and male infertility. Reprod. Biomed. Online 2004; 8(4):376–384. DOI: 10.1016/s1472-6483(10)60920-0.

[148] Gülçin I. Antioxidant and antiradical activities of L-carnitine. Life Sci 2006;78: 803–11. DOI: 10.1016/j. lfs.2005.05.103

[149] Farris PK. Cosmetical Vitamins: Vitamin C. In: Draelos ZD, Dover JS, Alam M, editors. Cosmeceuticals. Procedures in Cosmetic Dermatology. 2nd ed. New York: Saunders Elsevier; 2009. pp. 51–6. PMID: 29104718

[150] Thiele JJ, Friesleben HJ, Fuchs J, Ochsendorf FR. Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed

semen. Hum Reprod. 1995; 10(1):110–5. DOI: 10.1093/humrep/10.1.110.

[151] Rao A.V, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevent-ion of chronic diseases: A review. Nutr. Res. 1999, 19, 305–323. DOI: 10.1016/S0271-5317(98)00193-6.

[152] Kelkel M, Schumacher M, Dicato M, Diederich M. Antioxidant and antiprolifer-ative properties of lycopene. Free Radic Res. 2011; 45(8): 925–40. DOI:10.3109/ 107157 62.2011.564168.

[153] Crane FL. Biochemical functions of coenzyme Q10. J Am Coll Nutr. 2001; 20 (6):591–8. DOI: 10.1080/ 07315724.2001.10719063.

[154] Nadjarzadeh A, Shidfar F, Amirjannati N, Vafa M. R, Motevalian S. A, Gohari M. R, ... Sadeghi M. R. Effect of Coenzyme Q10 supplementation on antioxidant enzymes activity and oxidative stress of seminal plasma: a double-blind randomised clinical trial. Andrologia. 2014; 46(2):177–183. DOI: 10.1111/and.12062

[155] Balercia G, Mosca F, Mantero F, Boscaro M, Mancini A, Ricciardo-Lamonica G, Littarru G. Coenzyme Q (10) supplementation in infertile men with idiopathic astheno-zoospermia: an open, uncontrolled pilot study. Fertil Steril. 2004;81(1):93–8. DOI:10.1016/j. fertnstert.2003.05.009

[156] Majzoub A, Agarwal A. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol. 2018; 16(1):113–124. DOI: 10.1016/j.aju.2017.11.013.

[157] Fallah A, Mohammad-Hasani A, Colagar A.H. Zinc is an Essential Element for Male Fertility: A Review of Zn Roles in Men's Health, Germination, Sperm Quality, and Fertilization. J. Reprod. Infertil. 2018;19:69–81. PMID: 30009140.

[158] Powell SR. The antioxidant properties of zinc. J Nutr. 2000; 130(5S Suppl): 1447S–54S. DOI: 10.1093/jn/ 130.5.1447S.

[159] Ahsan U, Kamran Z, Raza I, Ahmad S, Babar W, Riaz M.H, Iqbal Z. Role of selenium in male reproduction – A review. Animal Reproduction Science. 2014; 146(1–2), 55–62. DOI:10.1016/j. anireprosci.2014.01.009.

[160] Tafuri S, Ciani F, Iorio EL, Esposito L, Cocchia N. Reactive oxygen species (ROS) and male fertility. In: Wu B, editor. Biochemistry, genetics and molecular biology "new discoveries in embryology". 2015. DOI: 10.5772/ 60632

[161] Evans HM, Bishop KS. On the existence of a hither to unrecognized dietary factor essential for reproduction. Science. 1922;8;56(1458):650–1. DOI: 10.1126/science.56. 1458.650.

[162] Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl. 1996; 17(5): 530–7. PMID: 8957697.

[163] Traber MG. Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R, eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore, MD: Lippincott Williams & Wilkins, 2006;396–411.

[164] Hsieh TC., Marinaro J., Shin P.R. Nutritional Pathways to Protect Male Reproductive Health. In: Parekattil S., Esteves S., Agarwal A. (eds) Male Infertility. Springer, Cham. 2020, pp.529–534. DOI:10.1007/978-3-030-32300-4_42.

[165] Matorras R, Pérez-Sanz J, Corcóstegui B, ... , Expósito A. Effect of vitamin E administered to men in

infertile couples on sperm and assisted reproduction outcomes: a double-blind randomized study. F&S Reports, 2020;1 (3): 219–226. DOI:10.1016/j.xfre. 2020.09.006.

[166] Ener K, Aldemir M, Işık E, Okulu E, Özcan MF, Uğurlu M, Tangal S, Özayar A. The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy: a randomised controlled study. Andrologia. 2016;48 (7):829–34. doi: 10.1111/and.12521.

[167] Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID, Barratt CL. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril. 1995;64(4):825–31. DOI: 10.1016/ s0015-0282(16)57861-3.

[168] Taylor K, Roberts P, Sanders K, Burton P. Effect of antioxidant supplementation of cryopreservation medium on post-thaw integrity of human spermatozoa. Reprod Biomed Online. 2009;18(2):184–9. DOI: 10.1016/s1472-6483(10)60254-4.

[169] Ghafarizadeh AA, Malmir M, Naderi Noreini S, Faraji T, Ebrahimi Z. The effect of vitamin E on sperm motility and viability in asthenoteratozoospermic men: In vitro study. Andrologia. 2021;53(1):e13891. doi: 10.1111/and.13891.

[170] Keskes-Ammar L, Feki-Chakroun N, Rebai T, ... , Bahloul A. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch Androl. 2003;49(2):83–94. DOI: 10.1080/01485010390129269.

[171] ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad A, Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenozoospermia: A prospective, randomized trial. Andrology. 2015;3(5): 864–7. DOI: 10.1111/andr.12086.

[172] Nori M, Farzadi L, Ghasemzadeh A, et al. The effect of antioxidant supplements on semen quality in men with asthenozoospermia. *Med J Tabriz Univ Med Sci.* 2006:127–132.

[173] National Academy of Sciences. Institute of Medicine. Food and Nutrition Board. Dietary guidance: DRI tables. US Department of Agriculture, National Agricultural Library and National Academy of Sciences, Institute of Medicine, Food and Nutrition Board. 2009.

[174] Weber P, Bendich A, Machlin LJ. Vitamin E and human health: rationale for determining recommended intake levels. Nutrition. 1997;13(5):450–60. DOI: 10.1016/s0899–9007(97)00110-x.

[175] Zhaku V, Beadini Sh, Beadini N, Xhaferi V, Milenkovic T. Oral antioxidants improve sperm motility, sperm concentration and reduce oxidative stress in males with oligo-asthenozoospermia. In: 22nd European Congress of Endocrinology; 5–9 September 2020; Prague, Czech Republic: Endocrine Abstracts (2020) 70 EP581. DOI:10.1530/endoabs.70. EP581

[176] Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxid-ative stress and antioxidants in male fertility. Cent European J Urol. 2013; 66(1):60–7. DOI: 10.5173/ ceju.2013.01.art19.

[177] Azzi A. Many tocopherols, one vitamin E. Mol Aspects Med. 2018;61: 92–103. DOI: 10.1016/j. mam.2017.06.004.