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REVIEW

# Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management

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## Abstract

Infertility is a global health problem involving about 15% of couples. Approximately half of the infertility cases are related to male factors. The oxidative stress, which refers to an imbalance in levels of reactive oxygen species (ROS) and antioxidants, is one of the main causes of infertility in men. A small amount of ROS is necessary for the physiological function of sperm including the capacitation, hyperactivation and acrosomal reaction. However, high levels of ROS can cause infertility through not only by lipid peroxidation or DNA damage but inactivation of enzymes and oxidation of proteins in spermatozoa. Oxidative stress (OS) is mainly caused by factors associated with lifestyle. Besides, immature spermatozoa, inflammatory factors, genetic mutations and altering levels of sex hormones are other main source of ROS. Since OS occurs due to the lack of antioxidants and its side effects in semen, lifestyle changes and antioxidant regimens can be helpful therapeutic approaches to overcome this problem. The present study aimed to describe physiological ROS production, roles of genetic and epigenetic factors on the OS and male infertility with various mechanisms such as lipid peroxidation, DNA damage, and disorder of male hormone profile, inflammation, and varicocele. Finally, the roles of oral antioxidants and herbs were explained in coping with OS in male infertility.

**Keywords** Male infertility · Oxidative stress · Reactive oxygen species · Genetic factors · Antioxidants

## Abbreviations

| | |
|---|---|
| ·OH | Hydroxyl |
| ·ROO | Proxyl |
| 4-HNE | 4-Hydroxynonenal |
| 8-OHdG | 8-Hydroxy-2-deoxyguanosine |
| ABP | Androgen-binding protein |
| ARE | Antioxidant responsive element |
| ARTs | Assisted reproductive techniques |
| ATP | Adenosine triphosphate |
| BER | Base excision repair |
| cAMP | Cyclic adenosine monophosphate |
| CAT | Catalase |
| CK | Creatine kinase |
| CYP2E | Cytochrome P450 enzymes |
| ETC | Electron transport chain |
| G6PD | Glucose-6-phosphate dehydrogenase |
| GnRH | Gonadotropin releasing hormone |
| GPx | Glutathione peroxidase |
| GRX | Glutaredoxin |
| GSH | Glutathione |
| GST | Glutathione s-transferase |
| $H_2O_2$ | Hydrogen peroxide |
| HPA | Hypothalamic–pituitary–adrenal |
| HPG | Hypothalamic–pituitary–gonadal |
| HPT | Hypothalamo–pituitary–thyroid |
| IL | Interleukin |
| LOOHs | Lipid hydroperoxides |
| MDA | Malondialdehyde |
| miRNAs | Micro-ribonucleic acids |
| MPO | Myeloperoxidase |
| NAC | N-Acetylcysteine |
| NADPH | Adenine dinucleotide phosphate |
| NO | Nitrogen oxide |
| NOS | Nitric oxide synthase |
| NRF2 | Nuclear factor erythroid 2-related factor 2 |
| $O_2^{-}$ | Superoxide anion |

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| | |
|---|---|
| ONOO- | Peroxynitrite |
| OS | Oxidative stress |
| P-Tyr | Phosphotyrosine |
| PGE2 | Prostaglandin E2 |
| PKA | Protein kinase A |
| NER | Nucleotide excision repair |
| PMN | Polymorphonuclear |
| P-PKA | Phospho-PKA |
| PRX | Peroxiredoxin |
| PUFA | Polyunsaturated fatty acids |
| RF | Radio frequency |
| ROOH | Hydroperoxide |
| ROS | Reactive oxygen species |
| SCI | Spinal cord injury |
| SDF | Sperm DNA fragmentation |
| SH- | Sulfhydryl groups |
| SOD | Superoxide dismutase |
| SOD3 | Extracellular SOD |
| TAC | Total antioxidant capacity |
| TNF-α | Tumor necrosis factor alpha |
| TRX | Thioredoxin |

## Introduction

Infertility is defined as an inability in pregnancy after 12 months of unprotected sexual intercourse. Almost 7% of couples worldwide are infertile, and half of the infertility is associated with male factors [1]. Various factors are involved in the pathophysiology of male infertility including OS. The male infertility is strongly associated with reactive oxygen species (ROS) [2]. ROS includes highly reactive free radicals including superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), proxyl (·ROO) and hydroxyl (·OH) [3]. Low levels of ROS are necessary for sperm capacitation, hyperactivation, acrosomal reaction, and sperm–ovule fertilization. However, high levels of ROS neutralize antioxidants in the seminal plasma and cause OS. Sperm damage by ROS is the main cause of infertility in 30–80% of infertile men; and these conditions ultimately damage DNA in the nucleus and mitochondria [4]. Sperm cells are highly sensitive to oxidative damages. On the other hand, these cells have cytoplasmic membranes rich in unsaturated fatty acids, and thus they are faced with the lipid peroxidation under the influence of ROS [2]. Moreover, these cells are incapable of repairing damage by oxidative stress because they suffer from lack of essential cytoplasmic enzymes. Finally, lower motility and death of sperm occurs due to the loss of adenosine triphosphate (ATP) caused by lipid peroxidation followed by axonemal injury [5]. OS indirectly affects hypothalamic axes and can disrupt the secretion of sex hormones. ROS reduces levels of male sex hormones and strikes their balance and can disrupt the reproductive system [6]. A wide range of factors including genetic, epigenetic, lifestyle-related factors such as over-consumption of alcohol, smoking, radiation, obesity and also infection and varicocele could increase the level of OS in the body and lead to infertility [7]. Therefore, the role of the antioxidant system is essential to cope with excessive-produced ROS. Antioxidant enzymes and molecules such as superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) are abundant in plasma or natural sperm cells [8]. Most of these enzymes are encoded by NRF2, SOD, CAT, glutathione S-transferase (GST), and glutathione peroxidase (GPx) genes. Genetic mutations in these genes, in turn, cause infertility caused by OS in men [9]. As a result of these injuries, rates of fertilization and implantation are reduced and fetal growth is disturbed, and the likelihood of abortion increases. Since the balance between antioxidant levels and ROS maintains the redox homeostasis, the use of oral antioxidants as well as herbal medicine can help treat men with infertility. These antioxidants remove extra ROS and provide the redox balance [10]. To overcome infertility, the mechanisms that cause sperm damage, as well as their therapeutic methods, should be considered more. Recently, some literature reviews describing the effect of OS on the male reproductive system were published. For instance, Bui et al. reported the role of excessive ROS levels in damage of genomic and mitochondrial DNA, shortening of telomere length, Y chromosomal microdeletions, and epigenetic changes [4]. Also, Agarwal et al. discussed about infection and inflammation-induced OS and male infertility [11]. In another report, Darbandi et al. described the mechanisms of ROS-mediated disturbance of male hormonal profiles [6]. But in the present review with a more comprehensive attitude, we discussed about different aspects of OS on male infertility including its pathophysiological and genetic aspects with an emphasis on the therapeutic use of antioxidants.

## Physiological role of ROS in the male fertility

Reactive oxygen species can have both beneficial and destructive effects on sperm functions and these effects depend on the ROS level. Reactive species of oxygen and nitrogen should be regulated at appropriate levels to ensure physiological functions. A small amount of ROS is a product of normal metabolism in stem cells, sperm and ovum; and these reactive species regulate important reproductive processes such as capacitation, acrosomal reaction and hyperactivation. Sperm capacitation is a necessary process to obtain fertilizing facility. ROS such as superoxide, $H_2O_2$, nitrogen oxide (NO), and superoxide anion radical are contributed in this procedure [3]. Superoxide anion generation in neutrophils is correlated with some proteins tyrosine phosphorylation. Two chief phosphotyrosine-containing

proteins, 81 and 105 kDa, are overexpressed in capacitating conditions. Catalase and superoxide dismutase eliminate both tyrosine phosphorylation of p81 and p105 and sperm capacitation, confirm the role of hydrogen peroxide and $O_2^{\cdot-}$ in these two procedures. ADPH oxidase inhibitors could reduce both tyrosine phosphorylation of p81 and p105 and sperm capacitation whereas hydrogen peroxide induces these two procedures [12]. NADH and NADPH activate phosphorylation of p81 and p105 and sperm capacitation, but these procedures were not inhibited by catalase or SOD, nor were they correlated with an elevated production of $O_2^{\cdot-}$. So, these cofactors seemed to apply their function by mechanisms altered from some other inducers [13]. Nitric oxide is another essential compound for sperm capacitation while inhibition of nitric oxide synthase (NOS) reduced the acrosome reaction percentage. In addition, nitric oxide could modulate tyrosine phosphorylation of proteins in sperm and there is a true association between nitric oxide-related tyrosine phosphorylation and sperm capacitation [14]. During human sperm capacitation, a double phosphorylation occurs in threonine–glutamine–tyrosine motif (P-Thr-Glu-Tyr-P) of 105 and 81 kDa proteins. This phosphorylation is specifically regulated by nitric oxide and the protein Tyr kinase, protein kinase C, and MEK or MEK-like kinase are involved in this process [15]. The phospho-MEK-like proteins are regulated by kinases and ROS and maybe display an intermediate phase among the initial events and the late tyrosine phosphorylation correlated with sperm capacitation [16]. However, The PI3K/Akt axis contributed in Tyr and Thr-Glu-Tyr phosphorylation and sperm capacitation but not MEK-like proteins [17]. Additionally, during capacitation, Ca2+ stimulates increased levels of cAMP. The cAMP, via an indeterminate serine/threonine phosphorylation, regulates the superoxide anion generation and this procedure could lead to elevation of p81 and p105 phosphorylation [18]. Cyclic adenosine monophosphate (cAMP) activates the protein kinase A (PKA) and could indirectly control the tyrosine phosphorylation of protein. Levels of cAMP are regulated by a balance of adenylyl cyclase (AC) and phosphodiesterases (PDEs) activities, which are responsible for its production and degradation, respectively. Lefièvre et al. [19] suggested that cAMP level is under the regulation of AC, since activity of PDE is steady throughout sperm capacitation. Moreover, they suggest that low cAMP concentrations are enough for PKA activation and sperm capacitation [19]. The above-mentioned contents strengthen the concept that differentially intricate pathways are required for sperm to develop capacitated. In addition, phosphorylation events may be involved in the acrosomal reaction which could be affected by OS. The acrosomal reaction is correlated with the generation of extracellular ROS by spermatozoa and both $H_2O_2$ and $O_2^{\cdot-}$ might be involved in the control of this procedure. A possible mechanism of ROS effects on acrosomal reaction may be explained by tyrosine phosphorylation of sperm proteins such as 70–105 kDa and this effect could partially disappear by some ROS scavengers [13]. Acrosomal reaction and a partial pathway involved in sperm capacitation are depicted in Fig. 1.

## ROS sources in seminal plasma

Various sources cause OS including genital tract infection, varicocele, metabolic syndromes, smoking, excessive consumption of alcohol and drugs, ionizing radiation, cell phone use, mental stress, severe exercise, spinal cord injury (SCI) and environmental pollution. In general, these sources are classified into endogenous and exogenous groups [20, 21].

### Endogenous sources

#### Immature sperm

During the spermatogenesis, the sperm release may occur along with additional cytoplasm of the germinal epithelium; and spermatozoa are usually immature and non-functional. Sperms with excess cytoplasmic residues in the mid-piece are known as immature sperm. Additional cytoplasm in the mid-part of the sperm contains glucose-6-phosphate dehydrogenase (G6PD) enzyme. This cytoplasmic enzyme controls the production of intracellular β-nicotinamide adenine dinucleotide phosphate (NADPH) through a shunt hexose monophosphate. The NADPH is used to fuel the production of ROS via NADPH oxidase situated within the sperm membrane [22, 23]. In addition, there are abundant numbers of mitochondria in mid-piece that store energy for motility of sperm. Diaphorase, an enzyme at the mitochondrial respiratory chain, controls the ratio between reduced and oxidized forms of NADH; thus it contributes to the sperm energy balance. This oxidoreductase is integrated with the mitochondrial respiratory chain and generates superoxide anions, affecting the level of ROS [24]. Mitochondrial dysfunction could lead to ROS production and the ROS can influence mitochondrial integrity in sperm cells within a mutual process; ROS leads to damage of mitochondrial membrane and this causes an elevation in ROS generation production. Indeed, mitochondria and sperm membrane are two main regions for ROS production in immature sperms [25].

#### Leukocytes

There are few leukocytes in each natural ejaculation. According to the WHO criteria (World Health Organization), if the white blood cell count is greater than 1 million per ml of semen, it is defined as leukocytospermia [26]. The increased ROS in semen due to leukocytospermia leads to a decrease

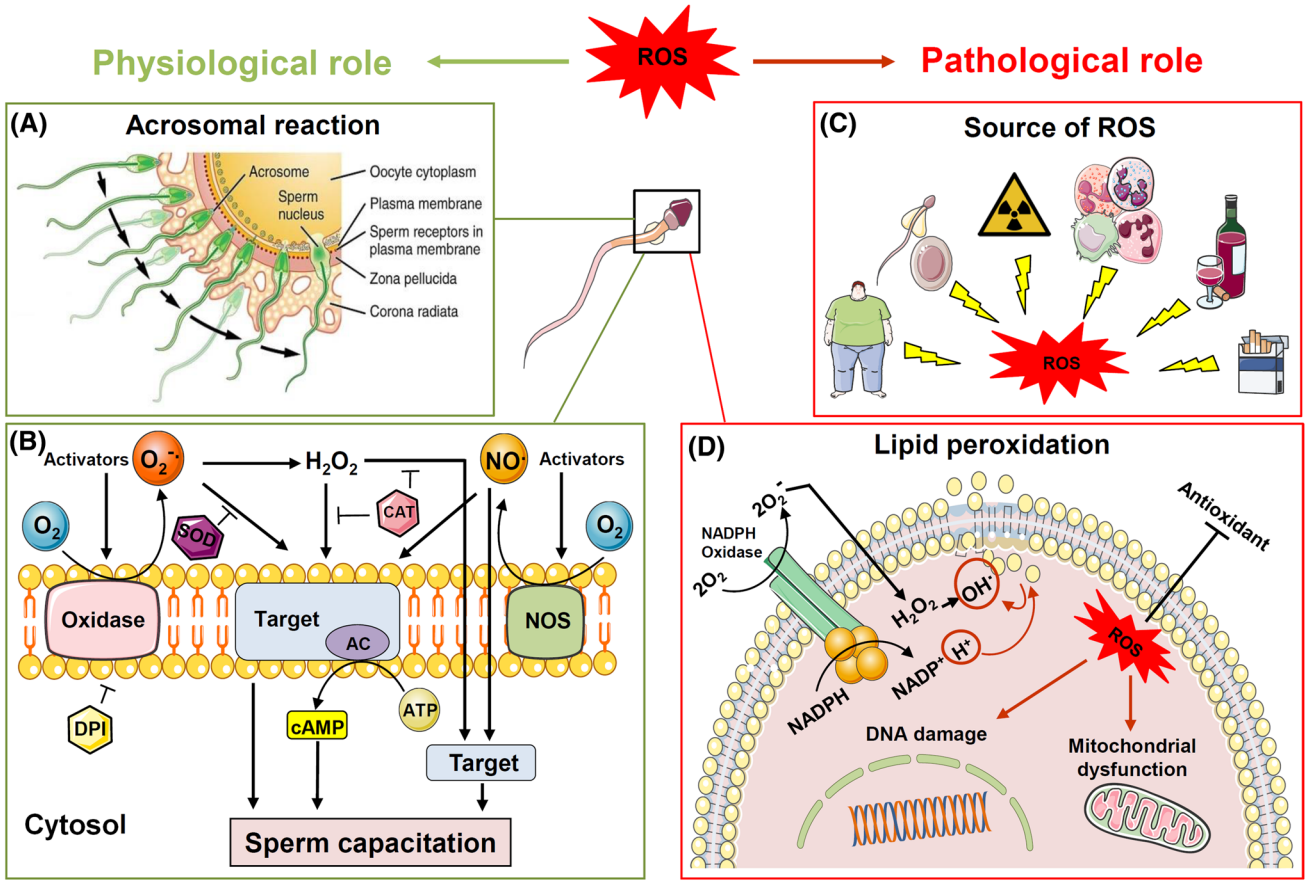


**Fig. 1** Physiological and pathological roles of ROS in male reproductive system. Small amount of ROS plays an important role in acrosomal reaction and penetration of spermatozoa to the zona pellucida through a mild lipid peroxidation (**a**). ROS production is an early procedure in sperm capacitation. In the plasma membrane, an oxidase produces $O_2^{\cdot-}$ in the extracellular region which helps sperm capacitation activators. Diphenyliodonium (DPI), an inhibitor of this oxidase could block production of $O_2^{\cdot-}$ in oxidative stress condition. In addition, superoxide dismutate (SOD) could block the extra $O_2^{\cdot-}$. Intration of $H_2O_2$ with a certain target in plasma membrane assists sperm capacitation and catalase could inhibit excessive $H_2O_2$. Nitric oxide (NO) produced by a NOS and induces capacitation. Adenylyl cyclase (AC) is a possible target for ROS which is responsible for producing cAMP (**b**). Sources of ROS in male infertility included obesity, immature sperm, radiation, leukocytosis, alcohol drinking, and smoking (**c**). The NADPH oxidase causes production of superoxide radical ($O_2^{\cdot-}$) in sperm lipid membrane. The $O_2^{\cdot-}$ turns into $H_2O_2$ in cytosol and this agent produces hydroxyl radical (OH·). $H^+$ and OH.could initiate lipid peroxidation in the sperm membrane and excessive ROS in the sperm causes mitochondrial dysfunction and DNA damage (**d**)

in concentration of antioxidants resulting in damage to DNA in sperms [27, 28]. The number of leukocytes increases in the seminal plasma due to the inflammation or infection of the reproductive tract. Positive peroxidase leukocytes can produce ROS 1000 times more than sperm by increasing the production of nicotinamide adenine dinucleotide phosphate (NADPH) through the transfer of hexose monophosphate [11]. Studies indicate that levels of ROS in fertile men are less than infertile men, and there is a direct relationship between OS and increased leukocyte count [29]. Seminal plasma leukocytes, except for macrophages, which are about 30%, are mainly polymorphonuclear (PMN). Leukocytes or white blood cells are created in response to stimuli during inflammation and infection and can create ROS up to 100 times more than inactive cells. Activation of the myeloperoxidase (MPO) system from PMN cells and macrophages leads to the respiratory burst and increases ROS products. Spermatic injury due to leukocyte occurs when the number of seminal plasma leukocytes such as leukocytospermia is significantly high [29]. The inflammation or infection should be taken into consideration in infertile men. Antibiotic treatment at low levels of leukocytospermia ($0.2–1 \times 10^6$) significantly increases spontaneous pregnancy [30].

## Exogenous sources

### Smoking

According to a study in Morocco in 2017, cigarette smoking is the most important factor affecting male infertility [31]. Nicotine and other chemicals in cigarettes disrupt levels of hormones and affect effective parameters in semen

[32]. Smoking can damage the DNA of sperm in male reproductive cells through high accumulation of ROS in the sperm production site. Cigarette smoke contains a lot of toxic substances, carcinogens, and mutagenic substances in addition to stable and unstable free radicals and ROS in its particles. It also increases the production of superoxide anions, $H_2O_2$ causing oxidative damage to lipid membranes of cells, proteins, enzymes, and DNA and subsequently leads to infertility [33]. Cigarette smoking reduces creatine kinase (CK) in sperm as an energy reservoir for rapid buffering and rebuilding ATP and plays an important role in the sperm motility [34]. In addition, ROS-induced damage of mitochondrial DNA results in reduced ATP and energy accessibility, impending sperm motility [5]. Furthermore, cigarette smoking reduces the quality of semen, reduces acrosin activity, protein phosphorylation, disturbance of expression of micro-ribonucleic acids (miRNAs) and histone-to-protamine transition and ultimately leads to infertility [35]. Damage to DNA and methylation patterns due to the increased level of ROS in tissues of people, who have been indirectly exposed to cigarette smoke, is also another side effect of cigarettes [36].

#### Alcohol intake

Excessive alcohol consumption also has devastating effects on the quantity and quality of sperm parameters. Excessive alcohol consumption is associated with a decrease in sperm motility and concentration and also reduces the percentage of normal morphology of sperm [37]. Alcohol causes spermatic chromatin disorders through apoptosis, thereby affecting the sperm motility, nuclear maturation and DNA integrity [25]. According to an animal study in 2017, the sperm motility and count in rats have received alcohol decreased significantly, as well as the level of MDA and SDF increased, in addition to a decrease glutathione and superoxide dismutase levels have been observed in the testicles of animals that received alcohol [38]. Ethanol causes changes in the structure and function of mitochondria, reduces respiration and ATP levels, and increases the ROS production through its metabolism in the liver [39]. Alcohol consumption increases activities of cytochrome P450 enzymes (CYP2E), which in turn increase the activity of NADPH oxidase, and then changes levels of certain metals (especially copper- and iron-free ions) in the body, and ultimately increases the production of superoxide anions [39] in men who constantly consume alcohol; and nitric oxide, NO production increases via inducible nitric oxide synthase (iNOS) [40]. Nitrite oxide and its metabolite, peroxynitrite (ONOO-) act as mediators of mitochondrial dysfunction [40, 41].

#### Radiation

Another exogenous factor is radiation that affecting male fertility and its biological consequences depend on the type of radiation, the amount of produced energy and duration of radiation [42]. Ionized and non-ionized radiation effectively affects spermatogenesis. Male fertility is heavily influenced by thermal, radioactive, radio frequency (RF) and other hazardous radiation [43]. In recent years, there is a significant increase in the use of mobile phones, laptops, wireless systems and microwave that have raised concerns about male infertility due to the electromagnetic radiation. Electromagnetic radiation leads to a lot of changes in reproductive parameters by an OS induction. Based on this hypothesis, the impact of 3G cell phone radiation was studied on the reproductive tract of male rats in 2018. After 3G cell phone radiation, decreased spermatogenic cells, sperm membrane changes, increased ROS rate and lipid peroxidation were observed along with a simultaneous reduction of sperm count and changes in morphology of sperm tail [44]. Cell phone radiation might affect cell plasma membrane and stimulates plasma membrane NADH oxidase which plays a key role in the various cellular harmful effects and leads to OS [45]. Another experimental study indicates that due to the electromagnetic radiation, the mitochondrial genome is damaged and leads to disturbances in the electron transport chain (ETC) resulting in OS [46]. Small changes in ROS may play an important role in the capacity of sperm, acrosome reaction, and fertilization, but the OS caused by radiofrequency radiation causes damage to spermatozoa [43]. Another study examined the impact of 2.45-GHz microwave on the histology and lipid peroxidation level in male rats. According to the present study, the exposure to microwave radiation for 2 hours per day for 35 days can cause oxidative changes in rats [47]. Other studies on the radio frequency radiation and its effects on male infertility reported the reduced glutathione level and disturbed sperm membrane integrity because of OS [48]. Therefore, researchers concluded that the ROS production could be a major pathway in men's infertility and radiation pathophysiology; hence, the antioxidant therapy is a useful way to minimize its harmful effects (Fig. 1) [46].

#### Environmental sources

One of the most important environmental ROS source is genital heat stress. Exposure to heat radiant for a long time leads to scrotal hyperthermia and excessive ROS production [49]. Animal studies demonstrate that spermatogenesis, motility of spermatozoa, sperm concentration and sperm viability could be affected by heat stress [50, 51]. Also, studies demonstrate that heat stress and subsequently increased ROS may result in over-expression of caspase 3 that lead

to apoptosis in various cell types including sertoli and leydig cells [52–54]. Another environmental source of ROS is pollution such as phthalates, air pollution, and heavy metals. Phthalates are the man-made chemical that exist in personal care products, plastics, and food packaging materials that influence the general population. Animal experiments demonstrated that a high level of ROS production, the lack of testicular antioxidant, and a decrease in hormone levels are the results of phthalates exposure [55, 56]. A human study shows that phthalates could increase the level of OS in the testis and subsequently cause mitochondrial dysfunction, and ultimately reduces the sperm function as a result of increased lipid peroxidation [57]. Air pollution can promote oxidative stress by affecting sperm lipid membrane and generate free radicals and decrease sperm parameters [58, 59]. In addition, heavy metals are considered to be another source of ROS. Cadmium and lead appear to cause testicular oxidative stress and also can be damaging to sperm DNA and the reduction of semen parameters [60–62].

## Roles of genetic and epigenetic factors in OS affecting the male infertility

Environmental, genetic, and lifestyle factors affect male fertility. Both factors are associated with levels of sex hormones, chromatin stability, and sperm quality. About 20% of observed changes in sperm count, hormonal levels, sperm morphology, and sperm chromatin parameters are influenced by hereditary factors [63–65]. Since the ROS is affected by endogenous and environmental factors and can cause great damage to sperm quality and DNA, antioxidant enzymes play important roles in counteracting OS during spermatogenesis and fertilization. A number of antioxidant genes involved in spermatogenesis include NRF2, SOD, CAT, GPX, PRX, GRX, TRX, and NOS. Enzymes, which are encoded by these genes, are widely used in antioxidant responses, syntheses, and reduction of GSH and redox cycle of reducing thiol during spermatogenesis. Furthermore, most of these genes have ARE (antioxidant responsive element) motifs in promoter sites, thereby facilitating the NRF2 transcription pathway in the OS [66]. NRF2 is a key gene in the antioxidant defense because it is a nuclear transcription factor that can induce antioxidant enzymes through the ARE element [67]. In response to OS, NRF2 binds with AREs and regulate defensive mechanisms against OS. A study in mice lacking Nrf2 showed an important role for the NRF2 in prevention of oxidative damage in spermatogenesis [8]. However, Chan et al. reported that the function of NRF2 gene is not essential for mouse fertility [68]. SODs and CATs are genes that are regulated by NRF2-ARE signal pathway, and they protect sperm from oxidative damage by superoxide and $H_2O_2$. SOD cause superoxide radical destruction in molecular oxygen and hydrogen peroxide. Three families of SOD isozymes are found in humans: soluble SOD (SOD1), mitochondrial SOD (SOD2) and extracellular SOD (SOD3) [69] among which, SOD2 isozyme is more expressed in human sperms [70]. SOD gene has several varieties, and studies indicate that rs4880 polymorphism is effectively associated with an increased risk of infertility in men [71]. CAT is a heme enzyme that is encoded by CAT genes. This enzyme converts $H_2O_2$ to oxygen and water, and eventually maintains normal levels of ROS and protects sperm against ROS [72, 73]. CAT gene also has a single-nucleotide C-262T mutation in the promoter site and is effectively associated with increased infertility in men [72]. However, an animal study revealed that the fertility rate was normal at 3 months in wild-type, $Sod^{-/-}$ or $Cat^{-/-}$ knockout mice. But, the fertility rate was significantly decreased in all mice at 12 months [74]. NOSs constitute a family of enzymes that catalyze the production of nitric oxide (NO) from L-arginine and confront with ROS at low levels. NO plays role in the sperm motility, survival and acrosome reaction [75]. Three NOS isozymes have been found in mammals including neuronal NOS (nNOS; NOS1), inducible NOS (iNOS; NOS2) and endothelial NOS (eNOS; NOS3) [76]. These three isoforms can be found in various testicular cells including sertoli cells, germ cells in seminiferous epididymis, leydig cells, myofibroblasts, endothelial cells, and spermatozoa. The effective presence of the above isoforms in testes indicates the importance of NOS in the spermatogenesis [77]. A study evaluated relationships of different nitrite oxide polymorphisms with male infertility. The results indicated significant relationships between T-786C and G894T polymorphisms, reduced sperm parameters, and increased OS [78]. Glutathione S-transferases (GSTs) are other proteins that catalyze the GSH binding to xenobiotic electrophilic substrates as causes of ROS [79]. GST family includes three large subgroups namely cytosolic, mitochondrial, and microsomal GSTs [80]. In humans, GSTs include mitochondrial GSTK1, microsomal MGST1-MGST3, and cytosolic GSTA1-GSTA5, GSTZ1, GSTM1-GSTM5, GSTO1-GSTO2, GSTP1, and GSTT1-GSTT4 [81]. Studies on different GST genotypes indicate that the removal of GSTT1 and GSTM1 genes, as well as point mutations in GSTP1 gene, is associated with an increase in the male infertility risk [82]. TPX, PRX, and GRX enzymes are involved in reducing cellular thiols. GRXs and TRXs jointly catalyze the reduction of protein-mixed disulfides [83]. TRX1 isozyme exists in the cytosol and nucleus, while TRX2 is specifically expressed in mitochondria [84]. PRX enzymes constitute a group of peroxidases that destroy organic hydroperoxides and $H_2O_2$. GPX family catalyzes the reduction of thiol with glutathione [85]. Among its isozymes, GPX4 is dominant in testes and is vital to spermatogenesis [86]. GPX5 is expressed in the epididymis caput and probably maintains

sperm DNA integrity [87]. As mentioned above, the genetic polymorphisms found in the genes involved in the OS system can affect male infertility. Genetic polymorphisms, depending on their position on the gene map, can alter the expression and structure of a protein [88, 89]. Genetic variations can occur on the coding or non-coding regions of the genes. Non-coding region mutations, as well as mutations in the coding regions, can affect the gene expression and RNA splicing. Mutations in promoter regions can be directly related to gene expression [90–92]. Studying the molecular effects of polymorphisms in vitro and in vivo conditions are time-consuming and costly. Some previous studies have shown that computational analyses with bioinformatics tools can be a quick and cost-effective way in molecular analyzes, especially in investigating the molecular effects of genetic mutations [93, 94]. The important genes associated with the OS system and the role of their genetic variation in male infertility are summarized in Table 1.

Epigenetic regulations are precise mechanisms that ensure that gene expression is regulated in normal growth [105]. Epigenetic changes regulate the gene expression and are not associated with the direct change in the DNA sequence. Epigenetic mechanisms including DNA methylation, histone changes, and performance of non-coding RNAs are essential for a number of biological processes including genomic imprinting and chromosome X inactivation [106]. Epigenetic markers, which play major roles in the gamete viability in sperms, are obtained during the cell growth and differentiation. Sperm DNA epigenetic planning happens at numerous important steps of spermatogenesis. In male rodents, expression of DNA methyltransferase 1 (DMT1) is upregulated in primary meiotic germ cells [107]. Testicular tissues from fertile male display restricted expression of DMT1 gene in pachytene spermatocytes, spermatogonia, and round spermatids [108]. It is reported that genes involved in spermatogenesis could experience epigenetic reprogramming during maturation in epididymis. So, testicular and epididymal pathology could play a possible role in disruption of normal DNA sperm methylation program [109]. ROS leads to hypomethylation which is associated with sertoli cell-only syndrome, testis cancer, and hypospermatogenesis in humans [110]. However, some possible mechanisms for disrupted methylation of sperm DNA may exist including failures in the folate metabolizing pathway and OS [111]. OS results in breaks of the DNA strands and the generation of DNA base adducts such as $O^6$-methylguanine and 8-OH-dG, both described to affect the interaction of the DNA and DNA methyltransferases [112]. The $O^6$-methylguanine [113] or 8-OHdG presence in CpG sequences [114], mainly prevents the adjacent cytosine methylation and eventually results in total DNA hypomethylation [111]. The folate/homocysteine metabolic pathway is necessary for the production of methyl donors and consequently is crucial for the DNA methylation process in all cell types [115]. Failings in the folate pathway could lead to reducing the levels of S-adenosylmethionine (SAM) and have been associated with hypomethylation of DNA [116]. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate pathway which converts the 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate. The latest compound provides methyl groups for conversion of homocysteine to methionine [117]. OS and hyper-homocysteinaemia have been associated with hypomethylation of somatic cell DNA. However, aberrations in metabolism of homocysteine could still possibly be correlated with changes in methylation of sperm DNA [111]. Further studies are necessary to access precise mechanisms of intermediary damage by ROS on epigenetic processes.

## Effect of OS in cellular components

### The role of OS in DNA damage

A high level of DNA damage usually occurs in sperms simultaneous with infertility [46, 118]. Undoubtedly, factors that enhance the level of ROS can affect the integrity of sperm chromatin. Guanine base (G) is the most common DNA's organic base that is exposed to oxidative attack by free radicals and converts to 8-hydroxyguanine (8-OHG). According to conducted surveys on DNA of infertile men's sperms and their comparison with fertile individuals, it is found that 8-OHG in infertile individuals is about 100 times more than fertile ones. OS leads to the production of oxidized DNA base compounds such as the 8-hydroxy-2-deoxyguanosine (8-OHdG) especially in genome locations that are less protamine [119].

Spermatozoon has 8-oxoguanine DNA glycosylase enzymes in the base excision repair (BER). This glycosylase is connected to the nucleus and mitochondria of sperm and can actively separate 8OHdG and release additional base compounds into the extracellular space. Spermatozoa do not have downstream compounds of BER pathway including apurinic endonuclease 1 (APE1) and XRCC1. Due to this incomplete repair capacity of DNA, abasic sites arise in locations that are under the influence of 8OHdG. These sites destabilize the ribose-phosphate and lead to the b-elimination or opening ribose cycle response and DNA strand fragment. This biochemical aspect of DNA is known as the main cause of the onset of cancer in other types of cells. Therefore, the base oxidative DNA damage not only is a mutagen but also indirectly fragments DNA [120]. The deficiency in the protection by antioxidants due to exogenous and endogenous factors leads to the oxidative damage in sperm and eventually DNA damage [121]. DNA damage causes a number of changes such as a single or double-strand fragment, DNA fragmentation, the creation of free-base

**Table 1** Common genetic polymorphisms in genes involved in OS and their role in male infertility

| Gene | Polymorphisms | Effects | Population | Sample size (case/control) | References |
|---|---|---|---|---|---|
| Paraoxonase 1 | rs662 | The variation is associated with decreased risk of male infertility | Iran | 450 (220/230) | [95] |
| Superoxide dismutase 2 | Ile58Thr | The rs1001179 is associated with increased risk of male infertility | Spain | 393 (313/80) | [96] |
| | rs4880 | | | | |
| Catalase | rs1001179 | | | | |
| Glutathione peroxidase 1 | rs1050450 | | | | |
| Glutathione S-transferase | GSTT1 (null or non-null) | | | | |
| | GSTM1 (null or non-null) | | | | |
| Nitric oxide synthase 3 | rs2070744 | Intron variant 4a/4b is associated with increased risk of male infertility | Serbia | 262 (131/131) | [97] |
| | rs1799983 | | | | |
| | Intron variant 4a/4b | | | | |
| Aryl hydrocarbon receptor | rs2066853 | The variation is associated with decreased sperm parameters as well as increased seminal OS | Egypt | 170 (50/120) | [97] |
| Glutathione peroxidase 1 | rs1050450 | There is no association between the variation and idiopathic male infertility | Iran | 250 (100/150) | [98] |
| Nitric oxide synthase 3 | rs1799983 | There is a significant association between rs1799983 and rs2070744 and decreased sperm parameters and increased seminal OS | Egypt | 300 (80/220) | [78] |
| | rs2070744 | | | | |
| Nitric oxide synthase 3 | rs2682826 | The rs1799983 polymorphism could cause oxidative sperm DNA damage, and contributing to male infertility | China | 1160 (580/580) | [99] |
| | rs1047735 | | | | |
| | rs2297518 | | | | |
| | rs10459953 | | | | |
| | rs1799983 | | | | |
| Human catalase | rs1001179 | This variation is associated with male infertility | Iran | 385 (90/95) | [72] |
| Superoxide dismutase 2 | rs4880 | The rs4880 and rs1799983 variations were significantly associated with male infertility | France | 179 (69/110) | [71] |
| Myeloperoxidase | rs2333227 | | | | |
| Glutathione peroxidase 1 | rs1050450 | | | | |
| Catalase | rs1001179 | | | | |
| Nitric oxide synthase | rs2070744 | | | | |
| | rs1799983 | | | | |
| 8-Oxoguanine DNA glycosylase 1 | rs1052133 | Interaction between rs1052133 polymorphism and smoking is related with male infertility | China | 1100 (480/620) | [100] |
| Glutathione S-transferase | GSTM1 (null or non-null) | The rs1695 is associated with idiopathic male infertility. Also, individuals carrying genotype GSTT1-null and GSTP1 (Ile/Val + Val/Val) were at a high risk for male infertility | China | 713 (234/479) | [82] |
| | GSTT1 (null or non-null) | | | | |
| | GSTP1 rs1695 | | | | |
| Glutathione S-transferase | GSTM1 | Men with GSTM1 (-/-)/GSTT1 (-/-) genotype are at a high risk for increased risk of reproductive problems | Russia | 264 (104/160) | [101] |
| | GSTT1 | | | | |
| Luteinising hormone β-subunit | Gly102Ser | This polymorphism is relatively common among infertile Egyptian men | Egypt | 89 (50/39) | [102] |
| Paraoxonase 1 | rs662 | The rs662 variation is not associated with male infertility | China | 732 (403/329) | [103] |
| N-Acetyltransferase-2 | rs1799929 | Male with homozygous and heterozygous variations of GSTP1 (rs1138272, rs1695) and NAT2 (rs1799929, rs1799930) genotypes have an increased risk of infertility | Vietnam | 300 (150/150) | [104] |
| | rs1799930 | | | | |
| Glutathione S-transferase | rs1138272 | | | | |
| | rs1695 | | | | |

sites, changes in purine, pyrimidine, deoxyribose, and DNA crosslinking [46]. These changes can lead to stopping or starting gene transcription, induction of final transduction pathways, increased degradation of telomeric DNA, replication errors, genomic instability, and transversion from CG to AT [122]. Sperms of patients with severe DNA oxidative damage are degenerated and absorbed through the spermatogenesis and sperm maturation in the epididymis resulting in reduced sperm count in these patients. DNA fragmentation is the final stage of ROS damage and is often seen in spermatozoa of infertile men [123]. There is a close relationship between ROS and sperm DNA fragmentation (SDF). SDF can occur for several reasons including varicocele, infection, aging, heat, lifestyle-related factors, incomplete protamination, and occasionally for no reason. A large number of them are mediated by ROS, and thus ROS is the main cause of SDF creation. ROS also can damage mitochondrial DNA of sperm. Since mtDNA have a circular structure with few

DNA base pairs, lack of histones and absence of nucleotide excision repair (NER) pathway is more susceptible to ROS attack. In addition, ROS can damage the inner mitochondrial membrane and thus directly damage mtDNA and simultaneously cause electron leakage from the electron transport chain, which in turn increases the production of OS [4].

## The role of OS in spermatic membrane lipid peroxidation

Sperms are particularly susceptible to injuries by ROS because, on the one hand, their plasma membranes are full of polyunsaturated fatty acids (PUFA) with multiple double bonds, and on the other hand, the cytoplasm rates of antioxidant enzymes in sperms are extremely low. Induction of lipid peroxidation cascades is a result of the exceeded level of ROS in stressed sperms leading to reduced sperm function [124]. Extensive lipid peroxidation changes the accumulation, structure, and dynamics of lipid membranes, and since these lipids are highly reactive compounds, it can produce a large amount of ROS [125]. Hydroxyl radical (·OH) is a powerful lipid peroxidation initiator (Fig. 1). Most of the unsaturated fatty acids of the sperm membrane are non-conjugated double bonds separated by methylene groups. The presence of double bonds near the methylene group weakens H–C bonds of methylene, thereby increasing the likelihood of hydrogen separation. When hydrogen is separated, free radicals are created and the location of double bonds changes in such a way that the created radical is stabilized. This radical contains two double bonds that are separated by a single bond. Therefore, lipids containing a large number of double bonds in the vicinity of methylene groups are highly susceptible to O2 peroxidation for radical creation. Conjugated radicals rapidly react with lipid peroxyl radicals (·ROO) that in turn separate hydrogen atoms from other lipid molecules to create lipid hydroperoxide (ROOH) [126]. Increased levels of ROS during a series of responses including the oxidation of sulfhydryl groups (SH-) decrease the phosphorylation of the axonal protein, thereby decreasing the sperm motility. Hydrogen peroxide (a kind of ROS) can pass through the membrane of sperm and enter the cytoplasm. Hydrogen peroxide in the sperm cytoplasm inhibits activities of certain enzymes including G6PD. This enzyme regulates the entry of glucose into the hexose monophosphate path (pentose phosphate pathway). Under normal conditions, this pathway produces reduced NADPH for cellular reduction responses. Inhibition of hexose monophosphate shunt reduces the production of NADPH as a reduction equivalent in sperm. Glutathione peroxidase (GPx) is a major antioxidant enzyme in the sperm and uses reduced glutathione (SH-G) for reduction of ROS. As a result of the action of glutathione peroxidase, the reduced glutathione is converted into its oxidized form (G-S-S-G). Cells need the NADPH for re-reduction of oxidized glutathione; hence, the reduced NADPH level due to the inhibition of G6PD enzyme reduces the activity of glutathione peroxidase enzyme as an antioxidant defense. Therefore, the level of phospholipids peroxidation increases in the sperm membrane, thereby reducing the fluidity of the membrane and reducing the sperm motility. Malondialdehyde (MDA) is a sub-product of lipid peroxidation that is considered as a measure of peroxidation damage to spermatozoa in various biochemical tests [124]. Also, removing electrons from lipids of plasmid membrane by ROS can cause lipid peroxidation. This leads to the creation of a series of reduction–oxidation reactions that ultimately result in highly mutagenic and genotoxic electrophilic aldehydes such as malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and acrolein. 4-HNE is derived from lipid hydroperoxides (LOOHs) of fatty acids ω-6 such as linoleic acid and arachidonic acid [4]. 4-HNE is a very genotoxic by-product, while MDA is very mutagenic. 4-HNE and acrolein significantly increase lipid peroxidation, ROS production in the mitochondria, DNA fragmentation and apoptosis [127, 128]. High levels of ROS could interrupt mitochondrial membranes, resulting in caspases' activation and eventually apoptosis. Cytochrome c release throughout the apoptosis further increases ROS levels, raising damage and fragmentation of DNA, and potentially enhances the apoptotic cycle [3, 103]. Therefore, the sperm plasma membrane is a key target for ROS, which by stimulating cascade signals can damage the genetic composition of these cells. A recent study investigated the comparison of sperm parameters with MDA level as a factor for measuring the lipid peroxidation, NO, ascorbic acid and GSH in both infertile and unknown reproductive potential groups. Comparison of variables indicates that the sperm quality decreases in the infertile group, while NO, GSH and ascorbic acid levels increase. The results indicate that GSH and ascorbic acid levels probably increased to cope with the harmful effects of ROS and prevention of lipid peroxidation [129]. Given the above cases, determination of MDA level and total antioxidant capacity (TAC) may be useful in the diagnosis and treatment of male infertility.

## The role of OS on male reproductive hormones

Testicles' OS, which may be due to the damage to Leydig cells and other endocrine structures such as the anterior pituitary, decreases testosterone production [130]. In normal steroidogenesis, ROS is mainly produced by mitochondrial respiration and catalytic responses of cytochrome steroidogenic enzymes P450 [131]. OS increases the number of immature sperms through indirect effects on the production of male hormones during spermatogenesis [132]. Systemic hormones (FSH, LH, testosterone, $E_2$, PRL) adjust the total antioxidant capacity [133]. Some hormones like testosterone and melatonin act

as antioxidants and protect sperm and other testosterone cells against damage by ROS [134]. Hypothalamic–pituitary–adrenal (HPA) axis is activated by the production of ROS and cortisol hormone is released in response to stress. This hormone negatively affects the anterior pituitary gland through cross-talk between hypothalamic–pituitary–gonadal (HPG) and HPA axes, and finally, decreases secretes of LH and FSH hormones. As a result of low LH, it cannot stimulate Leydig cells to secrete testosterone.

Furthermore, the reduced FSH decreases the release of androgen-binding protein (ABP) to sertoli cells; hence, a general decrease in testosterone level occurs during the OS. ROS also affects the hypothalamo–pituitary–thyroid (HPT) axis and reduces secretes of T3 and T4. Reduced T3 decreases StAR mRNA levels in Leydig cells and decreases testosterone production. Increased OS also decreases insulin secretion from the pancreas and negatively affects the secretion of $T_3$, thereby affecting the biosynthesis of testosterone. Conditions like obesity not only involve HPA and HPT axes but also cause the secretion or non-secretion of several metabolic hormones manifesting the sequence of ROS induction in male reproduction. ROS due to obesity can force adipocytes to secrete more leptin. This hormone together with insulin negatively adjusts the secretion of $T_3$, and thus inhibits the performance of testes. Secreted leptin by adipocytes inhibits the emission of gonadotropin-releasing hormone (GnRH) from the hypothalamus. After exposure to ROS, the aromatase activity increases resulting in excessive production of testicular $E_2$ hormones. OS also increases the secretion of inhibitory hormones; hence, $E_2$ hormones and the inhibitor prevent the secretion of testosterone. The exposure to ROS increases the PRL secretion from the anterior pituitary, and thus decreases GnRH secretion. The genital tract infection can produce some proinflammatory cytokines such as tumor necrosis factor alpha (TNF)-α, interleukin (IL)-1b and IL-6, thereby re-inhibiting the secretion of GnRH and testosterone hormones. Therefore, ROS can reduce the production of testosterone by affecting the hormonal axis alone or end up in cross-talk between different endocrine systems. Reducing testosterone disrupts spermatogenesis and produces immature sperm. Lack of testosterone as the main regulator of male reproductive behavior may lead to the suppression of sexual behavior among men. Therefore, ROS leads to male infertility by disrupting reproductive endocrine glands (Fig. 2) [6].

## Effect of OS in pathophysiological process and male infertility

### Inflammation and OS

Inflammation is the natural response of the host to microbial attack or tissue damage and ultimately leads to arterial regeneration and improvement of its function [135]. Following the inflammation, neutrophils are the first immune cells presenting at the infection area; though macrophages also play an important role in the inflammatory response. Macrophages and other stimulated inflammatory cells secrete a large amount of prostaglandin E2 (PGE2), cytokine, and NO. However, inflammation affects steroidogenesis and spermatogenesis. In addition to inflammation, there is a significant reduction in testosterone levels and luteinizing hormone in the blood [136]. Inflammation also stops spermatogenesis and inhibits the sperm maturation process. Spermatocytes and spermatids are the main targets of inflammation. Epididymitis may be also affected by inflammatory attacks on testes. The inflammatory response is caused by leukocytes that penetrate semen and secrete antisperm antibodies. Decreasing lipid components of sperm flagella membrane, the inflammation increases its hardiness and decreases sperm motility resulting in sperm agglutination and asthenozoospermia. As a result of these events, maturation processes such as acrosome reaction, capacitation, and oocyte penetration encounter problems [137]. However, evidence suggests that the relationship between inflammation and OS is very high in sperm. Semen of infertile men has ROS in addition to high levels of proinflammatory factors and cytokines. Despite the fact that some external pathogenic factors such as bacteria may produce ROS, leukocytes are also the most important sources of semen ROS [138]. There are direct and indirect ways to increase ROS level by leukocytes. Leukocytes indirectly increase ROS levels by producing proinflammatory cytokines. In general, cytokines are proteins that act as signal molecules to regulate cellular responses such as inflammation. These proteins activate the xanthine oxidase system, raise ROS levels, and create OS.

On the other hand, by stimulating phagocytes and initiating the phagocytosis process, a high level of ROS is produced that reacts with the spermatozoa membrane and increases the ratio of oxidants-to-antioxidants. The OS caused by these conditions continues even after the elimination of pathogens [136]. Studies indicate that an increase in TNF-α of semen is associated with decreased number and mobility of sperm and its morphological defect. Increased cytokine levels in semen due to the proliferation and differentiation of beta cells, the proliferation of T cells and natural lethal cells cause apoptosis. Interleukin 1 alpha and interleukin 1 beta also induce apoptosis through the proliferation and differentiation of beta cells, the chemical absorption of leukocytes into inflammation site, induction of neutrophils and production of monocytes. These cytokines also negatively affect the quality of semen. A study suggests that increased levels of interleukin 1 beta are associated with reduced sperm motility [139, 140]. These results are accompanied by a

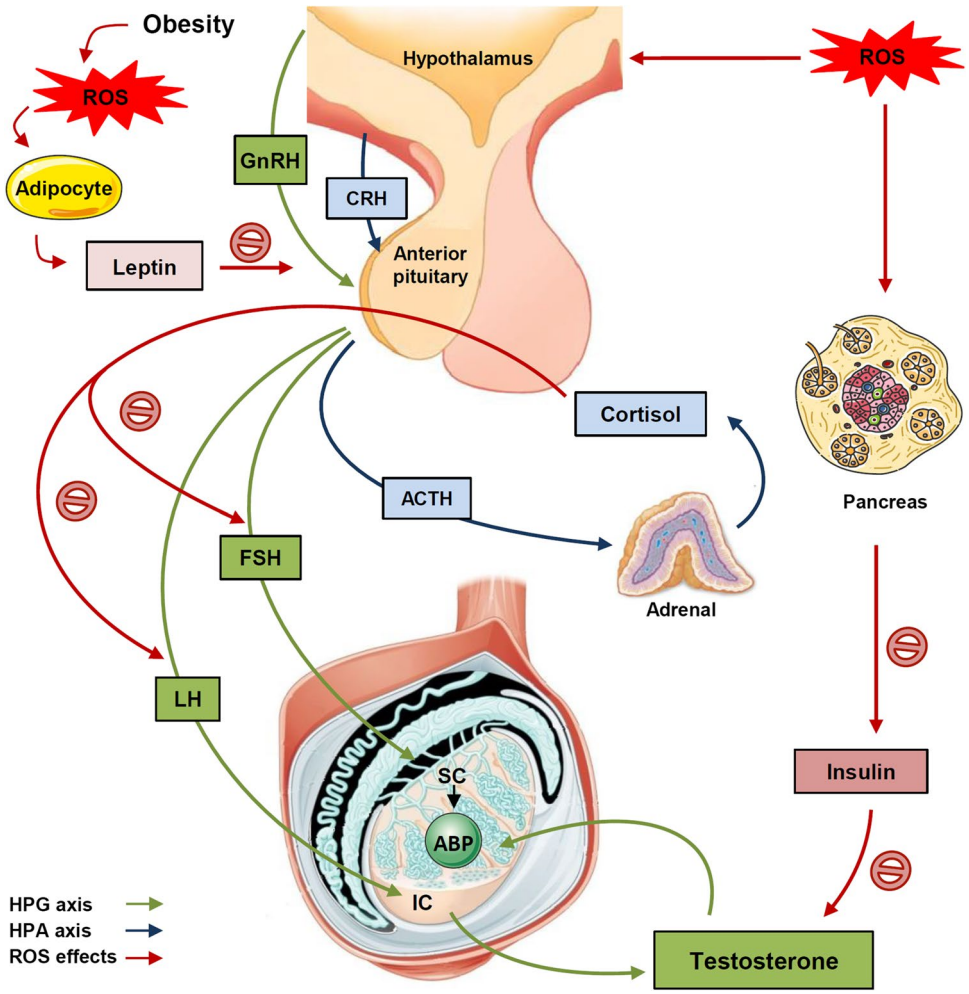


**Fig. 2** Effect of oxidative stress on production of testosterone. The hypothalamic–pituitary–gonadal axis (HPG; green arrow) regulates production of testosterone pathway in mammalian. The hypothalamus produces gonadotropin-releasing hormone (GnRH) and this hormone stimulates anterior lobe of the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). FSH affects sustentacular cells (SC) to release androgen-binding protein (ABP) which this protein concentrates testosterone near spermatogenic cells. In addition, LH affects interstitial cells (IC) to release testosterone. Excessive production of ROS activates hypothalamic–pituitary–adrenal axis (HPA; blue arrow) and affects hypothalamus to release corticotropin-releasing hormone (CRH) which stimulates the anterior lobe of pituitary gland to release adrenocorticotropic hormone (ACTH) and this hormone affects adrenal gland. The adrenal releases cortisol hormone in response to OS. This hormone negatively affects the anterior pituitary gland and finally decreases secretion of LH and FSH hormones. In another pathway, obesity increases production of ROS. Excessive ROS stimulates adipocyte cells to produce leptin hormone and this hormone inhibits the HPG axis. On the other hand, excess ROS negatively affect pancreas to decrease insulin production, which results in decrease of testosterone production

simultaneous increase in ROS and MDA levels. Concentrations of similar cytokines, which act as immune-regulating elements for gonads, increase with infection and damage to semen tissue. Participation of these cytokines in the inflammation has a direct relationship with leukocytospermia. In inflammatory conditions, white blood cells in semen penetrate the inflammation site. In normal conditions, leukocytes are prevented from going to the center of the seminal vesicle. In inflammatory sites, arteries which feed testes are dilated and allow leukocytes to go to the vascular center and exit the vascular endothelium. In addition to these changes, the increased permeability leads to the fluid accumulation followed by swelling, pain and also the accumulation of immunoglobulins and other semen proteins in the reproductive system. The excretion of leukocytes from the seminal vesicle depends on the crosslinking responses that are activated by the release of inflammatory mediators. Therefore, the inflammation or physical damage to the reproductive system causes phagocytic cells to be absorbed into the injury site. Activated neutrophils by TNF-α produce oxygen and oxide nitride radicals and release their stored granular content resulting

in the host defense and local tissue degradation. Since the male infertility is often caused by infection or damage to testes; and inflammatory response in the male reproductive system has a negative effect on the sperm quality and fertility, and the inflammation process produces ROS, all possible measures to eliminate or reduce inflammation reduce OS and improve pregnancy (Fig. 3) [141].

## Varicocele and oxidative stress

Varicocele is a main cause of infertility in men; and its incidence is 15% in the whole men and 40% in infertile men [142]. OS is a major contributor to the pathophysiology of this disease. Increased level of ROS and reduced concentrations of antioxidants simultaneously intensify varicocele [143]. In men with varicocele, ROS is released from three types of reproductive system cells under heat and hypoxic stress conditions. These cells include main epididymal cells, endothelial cells in the dilated pampiniform plexus and testicular cells such as developing germ cells, Leydig cells, macrophages, and peritubular cells. In mitochondria, heat and hypoxic stress can directly activate complex III of the electron transport chain and cause the release of ROS. On the other hand, NO is released from testicular and endothelial cells in testis with varicocele and can nitrosylate complexes I and IV, and simulates complex III to further release ROS [144]. OS plays an important role in causing damage to testicular tissue. A great number of studies have measured OS markers in semen of infertile men with varicocele and compared their values to men with idiopathic infertility. The results indicate that infertile men with varicoceles have higher levels of ROS, free radicals such as NO, nitric oxide synthase and $H_2O_2$, high levels of MDA and hexanoyllysine (another product of lipid peroxidation) [145]. Spermatic veins are the main components of the pathogenesis of varicocele. Endothelial cells can produce a lot of ROS by certain stimuli. Plasma levels of NO and iNOS are higher in spermatic veins of infertile men with varicocele than their other veins [146]. Enzymatic and non-enzymatic antioxidants have been also measured to investigate OS in infertile men with varicoceles. Results indicate lower levels of seminal TAC in infertile men with varicocele than healthy participants [144]. However, some studies have reported that an early increase in concentrations of antioxidants such as SOD and CAT is due to the application of a defense mechanism to prevent the disease [147]. ROS level is high both in infertile men with varicocele and infertile men without varicocele, but it is much higher in the first group indicating that varicocele increases the production of ROS and subsequently the oxidative damage. Varicocele is also associated with the severity of OS; and the highest degree of varicocele leads to the highest OS [145].

## Obesity and oxidative stress

Obesity is a metabolic disorder characterized by environmental, genetic, lifestyle and physical activity factors. Approximately 1.9 billion of the world's population are

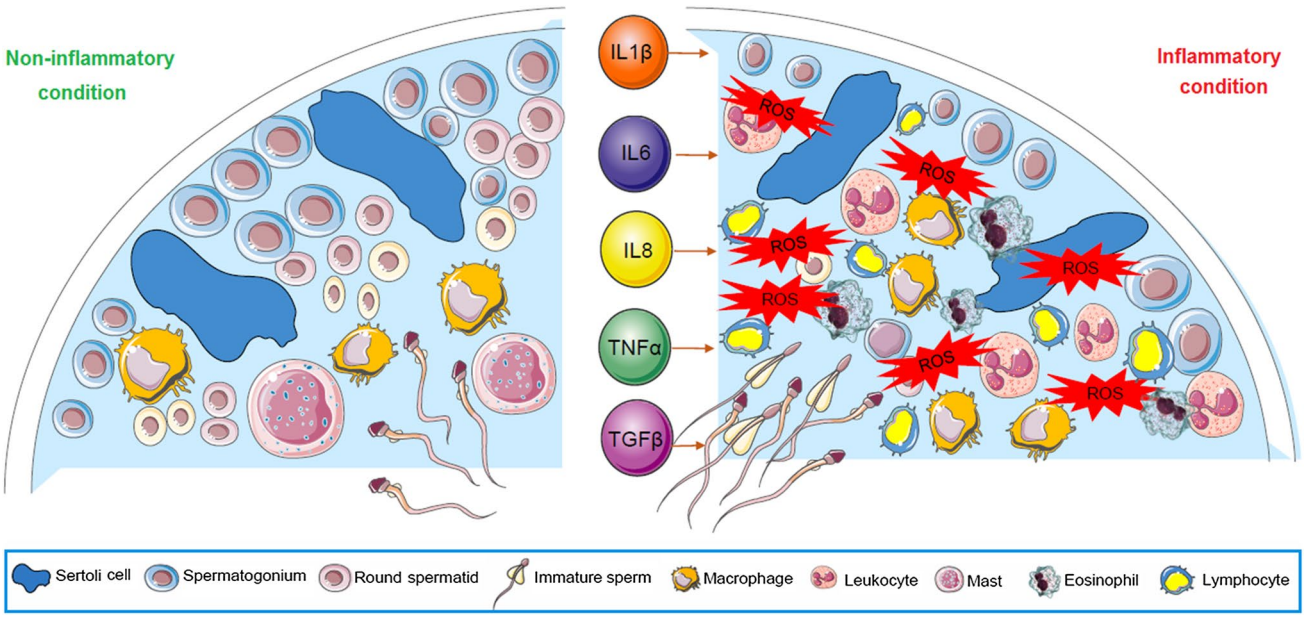


**Fig. 3** Schematic illustration of spermatogenesis in the seminiferous epithelium in an inflammatory condition. In the inflammatory reaction, sertoli cells, mast cells, and resident macrophages release chemokine and cytokines such as IL1β, IL6, IL8, TNFα, and TGFβ. These proinflammatory molecules cause the accumulation of leukocytes in the seminiferous tubules. Leukocytes also increase the level of ROS and OS up to 1000 times compared to normal conditions, and result in failed spermatogenesis

overweight (BMI > 25 kg/m$^2$) or obese (BMI > 30 kg/m$^2$). Laboratory and clinical data indicate the negative effect of obesity on the male reproductive function. Determinants of sperm quality including the sperm count and its motility are lower and the DNA damage is higher in obese or overweight people compared with those with normal BMIs [148]. Consequently, obesity increases the risk of infertility by more than 20% [36]. Obesity along with the chronic inflammation increases metabolic rate and ROS in the tissue of testes and sperm. OS in sperm and testis has a positive correlation with increasing BMI and sperm DNA damage, but a negative correlation with reduced sperm motility and acrosome reaction [149]. In addition, gonad temperature in obese men may also help to change sperm parameters. The optimum temperature of spermatogenesis is between 34 and 35 °C in humans, and the spermatogenesis process is put at risk at higher temperatures. In obese men, the increased scrotal fat directly increases the internal temperature of the gonad [150]. Typically, increasing testicular heat can significantly exacerbate OS, thereby reducing the mobility and concentration of sperm and increase DNA damage [151]. It has been recently found that the OS of testicular tissue in obese rats is used to the increased MDA level and reduced SOD level. Obesity is associated with an increase in serum-free fatty acids, and unsaturated fatty acids are sensitive to ROS attacks, and thus the SOD rate decreases and MDA increases by peroxidation. Therefore, the long-term effects of obesity can reduce fertility in men due to increased OS [152]. OS is a common complication in many diseases such as infertility in men, and reactive oxygen species play important roles in spermatogenesis and reproduction. The normal functioning of sperm requires a balance between ROS levels and antioxidants; hence, the use of natural antioxidants, healthy lifestyles, regular exercise, and proper diets lead to the weight loss and gradual BMI reduction that helps reduce OS and provide sperm health [153].

## Effect of antioxidants and herbal therapy on improvement of sperm quality

Antioxidant system including enzymatic factors (superoxide dismutase, catalase, and glutathione peroxidase), non-enzymatic factors, and low-molecular weight compounds (glutathione, n-acetylcysteine, vitamin E, A and C, coenzyme Q10, carnitines, myo-inositol, lycopene and …), and nutrients (selenium, zinc, and copper) can protect the body against OS. Lack of one of these leads to a reduction in the antioxidant capacity of the plasma [154]. Many urologists prescribe oral antioxidants for infertility [155], probably as the OS in semen is due to the lack of antioxidants and its side effects. Ideally, an oral antioxidant should reach a high concentration in the reproductive tract and restore vital elements for spermatogenesis. In addition, antioxidant supplementation should increase the seminal plasma clearance capacity and decrease ROS level in semen [156]. Nowadays, the use of herbal therapy has also been considered to prevent infertility because these antioxidants can reduce the destructive effects of oxidative stress [157].

### Enzymatic factors

SOD metalloenzymes are effective antioxidant enzymes in the body. These enzymes are the first body defense against the ROS. SODs convert the superoxide anion into $H_2O_2$, and then the produced $H_2O_2$ is eliminated by activities of glutathione peroxidase or catalase. SOD protects spermatozoa against $O_2$ toxicity, lipid peroxidation and ultimately OS [158]. There is no consensus on SOD consumption dosage; however, the most commonly proposed treatment for oral consumption is 150 IU per day for at least 3 months [159]. Catalase exists in peroxisomes and converts $H_2O_2$ into the water and molecular oxygen in two steps. This enzyme plays a major role in the capacitation of nitric oxide-induced sperm [154]. Glutathione peroxidase converts $H_2O_2$ to water or alcohol, provides the main protection against low levels of oxidative damage, and plays an important role in the defense of sperm [160]. Glutathione is a tripeptide containing sulfur and can be seen in GSH and GSSG. The decreased glutathione in the human seminal plasma leads to instability in the middle of spermatozoa and ultimately causes motion impairment [161]. Glutathione supplement plays a pathological role in infertile men in situations where reactive oxygen species or other toxic substances such as unilateral varicoceles or genital inflammation result in significant improvement in sperm parameters (number, mobility, and morphology). GSH also plays an important protective role against cell membrane lipid damage. Its effective dose is intramuscular 600 mg per day for 2–3 months [162]. N-Acetylcysteine (NAC) is a glutathione precursor and stimulates the sperm motility. It effectively improves the volume of seminal fluid and its viscosity and increases the sperm motility in humans [163]. The most commonly used dose of NAC is 600 mg per day as a combination with other antioxidants for at least 3 months [164].

#### Animal experiments

NAC protects sperm DNA against oxidative damage and can improve sperm parameters in mice [165]. The catalase present in the sperm of ram has a potential role in controlling oxidative stress by catalyzing $H_2O_2$ [166].

## Non-enzymatic factors

Carotenoids are natural antioxidants that protect the integrity of the cell membrane, regulate the differentiation of epithelial cells and contribute to the regulation of spermatogenesis. Lack of carotenoids in the diet can reduce sperm motility [154]. In humans, low serum levels of retinol reduce sperm quality [167]; hence the use of vitamin A can be used to treat male infertility [168]. The amount of vitamin C in seminal plasma is ten times more than serum [169]. In humans, ascorbic acid levels are positively correlated with percentages of normal sperms [170] and negatively with the DNA fragmentation [171]. These findings support the consumption of vitamin C for treating male infertility. Proper vitamin C dose for increasing sperm quality is oral 1 g per day [172]. Vitamin E protects the sperm cell membrane against the OS, prevents lipid peroxidation and traps free radicals of hydroxyl and superoxide [154]. A clinical trial indicates that sperm function improves after consuming 600 mg of vitamin E per day for 3 months. Other studies used lower doses (200 mg/300 mg) [173]. A study reported that the sperm motility increased in infertile men after oral consumption of vitamin E. Improvement of sperm motility is associated with the reduction of MDA production as the final product of lipid peroxidation. In addition, during 6 months of treatment, 21% of patients achieved pregnancy [174]. In general, these findings indicate that vitamin E can be a good choice for treating male infertility [175]. Coenzyme Q10 is a fat-soluble antioxidant that endogenously synthesizes and prevents the oxidation and lipid peroxidation. It also regulates the mitochondrial electron transfer chain [176]. A number of clinical studies found a positive effect of coenzyme Q10 on male fertility. This antioxidant improves all sperm parameters [177] and has a protective effect against OS and DNA damage [178]. Moreover, the treatment with CoQ10 improves sperm parameters in people with varicocele [176]. CoQ10 can regenerate other antioxidants such as vitamins E and C [179]. Selenium is a necessary nutrient for normal growth of testicles, spermatogenesis, and sperm motility and function. The selenium deficiency leads to the epithelium atrophy of seminiferous, spermatogenesis and sperm maturation impairment, reduced volume of testes, decreased sperm motility and changed sperm shape (especially in the head and middle part) [180]. There is no fully precise mechanism by which selenium reduces OS and improves sperm parameters, but is likely to be achieved by selenoenzyme such as GPX. An appropriate dose of selenium for oral use is 80–300 micrograms per day alone or with other antioxidants for 3 months [181]. Low amounts of ROS are essential for capacitation and acrosome reaction. Therefore, the excess reduction of ROS concentration due to the use of antioxidants may have reversible effects on fertility [182]. However, antioxidants neutralize the OS; and most studies indicate that improvement of sperm parameters occurs when several antioxidants are used in combination. However, there is no definitive consensus on dose and duration of use of these substances; and there is a need for further studies [10].

### Animal experiments

A study on cows indicated that retinol stabilizes sperm acrosome membrane in the OS condition due to the high temperature [167]. Vitamin C in mice in a dose equivalent to human dose (10 m/kg) can reduce MDA level and increase the number of sperms [183]. Another study shows that treatment with ascorbic acid reduces peroxidation levels and histological damage on seminiferous tubules in diabetic rats [184].

## Other nutrients

In addition to antioxidants, there are a variety of therapies around the world for male infertility management. The recent therapies as assisted reproductive techniques (ARTs) are not only costly but also have a success rate of only 10–30%. Accordingly, the use of herbal medicines as an alternative therapy or complementary technique has been taken into account more for coping with male infertility [157]. The active substances of plants, which improve fertility, include various chemical groups such as saponins, phytosterols, carotenoids, oxygenated volatile compounds, phenols, lignans and alkaloids [185]. *Withania somnifera* (ashwagandha) is the root of evergreen shrub in India. Studies indicate that the oral intake of this plant increases the quality of semen, prevents the lipid peroxidation, regulates levels of reproductive hormones, and also decreases the risk of infertility in men by reducing the level of OS [157]. Curcumin is a Chinese herb monomer that is extracted from yellow turmeric and has antioxidant and anti-inflammatory properties. According to a study on the effect of curcumin on sperm quality in patients with leukocytospermia, results indicate that curcumin significantly improves the sperm motility in patients and decreases $H_2O_2$. Therefore, it overcomes male infertility by reducing OS [186]. Cinnamon has antioxidant, anti-inflammatory and anti-diabetic properties. Therefore, the use of cinnamon has potential effects on the oxidative and antioxidant balance of testes and sperm quality; and its use is beneficial for men with asthenozoospermia [187]. *Crocus sativus* is a plant of iridaceae family and is traditionally used for diseases of the digestive system, reproductive tract, and depression. Saffron plays a protective role against DNA damage, chromatin anomalies and provides the membrane integrity [188, 189]. *Nigella sativa* (black caraway) is a herbal medicine that is applicable for a wide range of diseases such as pulmonary problems, headache, rheumatism, gastrointestinal problems, and male sexual disorders [185].

In one clinical trial, the use of seed oil for infertile men with different factors of infertility resulted in the enhancement of all semen parameters [190]. Pomegranate seeds contain fatty acids and phenolic compounds with antioxidant properties. Green tea can improve gametes in men and women by the ability of catechin polyphenol through eliminating ROS. Epigallocatechin-3-gallate (EGCG) is considered as an active antioxidant that can potentially improve reproductive health by reducing the OS [191]. There is little information about exact mechanisms of these plants and infertility, but most findings have pointed out the antioxidant properties of plants.

#### Animal experiments

A study examined the long-term effects of cinnamon crust oil on antioxidant levels of testes and sperm quality of mature adult rats. The results indicated a significant reduction in the level of MDA and a significant increase in the activity of glutathione peroxidase and catalase [187]. A study on rats demonstrated that dietary Zn deficiency decreases serum testosterone levels, increases OS and results in apoptosis of germ cells [192]. Catechins are the main components of green tea, which have higher antioxidant activity than vitamin C. Catechins in rats were observed to regulate gene expression and reduce ROS levels [193]. Animal studies indicate that the use of *Crocus sativus* (saffron) improves sperm parameters due to its antioxidant properties [194]. In addition, it promotes semen quality by increasing testosterone levels [195]. Animal studies show that the alcoholic extract of black caraway seed improves the sperm survival and also increases the sperm motility. The precise mechanism of black caraway function is still unknown, but it seems that it increases the activity of beta-hydroxy steroid dehydrogenase (a key enzyme in the synthesis of testosterone) due to the presence of unsaturated fatty acids, and thus it is useful for the fertility [196] *Sesamum indicum* (sesame) is an annual plant and old crop which could improve the quality of sperm parameters in rat. Furthermore, sesame helps to make better spermatogenesis process by affecting the hypothalamus–pituitary–testis axis and increasing the blood testosterone [197]. Pomegranate seeds can improve the integrity of the sperm membrane and protect it against oxidative damage, and prevent the DNA damage by antioxidant properties by eliminating free radicals [198]. *Ionidium suffruticosum* (L.) ging (Violaceae) is an important herbal medicine that is widely used as traditional medicine. According to a study on infertile rats treated with this herbal medicine, results indicated that spermatogenesis, sperm count, and oxidative biomarkers of SOD and CAT improved after a treatment period. Therefore, the use of this herbal medicine for the treatment of male infertility was recommended in traditional medicine [199]. Grape seed extract acts as an anti-inflammatory, antioxidant and antibacterial agent and also has protective effects on the heart, liver, and nerve. Grape seed contains a special flavonoid called anthocyanin oligomers. This compound increases intracellular vitamin C level and thus destructs ROS and free radicals, and it has more antioxidant properties than vitamins C and E. In a study, the impairment of testicular activity was induced by aluminum chloride, and the sperm number and motility increased after intervention with grape seed extract. It also protects sperm against DNA damage and protects testicles by reducing the NO activity [200].

## Conclusion

OS is now considered as the main cause of male infertility. Despite the need for low levels of ROS for the physiological function of sperm, its increased level disturbs sperm functions, thereby leading to male infertility by mechanisms such as lipid peroxidation and DNA damage. Therefore, the early diagnosis of infertility is necessary to prevent its irreversible damage due to the OS in the long term. There are several tests for early diagnosis of high levels of OS factors. Results of measuring levels of ROS in semen as well as the determination of MDA and TAC levels can affect the male infertility diagnosis and treatment. Besides, the lifestyle change is the primary action to balance levels of ROS and antioxidants in the body and prevent oxidative stress. Reduced smoking and alcohol, non-exposure to radiation, and selection of a proper diet along with planned physical activity have significant effects on reducing levels of OS and thus male infertility. Moreover, the use of oral antioxidants can also help to reduce OS, but more studies are necessary about their doses and duration.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR (2015) A unique view on male infertility around the globe. Reprod Biol Endocrinol 13(1):37

2. Agarwal A, Prabakaran S, Allamaneni SS (2006) Relationship between oxidative stress, varicocele and infertility: a meta-analysis. Reprod Biomed Online 12(5):630–633
3. Wagner H, Cheng JW, Ko EY (2018) Role of reactive oxygen species in male infertility: an updated review of literature. Arab J Urol 16(1):35–43
4. Bui A, Sharma R, Henkel R, Agarwal A (2018) Reactive oxygen species impact on sperm DNA and its role in male infertility. Andrologia 50(8):e13012
5. Gogol P, Szczęśniak-Fabiańczyk B, Wierzchoś-Hilczer A (2009) The photon emission, ATP level and motility of boar spermatozoa during liquid storage. Reprod Biol 9(1):39–49
6. Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, Sadeghi MR (2018) Reactive oxygen species and male reproductive hormones. Reprod Biol Endocrinol 16(1):87
7. Tremellen K (2008) Oxidative stress and male infertility—a clinical perspective. Hum Reprod Update 14(3):243–258
8. Nakamura BN, Lawson G, Chan JY, Banuelos J, Cortés MM, Hoang YD, Ortiz L, Rau BA, Luderer U (2010) Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. Free Radic Biol Med 49(9):1368–1379
9. Meseguer M, Antonio Martinez-Conejero J, Muriel L, Pellicer A, Remohí J, Garrido N (2007) The human sperm glutathione system: a key role in male fertility and successful cryopreservation. Drug Metab Lett 1(2):121–126
10. Alahmar AT (2018) The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia. Clin Exp Reprod Med 45(2):57–66
11. Agarwal A, Rana M, Qiu E, AlBunni H, Bui AD, Henkel R (2018) Role of oxidative stress, infection and inflammation in male infertility. Andrologia 50(11):e13126
12. Leclerc P, De Lamirande E, Gagnon C (1997) Regulation of protein-tyrosine phosphorylation and human sperm capacitation by reactive oxygen derivatives. Free Radic Biol Med 22(4):643–656
13. de Lamirande EHA, Gagnon C (1998) Human sperm capacitation induced by biological fluids and progesterone, but not by NADH or NADPH, is associated with the production of superoxide anion. J Androl 19(2):215–225
14. Herrero MB, de Lamirande E, Gagnon C (1999) Nitric oxide regulates human sperm capacitation and protein-tyrosine phosphorylation in vitro. Biol Reprod 61(3):575–581
15. Thundathil J, de Lamirande E, Gagnon C (2003) Nitric oxide regulates the phosphorylation of the threonine-glutamine-tyrosine motif in proteins of human spermatozoa during capacitation. Biol Reprod 68(4):1291–1298
16. Cn O'Flaherty, de Lamirande E, Gagnon C (2005) Reactive oxygen species and protein kinases modulate the level of phospho-MEK-like proteins during human sperm capacitation. Biol Reprod 73(1):94–105
17. O'Flaherty C, de Lamirande E, Gagnon C (2006) Reactive oxygen species modulate independent protein phosphorylation pathways during human sperm capacitation. Free Radical Biol Med 40(6):1045–1055
18. Leclerc P, de Lamirande EVE, Gagnon C (1998) Interaction between Ca2+, cyclic 3′, 5′adenosine monophosphate, the superoxide anion, and tyrosine phosphorylation pathways in the regulation of human sperm capacitation. J Androl 19(4):434–443
19. Lefièvre L, Jha KN, de Lamirande E, Visconti PE, Gagnon C (2002) Activation of protein kinase A during human sperm capacitation and acrosome reaction. J Androl 23(5):709–716
20. Condorelli R, Russo GI, Calogero A, Morgia G, La Vignera S (2017) Chronic prostatitis and its detrimental impact on sperm parameters: a systematic review and meta-analysis. J Endocrinol Invest 40(11):1209–1218
21. Harlev A, Agarwal A, Gunes SO, Shetty A, du Plessis SS (2015) Smoking and male infertility: an evidence-based review. World J Mens Health 33(3):143–160
22. Gomez E, Buckingham DW, Brindle J, Lanzafame F, Irvine DS, Aitken RJ (1996) 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 17(3):276–287
23. Said TM, Agarwal A, Sharma RK, Mascha E, Sikka SC, Thomas AJ Jr (2004) Human sperm superoxide anion generation and correlation with semen quality in patients with male infertility. Fertil Steril 82(4):871–877
24. Styrna J, Piasecka M, Malek P, Golas A (2010) Sperm mitochondria diaphorase activity–a gene mapping study of recombinant inbred strains of mice. Int J Dev Biol 54(4):667–673
25. Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR (2016) Etiologies of sperm oxidative stress. Int J Reprod Biomed 14(4):231
26. Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker H, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16(3):231–245
27. Fariello RM, Del Giudice PT, Spaine DM, Fraietta R, Bertolla RP, Cedenho AP (2009) Effect of leukocytospermia and processing by discontinuous density gradient on sperm nuclear DNA fragmentation and mitochondrial activity. J Assist Reprod Genet 26(2–3):151–157
28. Yadav SB, Suryakar AN, Huddedar AD, Shukla PS (2006) Effect of antioxidants and antibiotics on levels of seminal oxidative stress in leukocytospermic infertile men. Indian J Clin Biochem 21(1):152
29. Makker K, Agarwal A, Sharma R (2009) Oxidative stress & male infertility. Indian J Med Res 129(4):357–368
30. Hamada A, Agarwal A, Sharma R, French DB, Ragheb A, Sabanegh ES Jr (2011) Empirical treatment of low-level leukocytospermia with doxycycline in male infertility patients. Urology 78(6):1320–1325
31. Aboulmaouahib S, Madkour A, Kaarouch I, Sefrioui O, Saadani B, Copin H, Benkhalifa M, Louanjli N, Cadi R (2018) Impact of alcohol and cigarette smoking consumption in male fertility potential: looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage. Andrologia 50(3):e12926
32. Brand JS, Chan M-F, Dowsett M, Folkerd E, Wareham NJ, Luben RN, van der Schouw YT, Khaw K-T (2011) Cigarette smoking and endogenous sex hormones in postmenopausal women. J Clin Endocrinol Metab 96(10):3184–3192
33. Valavanidis A, Vlachogianni T, Fiotakis K (2009) Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. Int J Environ Res Public Health 6(2):445–462
34. Ghaffari MA, Rostami M (2013) The effect of cigarette smoking on human sperm creatine kinase activity: as an ATP buffering system in sperm. Int J Fertil Steril 6(4):258
35. Hamad M, Shelko N, Kartarius S, Montenarh M, Hammadeh M (2014) Impact of cigarette smoking on histone (H2B) to protamine ratio in human spermatozoa and its relation to sperm parameters. Andrology 2(5):666–677
36. Cui X, Jing X, Wu X, Wang Z, Li Q (2016) Potential effect of smoking on semen quality through DNA damage and the down-regulation of Chk1 in sperm. Mol Med Rep 14(1):753–761
37. Guthauser B, Boitrelle F, Plat A, Thiercelin N, Vialard F (2013) Chronic excessive alcohol consumption and male

fertility: a case report on reversible azoospermia and a literature review. Alcohol Alcohol 49(1):42–44
38. Akang EN, Oremosu AA, Osinubi AA, James AB, Biose IJ, Dike SI, Idoko KM (2017) Alcohol-induced male infertility: is sperm DNA fragmentation a causative? J Exp Clin Anat 16(1):53
39. Manzo-Avalos S, Saavedra-Molina A (2010) Cellular and mitochondrial effects of alcohol consumption. Int J Environ Res Public Health 7(12):4281–4304
40. Bailey SM, Robinson G, Pinner A, Chamlee L, Ulasova E, Pompilius M, Page G, Chhieng D, Jhala N, Landar A (2006) S-Adenosylmethionine prevents chronic alcohol-induced mitochondrial dysfunction in rat liver. Am J Physiol Gastrointest Liver Physiol 291(5):G857–G867
41. Radi R, Cassina A, Hodara R, Quijano C, Castro L (2002) Peroxynitrite reactions and formation in mitochondria. Free Radical Biol Med 33(11):1451–1464
42. Angelopoulou R, Lavranos G, Manolakou P (2009) ROS in the aging male: model diseases with ROS-related pathophysiology. Reprod Toxicol 28(2):167–171
43. Kesari KK, Agarwal A, Henkel R (2018) Radiations and male fertility. Reprod Biol Endocrinol 16(1):118
44. Gautam R, Singh KV, Nirala J, Murmu NN, Meena R, Rajamani P (2018) Oxidative stress-mediated alterations on sperm parameters in male Wistar rats exposed to 3G mobile phone radiation. Andrologia 51(3):e13201
45. Desai NR, Kesari KK, Agarwal A (2009) Pathophysiology of cell phone radiation: oxidative stress and carcinogenesis with focus on male reproductive system. Reprod Biol Endocrinol 7(1):114
46. Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P (2016) Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev 28(1–2):1–10
47. Chauhan P, Verma HN, Sisodia R, Kesari KK (2017) Microwave radiation (2.45 GHz)-induced oxidative stress: whole-body exposure effect on histopathology of Wistar rats. Electromagn Biol Med 36(1):20–30
48. Kesari KK, Kumar S, Behari J (2011) 900-MHz microwave radiation promotes oxidation in rat brain. Electromagn Biol Med 30(4):219–234
49. Du Plessis SS, Agarwal A, Sabanegh Jr ES (2014) Male infertility: a complete guide to lifestyle and environmental factors. Springer, New York, pp 1–268
50. Sabés-Alsina M, Tallo-Parra O, Mogas MT, Morrell JM, Lopez-Bejar M (2016) Heat stress has an effect on motility and metabolic activity of rabbit spermatozoa. Anim Reprod Sci 173:18–23
51. Pérez-Crespo M, Pintado B, Gutiérrez-Adán A (2008) Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol Reprod Dev 75(1):40–47
52. Paul C, Teng S, Saunders PT (2009) A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. Biol Reprod 80(5):913–919
53. Zhang M, Jiang M, Bi Y, Zhu H, Zhou Z, Sha J (2012) Autophagy and apoptosis act as partners to induce germ cell death after heat stress in mice. PLoS One 7(7):e41412
54. Li Y, Cao Y, Wang F, Li C (2014) Scrotal heat induced the Nrf2-driven antioxidant response during oxidative stress and apoptosis in the mouse testis. Acta Histochem 116(5):883–890
55. Pereira C, Mapuskar K, Rao CV (2006) Chronic toxicity of diethyl phthalate in male Wistar rats—A dose–response study. Regul Toxicol Pharmacol 45(2):169–177
56. Hauser R, Meeker J, Singh N, Silva M, Ryan L, Duty S, Calafat A (2006) DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Hum Reprod 22(3):688–695
57. Pant N, Shukla M, Patel DK, Shukla Y, Mathur N, Gupta YK, Saxena DK (2008) Correlation of phthalate exposures with semen quality. Toxicol Appl Pharmacol 231(1):112–116
58. Radwan M, Jurewicz J, Polańska K, Sobala W, Radwan P, Bochenek M, Hanke W (2016) Exposure to ambient air pollution-does it affect semen quality and the level of reproductive hormones? Ann Hum Biol 43(1):50–56
59. Kampa M, Castanas E (2008) Human health effects of air pollution. Environ Pollut 151(2):362–367
60. Hsu P-C, Guo YL (2002) Antioxidant nutrients and lead toxicity. Toxicology 180(1):33–44
61. Acharya U, Acharya S, Mishra M (2003) Lead acetate induced cytotoxicity in male germinal cells of Swiss mice. Ind Health 41(3):291–294
62. Xu D-X, Shen H-M, Zhu Q-X, Chua L, Wang Q-N, Chia S-E, Ong C-N (2003) The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. Mutat Res Genet Toxicol Environ Mutagen 534(1–2):155–163
63. Storgaard L, Bonde JP, Ernst E, Andersen CY, Spano M, Christensen K, Petersen HC, Olsen J (2006) Genetic and environmental correlates of semen quality: a twin study. Epidemiology 17(6):674–681
64. Mobasseri N, Babaei F, Karimian M, Nikzad H (2018) Androgen receptor (AR)-CAG trinucleotide repeat length and idiopathic male infertility: a case-control trial and a meta-analysis. Excli J 17:1167–1179
65. Rafatmanesh A, Nikzad H, Ebrahimi A, Karimian M, Zamani T (2018) Association of the c.-9C> T and c.368A> G transitions in H2BFWT gene with male infertility in an Iranian population. Andrologia 50(1):e12805
66. Yu B, Huang Z (2015) Variations in antioxidant genes and male infertility. Biomed Res Int 2015:513196
67. Holland R, Fishbein JC (2010) Chemistry of the cysteine sensors in Kelch-like ECH-associated protein 1. Antioxid Redox Signal 13(11):1749–1761
68. Chan K, Lu R, Chang JC, Kan YW (1996) NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proc Natl Acad Sci USA 93(24):13943–13948
69. Chyra-Jach D, Kaletka Z, Dobrakowski M (2018) The associations between infertility and antioxidants, proinflammatory cytokines, and chemokines. Oxidative Med Cell Longev 2018:8354747
70. Aitken RJ, Buckingham DW, Carreras A, Irvine DS (1996) Superoxide dismutase in human sperm suspensions: relationship with cellular composition, oxidative stress, and sperm function. Free Radic Biol Med 21(4):495–504
71. Faure C, Leveille P, Dupont C, Julia C, Chavatte-Palmer P, Sutton A, Levy R (2014) Are superoxide dismutase 2 and nitric oxide synthase polymorphisms associated with idiopathic infertility? Antioxid Redox Signal 21(4):565–569
72. Sabouhi S, Salehi Z, Bahadori M, Mahdavi M (2015) Human catalase gene polymorphism (CAT C-262 T) and risk of male infertility. Andrologia 47(1):97–101
73. Tefik T, Kucukgergin C, Sanli O, Oktar T, Seckin S, Ozsoy C (2013) Manganese superoxide dismutase Ile58Thr, catalase C-262 T and myeloperoxidase G-463 A gene polymorphisms in patients with prostate cancer: relation to advanced and metastatic disease. BJU Int 112(4):E406–E414
74. Selvaratnam JS, Robaire B (2016) Effects of aging and oxidative stress on spermatozoa of superoxide-dismutase 1-and catalase-null mice. Biol Reprod 95(3):60
75. Willoughby DS, Hwang P, Willoughby DS (2015) Intracellular mechanistic role of nitric oxide: a comparative analysis of the effectiveness of L-arginine and L-citrulline supplementation on

nitric oxide synthesis and subsequent exercise performance in humans. J Food Sci Nutr 2(1):1–8
76. Stuehr DJ, Griffith OW (1992) Mammalian nitric oxide synthases. Adv Enzymol Relat Areas Mol Biol 65:287–346
77. Doshi SB, Khullar K, Sharma RK, Agarwal A (2012) Role of reactive nitrogen species in male infertility. Reprod Biol Endocrinol 10(1):109
78. Mostafa T, Rashed LA, Nabil N, Fouad H, Sabry D, El-Saied DM (2015) Endothelial nitric oxide synthase gene polymorphism relationship with semen parameters and oxidative stress in infertile oligoasthenoteratozoospermic men. Urology 85(5):1058–1061
79. Oakley A (2011) Glutathione transferases: a structural perspective. Drug Metab Rev 43(2):138–151
80. Udomsinprasert R, Pongjaroenkit S, Wongsantichon J, Oakley AJ, Prapanthadara L-a, Wilce MC, Ketterman AJ (2005) Identification, characterization and structure of a new Delta class glutathione transferase isoenzyme. Biochem J 388(3):763–771
81. Safarinejad MR, Shafiei N, Safarinejad S (2010) The association of glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) with idiopathic male infertility. J Hum Genet 55(9):565
82. Xiong D-K, Chen H-H, Ding X-P, Zhang S-H, Zhang J-H (2015) Association of polymorphisms in glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) with idiopathic azoospermia or oligospermia in Sichuan. China Asian J Androl 17(3):481
83. Arnér ES, Holmgren A (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 267(20):6102–6109
84. Hansen JM, Go Y-M, Jones DP (2006) Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. Annu Rev Pharmacol Toxicol 46:215–234
85. Drevet JR (2006) The antioxidant glutathione peroxidase family and spermatozoa: a complex story. Mol Cell Endocrinol 250(1–2):70–79
86. Schneider M, Förster H, Boersma A, Seiler A, Wehnes H, Sinowatz F, Neumüller C, Deutsch MJ, Walch A, Hrabé de Angelis M (2009) Mitochondrial glutathione peroxidase 4 disruption causes male infertility. FASEB J 23(9):3233–3242
87. Chabory E, Damon C, Lenoir A, Kauselmann G, Kern H, Zevnik B, Garrel C, Saez F, Cadet R, Henry-Berger J (2009) Epididymis seleno-independent glutathione peroxidase 5 maintains sperm DNA integrity in mice. J Clin Invest 119(7):2074–2085
88. Salimi S, Keshavarzi F, Mohammadpour-Gharehbagh A, Moodi M, Mousavi M, Karimian M, Sandoughi M (2017) Polymorphisms of the folate metabolizing enzymes: association with SLE susceptibility and in silico analysis. Gene 637:161–172
89. Bafrani HH, Ahmadi M, Jahantigh D, Karimian M (2019) Association analysis of the common varieties of IL17A and IL17F genes with the risk of knee osteoarthritis. J Cell Biochem. https://doi.org/10.1002/jcb.29105
90. Karimian M, Aftabi Y, Mazoochi T, Babaei F, Khamechian T, Boojari H, Nikzad H (2018) Survivin polymorphisms and susceptibility to prostate cancer: a genetic association study and an in silico analysis. EXCLI J 17:479–491
91. Nejati M, Atlasi MA, Karimian M, Nikzad H, Tameh AA (2018) Lipoprotein lipase gene polymorphisms as risk factors for stroke: a computational and meta-analysis. Iran J Basic Med Sci 21(7):701–708
92. Mobasseri N, Nikzad H, Karimian M (2019) Protective effect of estrogen receptor alpha-PvuII transition against idiopathic male infertility: a case-control study and meta-analysis. Reprod Biomed Online 38(4):588–598
93. Noureddini M, Mobasseri N, Karimian M, Behjati M, Nikzad H (2018) Arg399Gln substitution in XRCC1 as a prognostic and predictive biomarker for prostate cancer: evidence from 8662 subjects and a structural analysis. J Gene Med 20(10–11):e3053
94. Tameh AA, Karimian M, Zare-Dehghanani Z, Aftabi Y, Beyer C (2018) Role of steroid therapy after ischemic stroke by N-methyl-d-aspartate receptor gene regulation. J Stroke Cerebrovasc Dis 27(11):3066–3075
95. Behrouzi S, Mashayekhi F, Bahadori MH (2018) The association of PON1 192 Q/R polymorphism with the risk of idiopathic male infertility in northern Iran. Avicenna J Med Biotechnol 11(1):253–256
96. Garcia Rodriguez A, de la Casa M, Johnston S (2019) Association of polymorphisms in genes coding for antioxidant enzymes and human male infertility. Ann Hum Genet 83(1):63–72
97. Vučić N, Nikolić Z, Vukotić V, Tomović S, Vuković I, Kanazir S, Savić-Pavićević D, Brajušković G (2018) NOS 3 gene variants and male infertility: association of 4a/4b with oligoasthenozoospermia. Andrologia 50(1):e12817
98. Mazjin M, Salehi Z, Mashayekhi F, Bahadori M (2016) Evaluation of GPx1 Pro198Leu polymorphism in idiopathic male infertility. Mol Biol 50(1):77–80
99. Yan L, Guo W, Wu S, Liu J, Zhang S, Shi L, Ji G, Gu A (2014) Genetic variants in nitric oxide synthase genes and the risk of male infertility in a Chinese population: a case-control study. PLoS One 9(12):e115190
100. Ji G, Yan L, Liu W, Qu J, Gu A (2013) OGG1 Ser326Cys polymorphism interacts with cigarette smoking to increase oxidative DNA damage in human sperm and the risk of male infertility. Toxicol Lett 218(2):144–149
101. Kolesnikova L, Kurashova N, Bairova T, Dolgikh M, Ershova O, Natyaganova L, Dashiev B, Gutnik I, Koroleva N (2017) Features of lipoperoxidation, antioxidant defense, and thiol/disulfide system in the pathogenesis of infertility in males, carriers of nonfunctional variants of GSTT1 and GSTM1 gene polymorphisms. Bull Exp Biol Med 163(3):378–380
102. Hashad D, Mohamed N, Hashad M (2012) Luteinising hormone β-subunit gene Gly102Ser variant and oxidative stress biomarkers in Egyptian infertile males. Andrologia 44:484–489
103. Liu H, Zhao J, Xu J, Zhu P, Yu M, Jiang W, Zhang J, Li W, Wu Q, Li Z (2018) Correlation of the single nucleotide polymorphism rs662 of PON1 with the risk of male infertility. Zhonghua Nan Ke Xue 24(8):708–712
104. Trang NT, Huyen VT, Tuan NT, Phan TD (2018) Association of N-acetyltransferase-2 and glutathione S-transferase polymorphisms with idiopathic male infertility in Vietnam male subjects. Chem Biol Interact 286:11–16
105. Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M, Weksberg R (2013) Basic concepts of epigenetics. Fertil Steril 99(3):607–615
106. Feinberg AP, Cui H, Ohlsson R (2002) DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms. Semin Cancer Biol 12(5):389–398
107. Jue K, Benoit G, Alcivar-Warren AA, Trasler JM (1995) Developmental and hormonal regulation of DNA methyltransferase in the rat testis. Biol Reprod 52:1364–1371
108. Omisanjo OA, Biermann K, Hartmann S, Heukamp LC, Sonnack V, Hild A, Brehm R, Bergmann M, Weidner W, Steger K (2007) DNMT1 and HDAC1 gene expression in impaired spermatogenesis and testicular cancer. Histochem Cell Biol 127(2):175–181
109. Ariel M, Cedar H, McCarrey J (1994) Developmental changes in methylation of spermatogenesis–specific genes include reprogramming in the epididymis. Nat Genet 7(1):59
110. Olszewska M, Barciszewska MZ, Fraczek M, Huleyuk N, Chernykh VB, Zastavna D, Barciszewski J, Kurpisz M (2017) Global methylation status of sperm DNA in carriers of chromosome structural aberrations. Asian J Androl 19(1):117

111. Tunc O, Tremellen K (2009) Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J Assist Reprod Genet 26(9–10):537–544
112. Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI (2008) Oxidative stress, DNA methylation and carcinogenesis. Cancer lett 266(1):6–11
113. Hepburn P, Margison G, Tisdale M (1991) Enzymatic methylation of cytosine in DNA is prevented by adjacent O6-methylguanine residues. J Biol Chem 266(13):7985–7987
114. Turk PW, Laayoun A, Smith SS, Weitzman SA (1995) DNA adduct 8-hydroxyl-2′-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase. Carcinogenesis 16(5):1253–1255
115. Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ (2000) Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. J Biol Chem 275(38):29318–29323
116. Jamaluddin MS, Chen I, Yang F, Jiang X, Jan M, Liu X, Schafer AI, Durante W, Yang X, Wang H (2007) Homocysteine inhibits endothelial cell growth via DNA hypomethylation of the cyclin Agene. Blood 110(10):3648–3655
117. Karimian M, Hosseinzadeh Colagar A (2018) Human MTHFR-G1793A transition may be a protective mutation against male infertility: a genetic association study and in silico analysis. Hum Fertil 21(2):128–136
118. Aitken RJ, Curry BJ (2011) Redox regulation of human sperm function: from the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line. Antioxid Redox Signal 14(3):367–381
119. Noblanc A, Damon-Soubeyrand C, Karrich B, Henry-Berger J, Cadet R, Saez F, Guiton R, Janny L, Pons-Rejraji H, Alvarez JG (2013) DNA oxidative damage in mammalian spermatozoa: where and why is the male nucleus affected? Free Radical Biol Med 65:719–723
120. Ohno M, Sakumi K, Fukumura R, Furuichi M, Iwasaki Y, Hokama M, Ikemura T, Tsuzuki T, Gondo Y, Nakabeppu Y (2014) 8-Oxoguanine causes spontaneous de novo germline mutations in mice. Sci Rep 4:4689
121. Muratori M, Tamburrino L, Marchiani S, Cambi M, Olivito B, Azzari C, Forti G, Baldi E (2015) Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity and oxidative stress. Mol Med 21(1):109–122
122. Bauer NC, Corbett AH, Doetsch PW (2015) The current state of eukaryotic DNA base damage and repair. Nucleic Acids Res 43(21):10083–10101
123. Dorostghoal M, Kazeminejad S, Shahbazian N, Pourmehdi M, Jabbari A (2017) Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. Andrologia 49(10):e12762
124. Aitken R, Smith T, Lord T, Kuczera L, Koppers A, Naumovski N, Connaughton H, Baker M, De Iuliis G (2013) On methods for the detection of reactive oxygen species generation by human spermatozoa: analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. Andrology 1(2):192–205
125. Gaschler MM, Stockwell BR (2017) Lipid peroxidation in cell death. Biochem Biophys Res Commun 482(3):419–425
126. Saleh RA, Ashok Agarwal HCLD (2002) Oxidative stress and male infertility: from research bench to clinical practice. J Androl 23(6):737–752
127. Aitken RJ, Whiting S, De Iuliis GN, McClymont S, Mitchell LA, Baker MA (2012) Electrophilic aldehydes generated by sperm metabolism activate mitochondrial reactive oxygen species generation and apoptosis by targeting succinate dehydrogenase. J Biol Chem 287(39):33048–33060
128. Talebi E, Karimian M, Nikzad H (2018) Association of sperm mitochondrial DNA deletions with male infertility in an Iranian population. Mitochondrial DNA A DNA Mapp Seq Anal 29(4):615–623
129. Moretti E, Collodel G, Fiaschi AI, Micheli L, Iacoponi F, Cerretani D (2017) Nitric oxide, malondialdheyde and non-enzymatic antioxidants assessed in viable spermatozoa from selected infertile men. Reprod Biol 17(4):370–375
130. Turner TT, Bang HJ, Lysiak JJ (2005) Experimental testicular torsion: reperfusion blood flow and subsequent testicular venous plasma testosterone concentrations. Urology 65(2):390–394
131. Hanukoglu I (2006) Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. Drug Metab Rev 38(1–2):171–196
132. Aitken RJ, Baker MA, Sawyer D (2003) Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reprod Biomed Online 7(1):65–70
133. Mancini A, Leone E, Festa R, Grande G, Silvestrini A, De Marinis L, Pontecorvi A, Maira G, Littarru GP, Meucci E (2008) Effects of testosterone on antioxidant systems in male secondary hypogonadism. J Androl 29(6):622–629
134. Shang X, Huang Y, Ye Z, Yu X, Gu W (2004) Protection of melatonin against damage of sperm mitochondrial function induced by reactive oxygen species. Zhonghua Nan Ke Xue 10(8):604–607
135. Adewoyin M, Mohsin SMN, Arulselvan P, Hussein MZ, Fakurazi S (2015) Enhanced anti-inflammatory potential of cinnamate-zinc layered hydroxide in lipopolysaccharide-stimulated RAW 264.7 macrophages. Drug Des Devel Ther 9:2475
136. Sarkar O, Bahrainwala J, Chandrasekaran S, Kothari S, Mathur PP, Agarwal A (2011) Impact of inflammation on male fertility. Front Biosci (Elite Ed) 3:89–95
137. Liew SH, Meachem SJ, Hedger MP (2007) A stereological analysis of the response of spermatogenesis to an acute inflammatory episode in adult rats. J Androl 28(1):176–185
138. Pasqualotto FF, Sharma RK, Potts JM, Nelson DR, Thomas AJ Jr, Agarwal A (2000) Seminal oxidative stress in patients with chronic prostatitis. Urology 55(6):881–885
139. Zamani-Badi T, Karimian M, Azami-Tameh A, Nikzad H (2019) Association of C3953T transition in interleukin 1β gene with idiopathic male infertility in an Iranian population. Hum Fertil 22(2):111–117
140. Zamani-Badi T, Nikzad H, Karimian M (2018) IL-1RA VNTR and IL-1α 4845G> T polymorphisms and risk of idiopathic male infertility in Iranian men: a case–control study and an in silico analysis. Andrologia 50(9):e13081
141. Azenabor A, Ekun AO, Akinloye O (2015) Impact of inflammation on male reproductive tract. J Reprod Infertil 16(3):123
142. Agarwal A, Esteves SC (2016) Varicocele and male infertility: current concepts and future perspectives. Asian J Androl 18(2):161
143. Mostafa T, Anis T, El Nashar A, Imam H, Osman I (2012) Seminal plasma reactive oxygen species–antioxidants relationship with varicocele grade. Andrologia 44(1):66–69
144. Agarwal A, Hamada A, Esteves SC (2012) Insight into oxidative stress in varicocele-associated male infertility: part 1. Nat Rev Urol 9(12):678
145. Hamada A, Esteves SC, Agarwal A (2013) Insight into oxidative stress in varicocele-associated male infertility: part 2. Nat Rev Urol 10(1):26
146. Türkyilmaz Z, Gülen Ş, Sönmez K, Karabulut R, Dinçer S, Can Başaklar A, Kale N (2004) Increased nitric oxide is accompanied by lipid oxidation in adolescent varicocele. Int J Androl 27(3):183–187
147. Altunoluk B, Efe E, Kurutas EB, Gul AB, Atalay F, Eren M (2012) Elevation of both reactive oxygen species and antioxidant enzymes in vein tissue of infertile men with varicocele. Urol Int 88(1):102–106

148. Soubry A, Guo L, Huang Z, Hoyo C, Romanus S, Price T, Murphy SK (2016) Obesity-related DNA methylation at imprinted genes in human sperm: results from the TIEGER study. Clin Epigenetics 8(1):51
149. Bakos H, Mitchell M, Setchell B, Lane M (2011) The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. Int J Androl 34(5pt1):402–410
150. Garolla A, Torino M, Miola P, Caretta N, Pizzol D, Menegazzo M, Bertoldo A, Foresta C (2015) Twenty-four-hour monitoring of scrotal temperature in obese men and men with a varicocele as a mirror of spermatogenic function. Hum Reprod 30(5):1006–1013
151. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A (2010) The effect of obesity on sperm disorders and male infertility. Nat Rev Urol 7(3):153
152. Jia Y-F, Feng Q, Ge Z-Y, Guo Y, Zhou F, Zhang K-S, Wang X-W, Lu W-H, Liang X-W, Gu Y-Q (2018) Obesity impairs male fertility through long-term effects on spermatogenesis. BMC Urol 18(1):42
153. Adewoyin M, Ibrahim M, Roszaman R, Isa M, Alewi N, Rafa A, Anuar M (2017) Male infertility: the effect of natural antioxidants and phytocompounds on seminal oxidative stress. Diseases 5(1):9
154. Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J (2013) The role of oxidative stress and antioxidants in male fertility. Cent Eur J Urol 66(1):60
155. Esteves SC, Agarwal A (2011) Novel concepts in male infertility. Int Braz J Urol 37(1):5–15
156. Zini A, San Gabriel M, Baazeem A (2009) Antioxidants and sperm DNA damage: a clinical perspective. J Assist Reprod Genet 26(8):427–432
157. Sengupta P, Agarwal A, Pogrebetskaya M, Roychoudhury S, Durairajanayagam D, Henkel R (2018) Role of *Withania somnifera* (Ashwagandha) in the management of male infertility. Reprod Biomed Online 36(3):311–326
158. Fujii J, Iuchi Y, Matsuki S, Ishii T (2003) Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. Asian J Androl 5(3):231–242
159. Kobayashi T, Miyazaki T, Natori M, Nozawa S (1991) Protective role of superoxide dismutase in human sperm motifity: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 6(7):987–991
160. Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40
161. Lenzi A, Lombardo F, Sgrò P, Salacone P, Caponecchia L, Dondero F, Gandini L (2003) Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil Steril 79(2):292–300
162. Opuwari CS, Henkel RR (2016) An update on oxidative damage to spermatozoa and oocytes. Biomed Res Int 2016:9540142
163. Ciftci H, Verit A, Savas M, Yeni E, Erel O (2009) Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. Urology 74(1):73–76
164. Kefer JC, Agarwal A, Sabanegh E (2009) Role of antioxidants in the treatment of male infertility. Int J Urol 16(5):449–457
165. Da Silva RF, Borges CdS, Villela e Silva P, Missassi G, Kiguti LRA, Pupo AS, Barbosa Junior F, Anselmo-Franci JA, Kempinas WDG (2016) The coadministration of N-acetylcysteine ameliorates the effects of arsenic trioxide on the male mouse genital system. Oxidative Med Cell Longev 2016:4257498
166. Bucak MN, Ateşşahin A, Varışlı Ö, Yüce A, Tekin N, Akçay A (2007) The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen: microscopic and oxidative stress parameters after freeze–thawing process. Theriogenology 67(5):1060–1067
167. Maya-Soriano MJ, Taberner E, Sabes-Alsina M, Lopez-Bejar M (2013) Retinol might stabilize sperm acrosomal membrane in situations of oxidative stress because of high temperatures. Theriogenology 79(2):367–373
168. Comhaire F, Mahmoud A (2016) The andrologist's contribution to a better life for ageing men: part 1. Andrologia 48(1):87–98
169. Jacob RA, Pianalto FS, Agee RE (1992) Cellular ascorbate depletion in healthy men. J Nutr 122(5):1111–1118
170. Thiele JJ, Friesleben HJ, Fuchs J, Ochsendorf FR (1995) Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. Human Reprod 10(1):110–115
171. Song GJ, Norkus EP, Lewis V (2006) Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. Int J Androl 29(6):569–575
172. Eskenazi B, Kidd S, Marks A, Sloter E, Block G, Wyrobek A (2005) Antioxidant intake is associated with semen quality in healthy men. Human Reprod 20(4):1006–1012
173. Geva E, Bartoov B, Zabludovsky N, Lessing JB, Lerner-Geva L, Amit A (1996) The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertil Steril 66(3):430–434
174. Suleiman SA, Ali ME, Zaki Z, El-Malik E, Nasr M (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl 17(5):530–537
175. Comhaire F (2010) The role of food supplementation in the treatment of the infertile couple and for assisted reproduction. Andrologia 42(5):331–340
176. Gvozdjáková A, Kucharská J, Dubravicky J, Mojto V, Singh RB (2015) Coenzyme Q10, α-tocopherol, and oxidative stress could be important metabolic biomarkers of male infertility. Dis Markers 2015:827941
177. Balercia G, Buldreghini E, Vignini A, Tiano L, Paggi F, Amoroso S, Ricciardo-Lamonica G, Boscaro M, Lenzi A, Littarru G (2009) Coenzyme Q10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. Fertil Steril 91(5):1785–1792
178. Safarinejad MR (2012) The effect of coenzyme Q 10 supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study. Int Urol Nephrol 44(3):689–700
179. Mancini A, Conte G, Milardi D, De Marinis L, Littarru G (1998) Relationship between sperm cell ubiquinone and seminal parameters in subjects with and without varicocele. Andrologia 30(1):1–4
180. Atig F, Raffa M, Ali HB, Abdelhamid K, Saad A, Ajina M (2012) Altered antioxidant status and increased lipid per-oxidation in seminal plasma of tunisian infertile men. J Hum Reprod Sci 8(1):139
181. Agarwal A, Sekhon LH (2011) Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: is it justified? Indian J Urol 27(1):74
182. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J (2005) Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 26(3):349–353
183. Mishra M, Acharya UR (2004) Protective action of vitamins on the spermatogenesis in lead-treated Swiss mice. J Trace Elem Med Biol 18(2):173–178
184. Aguirre-Arias MV, Velarde V, Moreno RD (2017) Effects of ascorbic acid on spermatogenesis and sperm parameters in diabetic rats. Cell Tissue Res 370(2):305–317
185. Tahvilzadeh M, Hajimahmoodi M, Toliyat T, Karimi M, Rahimi R (2016) An evidence-based approach to medicinal plants for the treatment of sperm abnormalities in traditional Persian medicine. Andrologia 48(8):860–879
186. Zhang L, Diao RY, Duan YG, Yi TH, Cai ZM (2017) In vitro antioxidant effect of curcumin on human sperm quality in leucocytospermia. Andrologia 49(10):e12760

187. Yüce A, Türk G, Çeribaşi S, Sönmez M, Ciftci M, Güvenç M (2013) Effects of cinnamon (*Cinnamomum zeylanicum*) bark oil on testicular antioxidant values, apoptotic germ cell and sperm quality. Andrologia 45(4):248–255
188. Heidary M, Vahhabi S, Nejadi JR, Delfan B, Birjandi M, Kaviani H, Givrad S (2008) Effect of saffron on semen parameters of infertile men. Urol J 5(4):255–259
189. Safarinejad MR, Shafiei N, Safarinejad S (2011) A prospective double-blind randomized placebo-controlled study of the effect of saffron (*Crocus sativus* Linn.) on semen parameters and seminal plasma antioxidant capacity in infertile men with idiopathic oligoasthenoteratozoospermia. Phytother Res 25(4):508–516
190. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF (2014) Effects of *Nigella sativa* L. seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. Phytomedicine 21(6):901–905
191. Roychoudhury S, Agarwal A, Virk G, Cho C-L (2017) Potential role of green tea catechins in the management of oxidative stress-associated infertility. Reprod Biomed online 34(5):487–498
192. Omu AE, Al-Azemi MK, Al-Maghrebi M, Mathew CT, Omu FE, Kehinde EO, Anim JT, Oriowo MA, Memon A (2015) Molecular basis for the effects of zinc deficiency on spermatogenesis: an experimental study in the Sprague-dawley rat model. Indian J Urol 31(1):57
193. Hijazi MM, Khatoon N, Azmi MA, Rajput MT, Zaidi H, Ijaz S, Azmi MA, Perveen R, Naqvi H, Naimul S (2015) Effects of *Camellia sinensis* L. (green tea) extract on the body and testicular weight changes in adult Wistar rate. Pak J Pharm Sci 28(1):249–253
194. Sakr SA, Zowail ME, Marzouk AM (2014) Effect of saffron (*Crocus sativus* L.) on sodium valporate induced cytogenetic and testicular alterations in albino rats. Anat Cell Biol 47(3):171–179
195. Modaresi M, Messripour M, Asadi Marghmaleki M, Hamadanian M (2008) Effect of saffron (*Crocus sativus*) extract on level of FSH, LH and testosterone in mice. J Adv Med Biomed Res 16(63):11–18
196. Parandin R, Yousofvand N, Ghorbani R (2012) The enhancing effects of alcoholic extract of *Nigella sativa* seed on fertility potential, plasma gonadotropins and testosterone in male rats. Iran J Reprod Med 10(4):355
197. Ashamu E, Salawu E, Oyewo O, Alhassan A, Alamu O, Adegoke A (2010) Efficacy of vitamin C and ethanolic extract of *Sesamum indicum* in promoting fertility in male Wistar rats. J Hum Reprod Sci 3(1):11
198. Zarepourfard H, Riasi A, Frouzanfar M, Hajian M, Esfahani MHN (2019) Pomegranate seed in diet, affects sperm parameters of cloned goats following freezing-thawing. Theriogenology 125:203–209
199. Chenniappan K, Murugan K (2017) Therapeutic and fertility restoration effects of Ionidium suffruticosum on sub-fertile male albino Wistar rats: effects on testis and caudal spermatozoa. Pharm Biol 55(1):946–957
200. Hala A, Khattab Z, Abdallah G, Kamel M (2010) Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats. J Am Sci 6(12):352–361