Chapter

Oxidative Stress and Reproduction Health: Physiology, Pathology, and Clinical Biomarkers

Ramona D'Amico, Salvatore Cuzzocrea, Marika Cordaro and Rosanna Di Paola

Abstract

Reactive oxygen species (ROS) are free radicals derived from oxygen during normal cellular metabolism. Cells, under aerobic conditions, have a defense system against ROS, and in normal circumstances, there is an appropriate balance between prooxidants and antioxidants. When an overproduction of ROS develops or the body fails to eliminate ROS in excess, oxidative stress arises, during which ROS accumulate and damage cells and tissues. ROS plays a crucial role in the physiological processes and signaling pathways associated in both male and female fertility. In females, oxidative stress acts as a mediator in the modulation of important ovarian functions, and its complications such as abortions, recurrent pregnancy losses, preeclampsia, and gestational diabetes. In males, ROS plays an important role in normal physiological processes such as capacitation, hyperactivation, acrosome reaction, and sperm-oocyte fusion to ensure appropriate fertilization. However, high concentrations of ROS cause sperm pathologies (ATP depletion) in the form of inadequate axonemal phosphorylation or lipid peroxidation, resulting in a loss of sperm motility and viability. This chapter will highlight the mechanisms, production, physiological, and pathophysiological roles of ROS in relation to the male and female reproductive system, and recent advances in diagnostic methods that use ROS as biochemical markers.

Keywords: oxidative stress, biomarkers, physiology, pathology, reproduction health, male, female

1. Introduction

The key criteria for successful fertilization are the formation of mature and normal spermatozoa from maturing spermatids. After immature germ cells are produced in the testis's seminiferous tubules, they travel to the epididymis, where they are stored and mature under the influence of the neighboring epithelial cells. This epididymal maturation phase converts nonmotile spermatozoa into motile germ cells capable of fertilizing an oocyte [1]. ROS formation is a feature shared by all cells, including spermatozoa, in all mammalian species. There are two hypothesized pathways for ROS generation by spermatozoa: (a) *via* the sperm membrane's nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system and/or (b) *via* the sperm's nicotinamide adenine dinucleotide-dependent redox reaction [2]. The second significant source of ROS detected in sperm is leukocytes, which operate as a vital element of the cellular defense mechanism against infection, varicocele, spinal cord damage, chronic sexual absentia, and inflammation. To combat infectious pathogens, leukocytic infiltration is increased during infection. Increased inflammatory cytokine production, such as Interleukin-8 (IL-8), combined with reduced SOD synthesis results in a respiratory burst and increased ROS generation, leading to oxidative stress [3]. Sertoli cells have also been shown to be capable of producing ROS [4]. Scavestrogens (synthetic steroidal estrogens with significant antioxidant capabilities) have been proven in studies to reduce the generation of ROS by Sertoli cells. *In vitro*, scavestrogens may scavenge free radical scavengers and limit iron-induced cell damage [5]. Thus, it is possible that Sertoli cells, under normal *in vivo* circumstances, assist spermatogenesis *via* ROS generation [6]. Mammalian sperm must have features in order to operate properly and reproduce, including correct morphology, motility, and the capacity to undertake certain processes such as capacitation and acrosomal response. According to research, physiological amounts of ROS operate as intracellular signaling molecules required for several physiological processes such as maturation, hyperactivation, capacitation, acrosomal response, and oocyte-sperm union [7]. ROS activities are mostly carried out through regulating the redox state of cysteine residues. ROS not only enhances protein tyrosine kinase activity but it also suppresses phosphotyrosine activity, which typically dephosphorylates tyrosine. Tyrosine in the fibrous sheath enclosing the axoneme of the sperm flagellum is phosphorylated by protein tyrosine kinase. Capacitation results in increased phosphorylation of tyrosine. A-kinase anchoring proteins are phosphorylated proteins that bind PKA to the fibrous sheath of the sperm, suggesting a possible function in sperm hyperactivity. Spermatozoa that emerge from the testis are not fully developed, and hence cannot fertilize an egg. In addition to storage and transportation, the epididymis is important in spermatozoa obtaining the capacity to fertilize. In the epididymal environment, spermatozoa undergo changes such as progressive motility, chromatin condensation, and plasma membrane remodeling, all of which are required for capacitation and fertilization in the female reproductive tract. Despite the fact that spermatozoa are extremely vulnerable to oxidative stress-mediated destruction, normal amounts of ROS are required for epididymal sperm maturation [8]. Several antioxidants help to maintain a physiological redox equilibrium in the epididymis. As a result, in addition to functioning as ROS scavengers, antioxidants control the bioavailability of oxidants, which are then employed to catalyze protamine and flagellar sulfoxidation. While the testis partially completes disulfide production on cysteine sulfhydryls of sperm protamines, the majority of sulfoxidation occurs in the epididymal environment [9]. During capacitation, spermatozoa undergo molecular changes that guarantee the continuation of the acrosome reaction and fusion with the egg membrane. These include (a) basification of intracellular pH, (b) activation of cAMP-dependent pathways, (c) removal of cholesterol from the sperm membrane, and (d) protein phosphorylation by cAMP-dependent kinases at serine, threonine, and tyrosine residues. ROS are essential regulators of these activities [10]. Suppression of capacitation has been reported in epididymal spermatozoa incubated with catalase, which inhibits H_2O_2 synthesis. Several studies have highlighted the significance of ROS in regulating the cAMP pathway, which includes PKA activation and phosphorylation of PKA substrates. Indeed, elevated amounts of the second messengers cAMP and Ca^{2+}, as well as a high rate of protein phosphorylation, have been linked to ROS generation during the capacitation process [11]. The oxidation of cholesterol and subsequent efflux of cholesterol from the plasma membrane is required for the preparation of spermatozoa for the acrosome reaction [12]. The basic condition for successful fertilization is sperm-oocyte fusion, which is followed by sperm head penetration through the zona pellucida. To do the same, hyperactivated spermatozoa must overcome the layer of cumulus cells to reach the oocyte's zona pellucida. The completion of an acrosome reaction (AR) refers to the conclusion of the spermatozoon's maturational phases at which time it obtains the final fertilizing capacity. ROS may aid the acrosome process by inducing phosphorylation of certain plasma membrane proteins [5]. The AR can be induced by both physiological and nonphysiological stimuli such as ROS, the zona pellucida, or progesterone [13]. The large amount of membrane fatty acids released during AR increases the sperm plasma membrane fluidity essential for fusion with the oocyte [14]. ROS plays an important role in enhancing plasma membrane fluidity during sperm-oocyte fusion by mediating the activation of metabolic pathways for capacitation, which is followed by a successful acrosome response. ROS suppresses PLA2 deactivation by inhibiting protein tyrosine phosphatase activity throughout spermatozoa capacitation. Thus, active PLA2 may cleave secondary fatty acids from membrane phospholipid triglycerides, increasing plasma membrane fluidity [15].

2. ROS physiology in women

OS plays an important role in a variety of physiological and pathological processes of the female reproductive system. As ROS influences many physiological and pathological processes in the ovaries, as well as the peritoneal environment, there are methods through which OS may impair female fertility. The main sources of ROS in the follicular fluid microenvironment include cytokines, macrophages, and leukocytes. Because ROS are regarded key inducers of ovulation, a particular level of ROS is required [16]. However, one way that OS may impair female fertility is by the action of ROS on female germ cells. The fundamental mechanism by which the postovulatory oocytes lose their developmental ability after ovulation appears to be OS. Postovulatory oocytes enter apoptosis and lose functioning as a result of a complicated chain of events caused by an increase in oxidative stress [14]. One common marker of postovulatory oocyte aging is zona pellucida induration, which can be caused by OS exposure [14]. Ovoperoxidase, which is present in the cortical granules that are ejected from the oocyte's surface during an exocytotic process, is fueled by OS and aids in oocyte aging. Furthermore, excessive ROS production might damage oocyte DNA, resulting in incorrect fertilization [14]. According to research, psychological stress and aging may produce oxidative imbalance [17]. Furthermore, oxidative stress may impair granulosa cells' capacity to produce steroid hormones such as follicle-stimulating hormone (FSH) and estradiol (E2), potentially affecting oocyte quality [18]. The increased OS in the granulosa cells, which is coupled with a reduction in the expression of the follicle-stimulating hormone receptor (FSHR) and a dysregulation of the FSHR signaling pathway, may be linked to the poor response to FSH and impaired steroidogenic activity in older women [19]. O_2 and its scavenging molecules have been extensively studied in terms of their physiologic and pathologic effects. The presence of O_2 and SOD antioxidant enzymes in the mammalian female reproductive system has been found in the uterus [20]. Throughout the peri-implantation stage, SOD levels and activity rise, ostensibly to protect the embryo throughout the implantation process. Copper, zinc SOD isoenzyme, and SOD activity have also been found in rabbit and human fallopian tube epithelium and oviduct fluid [21]. During the estrous cycle, O_2 and SOD levels in the rat ovary alter inversely. Superoxide dismutase activity and the copper, zinc SOD isoenzyme is seen in developing follicles, granulosa cell membranes in Graafian follicles, and postovulatory follicles [22]. Further research on rat and human ovaries suggests that O_2 and SOD enzymes may play a function in the ovulation process and oocyte development, and current results reveal that O_2 and its scavenging molecules have a regulatory role in progesterone generation by the mammalian corpus luteum [23]. The oocyte maturation process needs a steady supply of energy in the form of adenosine triphosphate (ATP) to feed the transcription process and expand the size of the follicles, as well as the oocyte. The content of ATP during metaphase II (MII) arrest in the human egg is positively connected with successful fertilization and IVF result. During the maturation process, the mitochondrial electron transport chain generates ATP, which leads in the creation of ROS. This high quantity of ROS is oxidized by antioxidant enzymes found in the follicular fluid, including as catalase, SOD, glutathione transferase (GST), heat shock protein, and protein isomerase, which finally protect the oocytes from the detrimental effects of ROS [24]. Aside from mitochondrial respiration and oxidative phosphorylation, various other processes contribute to ROS emission in follicles. The corpus luteum is signaled by luteinizing hormone (LH) to generate and release progesterone. The procedure is quite complex and is controlled at various stages, which are detailed below. Following LH signaling, cholesterol is first transformed to pregnenolone in the mitochondria by cytochrome P450 side-chain breakage, which produces ROS [25]. Pregnenolone is further converted to progesterone in the endoplasmic reticulum by 3-β-hydroxysteroid dehydrogenase, which needs oxidized nicotinamide adenine dinucleotide (NAD^+) as the hydrogen acceptor. NAD^+ is produced by the oxidation of NADH by ascorbic radicals *via* a free radical process mediated by ROS [26]. This demonstrates the critical function that ROS plays in the process of LH-induced progesterone release. According to one study, LH increases the intraluteal expression of copper/zinc-dependent SOD (Cu/Zn-SOD), manganese-dependent SOD (Mn-SOD), and catalase to preserve luteal cell viability and ROS equilibrium. According to this study, LH not only stimulates ROS generation but also controls it by promoting the expression of antioxidant enzymes, hence preserving the redox state [27]. In rat preovulatory granulosa cells, LH increases P4 synthesis in part by activating P450scc, which generates ROS. It has been demonstrated that ROS cause the apoptosis of bovine luteal cells. As a result, pulsatile LH secretion appears to both boost P4 and cause the formation of luteolytic ROS throughout the luteal phase. Moreover, P4 production in the bovine CL peaks around the middle of the luteal phase, which is also when ROS generation in the CL may be at its maximum. Due to LH's stimulation of antioxidant enzyme synthesis, CL function may be preserved at the mid luteal phase. The overall findings of that investigation show that LH increases the viability of luteal cells by promoting intraluteal expressions of and by promoting SOD activity to preserve luteal function [27, 28].

3. ROS pathology in male

Leukocytospermia, a condition that is closely associated to problems in sperm function, is characterized by the presence of more than a million peroxidase-positive leukocytes per milliliter of semen [29]. The physiological process of excess cytoplasm being extruded during sperm maturation is common. However, when spermiogenesis is disrupted, spermatozoa retain excess cytoplasm around the midpiece, which impairs the midpiece's capabilities. Most immature spermatozoa with a distorted head shape and cytoplasmic retention produce seminal ROS [30]. The accumulation of metabolic enzymes, such as glucose-6-phosphate dehydrogenase (G6PD) and NADPH oxidase, which are directly implicated in the synthesis of free radicals through the intermediate NADPH creation, is brought on by the preservation of extra cytoplasm in immature sperm [30]. Both NADPH oxidase in the plasma membrane and NADH-dependent oxidoreductase (diaphorase) in the mitochondria allow normal spermatozoa to produce ROS [31]. Thus, it is possible to infer that Sertoli cells support spermatogenesis through ROS generation under normal *in vivo* settings. To fully understand Sertoli cells' contribution to the creation of ROS, more research is necessary. One of the main reasons of male subfertility and infertility is varicocele, an aberrant venous dilatation in the pampiniform plexus around the spermatic cord that affects roughly 40% of male partners in overall infertile couples [32]. The pathophysiology of changes in sperm functions brought on by varicocele is explained by a number of processes. However, it has been shown that the most frequent mechanism for OS-induced sperm dysfunctions involves varicocele-mediated testicular heat and hypoxia [33]. In comparison to healthy fertile donors, oxidative stress indices, including ROS and lipid peroxidation, are more common in semen samples from varicocele-affected infertile individuals, according to a meta-analysis [34]. The degree of varicocele has been suggested to directly correlate with seminal ROS levels [35]. The inflammatory condition affecting the male genital system that has received the most research to date is prostatitis. Depending on the pathogen strain, pathogens can directly impact sperm in cases of acute and chronic bacterial prostatitis or indirectly through the activation of cytokines such as IL6, IL8, or tumor necrosis factor (TNF) [36]. OS is caused by elevated cytokine levels, which can also influence spermatozoa [37] and could cause a systemic reaction by reducing testosterone hormone levels. Elevated IL6, IL8, and TNF-levels may worsen sperm transit during ejaculation when the infection spreads to the testis [38]. The paternal genetic contribution to the embryo is typically decreased by increased OS, which frequently has a direct impact on sperm DNA [39]. Leukocytes are stimulated by prostate infection and inflammation and can generate ROS. Because a high level of ROS can affect up to 35% of men seeking infertility treatment, infections must be treated very away [40]. This is mostly as a result of the possibility of oligozoospermia, azoospermia, or asthenozoospermia as a result of untreated prostatitis [41]. Infections can also lead to chronic illnesses, which are more challenging to cure than acute illnesses. For the treatment of bacterial prostatitis, fluoroquinolones, tetracyclines, macrolides, and trimethoprim (singly or in combination with sulfamethoxazole) were all specifically mentioned [42]. The mainstay of treatment for bacterial prostatitis continues to be antibiotic medication, and numerous studies have shown that antibiotics can considerably improve sperm characteristics and conception rates. However, given that recent research has concentrated on multidrug resistance, antibiotic treatment must be administered with caution and after the proper drug resistance testing [43]. When a patient's first-line therapy is ineffective, combination therapy is frequently recommended [44]. Ciprofloxacin and rifampin appear to be effective against Staphylococcus aureus, and fluoroquinolones bind with metal cations such as aluminum, magnesium, calcium, iron, and zinc, while the majority of combinations are ineffective or additive. The serum medication concentrations that are accessible for tissue penetration are significantly reduced by this pharmacological interaction [45]. The amount of zinc produced by prostate epithelial cells can be three times that of the majority of other mammalian cells [46]. Despite what was just said, zinc buildup in the prostate may prevent fluoroquinolones from working to their full potential. Due to the direct effect of colonized bacteria or other pathogens on spermatozoa when they travel through the urethra during ejaculation, infection or inflammation of the urethra may result in infertility. By reducing the volume and/or the number of sperm, tissue scarring as a result of infection may prevent sperm from depositing in the female reproductive tract [47]. A genitourinary condition known as urethritis is frequently seen in males. This inflammatory disease has been associated with a number of infections, including Mycoplasma genitalium (MG), Neisseria gonorrhoeae (GC), Trichomonas vaginalis (TV), Chlamydia trachomatis (CT), Ureaplasma urealyticum (UU), Herpes simplex virus (HSV), and adenovirus [48]. The Staphylococcus with coagulase-negative. In women, Staphylococcus saprophyticus frequently causes urinary tract infections. However, it has been demonstrated to cause urethral infection in males, albeit its relevance in this context is uncertain [49]. Numerous problems have been connected to it, including acute epididymitis, orchitis, and prostatitis. Male infertility has an unclear etiology in roughly 50% of cases; however, it is evident that 30–80% of infertile men have a high level of ROS in their ejaculate. The term "male oxidative stress infertility (MOSI)" was created by Agarwal et al. to refer to male infertility brought on by OS because of the close association between OS and male infertility [50]. Leukocytes in the seminal fluid and immature sperm with a morphologically defective head and cytoplasmic retention are the two main sources of endogenous ROS in human semen [51]. Extrinsic ROS are created during male genital tract infection, and leukocyte chemotaxis and activation encourage further inflammatory responses. Leukocytes activate the myeloperoxidase system, which generates ROS, to fight infections [52]. The overproduction of ROS by leukocytes can result in OS in the seminal fluid. However, the origins of intrinsic ROS are defective and immature spermatozoa. It has been demonstrated to have harmful effects on sperm, including sperm DNA fragmentation (SDF), LPO, and apoptosis in germ cells. SDF may be caused by increased ROS generation and inadequate antioxidant protection in sperm [53]. Through the activation of sperm caspases and the generation of endonuclease, OS has the capacity to harm sperm DNA either directly or indirectly. The failure of the chromatin structure substitution from histone to protamine during the process of spermiogenesis is thought to be the cause of SDF since it results in DNA vulnerability. The migration of spermatozoa from the seminiferous tubules to the cauda epididymis *via* the rete testis causes excessive ROS exposure, which has been reported to cause DNA damage [54]. This reaction results in the formation of 8-OH-guanine and 8-OH-20-deoxyguanosine (8-OHdG), an oxidized guanine adduct. In the lab, increased levels of 8-OHdG have been connected to DNA fragmentation and strand breaks [55]. DNA fragmentation can happen to both single- and double-stranded (ds-) DNA [56]. DNA repair is only possible during specific stages of the spermiogenesis process, and it ceases to be active during the nuclear condensation of the epididymis. The human oocyte represents the next opportunity for ss-DNA break repair; however, SDF repair efficiency decreases with increasing maternal age [57]. A break in the ds-DNA causes genomic instability and ultimately causes cellular death when DNA repair does not take place [58]. The "late paternal effect" is hypothesized to be induced by the presence of unrepaired SDF above a specific threshold, which has a negative impact on embryo development and pregnancy outcomes [59]. A cleavage-stage embryo experiences a major activation of embryonic genome expression on the second day of human embryo development (4 cells), and the dependence of embryogenesis on maternal factors is replaced by the dependence on the embryo's own genome [60]. As a result, after fertilization, SDF in a spermatozoon may have a deleterious impact on the outcomes of blastulation, implantation, and pregnancy. The "early paternal effect" [61] was the term coined by the researchers to describe how OS negatively affects the growth of cleavage embryos. The effect of SDF on the results of ART has been the subject of numerous investigations [62]. A meta-analysis found a positive correlation between SDF and miscarriage and an inverse correlation between SDF with pregnancy outcome [62]. SDF is one of the elements that might lead to repeated pregnancy losses, thus accurate assessment and control could help couples with their issue. Apoptosis is acknowledged as a biologically designed cell death due to the DNA fragmentation that is brought on by a number of cell death signaling and regulatory mechanisms [63]. Ds-DNA breakage brought on by ROS may result in apoptosis. Additionally, ROS leads to mitochondrial membrane rupture, which releases the signaling molecule cytochrome-C, which can then activate apoptotic caspases and annexin-V phosphatidylserine-binding activity [64]. High amounts of cytochrome-C seen in the seminal plasma of infertile men may cause severe mitochondrial damage [65].

4. ROS pathology in female

The vascular endothelium-produced ROS may contribute to regular cellular signaling processes. They might also have a significant role in the development of endothelial dysfunction. Endometrial tissue can be found outside the uterus in endometriosis, a benign, estrogen-dependent, and persistent gynecological condition. According to a report, erythrocytes and apoptotic endometrioma cells produce high ROS that cause OS, as do activated macrophages that are brought in to phagocytize apoptotic cells [66]. In addition, endometriosis-afflicted women produce higher levels of the ROS-producing enzyme xanthine oxidase, which is thought to be another cause of excessive ROS [67]. Additionally, OS causes local inflammation that raises cytokine levels, as well as other variables that support endometriosis [68]. Phagocytic cells, which are the primary sources of ROS and RNS, are recruited and activated by pro-inflammatory and chemotactic cytokines. Endometriosis patients' peritoneal macrophages and endometriotic lesions have been found to be activated by OS [69]. Mitogen-activated protein kinase (MAPK) and extracellular regulated kinase (ERK1/2) are activated in endometriotic cells in a manner similar to tumor cells by elevated ROS and consequent cellular proliferation [70]. More gravely, the rise in ROS in endometriosis patients can have a negative impact on the development of the fetus, causing fetal dysmorphogenesis, IUGR, or spontaneous miscarriage [71]. A vascular pregnancy disease called preeclampsia frequently involves poor placental growth. It is a multisystem illness that can affect women with normal blood pressure. Because OS increases p38 MAPK nitration, which lowers its catalytic activity, it can result in the poor implantation and growth restriction seen in preeclampsia. The increased levels of MDA, a marker of lipid peroxidation, in preeclampsia patients have been used to demonstrate elevated ROS concentrations [72]. Under normal circumstances, preeclampsia's vascular endothelial dysfunction is the main factor contributing to the disruption of circulatory homeostasis. Low anticoagulant activity and a propensity to promote vasoconstriction define it. The endothelial dysfunction related to preeclampsia appears to be significantly influenced by ROS [73]. Injury to the vascular endothelium caused by increased placental ROS or impaired antioxidant activity [74] is the pathogenic event in preeclampsia. The rise of ROS can be attributed to a variety of factors. A prominent source of ROS, for instance, is neutrophil regulation in preeclampsia, which increases the production of the SO anion and decreases NO levels, ultimately damaging endothelial cells in preeclampsia patients. Preeclampsia is associated with elevated levels of TNF and oxLDL, which have been found to activate the endothelium isoform of NAD(P)H oxidase and consequently raise SO anion levels. According to these findings, consuming antioxidants to combat increased lipid peroxidation may harm the vascular endothelium and contribute to the pathophysiology of preeclampsia [75]. In comparison to women without the condition, preeclamptic women create more ROS and have higher NAD(P)H expression [76]. More particular, it has been noted that early-onset preeclampsia causes women to create more SO anion than late-onset preeclampsia does [77]. Affected women also have reduced levels of vitamins C and E and a lowered total antioxidant status (TAS), as well as placental GPx [78]. Preeclampsia seems to be more likely in people who do not consume enough vitamin C, and some research suggests that multivitamin supplementation during pregnancy can reduce preeclampsia risk in women who are normal weight or underweight [79]. Studies on the effects of restraint stress on uterine and embryo implantation in pregnant mice have been conducted. These studies focused on uterine histomorphology study and changes to the uterine local microenvironment. The mice were treated to restraint stress beginning on embryonic day 1 (E1), according to Liu Guanhui et al. This study showed that restraint stress raised corticosterone (CORT) levels in plasma and dramatically increased uterine natural killer (uNK) cells in the endometrium, along with a decrease in the density of mast cells in the myometrium. In addition, the CD3$^+$CD4$^+$/CD3$^+$CD8$^+$ T cell ratio was significantly reduced by constraint stress. Additionally, the content of MDA rose, and antioxidant capacity was weakened [41]. Restraint stress also decreased the weight of the uterus, ovary, and food consumption with weight loss, while it also decreased the relative endometrial area and uterine gland area. Restraint stress also reduced VEGF expression and micro-vessel density [80]. During reproductive life, only a tiny percentage of the ovarian follicles present at birth mature to ovulation, while the remainder undergo a degenerative process known as "atresia." Follicular atresia is caused by granulosa cell death, according to research [81]. Excessive ROS production causes antral follicular atresia by triggering granulosa cell death, according to mounting data. ROS are also implicated in the decrease of granulosa cell sensitivity to gonadotrophin hormones and the loss of steroidogenic activity, both of which are symptoms of follicular atresia. Furthermore, ROS concentrations rose throughout the regression phase in the rat ovarian corpus luteum. Thus, ROS might initiate apoptosis in the luteal cells and inhibit their function at an appropriate time during the female menstrual cycle [82].

5. ROS as biomarkers

Biomarkers of oxidative stress (OS) can be classified as molecules that are modified by interactions with ROS in the microenvironment, and molecules of the antioxidant system that change in response to increased redox stress. DNA, lipids (including phospholipids), proteins, and carbohydrates are examples of molecules that can be modified by excessive ROS *in vivo*. There are several methods to measure ROS in the laboratory setting, which can be classified into direct and indirect assays [83]. The indirect measurements of ROS include enzymatic antioxidant levels such as SOD, CAT, GPx, and reduced glutathione (GSH) *via* means of spectrophotometric measurement. As regards the direct assays of the ROS, these include the direct measurement of total antioxidant capacity (TAC), detection of ROS species *via* chemiluminescence, and the use of florescent markers for ROS, which can be measured using fluorescence microscopy and flow cytometry [83]. Some of the most widely used methods to detect ROS levels in both male and female reproductive systems are described below.

5.1 ROS measurement by chemiluminescence assay

Chemiluminescence measures the light that is emitted in a reaction when reagents are added to a biological sample [83]. Luminol and Lucigen are two probes that are used in chemiluminescent assays to detect ROS [84]. Lucigenin is best suited to detect $O_2^{-\bullet}$ as it is positively charged, which renders it membrane-impermeable and allows it to react with $O_2^{-\bullet}$ in the extracellular space [85]. Unlike Lucigen, Luminol is uncharged and is, therefore, membrane permeable; this allows it to react with ROS in both the intra- and extracellular spaces. Luminol reacts with a variety of ROS, including $O_2^{-\bullet}$, H_2O_2, and hydroxyl radicals ($OH^\bullet$) [86]. This probe, however, is unable to differentiate between the types of ROS and therefore measures global ROS [86].

5.2 ROS measurement by flow cytometry

Flow cytometry involves the use of florescent markers to measure ROS and RNS within the cells [87]. Contradictory to chemiluminescence, florescent techniques have a higher accuracy, specificity, and reproducibility rate for intracellular ROS [88]. However, the utilization of florescent probes requires expensive equipment. The data generated does not quantify ROS but rather is indicative of the percentage of cells displaying a high ROS activity. An example of florescent probe used is 2,7-dichlorofluorescein diacetate (H2DCFDA) that penetrates the cells and indicates H_2O_2 concentrations, as H_2O_2 de-esterifies in the presence of DCFH and forms highly fluorescent 2,7-dichlorofluorescein (DCF) [89]. DCF fluoresces and this can be measured and indicates of formation of intracellular levels of hydrogen peroxide. Dihydroethidium/hydroethidine (DHE) is a non-florescent probe that is oxidized by a variety of reactive oxygen and nitrogen species. This probe is primarily used to visualize $O_2^{-\bullet}$ production [90].

5.3 Measurement of total antioxidants

Total antioxidant capacity (TAC) highlights the crucial role of antioxidant enzymes in counterbalancing ROS generation, and therefore can be a powerful tool in determining the redox status of a sample [91]. This measurement may give more relevant biological information compared to that obtained by the measurement of individual components (e.g., SOD, CAT, GPx) as it considers the cumulative effect of all antioxidants present in plasma and body fluids [92]. The total antioxidant assay relies on the ability of antioxidants in the sample to inhibit the oxidation of 2,20-azino-di-3-ethylbenzthiazoline sulphonate (ABTS) to ABTS+ by metmyoglobin. Briefly, metmyoglobin and hydrogen peroxide produced ferryl-myoglobin radical, which oxidized the ABTS (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS+, a soluble chromogen that is green in color [93]. Then, the oxidized ABTS radical cation ($ABTS$•+) reacts with antioxidants to reduce the ABTS radical and lose its bluish-green color [94]. This reaction may be monitored spectrophotometrically with a spectrophotometer, a common laboratory instrument. $ABTS$• + has several characteristics that make it suitable for colorimetric assays; it has several absorbance peaks at different wavelengths [94], it has a high extinction coefficient, its solubility in water is high, and it is Also soluble in organic media. This assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay. The reactivity of the various antioxidants tested is compared to that of Trolox, which is a water-soluble analog of vitamin E [95].

6. ROS in male reproductive diseases

Sperm require an endogenous level of ROS for normal sperm function and "optimum" concentrations are not yet fully determined; therefore, a better approach could be suggested, which could be to measure the downstream effects of OS on sperm, such as oxidative DNA damage or lipid peroxidation (LPO), since these have been shown to directly inhibit sperm function and fertilization [96]. Below, we outline the strengths and limitations of measuring of some assays used to measure the activity of these downstream markers.

6.1 ROS and LPO in sperm

LPO is one of the main consequences of OS and high concentrations of seminal ROS, this is because the plasma membranes of spermatozoa contain abundant polyunsaturated fatty acids (PUFA) [97]. LPO has a two-fold effect on fertility: it decreases motility, thereby reducing the number of sperm that reach the oocyte and decreases membrane fluidity necessary for sperm-oocyte fusion [98]. The lipid peroxidation cascades result in the formation and accumulation of lipid aldehydes, including malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4HNE) that are capable of disrupting sperm function through the formation of adducts with key proteins and DNA [96].

6.1.1 Thiobarbiuric acid-malondialdehyde (TBA-MDA)

In particular, MDA, one of the by-products of lipid breakdown, reacts with TBA and can be used in biochemical assays as a marker to monitor the degree of peroxidative damage sustained by spermatozoa [99]. Although its clinical use has been questioned, the results of this test show an excellent correlation with the degree of impairment of sperm function in terms of motility and sperm-oocyte fusion ability, thus deeming the test reliable and accurate in assessing male infertility [96].

6.1.2 Hydroxy-2-nonenal (4HNE)

Another cytotoxic break down product of LPO is 4HNE, a highly reactive lipid aldehyde that can react with proteins and nucleic acids, as well as other lipids present in spermatozoa [100]. Increased concentrations of 4HNE in human sperm have been shown to induce impaired motility, compromised membrane integrity, and reduced oocyte-binding ability, with a significant negative impact on fertilization potential [101]. 4HNE can be quantified in semen samples using antibodies that detect and attach to the lipids, which are then quantified using ELISA assay or using either western blot and immunohistochemistry analysis [96]. Although quantifying 4HNE in sperm holds some promise in infertility diagnostics, the need for highly specialized and pricey equipment prevents its clinical application. Therefore, further attempts should be made to develop stable, highly specific probes for the detection of lipid peroxidation in sperm. In this regard, probe BODIPY-C11 has recently been developed for determining peroxidative damage in human sperm. Elevated fluorescence by BODYIP-C11 has been correlated with increased ROS generation and genital urinary tract infection and has been negatively associated with sperm motility [102]. However, BODPIY-C11 may overrepresenting lipid peroxidation of cells [96].

6.2 ROS: induced sperm DNA damage

In general, DNA damage has been shown to decrease semen parameters (motility, viability, etc.) and reduce the success of *in vitro* fertilization (IVF). The main tests used for the assessment of DNA damage in spermatozoa induced by oxidative stress are summarized in **Table 1**; however, 8-hydroxy-2′-deoxyguanosine (8OHdG) deserves a special mention. 8-hydroxy-2′-deoxyguanosine (8OHdG) is a DNA base adduct, commonly used as a biomarker of oxidative stress due to its association with nuclear and mitochondrial DNA damage [103]. Formation of 8OHdG in semen samples has been correlated with increases in DNA fragmentation, chromatin retention, decreases in sperm motility, and fertilization rates, thus making it a promising potential biomarker for oxidative stress damage and male infertility [104]. Currently, 8OHdG can be quantified in semen samples using light microscopy, fluorescence microscopy and flow cytometry [105]. The main limitations are due to long and difficult protocols, as well as the need for large and expensive equipment.

6.3 ROS and TAC of semen

TAC in the seminal plasma can be measured using an enhanced chemiluminescence assay [91]. The fact that neither ROS alone nor TAC alone can adequately quantify seminal OS led to the logical conclusion that combining these two variables

Technique.	Assay principle	Detection method
TUNEL assay	DNA fragmentation, adds labeled nucleotides to free DNA ends, and single- and double-strand DNA breaks	Flow cytometry/fluorescence microscopy
Comet assay	Intact DNA stays in the head, DNA fragments form tails, evaluates DNA integrity, and single- and double-strand DNA breaks	fluorescence microscopy
Sperm chromatin structure assay	Acid DNA denaturation, single- or double-strand breaks	Flow cytometry
DNA breakage Detection-fluorescence in situ hybridization	DNA breaks and denatures nicked DNA	fluorescence microscopy and image analyzer

Table 1.
Principal assays for the assessment of ROS-induced DNA damage in spermatozoa.

may be a better index for the diagnosis of the overall OS affecting spermatozoa. The ROS-TAC score is an accurate measure of seminal OS, and low ROS-TAC scores indicate high seminal OS [91].

6.4 Current clinical measures of ROS in semen

The advanced examinations section of the most recent sixth edition of the WHO manual for the assessment of human semen has been updated to include two of the three currently known clinically available measurements of ROS in semen, Luminol and MiOXYSIS [96]. Currently, these measures are only performed in men if abnormalities are observed during standard semen analysis, or when couples undergoing ART have experienced reduced fertilization and embryo development rates, or repeated implantation failure [106].

6.4.1 Luminol chemiluminescence

Chemiluminescent assays have been used to show a negative association between an increase in ROS levels and sperm parameters. These parameters include sperm motility, viability, morphology, and concentration [107]. A variety of factors may influence the data generated by the chemiluminescence assays, which include the presence of leukocytes in the sperm sample, sperm incubation time, the pH, and contamination of the seminal plasma [108]. It should also be noted that both sample and probe concentrations also affect luminescence; thus, it is important to have a fixed probe concentration for varying concentrations of sperm. However, chemiluminescence assays are sensitive, which is ideal as sperm generally produce low concentrations of ROS [109].

6.4.2 MiOXSYS system™

The MiOXSYS™ is a highly specific *in vitro* diagnostic tool used to measure static oxidation-reduction potential (sORP) in human semen [110]. The system works by measuring the transfer of electrons between oxidants and reductants within fresh semen samples to ultimately calculate total oxidant and antioxidant activity present within the ejaculate. Samples of high sORP levels indicate an imbalance that suggests the presence of OS [110]. Unlike chemiluminescence, where ROS levels have a significantly short half-life, ORP measurements are stable for up to 120 min. The assay requires only 30 μL of fresh or frozen semen sample and produces results in less than 5 min, making it a popular choice in both clinical and research settings [83]. Additionally, MiOXSYS sORP's ability to measure all oxidants and reductants makes it clinically meaningful in the diagnosis of cases of male infertility that is associated with high ROS levels [111].

6.4.3 OxiSperm®

OxiSperm® measures the presence of excess O_2^{-} in sperm, seminal plasma, and whole semen. The assay is largely based on nitroblue tetrazolium (NTB) staining in which the yellow NBT molecule is reduced into the insoluble blue crystals, called formazan, in the presence of O_2. This assay uses a gel that shows varying intensities once reacted that can be categorized as low, medium, or high as per the color scale [112]. However, the semi-qualitative measurement of OxiSperm® creates considerable opportunity for introducing bias into results while also showing low assay precision, as differences in color perception between scientists could result in different interpretations [96]. This may partially explain the absence of OxiSperm kits from inclusion in the latest sixth edition WHO guidelines under the recommended assessments of ROS.

7. ROS in female reproductive diseases

OS also affects the body fluids, tissues, and organs of the female reproductive system; not only, a host of female infertility diseases are attributed to the production of pathological ROS levels [113]. Concentrations of ROS may also play a major role both in the implantation and fertilization of eggs [114]. A number of OS biomarkers have been investigated, including SOD, LPO, oxidative DNA adducts, and TAC, both in peritoneal fluid and in whole blood and in the placenta. The principal biomarkers are summarized in **Table 2**.

7.1 ROS in ovaries and follicular fluid

Various studies have confirmed the role of ROS in follicular maturation, folliculogenesis, function of the corpus luteum, as well as ovulation [22]. The environment of the follicular fluid is thought to play a critical role in oocyte maturation and the eventual development of an embryo [16]. The main sources of ROS in the follicular fluid microenvironment are macrophages, leukocytes, and cytokines [115]. At the same time, a crosstalk, mediated by ROS, cytokines, and vascular endothelial growth factor (VEGF), important for ovarian folliculogenesis and embryo formation has been demonstrated [16]. Accordingly, ROS are involved in follicular growth in part by regulating angiogenesis. Increased ROS levels have been associated with poor oocyte quality, low fertilization rate, and impaired embryo development [115]. Furthermore, any imbalance between the cytokines and angiogenesis factors could result in implantation failure and pregnancy loss [116]. Normal ovaries express many of the common biomarkers of OS [117]. Markers of OS such as Cu-SOD, Zn-SOD, Mn-SOD, GPx, γ glutamyl synthetase, and lipid peroxides have been measured by immunohistochemical staining, mRNA expression, and thiobarbituric acid methods [118]. Additionally, 8-OHDG has become an important marker for OS in ovaries, as causes base mutation and mismatches in DNA replication [16].

Biomarkers	Methodology
SOD, CAT, GPx	Reverse transcription-polymerase chain reaction
TAC	Chemiluminescence assay
LPO, MDA, coniugated dienes	Thiobarbituric acid method
Oxidative DNA adducts	8-hydroxy-2′-deoxyguanosine measurement
Plasma and red blood cell, glutathione content	Colorimetric assay, fluorometric assay
Superoxide anion, hydrogen peroxide peroxynitrite	Spectrophotometry/flow cytometry

Table 2.
Principal biomarkers of ROS in the female reproductive system.

7.2 ROS in amniotic/placental fluid

During pregnancy, the mother and fetus can be exposed to high levels of OS due to the higher metabolic demand of the growing fetus [16]. Superoxide anions produced by the placental mitochondria appear to be a major source of ROS and LPO contributing to OS in the placenta [119]. However, the placenta gradually adapts to this environment and returns to normal under the action of antioxidant activity [120]. These studies showed that limited levels of ROS are necessary to maintain physiological function, but when present in higher concentrations, ROS can have deleterious effects. The placenta experiences a heightened level of OS in certain pathologic conditions of pregnancies, including gestational diabetes, fetal growth restriction, preeclampsia, and miscarriage [119]. The biomarkers of OS in the placenta and amniotic fluid include LHP, intracellular ROS, TAC, and DNA adducts-8-OHDG [71]. Another biomarker of ROS is LPO, which is the oxidative destruction of polyunsaturated fatty acids in the plasma membrane [16]. This leads to increased membrane permeability, degraded membrane integrity, structural damage of the DNA, and cell death.

7.3 ROS in peritoneal fluid

Peritoneal fluid bathes the pelvic cavity, uterus, fallopian tubes, and ovaries. It may be an important factor controlling the peritoneal microenvironment influencing the development of some pathologies such as preeclampsia ad endometriosis [121]. The peritoneal fluid of patients with endometriosis has been found to contain high concentrations of MDA, pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β), angiogenic factors (IL-8 and VEGF), monocyte chemoattractant protein-1 (MCP-1), and oxidized LDL (ox-LDL) [122]. Pro-inflammatory and chemotactic cytokines play central roles in the recruitment and activation of phagocytic cells, which are the main producers of ROS and RNS [73]. ROS also appears to play a critical role in the endothelial dysfunction associated with preeclampsia. Increased ROS concentrations in patients with preeclampsia have been proved by the increased levels of MDA, an index of lipid peroxidation [16]. Additionally, levels of TNF-α and oxLDL are increased in preeclampsia and have been shown to activate the endothelial isoform of NAD(P)H oxidase, ultimately resulting in increased levels of the SO anion [123]. Affected women also have decreased TAC and placental GPx [16].

8. Conclusion

In conclusion with this chapter, we hope to have given a broad description of the scientific bibliography on the role played by ROS in physiological and pathological conditions in both men and women. New studies aimed at understanding whether it is possible to reduce oxidative stress with nutraceuticals, physical exercise, or diet, for example, in order to delay and/or limit the onset of pathologies of the reproductive system, are obviously indispensable. Some studies for example have evaluated the effects of diet in mice model endometriosis, testicular damage cyclofasphamide-induced, testicular growth, urethral syndrome, chronic prostatitis/chronic pelvic pain syndrome, and more [124–128]. Further, studies will also be essential to discover new and advanced methods of diagnosis through biomarkers in order to make the diagnosis ever earlier and more precise.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Ramona D'Amico[1], Salvatore Cuzzocrea[1], Marika Cordaro[2]* and Rosanna Di Paola[3]

1 Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

2 Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

3 Department of Veterinary Sciences, University of Messina, Messina, Italy

*Address all correspondence to: cordarom@unime.it

References

[1] Ozkocer SE, Konac E. The current perspective on genetic and epigenetic factors in sperm maturation in the epididymis. Andrologia. 2021;**53**(3):e13989

[2] Das A, Roychoudhury S. Reactive Oxygen Species in the Reproductive System: Sources and Physiological Roles. In: Kesari KK, Roychoudhury S, editors. Oxidative Stress and Toxicity in Reproductive Biology and Medicine. Advances in Experimental Medicine and Biology. Vol. 1358. Cham: Springer; 2022. DOI: 10.1007/978-3-030-89340-8_2

[3] Beygi Z et al. Role of oxidative stress and antioxidant supplementation in male fertility. Current Molecular Medicine. 2021;**21**(4):265-282

[4] Kongmanas K et al. Accumulation of seminolipid in sertoli cells is associated with increased levels of reactive oxygen species and male subfertility: Studies in aging arsa null male mice. Antioxidants. 2021;**10**(6):912

[5] Dutta S, Henkel R, Sengupta P, Agarwal A, et al. Physiological role of ROS in sperm function. Male infertility: Contemporary clinical approaches, andrology. ART and Antioxidants. 2020:337-345

[6] Naseer Z, Raza S, Ahmad E, Aksoy M. Use of antioxidants to improve the reproductive performance in model laboratory animals. The Role of Exogenous Antioxidants in Enhancing Reproductive Function and Performance, Chapter 1. 2022

[7] Baskaran S et al. Reactive oxygen species in male reproduction: A boon or a bane? Andrologia. 2021;**53**(1):e13577

[8] Sullivan R, Mieusset R. The human epididymis: Its function in sperm maturation. Human Reproduction Update. 2016;**22**(5):574-587

[9] Peña FJ et al. Redox regulation and oxidative stress: The particular case of the stallion spermatozoa. Antioxidants. 2019;**8**(11):567

[10] Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Molecular Reproduction and Development. 2017;**84**(10):1039-1052

[11] Takei GL et al. Activation of cAMP-dependent phosphorylation pathways is independent of ROS production during mouse sperm capacitation. Molecular Reproduction and Development. 2021;**88**(8):544-557

[12] GONZÁLEZ S, Ruturi S, et al. Cortisol modulates Ca^{2+} signaling and acrosome reaction in human sperm. Andrology. 2023;**11**(1):134-142

[13] Balestrini PA et al. Membrane hyperpolarization abolishes calcium oscillations that prevent induced acrosomal exocytosis in human sperm. The FASEB Journal. 2021;**35**(6):e21478

[14] Aitken RJ. Impact of oxidative stress on male and female germ cells: Implications for fertility. Reproduction. 2020;**159**(4):R189-R201

[15] Engel KM et al. Metabolomic profiling reveals correlations between spermiogram parameters and the metabolites present in human spermatozoa and seminal plasma. PLoS One. 2019;**14**(2):e0211679

[16] Lu J et al. A novel and compact review on the role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology. 2018;**16**:80

[17] Alam F et al. Association of oxidative stress with female infertility-a case control study. JPMA. The Journal of the Pakistan Medical Association. 2019;**69**(5):627

[18] Ávila J et al. Oxidative stress in granulosa-lutein cells from in vitro fertilization patients. Reproductive Sciences. 2016;**23**:1656-1661

[19] Ahmed TA et al. Oocyte aging: The role of cellular and environmental factors and impact on female fertility. Cell Biology and Translational Medicine. Stem Cells in Regenerative Medicine. 2020;**8**:109-123

[20] Campos-Rentería AC et al. Evaluation of antioxidant enzymes in the female genital fluid of the bat Corynorhinus mexicanus during sperm storage. Animal Biology. 2023;**1**(aop):1-14

[21] Goutami L, Jena SR, Swain A, Samanta L, et al. Pathological role of reactive oxygen species on female reproduction. In: Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility Volume II. Cham: Springer International Publishing; 2022. pp. 201-220

[22] Wang L et al. Oxidative stress in oocyte aging and female reproduction. Journal of Cellular Physiology. 2021;**236**(12):7966-7983

[23] Liang J et al. Reactive oxygen species and ovarian diseases: Antioxidant strategies. Redox Biology. 2023:102659

[24] Podolak A, Woclawek-Potocka I, Lukaszuk K. The role of mitochondria in human fertility and early embryo development: What can we learn for clinical application of assessing and improving mitochondrial DNA? Cell. 2022;**11**(5):797

[25] Shrestha HK. Loss of luteal sensitivity to luteinizing hormone underlies luteolysis in cattle: A hypothesis. Reproductive Biology. 2021;**21**(4):100570

[26] Mahmoud AA, Elfiky AM, Abo-Zeid FS. The anti-androgenic effect of quercetin on hyperandrogenism and ovarian dysfunction induced in a dehydroepiandrosterone rat model of polycystic ovary syndrome. Steroids. 2022;**177**:108936

[27] Przygrodzka E, Plewes MR, Davis JS. Luteinizing hormone regulation of inter-organelle communication and fate of the corpus luteum. International Journal of Molecular Sciences. 2021;**22**(18):9972

[28] Kawaguchi S, Sakumoto R, Okuda K. Induction of the expressions of antioxidant enzymes by luteinizing hormone in the bovine corpus luteum. The Journal of Reproduction and Development. 2013;**59**(3):219-224

[29] World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. Geneva: World Health Organization; 2010

[30] Agarwal A, Tvrda E, Sharma R. Relationship amongst teratozoospermia, seminal oxidative stress and male infertility. Reproductive Biology and Endocrinology. 2014;**12**:45

[31] Du Plessis SS et al. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. Journal of Assisted Reproduction and Genetics. 2015;**32**(4):509-520

[32] Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: A meta-analysis. Reproductive Biomedicine Online. 2006;**12**(5):630-633

[33] Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. The Indian Journal of Medical Research. 2009;**129**(4):357-367

[34] Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian Journal of Andrology. 2016;**18**(2):186-193

[35] Agarwal A et al. Effect of cell phone usage on semen analysis in men attending infertility clinic: An observational study. Fertility and Sterility. 2008;**89**(1):124-128

[36] Alshahrani S, McGill J, Agarwal A. Prostatitis and male infertility. Journal of Reproductive Immunology. 2013;**100**(1):30-36

[37] Irez T et al. Cytokines and adipokines in the regulation of spermatogenesis and semen quality. Chemical Biology Letters. 2020;**7**:131-140

[38] Sanocka D, Kurpisz M. Reactive oxygen species and sperm cells. Reproductive Biology and Endocrinology. 2004;**2**(1):12

[39] Tremellen, K., Oxidative stress and male infertility—A clinical perspective. Human Reproduction Update, 2008. 14(3): p. 243-258.

[40] Okonofua FE et al. Causes and risk factors for male infertility: A scoping review of published studies. International Journal of General Medicine. 2022;**15**:5985-5997

[41] Liu G et al. Restraint stress delays endometrial adaptive remodeling during mouse embryo implantation. Stress. 2015;**18**(6):699-709

[42] Lipsky BA, Byren I, Hoey CT. Treatment of bacterial prostatitis. Clinical Infectious Diseases. 2010;**50**(12):1641-1652

[43] Krupp K, Madhivanan P. Antibiotic resistance in prevalent bacterial and protozoan sexually transmitted infections. Indian Journal of Sexually Transmitted Diseases and AIDS. 2015;**36**(1):3-8

[44] Magri V et al. Efficacy of repeated cycles of combination therapy for the eradication of infecting organisms in chronic bacterial prostatitis. International Journal of Antimicrobial Agents. 2007;**29**(5):549-556

[45] Oliphant CM, Green GM. Quinolones: A comprehensive review. American Family Physician. 2002;**65**(3):455-464

[46] Costello LC et al. Role of zinc in the pathogenesis and treatment of prostate cancer: Critical issues to resolve. Prostate Cancer and Prostatic Diseases. 2004;**7**(2):111-117

[47] Krause W, Bohring C. Male infertility and genital chlamydial infection: Victim or perpetrator? Andrologia. 2003;**35**(4):209-216

[48] Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. Morbidity and Mortality Weekly Report. 2010;**59**(Rr-12):1-110

[49] Dutta S et al. Staphylococcal infections and infertility: Mechanisms and management. Molecular and Cellular Biochemistry. 2020;**474**(1-2):57-72

[50] Agarwal A et al. Male oxidative stress infertility (MOSI): Proposed terminology and clinical practice guidelines for Management of Idiopathic Male Infertility. World Journal of Men's Health. 2019;**37**(3):296-312

[51] Koppers AJ, Garg ML, Aitken RJ. Stimulation of mitochondrial reactive oxygen species production by unesterified, unsaturated fatty acids in defective human spermatozoa. Free Radical Biology & Medicine. 2010;**48**(1):112-119

[52] Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertility and Sterility. 1992;**57**(2):409-416

[53] Panner Selvam MK, Sengupta P, Agarwal A. In: Arafa M et al., editors. Sperm DNA Fragmentation and Male Infertility, in Genetics of Male Infertility: A Case-Based Guide for Clinicians. Cham: Springer International Publishing; 2020. pp. 155-172

[54] Dutta S et al. Physiological role of ROS in sperm function. In: Parekattil SJ, Esteves SC, Agarwal A, editors. Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants. Cham: Springer International Publishing; 2020. pp. 337-345

[55] Sengupta P, Dutta S. Hormones in male reproduction and fertility. Asian Pacific Journal of Reproduction. 2019;**8**(5):187-188

[56] Dutta S et al. Oxidative stress, testicular inflammatory pathways, and male reproduction. International Journal of Molecular Sciences. 2021;**22**(18)

[57] Wells D et al. Expression of genes regulating chromosome segregation, the cell cycle and apoptosis during human preimplantation development. Human Reproduction. 2005;**20**(5):1339-1348

[58] Sakkas D, Alvarez JG. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. Fertility and Sterility. 2010;**93**(4):1027-1036

[59] Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Human Reproduction. 2004;**19**(3):611-615

[60] Tesarík J et al. Activation of nucleolar and extranucleolar RNA synthesis and changes in the ribosomal content of human embryos developing in vitro. Journal of Reproduction and Fertility. 1986;**78**(2):463-470

[61] Kuroda S et al. Early and late paternal effects of reactive oxygen species in semen on embryo development after intracytoplasmic sperm injection. Systems Biology in Reproductive Medicine. 2020;**66**(2):122-128

[62] Zhao J et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: A systematic review and meta-analysis. Fertility and Sterility. 2014;**102**(4):998-1005.e8

[63] Trevelyan SJ et al. Structure-based mechanism of preferential complex formation by apoptosis signal-regulating kinases. Science Signaling. 2020;**13**(622):eaay6318

[64] Shukla KK, Mahdi AA, Rajender S. Apoptosis, spermatogenesis and male infertility. Frontiers in Bioscience (Elite Edition). 2012;**4**(2):746-754

[65] Calivarathan L et al. Environmental Contaminants and Male Reproductive Health: Past, Present and Future. In: Environmental Studies and Climate Change. CRC Press; 2022. p. 361-372

[66] Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: A systematic review. Obstetrical & Gynecological Survey. 2007;**62**(5):335-347

[67] Ota H, Igarashi S, Tanaka T. Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertility and Sterility. 2001;**75**(4):785-790

[68] Gupta S et al. Role of oxidative stress in endometriosis. Reproductive Biomedicine Online. 2006;**13**(1):126-134

[69] Kajihara H et al. New insights into the pathophysiology of endometriosis: From chronic inflammation to danger signal. Gynecological Endocrinology. 2011;**27**(2):73-79

[70] McCubrey JA, Lahair MM, Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. Antioxidants & Redox Signaling. 2006;**8**(9-10):1775-1789

[71] Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Current Opinion in Obstetrics & Gynecology. 2006;**18**(3):325-332

[72] Madazli R et al. The plasma and placental levels of malondialdehyde, glutathione and superoxide dismutase in pre-eclampsia. Journal of Obstetrics and Gynaecology. 2002;**22**(5):477-480

[73] Matsubara K et al. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. International Journal of Molecular Sciences. 2015;**16**(3):4600-4614

[74] Hubel CA et al. Lipid peroxidation in pregnancy: New perspectives on preeclampsia. American Journal of Obstetrics and Gynecology. 1989;**161**(4):1025-1034

[75] Uzun H et al. Circulating oxidized low-density lipoprotein and paraoxonase activity in preeclampsia. Gynecologic and Obstetric Investigation. 2005;**60**(4):195-200

[76] Behrman HR et al. Oxidative stress and the ovary. Journal of the Society for Gynecologic Investigation. 2001;**8**(1 Suppl Proceedings):S40-S42

[77] Raijmakers MTM et al. NAD(P)H oxidase associated superoxide production in human placenta from normotensive and pre-eclamptic women. Placenta. 2004;**25**:S85-S89

[78] Walsh SW. Eicosanoids in preeclampsia. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2004;**70**(2):223-232

[79] Klemmensen A et al. Intake of vitamin C and E in pregnancy and risk of pre-eclampsia: Prospective study among 57 346 women. BJOG. 2009;**116**(7):964-974

[80] Liu G et al. Restraint stress alters immune parameters and induces oxidative stress in the mouse uterus during embryo implantation. Stress. 2014;**17**(6):494-503

[81] Xiaoyan S et al. Follicle stimulating hormone inhibits the expression of p53 up-regulated modulator of apoptosis induced by reactive oxygen species through PI3K/AKT in mouse granulosa cells. Physiological Research. 2020;**69**(4):687

[82] Denefil OV et al. The peculiarities of morpological changes of rats'ovary and biochemical state under the damage with different doses of lead acetate. Wiadomości Lekarskie. 2022;**75**(2):377-382

[83] Ayad B et al. Oxidative stress and male infertility: Evidence from a research perspective. Human Reproductive Health. 2022;**4**:822257

[84] Agarwal A, Qiu E, Sharma R. Laboratory assessment of oxidative

stress in semen. Arab Journal of Urology. 2018;**16**(1):77-86

[85] Gosalvez J, Tvrda E, Agarwal A. Free radical and superoxide reactivity detection in semen quality assessment: Past, present, and future. Journal of Assisted Reproduction and Genetics. 2017;**34**(6):697-707

[86] Agarwal A, Allamaneni SS, Said TM. Chemiluminescence technique for measuring reactive oxygen species. Reproductive Biomedicine Online. 2004;**9**(4):466-468

[87] Zhang X et al. ROS/RNS and base dual activatable merocyanine-based NIR-II fluorescent molecular probe for in vivo biosensing. Angewandte Chemie (International Ed. in English). 2021;**60**(50):26337-26341

[88] Hans C et al. 2′,7'-Dichlorofluorescein (DCF) or 2′,7′-dichlorodihydrofluorescein diacetate (DCFH2-DA) to measure reactive oxygen species in erythrocytes. Biomedicine & Pharmacotherapy. 2021;**138**:111512

[89] Yao H, Jockusch RA. Fluorescence and electronic action spectroscopy of mass-selected gas-phase fluorescein, 2′,7′-dichlorofluorescein, and 2′,7′-difluorofluorescein ions. The Journal of Physical Chemistry. A. 2013;**117**(6):1351-1359

[90] Kalyanaraman B et al. HPLC-based monitoring of products formed from hydroethidine-based fluorogenic probes--the ultimate approach for intra- and extracellular superoxide detection. Biochimica et Biophysica Acta. 2014;**1840**(2):739-744

[91] Gupta S et al. Total antioxidant capacity-relevance, methods and clinical implications. Andrologia. 2021;**53**(2):e13624

[92] Apak R et al. Antioxidant activity/capacity measurement. 3. Reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/chemometric assays. Journal of Agricultural and Food Chemistry. 2016;**64**(5):1046-1070

[93] Hosen MB et al. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. International Journal of Reproductive BioMedicine. 2015;**13**(9):525-532

[94] Cano A et al. ABTS/TAC methodology: Main milestones and recent applications. PRO. 2023;**11**(1):185

[95] Barclay LRC, Locke SJ, MacNeil JM. Autoxidation in micelles. Synergism of vitamin C with lipid-soluble vitamin E and water-soluble Trolox. Canadian Journal of Chemistry. 1985;**63**(2):366-374

[96] Castleton PE, Deluao JC, Sharkey DJ, McPherson NO. Measuring reactive oxygen species in semen for male preconception care: A scientist perspective. Antioxidants (Basel). 2022;**11**(2):264

[97] Agarwal A et al. Sperm vitality and necrozoospermia: Diagnosis, management, and results of a global survey of clinical practice. World Journal of Men's Health. 2022;**40**(2):228-242

[98] Walters JLH et al. Mechanistic insight into the regulation of lipoxygenase-driven lipid peroxidation events in human spermatozoa and their impact on male fertility. Antioxidants (Basel). 2020;**10**(1):43

[99] du Plessis SS et al. Oxidative phosphorylation versus glycolysis: What fuel do spermatozoa use? Asian Journal of Andrology. 2015;**17**(2):230-235

[100] Walters JLH et al. Oxidative stress in the male germline: A review of novel strategies to reduce 4-Hydroxynonenal production. Antioxidants (Basel). 2018;**7**(10):132

[101] Nowicka-Bauer K, Nixon B. Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants (Basel). 2020;**9**(2):134

[102] Ghantabpour T et al. The effect of astaxanthin on motility, viability, reactive oxygen species, apoptosis, and lipid peroxidation of human spermatozoa during the freezing-thawing process. Biopreservation and Biobanking. 2022;**20**(4):367-373

[103] Rahman MF et al. Effects of elevated temperature on 8-OHdG expression in the American oyster (*Crassostrea virginica*): Induction of oxidative stress biomarkers, cellular apoptosis, DNA damage and gammaH2AX signaling pathways. Fish and Shellfish Immunology Reports. 2023;**4**:100079

[104] De Iuliis GN et al. DNA damage in human spermatozoa is highly correlated with the efficiency of chromatin remodeling and the formation of 8-hydroxy-2′-deoxyguanosine, a marker of oxidative stress. Biology of Reproduction. 2009;**81**(3):517-524

[105] Aitken RJ, Bakos HW. Should we be measuring DNA damage in human spermatozoa? New light on an old question. Human Reproduction. 2021;**36**(5):1175-1185

[106] Korneyev IA. Oxidative stress and male infertility a clinical perspective. Urologiia. 2022;**1**:102-108

[107] Mannucci A et al. The impact of oxidative stress in male infertility. Frontiers in Molecular Biosciences. 2021;**8**:799294

[108] Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab Journal of Urology. 2019;**17**(2):87-97

[109] Agarwal A, Ahmad G, Sharma R. Reference values of reactive oxygen species in seminal ejaculates using chemiluminescence assay. Journal of Assisted Reproduction and Genetics. 2015;**32**(12):1721-1729

[110] Homa ST et al. A comparison between two assays for measuring seminal oxidative stress and their relationship with sperm DNA fragmentation and semen parameters. Genes (Basel). 2019;**10**(3):236

[111] Liu K et al. Correlation analysis of sperm DNA fragmentation index with semen parameters and the effect of sperm DFI on outcomes of ART. Scientific Reports. 2023;**13**(1):2717

[112] Castleton P et al. MiOXSYS® and OxiSperm® II assays appear to provide no clinical utility for determining oxidative stress in human sperm-results from repeated semen collections. Andrology. 2022

[113] Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology. 2005;**3**:28

[114] Agarwal A et al. The effects of oxidative stress on female reproduction: A review. Reproductive Biology and Endocrinology. 2012;**10**:49

[115] Valsamakis G, Chrousos G, Mastorakos G. Stress, female reproduction and pregnancy. Psychoneuroendocrinology. 2019;**100**:48-57

[116] Rizov M, Andreeva P, Dimova I. Molecular regulation and

role of angiogenesis in reproduction. Taiwanese Journal of Obstetrics & Gynecology. 2017;**56**(2):127-132

[117] Steller JG, Alberts JR, Ronca AE. Oxidative stress as cause, consequence, or biomarker of altered female reproduction and development in the space environment. International Journal of Molecular Sciences. 2018;**19**(12):3729

[118] Victoria M et al. Anti Mullerian hormone: More than a biomarker of female reproductive function. Journal of Gynecology Obstetrics and Human Reproduction. 2019;**48**(1):19-24

[119] Ruano CSM et al. The impact of oxidative stress of environmental origin on the onset of placental diseases. Antioxidants (Basel). 2022;**11**(1):106

[120] Bisson C et al. Preeclampsia pathophysiology and adverse outcomes during pregnancy and postpartum. Frontiers in Medicine (Lausanne). 2023;**10**:1144170

[121] Guo M et al. Mass cytometry analysis reveals a distinct immune environment in peritoneal fluid in endometriosis: A characterisation study. BMC Medicine. 2020;**18**(1):3

[122] Sipak-Szmigiel O et al. Serum and peritoneal fluid concentrations of soluble human leukocyte antigen, tumor necrosis factor alpha and interleukin 10 in patients with selected ovarian pathologies. Journal of Ovarian Research. 2017;**10**(1):25

[123] Negi R et al. Biochemical markers in the pathogenesis of preeclampsia: Novel link between placental growth factor and interleukin-6. Molecular and Cellular Biochemistry. 2022;**477**(6):1765-1774

[124] Krisiloff M. A dietary cure for prostatitis and the urethral syndrome. Infectious Diseases in Clinical Practice. 2002;**11**(3):107-110

[125] Martin GB et al. Effects of dietary zinc deficiency on the reproductive system of young male sheep: Testicular growth and the secretion of inhibin and testosterone. Journal of Reproduction and Fertility. 1994;**101**(1):87-96

[126] Hansen SO, Knudsen UB. Endometriosis, dysmenorrhoea and diet. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2013;**169**(2):162-171

[127] Parazzini F et al. Diet and endometriosis risk: A literature review. Reproductive Biomedicine Online. 2013;**26**(4):323-336

[128] Herati AS, Moldwin RM. Alternative therapies in the management of chronic prostatitis/chronic pelvic pain syndrome. World Journal of Urology. 2013;**31**(4):761-766