Oxidative stress and male infertility

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Abstract | DNA damage, largely owing to oxidative stress, is a leading cause of defective sperm function. High levels of oxidative stress result in damage to sperm DNA, RNA transcripts, and telomeres and, therefore might provide a common underlying aetiology of male infertility and recurrent pregnancy loss, in addition to congenital malformations, complex neuropsychiatric disorders, and childhood cancers in children fathered by men with defective sperm cells. Spermatozoa are highly vulnerable to oxidative stress owing to limited levels of antioxidant defence and a single, limited DNA-damage detection and repair mechanism. Oxidative stress is predominantly caused by a host of lifestyle-related factors, the majority of which are modifiable. Antioxidant regimens and lifestyle modifications could both be plausible therapeutic approaches that enable the burden of oxidative-stress-induced male factor infertility to be overcome. Lifestyle interventions including yoga and meditation can substantially improve the integrity of sperm DNA by reducing levels of oxidative DNA damage, regulating oxidative stress and by increasing the expression of genes responsible for DNA repair, cell-cycle control and anti-inflammatory effects. Oxidative stress is caused by various modifiable factors, and the use of simple interventions can decrease levels of oxidative stress, and therefore reduce the incidence of both infertility and complex diseases in the resultant offspring.

Free radical
A free radical is an atom or molecule that is highly reactive because it contains an unpaired electron in the outer shell.

Capacitation
A process that sperm undergo as they travel through the woman's reproductive tract. Capacitation enables the sperm to penetrate the egg.

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Infertility is defined as the inability of a couple to achieve spontaneous pregnancy after 1 year of regular, unprotected sexual intercourse[1]. This issue reportedly affects 8–12% of couples of reproductive age, globally[2]. Male factor infertility contributes to approximately half of all cases of infertility and affects around one in 20 men in the reproductive age group (defined here as between puberty and 40 years of age)[3]. Reactive oxygen species (ROS) are highly reactive oxidizing free radical agents and include superoxide anions (O_2•), hydrogen peroxide (H_2O_2), peroxyl (ROO•), and hydroxyl (OH•) radicals[4]. The major sources of ROS in sperm include activated leukocytes in the seminal plasma and the mitochondria in the spermatozoa. A plethora of evidence suggests that ROS-mediated damage to spermatozoa is a major contributor to the pathology of infertility in 30–80% of infertile men[5,6].

From a physiological perspective, reactive oxygen metabolites are indispensable to apoptotic processes and are involved in the capacitation of these highly differentiated and polarized cells[7]. Oxidative stress is defined as a condition in which the antioxidant scavenging system of the cell is overwhelmed by the overproduction of ROS, resulting in a state of oxygen paradox, whereby free radicals are required for cellular processes, but, at increased concentrations, can also interfere with essential metabolic processes[8]. Oxidative stress is a major cause of sperm cell dysfunction and a major contributor to the aetiology of male infertility[9] owing to impairment of both the structural and functional integrity of spermatozoa[10–12]. Low levels of ROS are required for several redox-sensitive physiological processes, such as sperm capacitation and hyperactivation, although supraphysiological ROS levels impede sperm membrane fluidity and permeability[13]. The exact mechanism of oxidative-stress-induced decline in sperm function remains unknown but is mainly attributed to peroxidative damage to axoneme and depletion of intracellular ATP levels, followed by generation of 4-hydroxynonenal and malondialdehyde owing to oxidation of lipid membrane components and fragmentation of both nuclear and mitochondrial DNA[13,14].

Spermatozoa with damaged DNA have very limited potential for natural fertilization, and the presence of high levels of DNA damage in human spermatozoa has been correlated with adverse clinical outcomes including infertility, recurrent pregnancy loss (RPL), childhood mortality and high rates of morbidity in the offspring including dominant genetic disorders, complex polygenic disorders and childhood cancers[15]. The integrity of spermatozoa, as indicated by the DNA fragmentation index (DFI), which can be assessed using a sperm chromatin structure assay (SCSA), provides an indication of likely reproductive outcomes. A DFI >30 is associated

Key points

- Male infertility is a complex lifestyle-related disorder
- Oxidative stress has adverse effects on the structural and functional integrity of sperm and is a major cause of defective sperm function and male infertility
- Oxidative stress causes damage to both mitochondrial and nuclear DNA and also affects the sperm epigenome, resulting in infertility, recurrent pregnancy loss, poor pregnancy outcomes and an increased disease burden in the offspring
- Spermatozoa are most vulnerable to oxidative stress and oxidative DNA damage (ODD) as these cells have limited antioxidant defence mechanisms and a limited capacity for detection and repair of DNA damage
- A number of intrinsic and extrinsic factors can regulate oxidative stress, and these must be maintained at moderate levels for optimal sperm function and the maintenance of cellular homeostasis and redox-sensitive signal-transduction pathways
- Simple lifestyle modifications and interventions can substantially reduce levels of testicular inflammation, oxidative stress and ODD and improve the quality of life of infertile couples

with failure of spontaneous conception and infertility, whereas DFI >26 is associated with conception but often results in RPL[16]. The use of assisted reproductive techniques (ART), such as *in vitro* fertilization, circumvents the natural selection process that occurs during fertilization and might result in an increased risk of birth defects and genetic and/or epigenetic abnormalities in the child[17]. Simple lifestyle modifications, such as meditation and/or yoga have been demonstrated to reduce seminal oxidative stress levels within 10 days and levels of oxidative DNA damage (ODD) after 6 months of practice, therefore improving both male reproductive health and also quality of life (QOL)[18,19].

This Review provides an overview of the physiology of oxidative-stress-induced damage in human spermatozoa, followed by a description of the mechanisms that induce oxidative stress in the male germ line. Subsequently, we focus on the clinical implications of oxidative-stress-induced sperm DNA damage and the effects of such damage on reproductive outcomes and disease burdens in the next generation.

Human spermatozoa: cells in crisis

Human spermatozoa are polarized cells with an exceptional ability to escape recognition by the female immune system for a length of time sufficient to enable fertilization of the egg. Therefore, sperm dysfunction is a major contributor to poor fertility[20]. Spermatozoa were previously considered to be mere vectors that enable delivery of the paternal genome whose function ended at the time of fertilization. However, data from studies published in the past two decades have highlighted that spermatozoa function is a critical determinant of both reproductive outcome and embryonic viability[9]. Damaged, or defective spermatozoa have a major influence not only on the outcomes of pregnancy but also on the health trajectory of the offspring, resulting in a paternally mediated increase in the risk of miscarriage and a wide range of dominant genetic disorders in the progeny, including neuropsychiatric disorders such as autism and schizophrenia, and childhood cancers[9,21]. Therefore, explorations of the factors that cause a decline in sperm function and factors that aid in its maintenance are vital in order to fully understand both infertility and the risks of genetic disorders in the progeny[22].

Mammalian sperm DNA has a sixfold greater degree of compaction in comparison to the loose chromatin structure of somatic cells, in which the DNA is first packed into nucleosomes[23] and then into a solenoid[24]. This hierarchical packaging increases the volume of chromatin in somatic cells, relative to those of the germ line, owing to the added histone volume and solenoid core. However, such packaging is not feasible in sperm chromatin as their nuclei do not possess the volume required for such a level of DNA compaction.

Sperm nuclear DNA is tightly wrapped around nuclear proteins known as protamines (approximately 50 kbp of sperm nuclear DNA is wrapped around one protamine molecule). Protamines are highly basic (rich in arginine) and are the most abundant nuclear proteins in many species, including mammals, and provide extensive packaging of the sperm genome[25]. Protamines enable the condensation and decondensation of the same DNA molecule with the help of a series of arginine-rich anchoring domains that bind to the DNA phosphodiester backbone in a base-sequence-independent manner[25]. During the later stages of spermatogenesis, both histone and non-histone proteins in the round spermatids are replaced by transition proteins, and in elongated spermatids the transition proteins are replaced by protamines[26,27]. The compaction of genetic material provided by protamines protects the sperm genome from both external and internal insults[28]. The majority of the sperm genome (~85%) is bound to central nucleoprotamines and the rest (~15%) to peripheral nucleohistones. The histone-bound DNA sequences include the telomeres and promoters of genes that regulate early embryonic development[29]. This nucleohistone compartment is particularly vulnerable to oxidative stress and also aids in the maintenance of genomic imprints. An increase in sperm histone:protamine ratio indicates defective chromatin compaction and an increased risk of male infertility[30,31]. Furthermore, an altered relative ratio of protamine 1:protamine 2, skewed in the direction of a relative reduction in protamine 2 levels, at the level of mRNA and protein has also been demonstrated to lead to infertility[32,33]. Data from a number of case-control studies highlight that polymorphisms or mutations in genes encoding protamines result in conformational changes in sperm chromatin structure, and hence impaired spermatogenesis[34,35].

Oxidative stress in the male germ line

Seminal oxidative stress

Oxidative stress describes the condition in which levels of oxygen and oxygen-derived free radicals overwhelm the natural antioxidant defences of the cell[10]. Oxidative stress has a negative effect on sperm function by disrupting the integrity of the DNA as a result of concurrent damage to proteins and lipids present in the sperm-cell plasma membrane, therefore affecting cell membrane fluidity and permeability[20]. The first observation

Oxidative stress
Results from an imbalance between the intracellular production of free radicals and the cellular defence mechanisms.

Antioxidant
Any substance that prevents or reduces damage caused by free-radicals (highly reactive chemicals containing oxygen) that attack other molecules and modify their chemical structure.

DNA fragmentation
Splitting of DNA strands into shorter pieces by endonucleolytic DNA cleavage at multiple sites. This process includes internucleosomal DNA fragmentation, which, along with chromatin condensation, is considered a hallmark of apoptosis.

Assisted reproductive techniques
(ART). All treatments or procedures that include the *in vitro* handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy.

Meditation
A practice of concentrated focus upon a sound, object, visualization, such as the breath, movement, or attention itself in order to increase awareness of the present moment, reduce stress, promote relaxation, and enhance personal and spiritual growth.

of oxidative-stress-induced defective sperm function dates back to 1943 when Dr John MacLeod[36] reported that human spermatozoa, when incubated under conditions of high oxygen tension, lose their motility through a mechanism that could be inhibited by the concomitant presence of catalase — an enzyme that catalyses the decomposition of H_2O_2[37]. The various milestones in our understanding of the effects of oxidative stress on sperm function (BOX 1) prompted many andrologists to further investigate the causes and consequences of oxidative-stress-induced damage to human sperm and its association with the rising burden of male infertility.

ROS can originate from both endogenous and exogenous sources. Endogenous sources of ROS include oxidative phosphorylation, cytochrome P450-catalysed drug metabolism, peroxisomes and inflammatory cell activation. Research by Gomez *et al.*[38] demonstrated that ROS reduce sperm motility in a manner that is directly proportional to the level of lipid peroxidation. These researchers also reported that sperm motility declines extensively after overnight incubation of the tested semen samples, which is associated with activation of a lipid peroxidation cascade initiated by ROS-mediated (mainly H_2O_2) oxidation of lipids in the sperm plasma membrane. In another independent study, Aitken and colleagues[39] reported that oxidative damage to lipids of the sperm plasma membrane could be reversed by the inclusion of α-tocopherol, an antioxidant that disrupts the peroxidation cascade.

ROS are naturally generated by sperm in order to induce tyrosine phosphorylation of nuclear factor NF-kappa-B p105 subunit and ezrin proteins, which is required for sperm capacitation and activation. The extent of ROS-induced tyrosine phosphorylation is largely dependent upon the ability of H_2O_2 to suppress tyrosine-phosphatase activity and the ability to induce a twofold increase in cAMP levels, which is comparable to the effects of bicarbonate, a known activator of soluble adenylyl cyclase in sperm[40]. H_2O_2 activates adenylyl cyclase to produce cAMP, leading to protein kinase A-dependent tyrosine phosphorylation. Oxidative phosphorylation occurs in the inner mitochondrial membrane, commencing with a redox reaction between oxygen and hydrogen. This energy-generating metabolic process generates ROS as byproducts, which act in two ways[8]. Firstly, by oxidation of lipids in the sperm plasma membrane, resulting in a decrease in both sperm motility and in the ability of sperm to fuse with the vitelline membrane of the oocyte, and secondly, by damaging sperm nuclear DNA and RNA, thus affecting the contribution of the paternal genome to the embryo. Spermatozoa are susceptible to oxidative stress because their plasma membrane contains an abundance of polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid, which has six carbon–carbon double bonds per molecule, therefore providing numerous potential sites for oxidation by free radicals. Furthermore, the carbon–hydrogen dissociation energies of PUFAs are lowest in the bis-allylic methylene position, thus, rendering these lipids particularly vulnerable to oxidation. The lipid peroxidation cascade, which is initiated by oxidation of NADPH, leading to hydrogen abstraction, generates carbon-centred lipid radicals, which combine with oxygen to produce peroxyl (ROO•) or alkoxyl (RO•) radicals, which then sequester hydrogen from the adjacent carbon atoms in order to stabilize[41]. These chemical reactions generate additional lipid radicals that maintain the lipid peroxidation reactions and culminate in the formation of low-molecular-mass electrophilic aldehydes such as acrolein, 4-hydroxynonenal, and malondialdehyde. This end product is both electrophilic and nucleophilic and forms malondialdehyde homodimers, which are

Box 1 | **Key breakthroughs in understanding the effects of seminal oxidative stress**

John Macleod (1943)[36]
Provided the first reported observation of oxidative-stress-induced defective sperm function, finding that human spermatozoa, when incubated under high oxygen tension, lose their motility through a mechanism that can be reversed by catalase — a specific scavenger of H_2O_2.

Tosic and Walton (1946)[182]
Confirmed the notion that spermatozoa can generate reactive oxygen species (ROS), specifically H_2O_2, and demonstrated that bovine spermatozoa possess an enzyme system that generates H_2O_2 at concentrations that are toxic to the respiration and motility of the spermatozoa.

Yanagimachi (1976)[183]
Introduced a novel 'zona-free hamster oocyte penetration assay' technique to selectively assess the fertilizing potential of human spermatozoa using zona pellucida free (hamster) ova as a substitute for human ova.

Shannon and Curson (1982)[184]
Confirmed the presence of L-amino acid oxidase enzymes in bovine spermatozoa, which are active only in dead spermatozoa and actively generate H_2O_2 as a toxic byproduct of phenylalanine oxidation under conditions of high oxygen tension.

Aitken & Clarkson[185] and Alvarez[186] (1987)
Independently reported that human spermatozoa have the capacity to generate ROS in the male germ line.

Aitken (1993)[187]
Demonstrated that sperm cells from infertile men have reduced fertility potential compared with those from fertile men. This observation remained significant even when their spermatozoa were treated with A23187, a cation ionophore that induces calcium influx across the sperm-cell plasma membrane, resulting in an acrosome reaction and sperm–oocyte fusion.

Yoga
A Hindu spiritual and ascetic discipline, a part of which, including breath control, simple meditation, and the adoption of specific bodily postures, is widely practiced for health and relaxation purposes.

Protamines
Proteins that bind with DNA in sperm cells, replacing histones and allowing chromosomes to become more highly condensed than is possible with histones.

Spermatogenesis
The production of sperm within the seminiferous tubules.

mutagenic, but can also form DNA adducts, which are known to induce mutations in tumour suppressor genes and oncogenes[42]. These lipid aldehydes are electrophilic and bind to mitochondrial proteins, therefore altering their conformation and triggering the production of ROS, leading to electron leakage and superoxide formation.

Mitochondria are the source, and often also the targets of free-radical oxidation. Mitochondrial DNA is particularly vulnerable in that it is not protected by histones and has a very limited capacity for DNA repair, owing to a complete lack of nucleotide-excision repair pathways[43]. Thus, the mutation rate of mitochondrial DNA is estimated to be two orders of magnitude higher than that of nuclear DNA. Sperm cells containing large numbers of damaged mitochondria are incapable of completing apoptosis owing to this damage, therefore resulting in the presence of sperm with damaged DNA in the ejaculate[44]. Dysfunctional mitochondria harbouring mutations produce more free radicals and less ATP than their fully functional counterparts and the accumulation of large numbers of such mitochondria might result in hypospermatogenesis, owing to meiotic arrest during sperm-cell development and/or partially formed and/or disorganised axonemal apparatus resulting in asthenozoospermia[45]. A decrease in intracellular ATP levels can inhibit the progression of stem cell precursors through the cell cycle and can therefore result in hypospermatogenesis or maturation arrest. Furthermore, fragments of mitochondrial DNA can become inserted into the nuclear genome and cause activation of oncogenes[46]. Thus, genomic DNA adducts resulting from oxidative stress, mitochondrial mutations, mitochondrial DNA fragmentation and insertion into the nuclear genome not only damage sperm, but also might promote the initiation of carcinogenesis, which possibly explains the link between male infertility and an increased risk of gonadal and extragonadal tumours[47].

Oxidative stress and the sperm genome

ROS are generated by spermatozoa themselves[4] and also by leukocytes in the seminal plasma[48]. Mild oxidative stress is essential in mediating tyrosine phosphorylation, which is required for capacitation of sperm cells and also supports the maintenance of telomere length[40]. However, severe oxidative stress, owing to seminal ROS levels >35 relative light units (RLU)/s per million sperm cells results in accelerated telomere shortening whereas mild oxidative stress, owing to seminal ROS levels of 21.3–35 RLU/s per million sperm cells, promotes the maintenance of telomere length[49]. The functional capacity of spermatozoa is defined not only by the ability to fertilize oocytes but also by the ability to transfer the intact paternal genome[50].

DNA fragmentation is most commonly observed in the spermatozoa of infertile men and a substantial amount of evidence exists that this damage is free-radical mediated[51–53]. Spermatozoa with extensive DNA damage will, obviously, encounter biological and molecular barriers that most likely preclude fertilization, such as a lack of viable DNA and this, as a result, prevents the transmission of damaged DNA during fertilization. However, these stages are bypassed by the use of ARTs and might have adverse effects on the health of the offspring to the extent of doubling the incidence of infertility and that of several childhood and genetic disorders[54,55].

The oxidative DNA damage observed in spermatozoa includes single-strand and double-strand breaks, DNA fragmentation, the introduction of abasic sites, purine, pyrimidine and deoxyribose modifications and DNA crosslinking, which can result in arrest or induction of gene transcription, induction of signal transduction pathways, accelerated telomeric DNA attrition, replication errors, genomic instability and GC to TA transversions[56–58]. These changes are also observed during carcinogenesis and might explain the link between infertility and cancer. Many ROS lead to oxidation of bases — chief among them being hydroxyl radicals, which have a high level of reactivity. Peroxynitrite, another product of oxidative stress, is formed by the coupling of nitric oxide and superoxide and is a strong cellular oxidant that induces both inflammation and mutation[59]. ROS-induced DNA damage might accelerate the process of germ-cell apoptosis, leading to the decline in sperm count associated with male infertility[60]. Research by Koppers and colleagues[41] showed that exogenous supplementation of human spermatozoa with unsaturated fatty acids stimulates mitochondrial superoxide production via mechanisms that are independent of lipoxygenase and cyclooxygenase activity. The application of these fatty acids results in a concomitant loss of motility and an increase in oxidant-induced damage to nuclear and mitochondrial DNA, two key aspects of male infertility. The authors[41] demonstrated that defective human spermatozoa have high levels of both esterified and unesterified fatty acids and a decrease in the proportion of the total fatty acid pool derived from docosahexenoic acid. The defective spermatozoa have high total fatty acid levels and/or free fatty acid levels compared with those of their functional counterparts, which promotes ROS generation by the sperm mitochondria, leading to oxidative stress and loss of sperm function.

During spermatogenesis, the total and free fatty acid content is reduced, with a parallel increase in the docosahexaenoic acid content. This morphogenetic transformation does not occur in defective spermatozoa, which are characterized by high fatty acid levels (both free and total) and low docosahexaenoic acid levels[61]. In spermatozoa, high fatty acid levels are associated with a decline in membrane fluidity, and hence a decline in the ability to fuse with the oolema. An increase in unsaturated fatty acid content is associated with free-radical generation by sperm mitochondria, which can trigger lipid peroxidation and ultimately drive these cells into a state of oxidative stress[62]. Mitochondrial ROS generation is based upon the ability of unsaturated fatty acids to inhibit complex I of the electron transport chain, with minor inhibition of complex III, which results in electron leakage from complex I and ultimately transfers electrons to O_2 molecules, therefore, posing a major threat to human spermatozoan physiological function[63].

Asthenozoospermia
Low sperm motility.

Telomere length
A telomere is a region of repetitive nucleotide sequences at each end of a chromosome, which protect the end of the chromosome from deterioration or from fusion with neighbouring chromosomes. Telomere length decreases with advancing cellular age, thus strategies that reduce the rate of telomere shortening might delay the cellular ageing process.

Infiltrating leukocytes in seminal plasma produce 1,000 times more ROS than the spermatozoa[64]. These high levels of ROS generated by seminal leukocytes act as a response to a wide range of infection-related and/or inflammation-related stimuli in the male genital tract, especially in the accessory sex glands, including the seminal vesicles and prostate. The phenomenon referred to as 'respiratory burst of the leukocyte', a situation in which ROS release by activated leukocytes rapidly increases to around 100 times that of the inactivated leukocytes, can occur in response to infection-related stimuli[65,66]. According to the WHO guidelines, leukocytospermia is defined as a concentration of seminal leukocytes $>1 \times 10^6$ leukocytes/ml (REFS 67,68). Leukocytes are the major contributor to oxidative stress because, compared with that of spermatozoa, the rate of ROS production in leukocytes is 1,000 times greater[53] and other researchers reported that exposure to excessive levels of polymorphonuclear-leukocyte-generated ROS is associated with a decline in fertility[69,70]. Thus, prompt treatment of inflammation and/or infection substantially reduces seminal oxidative stress levels, and might improve fertility outcomes.

DNA damage repair

Oxidative stress causes damage to biomolecules including DNA, lipids and proteins and contributes to the pathology of many diseases including neurodegenerative disorders, autoimmune diseases, cardiovascular dysfunction, accelerated ageing, cancer and diseases of the reproductive system (including both male and female infertility)[8,71–73]. Oxidative stress induces reversible and irreversible modifications of cellular proteins, thereby causing cellular dysfunction and a predisposition to a wide range of disorders. The identification of markers of cellular oxidative stress would enable the pathobiology, prognosis and/or responses to treatment of patients with various diseases to be identified[74]. A wide range of tests are available to measure the extent of oxidative stress and these generally involve quantification of ROS levels, total antioxidant capacity (TAC) and/or levels of biomarkers associated with oxidative damage to biomolecules[75] (TABLE 1).

Sperm cells are most vulnerable to ODD following spermiogenesis, especially when they are stored in the epididymis for 12–14 days and thus lack the protection provided by the antioxidant-rich seminal plasma[76]. Oxidative stress in the male germ line is associated with damage to nuclear and mitochondrial genomes and epigenomes. Telomeres are hexameric guanine-rich repeats, which are preferential targets of free-radical attack owing to the fact that guanine has a low oxidation potential and is, therefore, more susceptible to oxidative stress than other nucleotides[77,78]. Oxidative damage to telomeres typically results in the generation of highly mutagenic 8-hydroxy-deoxyguanine (8-OHdG) adducts, by virtue of the ability to form stable pairs with adenine, resulting in G:C to T:A transversions during DNA replication, leading to single-strand and double-strand breaks[79]. Spermatozoa have a limited capacity for DNA damage detection and repair, owing to the expression of just a single base-excision repair (BER) enzyme, *N*-glycosylase/DNA lyase (also known as 8-oxoguanine DNA glycosylase (OGG1)), with a lack of both DNA (apurinic or apyrimidinic site) lyase (also known as apurinic-apyrimidinic endonuclease 1; APEX1) and DNA repair protein XRCC1 (also known as X-ray repair cross-complementing protein 1; XRCC1) expression, requiring spermatozoan BER mechanisms to function in concert with those of the ova, which express both APEX1 and XRCC1 (REF. 80). However, the extent of damage, and the age of the oocyte will ultimately determine its capacity to repair sperm DNA damage[81]. Persistence of this damage and of mutagenic bases might predispose the sperm to develop *de novo* germ line mutations and might also be the cause of several dominant genetic disorders and even certain cancers such as leukaemia and retinoblastoma in children[19]. Various direct and indirect assays are available to measure ROS levels, although the presence of high levels of ROS in semen samples is also indicated by asthenozoospermia, teratozoospermia, increased numbers of round cells in the semen, increased semen viscosity and poor sperm membrane integrity.

Similar to the detection of ROS, a variety of tests have been designed to detect DNA damage in human spermatozoa (TABLE 2). These tests include both direct tests, in which the extent of DNA fragmentation and/or oxidation is measured directly by incorporating probes at the site of DNA damage, and indirect tests, in which DNA fragmentation is measured indirectly by measuring the degree of chromatin compaction.

Factors causing oxidative stress

Male infertility is a multifactorial disorder involving a wide range of factors including genetic, epigenetic, environmental and lifestyle-related factors. Unhealthy lifestyle-related factors such as smoking, excessive alcohol intake, a diet rich in saturated fats and proteins, a sedentary lifestyle, psychological stress, obesity and advanced paternal age (>40 years of age) all contribute to the risk of infertility. Furthermore, environmental factors such as exposure to air pollution or to persistent organic pollutants, exposure to high temperatures, plasticizers, metals (particularly transition metals such as cadmium, lead, iron and copper), chemotherapeutic agents, environmental toxicants (acrylamide, endosulfan, bisphenol A and phthalates), electromagnetic radiation, systemic and testicular infection and varicocele can all increase the levels of oxidative stress in spermatozoa[64,82] (FIG. 1). Thus, adoption of a healthy lifestyle, an increased intake of fruits and vegetables, and regular meditation and yoga all might reduce levels of oxidative stress in spermatozoa, and therefore reduce the incidence of male infertility and the burden of hereditary childhood disease[83,84]. Several antioxidants are available as dietary supplements such as vitamins E and C, glutathione, albumin and superoxide dismutase. Some of these agents increase sperm concentrations and others motility, although few have been proven to have ameliorative effects on damaged DNA at therapeutic doses[85]. Additionally, several

Table 1 | **Advantages and disadvantages of the available biomarkers of oxidative stress**

Biomarker	Characteristics	Advantages	Disadvantages	Refs
8-OHdG	• One of the most widely studied markers of oxidative stress and DNA damage • Changes in tissue expression and serum levels of 8-OHdG are associated with the prognosis of several diseases including cancer. • Mutations associated with 8-OHdG reflect total ROS-derived DNA damage	Can be detected and/or measured in various samples (plasma and/or serum, urine, tissue, saliva, and others) and has been shown to be elevated in response to oxidative damage to cellular DNA	• None of the methods (HPLC, GC–MS, LC–MS, antibody-based techniques) used to measure cellular 8-OHdG levels serves as a 'gold standard' for measurement • The techniques used during experimentation (isolation, hydrolysis) have the potential to cause DNA oxidation (especially of guanine residues), which will likely interfere with the basal level of 8-OHdG and might invalidate the results	188,189
Isoprostanes	Isoprostanes are the products of nonenzymatic lipid peroxidation formed by the free-radical-mediated oxidation of arachidonic acid	Can be detected in various samples (plasma and/or serum, urine) as byproducts of the peroxidation of various PUFAs (arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid)	• Isoprostanes are the minor products of lipid peroxidation and their levels are influenced by O_2 concentrations • Isoprostanes are rapidly metabolized and show rapid turnover in the body, therefore, isoprostanes do not qualify as the ideal biomarkers of cellular oxidative stress	190
Malondialdehyde	Malondialdehyde is considered the most mutagenic product of lipid peroxidation and plasma concentrations of malondialdehyde are a predictive indicator of lipid peroxidation	Technically easy to quantify in biological samples (plasma, urine) spectro-photometrically using the TBARS assay or can be quantified using HPLC to isolate malondialdehyde–thiobarbituric acid conjugates	• One of the many aldehydes formed during the lipid peroxidation cascade • Unsaturated aldehydes (4-hydroxynonenal and acrolein) cause considerably more cytotoxicity *in vivo* than malondialdehyde, therefore, their assessment provides a more relevant indication of oxidative stress	191
4-Hydroxenonenal	• 4-hydroxynonenal is also a product of lipid peroxidation and is considered highly toxic owing to its ability to rapidly react with thiols and amino groups • 4-hydroxynonenal is also considered one of the most potent generators of oxidative stress and is the major and most toxic lipid peroxidation product	• Most-potent biomarker for the assessment of oxidative stress as they are key modulators of numerous biological processes including cell signalling, cell proliferation, apoptosis and other processes • Potent indicators of a state of cellular oxidative stress as 4-hydroxynonenal has long-lasting biological consequences, in particular the covalent modification of macromolecules	Kit-based assays for 4-hydroxynonenal have less sensitivity than HPLC or GC-MS because many reactive species are bound to proteins and need to be released before conducting this assay.	192,193
ROS-mediated changes in gene expression	Cellular ROS levels affect multiple signal-transduction pathways by altering the activity of target molecules which might include inflammatory cytokines, growth factors coupled to receptor tyrosine kinases, as well as ligands transduced by G-protein coupled receptors	Altered gene-expression profile (owing to oxidative stress) can be quantified using various approaches such as microarrays and next-generation sequencing	• The techniques used for quantifying gene expression are often labour-intensive and require expensive software programmes • Expression profile of cells in the biological sample might not reflect the actual gene expression profile of the tissue and/or organ concerned	194
TAC	TAC in plasma and/or serum reflects the activity of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase-1), which reflects the cellular oxidative stress levels	Various commercial kits are available that enable quantification and measurement of TAC levels in plasma and/or serum; such kits provide highly reproducible results, despite use of frozen samples	• TAC measurement involves mainly contributions from ascorbate, urate and sometimes albumin; TAC levels are also influenced by diet, as consumption of certain food items could lead to changes in plasma ascorbate or urate levels thereby affecting TAC • TAC levels, as measured using commercially available kits, also require further validation and/or investigation before predicting any disease risk or disease pathology	195

8-OHdG, 8-hydroxy-2′-deoxyguanosine; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive species.

Table 2 | **Tests for detection of DNA damage in human spermatozoa**

Test	Measurement and/or detection	Characteristic features	Refs
TUNEL	Direct quantification of sperm DNA breaks (single-stranded and double-stranded)	Direct assay, involving fluorescence-based quantification of DNA-damage, which can be monitored using flow-cytometry	196,197
Comet	Double-stranded DNA breaks	Direct assay, based on single-cell gel electrophoresis and involves the use of staining dyes such as propidium iodide, SYBR-green and YOYO-1 iodide to visualize DNA	198,199
ISNT	Single-stranded DNA breaks	Direct assay, template-dependent DNA polymerase I, less sensitive than most other techniques	200
DNA oxidation	8-OHdG adducts in DNA bases	Direct assay, ELISA-based and labour intensive	201
SCSA	Based on susceptibility of sperm DNA to acid-induced denaturation *in situ*	Indirect assay, flow-cytometry based, involves intercalation of acridine orange dye	202
Nuclear protein composition	Protamine-to-histone ratio	Indirect assay, ratio assessed using protein extraction, gel separation, and immunoblotting with specific antibodies	203
Sperm nuclear maturity test	Chromatin integrity, protamine composition of sperm DNA	Indirect assay, simple and inexpensive, with slide-based quantification	204
Sperm chromatin dispersion	Sperm DNA fragmentation basically single-stranded DNA fragments	Indirect assay, simple, based on characteristic halo produced by sperm depending upon the integrity of the DNA	205

8-OHdG, 8-hydroxy-2′-deoxyguanosine; ISNT, *in situ* nick translation; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labelling

controversies exist regarding the effects of various antioxidants, which advocates the intake of a combination, rather than a single type of antioxidant[86]. To this extent, data from our laboratory show that regular meditation and yoga are therapeutic and decrease levels of oxidative stress and ODD in seminal fluid[87]. Regular meditation and yoga not only decreases ROS levels but also ameliorate levels of oxidative stress by both regulating the expression of antioxidant genes, depending on the levels of free radicals, and also decreasing the expression of proinflammatory genes[88].

Age

Unlike women, whose fertility declines with advancing age, men produce spermatozoa throughout their life, from puberty onwards. Advanced paternal age (>40 years), however, is associated with deterioration in gamete quality, increased levels of ODD and a decline in semen quality, which is further precipitated by the age-associated accumulation of mutations in the nuclear and mitochondrial genome[89]. Sperm DNA accumulates mutations owing to increased rounds of cell division, reduced fidelity of DNA replication, inefficient DNA repair and accumulation of mutagens from external and internal sources, including from exposure to oxidative stress[90]. Ageing is also associated with reduced nuclear *OGG1* expression, with increased *OGG1* expression in mitochondrial DNA[91]. Thus, sperm from men of an advanced age have a higher mutational load than sperm from younger men and are more dependent on the oocyte for removal of DNA lesions[92]. However, ageing oocytes also have suboptimal repair capabilities, resulting in inefficient, aberrant and incomplete removal of DNA lesions, therefore leading to the persistence of mutagenic bases. Once the mutational load of the fertilized ovum crosses a critical threshold, the risk of pathologies such as autism and schizophrenia in the next generation increases substantially[93].

Senescence-accelerated mouse prone 8 (SAMP8) is a mouse model containing a suite of naturally occurring mutations resulting in an accelerated senescence phenotype mainly caused by exposure to oxidative stress, which is further enhanced by mutations in *OGG1*, which greatly reduce the ability of the enzyme to excise 8-OHdG adducts[94]. Therefore, SAMP8 provides an excellent model of oxidative-stress-induced, age-dependent increases in the extent of DNA damage. Research by Kong and colleagues[95] indicates that in humans the number of *de novo* germ line mutations present in the offspring is dependent on the age of the father during conception, and that this effect increases by about two mutations per year, and doubles after every 16.5 years. Kumar and colleagues[19] reported that the last-born child is the most likely to be affected by sporadic retinoblastoma, which might also explain the increased incidence of childhood cancers in children conceived via ART[96]. In an ongoing study conducted in our lab, we observed that the majority of infertile couples, with a mean duration of infertility of 6–10 years, both partners had mild-to-moderate depression, and on microarray analysis, we found that such couples had a twofold lower level of retinoblastoma-associated protein expression, with dysregulation of several transcripts including lower levels of expression of antioxidant genes. Age-associated susceptibility to paternally mediated disorders is mainly attributed to epigenetic alterations in the sperm genome[97,98]. Advanced paternal age (>40 years) is associated with deterioration in the integrity of the DNA in gametes and an increased disease susceptibility in the offspring (including susceptibility to neuropsychiatric disorders such as schizophrenia and autism, trinucleotide-expansion-associated diseases, such as myotonic dystrophy, Huntington disease and certain forms of childhood cancers)[99].

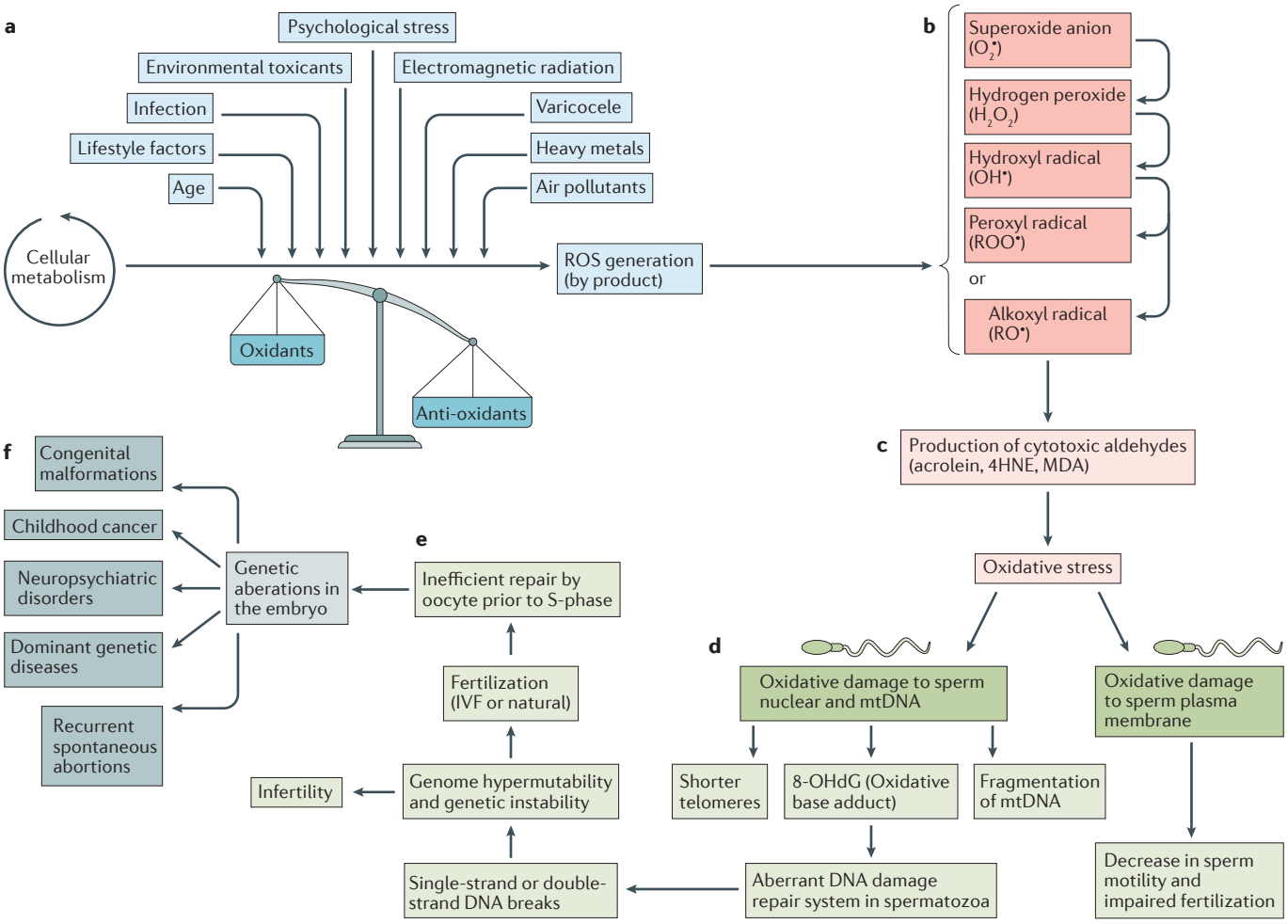

Figure 1 | **Causes and consequences of seminal oxidative stress and oxidative DNA damage. a** | A variety of factors can lead to, or affect the generation of reactive oxygen species (ROS) in the male germ line, which creates oxidative stress. **b** | High ROS levels in the spermatozoa perpetuates a lipid peroxidation cascade in the spermatozoa. **c** | The oxidative stress created by lipid peroxidation and production of free radicals induces the production of lipid aldehydes such as acrolein, 4-hydroxynonenal (4HNE), and malondialdehyde (MDA). **d** | Oxidative stress can then affect sperm function in two ways: by damaging the sperm nuclear and mitochondrial DNA (mtDNA), which is associated with shorter telomere length, formation of the oxidative base adduct 8-hydroxy-deoxyguanine (8-OHdG) and fragmentation of mitochondrial DNA; or by damaging the sperm plasma membrane and thus affecting sperm motility and its ability to fuse with the oocyte. The effects of 8-OHdG adducts in sperm DNA are more likely to lead to DNA damage owing to the limited capacity of spermatozoa to undergo DNA repair **e** | Single-strand or double-strand DNA breaks are generated. Owing to the high mutagenic load in the sperm DNA, oocytes might skip the completion of base-excision repair and repair of 8-OHdG lesions, which will persist in each and every cell of the embryo after fertilization, often leading to genome hypermutability, genetic instability and infertility. Fertilization of the oocyte (either through *in vitro* fertilization or spontaneously) by a sperm cell with a high mutational load may lead to persistence of DNA damage due to inefficient or aberrant repair by the oocyte before S phase. **f** | ROS-induced oxidative stress in the male germ line is associated with increased incidences of genetic aberrations in the embryo, including those that lead to childhood cancers, neuropsychiatric disorders (such as autism and schizophrenia) and diseases arising from a dominant genetic mutation (such as Apert syndrome and achondroplasia).

Jenkins and colleagues[100] reported hypomethylation of 139 regions in sperm DNA and eight regions that were significantly hypermethylated with advancing age. These investigators reported that 117 genes are located within these affected regions and that a proportion of age-related changes in sperm DNA methylation are located in genes associated with schizophrenia and/or bipolar disorders. Furthermore, Feinberg and colleagues[101] reported the existence of 193 differentially methylated regions in paternal spermatozoa that are associated with the autism-spectrum-disorder phenotype. Published data from our laboratory indicate that regular yoga and meditation can slow the rate of ageing, by decreasing levels of oxidative stress and DNA damage, and upregulating the expression of telomerase enzymes. This increase in telomerase expression might enable the maintenance of telomere length and promote spermatogonial proliferation and production of sperm with low levels of DNA damage[18].

DNA methylation
Attachment of methyl (–CH3) groups to DNA, most commonly to cytosine bases.

Smoking

The gaseous, as well as particulate contents of cigarette smoke, which include several notable carcinogens, such as cadmium, radioactive polonium, benzopyrenes, dimethylbenzanthracene, naphthalene, methylnaphthalene, and, among others, polycyclic aromatic hydrocarbons, has a profound negative effect on sperm parameters (including the motility, morphology and sperm count), causes oxidative stress and therefore has negative effects on reproductive outcomes[102]. Furthermore, the constituents of cigarette smoke also decrease the level of sperm mitochondrial activity and damage the chromatin structure of both nuclear and mitochondrial sperm DNA, resulting in a decline in fertilization capacity through both traditional methods of conception, and when using ART[103]. Smoking also causes testicular inflammation and is associated with a 48% increase in seminal leukocyte levels, with a 107% increase in seminal ROS levels, and is also associated with shorter telomere lengths[104].

Zenzes and colleagues[105] reported that cigarette smoke is both a mutagen and a carcinogen that negatively affects male fertility[105]. Furthermore, Trummer and colleagues[106] reported a higher percentages of leukocytes in the semen of infertile smokers compared with that of infertile non-smokers and ex-smokers. These investigators also reported that free and total serum testosterone levels were high, and that prolactin levels were low in infertile smokers[106]. Paternal smoking is associated with 13.3-fold increase in the risk of developing retinoblastoma in the offspring, and also predisposes to *de novo* germ line mutations ('first hit') with a postzygotic 'second hit' commonly observed in retinal cells[19].

Increased numbers of both apoptotic sperm and sperm with ODD are found in men who consume tobacco in any form[107]. Nicotine, cadmium, carbon monoxide, benzopyrene, and pyrolysis-derived compounds in cigarette smoke result in ODD to sperm and testicular inflammation. Cigarette smoke is an established source of cadmium, and cadmium has been shown to inhibit OGG1, thereby affecting BER — the only repair mechanism active in spermatozoa[108].

Data from many studies, including meta-analyses, have been published on the effects of smoking on male infertility, although the results of these studies are often contradictory as some studies indicate adverse effects of smoking on sperm parameters, while others have failed to demonstrate such effects. The contradictory results of various studies might be attributed to variations in methodology, variations in the underlying mechanisms investigated and to the involvement of confounding factors (such as diet, exposure to toxins, alcohol consumption and other lifestyle-related variations)[109]. In 2016, Sharma and colleagues[110] published a meta-analysis of data from 20 studies, including a total of 5,865 participants, demonstrating a functional negative effect of smoking on conventional semen parameters (motility, morphology and sperm count). This meta-analysis[110] was the first to provide evidence of an association between cigarette smoking and sperm quality based on 2010 WHO Laboratory Methods for the Examination of Human Semen[110]. The findings of this analysis[110] suggest that cigarette smoking has an overall negative effect on conventional semen parameters such as sperm count, sperm motility, and sperm morphology, but the effects on semen volume were equivocal. These effects were more pronounced in infertile men than in the general male population, and deterioration of semen quality was particularly associated with moderate and heavy smoking. However, use of the 2010 WHO method[110] decreased the effect size for sperm morphology, but the results were not significantly different for volume, count, and motility (compared with previous WHO examination methods).

Excessive alcohol intake

Excessive alcohol intake is associated with alterations in testosterone secretion, reductions in spermatogenesis, a decrease in seminal fluid volume, hypotestosteronaemia, altered circulating gonadotropin levels, and increased leukocyte concentrations in seminal fluid[111,112]. Some studies describe the presence of resveratrol in red wine, which has proven chemopreventive effects, although resveratrol is also present in many other fruits and vegetables. For example, red grape juice is a richer source of resveratrol than red wine. Additionally, red wine contains ethanol, which acts through the cathepsin pathway to induce various biologically harmful effects[113,114].

Environmental factors

Insecticides, pesticides and phthalates not only induce oxidative stress but also disrupt the hypothalamo–pituitary–gonadal axis[115]. This disruption inhibits the release of gonadotrophin-releasing hormone, which in turn inhibits the release of luteinizing hormone and follicle-stimulating hormone. This results in inhibition of gametogenesis and steroidogenesis, therefore, exposure to insecticides can have negative effects on sperm function[115]. Furthermore, incubation of spermatozoa in media containing heavy metal ions, particularly iron and copper, results in a reduction in sperm motility and viability, with an increase in ODD[19,116,117]. Bisphenol A (BPA) is an environmental endocrine toxicant used mainly in the production of polycarbonate plastics and epoxy resins and is the most abundant environmental endocrine disruptor worldwide. BPA has also been shown to induce ODD and alterations in hormonal milieu, leading to epigenetic modifications of sperm cells that can cause azoospermia and/or male infertility[118].

Apoptosis in the male germ line

Programmed cell death, more commonly known as apoptosis, is defined as the process by which the number of cells in a multicellular organism is tightly regulated not simply by controlling the rate of cell division, but also by controlling the rate of cell death. During apoptosis, a suicide programme is activated within the cell that leads to DNA fragmentation, shrinkage of the cytoplasm, nuclear membrane blebbing and, ultimately, cell death without damage to neighbouring cells. Apoptosis is an important biological process by which the spermatogonial stem-cell pool is established in the male germ line during the embryonic stage of development by

Azoospermia
Semen containing no sperm, either because the testicles cannot produce sperm or because of a blockage in the reproductive tract.

regulating the germ cell:sertoli cell ratio, which ultimately defines the extent of male fertility[119]. During the end stages of spermatogenesis in the testis, large numbers of premeiotic spermatogonia undergo apoptosis during the first round of spermatogenesis in order to regulate their numbers to appropriate levels[114]. Deletion of the genes that regulate apoptosis, such as bcl-2-like protein 1 and apoptosis regulator BAX[120], growth arrest and DNA damage-inducible protein GADD45α, and cytochrome C[121] leads to perturbation in germ cell:sertoli cell ratios in the direction of decreased germ cell apoptosis, which leads to male factor infertility[122]. Crucially, apoptosis enables the selective removal of damaged or defective germ cells from the seminiferous tubules, therefore preventing participation in spermatogenesis and hence precluding differentiation into mature spermatozoa. Regulation of apoptosis is also a critical factor in determining germ-cell development, differentiation and function. A majority of factors, including unhealthy lifestyles, exposure to electromagnetic radiation, exposure to environmental toxins, use of chemotherapeutic agents such as cisplatin, exposure to BPA and deletions in spermatogenesis regulatory genes, in both human and experimental animals, might also lead to induction of apoptosis[123,124]. All of these factors can promote the generation of oxidative stress, which has adverse effects on spermatogenesis and generates spermatozoa with poorly remodelled chromatin. Infertile men have higher levels of seminal oxidative stress and enhanced apoptosis compared with their fertile counterparts. Spermatozoa with poorly compacted chromatin (owing to elevated ROS levels and high levels of oxidative stress during development) enter the apoptotic cascade, as demonstrated by the large number of testicular apoptotic germ cells, or cells with phosphatidylserine exteriorization, high levels of caspase activation and high levels of DNA damage in infertile men, and are considered a major cause of male infertility[125].

The majority of the DNA damage encountered by spermatozoa is caused by oxidative stress. The effects of oxidative stress are compounded by the physical architecture of human spermatozoa, which prevents nucleases from entering the sperm nuclear DNA and inducing fragmentation. The apoptotic cascade, which is initiated as a result of excessive generation of ROS and oxidative stress impedes essential sperm function, thus rendering the sperm inactive and immotile. Thus, the development of therapies that ameliorate or inhibit oxidative-stress-induced apoptosis in sperm cells is an immediate requirement[126]. In addition, oxidative-stress-induced accumulation of free radicals causes binding of tumour necrosis factor (TNF) to its receptor and increased inflammatory cytokine and nuclear factor-κB (NF-κB) levels. This increase in inflammatory cytokine levels inhibits apoptosis, promotes cell survival and proliferation and might explain the presence of sperm with damaged DNA in the ejaculate[127]. These sperm in the ejaculate have entered the apoptotic cascade but, owing to dysfunctional mitochondria and inhibition of apoptosis caused by elevated NF-κB expression, have not completed apoptosis and are therefore present in the ejaculate[128]. Thus, the maintenance of an optimal gene-expression signature, which is also mediated by levels of free radicals that regulate the sperm epigenome (methylome) and maintain nuclear and mitochondrial DNA integrity, is vital to continued healthy sperm function[47].

ODD and the sperm epigenome

Epigenetics refers to the study of changes in gene expression that arise from nuclear chromatin modifications, or covalent modifications of bases associated with DNA that result in a change in phenotype without any apparent alterations in genotype. Methylation of cytosine residues in DNA by DNA-methyltransferase enzymes is considered one of the most important epigenetic mechanisms. Protamine-bound sperm DNA is both transcriptionally and translationally inert. However, the nucleosomal component (comprising around 15% of the sperm genome) contains highly acetylated histones, which are an essential feature of transcriptionally active cells[129].

ODD leads to aberrant sperm DNA methylation patterns and results in global hypomethylation and genomic instability, owing to unmasking and expression of repetitive elements, and is associated with male infertility[130]. Changes in histone acetylation patterns are associated with impaired spermatogenesis and predispose to Sertoli-cell-only syndrome or testicular cancers[131]. Children conceived using ARTs have a higher incidence of genomic imprinting disorders, such as Angelman syndrome, Beckwith–Wiedeman syndrome, Russell–Silver syndrome, and retinoblastoma than those conceived naturally[132,133]. Sperm retrieved directly from the testicles are epigenetically immature, whereas epididymal spermatozoa gain epigenetic maturity but also accumulate oxidative damage. Oxidative-stress-induced *de novo* epigenetic modifications, such as global hypomethylation and hypermethylation of tumour-suppressor genes might also affect the trajectory of subsequent embryonic development and have lifelong implications for the health of the offspring[134,135]. Data from recent studies conducted over the past decade indicate that oxidative stress is associated with upregulation of DNA methyltransferase expression, thus leading to hypermethylation[130,136]. Oxidative stress can affect several signal-transduction pathways, and therefore modulates various biological processes. Free radicals are second messengers, which are able to modulate the expression of various genes and signal transduction cascades upstream of transcription factors. ROS levels modulate calcium signalling pathways, protein phosphorylation and various protein-kinase signalling pathways. In summary, the various signalling pathways are redox sensitive, therefore highlighting the need to maintain optimal ROS levels in men seeking to maintain optimal levels of sperm function.

Sedentary lifestyles[137], smoking and excessive alcohol intake are all associated with oxidative stress and thus might all lead to aberrant global methylation of sperm DNA. Furthermore, poor-quality spermatozoa have an abnormal methylation pattern that is not merely

Sertoli cell
A testicular cell responsible for nurturing the spermatids (immature sperm). These cells secrete inhibin, a hormone that regulates follicle-stimulating hormone (FSH) production by the pituitary gland. When stimulated by FSH, the Sertoli cell initiates spermatogenesis.

confined to differentially methylated regions, but can also be observed in other regions of the genome. Oxidative stress induces expression of TNF and interleukins, followed by increased expression of NF-κB, which inhibits apoptosis and promotes cell survival, a mechanism explaining the link between oxidative stress, male infertility and carcinogenesis. Meditation and yoga are both techniques that can upregulate the expression of antioxidant, anti-inflammatory genes and that of telomerase enzymes, which both aid in maintenance of telomere length and genomic integrity[138].

Oxidative stress and testicular ageing

Infertile men with abnormal semen parameters (such as reduced sperm motility, morphology and sperm count, with sperm cells showing signs of DNA fragmentation) are at a 20-fold higher risk of developing testicular cancer and a 4–5-fold increased risk of extragonadal tumours compared with that of male members of the general population[139]. This increased risk of cancer is associated with accelerated testicular ageing and infertility[92]. We believe that infertility is, in fact, accelerated testicular ageing characterized by increased oxidative stress levels, DNA damage and shortened telomeres[140]. Shortened telomeres, age-associated accumulation of mutations in mitochondrial DNA, and oxidative-stress-induced genome-wide hypomethylation all contribute to genome hypermutability and genetic instability, and therefore, might predispose to cancer[95,98] (FIG. 1). Male infertility and testicular cancer can both arise from mutations in genes involved in DNA repair and tumour suppression[141]. Furthermore, Jacobson and colleagues[142], reported that a low seminal volume, poor motility and a high proportion of morphologically abnormal sperm are all associated with an increased risk of developing extragonadal germ-cell tumours.

Mutations in DNA-repair genes and lower expression of *OGG1*, owing to exposure to mutagens such as those found in cigarette smoke result in the persistence of mutagenic lesions in sperm DNA[143]. Persistence of this lesion is indicative of a deficiency in DNA-mismatch repair genes[144]. These genes have an important role in meiotic recombination. DNA mismatch repair protein Mlh1-deficient men are infertile and DNA mismatch repair protein Msh2 has also been shown to cause infertility in mouse models owing to disruption of chromosomal synpasis. *P53*, also known as *TP53*, is a tumour-suppressor gene that acts as the guardian of the cell cycle[145]. Loss of function mutations in *P53* predispose to a range of spontaneous and induced tumours, highlighting the role of this protein as a barrier to tumour development[146]. P53 has a crucial role in spermatogenesis, especially during the prophase of meiosis, where P53 mRNA and protein are present in the primary spermatocytes. This expression is mediated by the induction of genome polyprotein, (also known as P21), which is expressed during the prophase of meiosis[147]. P53 has an important role in the upregulation of certain antioxidant genes such as sestrins 1 and 2, nuclear factor, erythroid 2 like 2, glutathione peroxidase 1 (REF. 148), aldehyde dehydrogenase 4 (REF. 149), glutaminase 2, TP53-induced glycolysis regulatory phosphatase, and tumour protein p53-inducible nuclear protein 1. Therefore, loss-of-function mutations in *P53* are associated with a decrease in antioxidant capacity, resulting in ODD and testicular cancers[148,150]. Studies from our lab have shown that testicular ageing can be partially reversed by regular meditation and yoga[151]. Using a microarray approach to evaluate gene expression patterns, followed by validation using real-time PCR and ELISA, we found that this simple intervention resulted in a decline in levels of peripheral oxidative stress and DNA damage, with upregulation of telomerase activity and a decline in levels of interleukin 6, and in expression of mitogen-activated protein kinases 10 and 15, with upregulation of the anti-inflammatory cytokines IL-2 and IL-4 in patients with primary open angle glaucoma, indicating that this systemic effect might also be relevant to seminal oxidative stress[87,152]

Antioxidant therapy

Oxidative stress is the main cause of the loss of structural and functional integrity of sperm cells. Therefore, early diagnosis and prompt treatment form the mainstay of the management of infertile men. However, indiscriminate use of antioxidants and inadequate monitoring of ROS levels might result in reductive stress, whereby the cell becomes more reduced than in the healthy resting state, resulting in disruption of cellular homeostasis. Conflicting reports exist regarding the role of antioxidants in infertile men and the dosage required to reduce the risk of ODD[153,154]. The majority of antioxidants only decrease ROS levels and improve sperm motility with little or no effect on the integrity of sperm DNA[155], thus highlighting a need for early intervention. This observation has repercussions for those considering use of ARTs as sperm with high levels of DNA damage might affect the outcome of ART.

Antioxidant scavenging systems have an important role in the inactivation of ROS and various antioxidant therapies and combinations of regimens, such as vitamin C, vitamin E, selenium, zinc and glutathione, have long been used as treatments of male infertility[156–158]. A large number of studies have already been conducted to determine the efficacy of antioxidant supplementation in the treatment of male infertility, in terms of improving both the rates of fertilization and pregnancy outcomes[159].

Seminal plasma is enriched with enzymatic antioxidants (such as superoxide dismutase and catalase) and nonenzymatic antioxidants (such as pyruvate and acorbic acid), which scavenge ROS and protect spermatozoa from oxidative stress, specifically after leaving the testicles. Intake of zinc is also recommended as it prevents copper-induced and iron-induced lipid peroxidation and has a synergistic effect when used in combination with vitamin E[160]. Selenium is found in selenoproteins such as glutathione peroxidase and is therefore a key component of antioxidant homeostatic systems[161]. Deficiencies in enzymatic or nonenzymatic antioxidant systems are associated with male infertility as the absence of either of these systems leads to the accumulation of excessive levels of ROS,

Genomic integrity
The active maintenance of all the genetic elements in the cells of an organism (including DNA, RNA and epigenetic determinants and appropriate developmental gene expression) for proper dynamic function.

Table 3 | **Antioxidants, their characteristics and effects on semen parameters**

Antioxidant	Characteristics	Impact on semen parameters	Refs
Vitamin E	Major chain-breaking antioxidant in sperm plasma membrane	Preserves and restores sperm motility and morphology by suppressing lipid peroxidative damage to sperm plasma membrane	206
Vitamin C	Principal hydrophilic antioxidant in seminal plasma, contributes to 65% of chain-breaking antioxidant capacity	• Increases sperm motility in a dose-dependent manner • High doses can damage chromatin structures by disrupting disulphide bridges and therefore increasing the cellular density of staining from 17.5–21.5% following a 3 mM dose	70
Carnitine	Water-soluble antioxidant primarily derived from human diet, found at high concentrations in the epididymis	Has a key role in sperm maturation and metabolism, decreases fatty acid oxidation by enhancing the cellular energetics of mitochondria, protects from ROS-induced damage to sperm DNA and to the sperm plasma membrane, improves sperm motility and count	207
Glutathione	Most abundant reducing agent in the body and protects proteins, lipids and nucleic acid against oxidative damage	• Glutathione administration increases forward progressive sperm motility in infertile men • Oral glutathione has poor bioavailability	208
CoQ10	Abundant in the sperm mid-piece and has important antioxidant and metabolic functions	• Involved in energy generation in spermatozoa • Incubation of human spermatozoa of infertile men with appropriate doses (50 mM) of CoQ10 significantly improves sperm motility as well as function	209

CoQ10, coenzyme Q10; ROS, reactive oxygen species.

which damage the sperm genome, therefore rendering it unable to fertilize the oocyte[155]. Other dietary antioxidants include vitamins C and E, carotenoids and flavonoids, which are chain-breaking antioxidants that act by cleavage of the lipid peroxidation chain and hence scavenging of free radicals[162,163] (TABLE 3). Metal-binding proteins (myoglobin, albumin, cerruloplasmin and metallothionein) bind with and inactivate transition metal ions such as zinc and iron, and therefore inhibit the enzymatic production of ROS. Thus, antioxidants reduce cellular levels of ROS in two ways: firstly, by inactivating the ROS produced by various metabolic activities and rendering it inactive and unable to generate lipid peroxidative damage to the sperm plasma membrane; and secondly, by decreasing the level of enzymatic ROS production. Thus, it is recommended to consume a diet containing a combination of antioxidants including zinc and selenium.

Lifestyle-related factors, such as excessive mobile phone usage, a stressful, sedentary lifestyle, smoking, alcohol consumption, a lack of exercise and regular intake of nutritionally depleted foods result in oxidative stress. Thus, men seeking to improve their fertility must improve their lifestyle by cessation of smoking, the avoidance of excessive alcohol consumption and excessive use of mobile phones, exercise in moderation, possibly including regular yoga and meditation, minimizing exposure to xenobiotics and increased dietary intake of fruits and vegetables that are rich in antioxidants[84].

Yoga and meditation

Meditation is a set of cognitive practices involving intentional and self-regulated focussing of attention with an aim to relax and pacify both the mind and the body[164]. Regular meditation reduces levels of psychological stress, anxiety, depression[165] and systemic inflammation[166] and improves QOL[167], cognitive abilities[168], and immediate, and long-term cardiovagal tone[169,170]. Regular meditation can bring about these changes through regulation of ROS levels[171,172]. A delicate balance between ROS and antioxidants is formed in cells in order to maintain a healthy oxidative pool. ROS levels are imperatively associated with the extent of progression of many diseases, although data from epidemiological studies indicate that antioxidants might, in fact, shorten lifespan[173] and might be detrimental to ROS-mediated immune responses, and thus warrant against the indiscriminate use of antioxidants[174]. Overuse of antioxidants is an important concept, although it does not deny the implication of ROS in the aetiology and pathology of numerous diseases. Antioxidant therapy can decrease ROS levels but does not regulate ROS levels, therefore, such approaches might not restore a healthy antioxidant balance. Meditation regulates ROS levels rather than simply lowering them[138]. An important mechanism for the effects of meditation on ROS levels was described in 2013: specifically that meditation buffers and reverses many stress-related processes and alters the expression of genes that regulate oxidative stress[175]. This effect is mediated through activation of the parasympathetic nervous system and depression in levels of pro-oxidants such as cortisol[176], and elevation in levels of antioxidants such as melatonin[177]. Furthermore, long-term effects on gene expression patterns can be brought about by histone modification in experienced meditators[178]. Elsewhere, meditation has been shown to influence human biophoton emission immediately (within 10 minutes of commencing this activity),

elevates total glutathione levels within 65 minutes[179] significantly increases superoxide dismutase (SOD) activity within 65 minutes[179], causes decline in the levels of many ROS metabolites within 8–24 h and precipitates an increase in total antioxidant capacity within 24 h (REF. 178). Investigators also reported a significant decrease in levels of thiobarbituric acid ROS within 9 days. Elsewhere, 5 months of regular yoga practice has been show to be associated with significantly increased total glutathione, catalase and SOD levels[180]. These changes result in elevated serum nitric oxide levels, blood glutathione reductase levels, glutathione reductase:reduced glutathione ratios and TAC levels within 6 months[114], and also lead to increased telomerase activity and decreased levels of ODD[18,19,181].

Conclusions

The available evidence suggests that oxidative stress is central to the aetiology of male infertility but the exact effects of oxidative stress on ODD in spermatozoa have not yet been comprehensively investigated. Standard semen parameters are poor predictors of reproductive outcomes. Therefore, a need exists to develop other cost-effective laboratory techniques for the estimation of seminal levels of oxidative stress and ODD. ODD is associated with poor lifestyle habits and environmental exposures, therefore adoption of a healthy lifestyle, possibly including lifestyle-related interventions like meditation and yoga, might help to reduce the risk of ODD and improve sperm function and thereby, reduce the incidence of male factor infertility and childhood disease burden.

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This study suggests that Coenzyme Q_{10} (CoQ_{10}) in human seminal fluid, has important metabolic and antioxidant functions; and shows a direct correlation with seminal parameters (count and motility), thus exogenous administration of CoQ_{10} increases both ubiquinone and ubiquinol levels in semen and can be effective in improving sperm kinetic features in patients affected by idiopathic asthenozoospermia.

Author contributions
All authors contributed equally to all aspects of the preparation of this manuscript.

Competing interests statement
The authors declare no competing interests.

Review criteria
We performed a search of the PubMed and Google Scholar databases for full-text English-language articles published between 1943 and 2016 using various combinations of the following search terms: 'spermatozoa', 'infertility', 'male infertility', 'oxidative stress', 'seminal oxidative stress', 'cellular oxidative stress', 'reactive oxygen species', 'lifestyle interventions', 'sperm DNA damage', 'recurrent pregnancy loss', 'peroxidative damage', 'lipid peroxidation', 'antioxidants', 'apoptosis in sperm', 'sperm genome', 'sperm epigenome', 'sperm DNA fragmentation', 'oxidative DNA damage', 'advanced paternal age', 'testicular aging', 'testicular cancers', 'yoga', and 'meditation'. The reference lists of selected articles were searched for further relevant publications. Relevant primary research papers, reviews and meta-analyses were then classified and analysed for coherent theoretical explanations. All relevant literature reports were taken into consideration when writing the manuscript.