Redox Regulation of Fertility in Aging Male and the Role of Antioxidants: A Savior or Stressor

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Abstract: Oxidative stress (OS), an imbalance between reactive oxygen species (ROS) and antioxidant scavengers, is considered an important underlying mechanism associated with ageing, age-related chronic diseases and reproductive decline in males. However, OS is an important regulator of numerous molecular pathways essential for homeostasis within cells, tissues and systems. A fine balance between factors promoting OS and those that scavenge ROS is critically important. In the male reproductive tract and ejaculate, ROS are critical molecular players in essential reproductive processes, including spermatogenesis, epididymal transport, spermatozoa maturation and post-ejaculation processes such as motility, capacitation and the acrosome reaction. Increasing evidence suggests there is an overlap between age-related OS and male reproductive tract dysfunction correlating with reduced fertility potential due to semen quality decline, endocrine changes and sexual dysfunction. With increasing life expectancy in many regions of the world, together with an increasing paternal age and associated risk of for infertility, pregnancy complications and reduced health potential in the offspring, it is becoming increasingly important to fully understand the mechanism linked with these associations. This review considers the role of ROS and OS in fertility regulation, the ageing process, the role for OS in age-related reproductive decline, the approach to assessment in male infertility and the current use of antioxidants as a therapeutic option. Although evidence suggests exogenous antioxidant supplementation is useful in male infertility, caution to excessive or unnecessary use of these approaches is required to avoid the paradox of antioxidants inhibiting essential and beneficial ROS activities in male reproductive biology.

Keywords: Oxidative stress, reactive oxygen species, antioxidants, aging males, male infertility, redox balance.

INTRODUCTION

Life expectancy is increasing worldwide, now reaching an average of up to 80 years in many developed countries [1]. Ageing can be defined as a time-dependent decline in biological functions on the cellular, tissue and system level, related to a progressive decline in quality of life (QoL) alongside an increased probability of various functional and organic co-morbidities and risk of mortality [2]. There is, however, much variation in how advanced co-morbidity processes are within a specific chronological age group [3]. The rate of ageing depends on genotypic inheritance, epigenetic expressions, underlying disease processes and accumulated effects of lifestyle, environmental and socioeconomic factors [4]. Some people also enter an increased risk category defined as frailty, in which advancing age is the underlying cause [3]. Although ageing is not in itself a disease, this natural biological phenomenon is further considered a major risk factor for numerous degenerative diseases, including various common malignancies, neurodegeneration and dementia, cardiovascular disease, osteoarthrosis, osteoporosis and type-2 diabetes in the context of obesity and metabolic syndrome [1; 2; 5]. Ageing is also associated with a shift in the balance between pro-oxidants and anti-oxidants towards oxidative stress [6]. It is therefore becoming increasingly important to understand the mechanisms of ageing associated with all clinical specialities, as well as within interdisciplinary practice, in order to develop and improve strategies associated with prevention and therapeutic potentially increasing life span and improving quality of life.

From a biological science perspective, ageing is a complex, multifactorial and relatively poorly understood phenomenon, with no clear biological basis [7]. Although still an elusive area of study, numerous biomarkers of ageing have been proposed, as well as correlated to various frailty indices and pathophysiology of well-defined co-morbidities of ageing (Table 1) [8]. Within this context, oxidative stress (OS) and the broader focus of redox biology has been receiving increased scientific attention in recent decades [9]. Although the OS theory has risen to prominence in recent decades, the role of other theories of ageing, relating to immunosenescence, neuroendocrine decline, somatic mutation and error catastrophe, is less well defined [7]. The immune and neuroendocrine systems clearly deteriorate with age. However, underlying the phenotypic and clinical changes of ageing, genomic factors and OS are considered important mediators that drive the cellular senescence underpinning the ageing process [1-2]. OS is triggered by various pathological conditions, including inflammation, ischemia, heat-stress and ageing, as well as being an underlying cause of age-related fertility decline [10]. It is also interesting to note that more than half of all males that present with infertility and undergo relevant assessments are found to have excessive OS in the male reproductive tract [11]. Furthermore, evidence suggests that antioxidant defenses decline with age [12]. This provides a basis for OS being involved in the pathogenesis of male reproductive changes associated with ageing, highlighting the importance of understanding these mechanisms for therapeutic targets in ageing males.

Although there may be a gradual decline in male reproductive potential with age, the average paternal age has been increasing in recent years for various socioeconomic reasons. Furthermore, many males want to father children at older ages, and a detailed understanding of changes in the male reproductive system associated with ageing is of critical importance [13]. It is generally accepted that females live longer on average than males (although they have
Table 1. Proposed age associated biomarkers and direction of change [8].

<table>
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<tr>
<th>Category</th>
<th>Marker</th>
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<tr>
<td>Immune and Inflammatory Markers</td>
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<td>Adiponectin</td>
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<td>T₇₇-Lymphocytes (CD4+) (%T-Cells)</td>
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<td>T₄-Lymphocytes (CD8+) (%T-Cells)</td>
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<td>CD4+: CD8+ Ratio</td>
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<td>Memory: Naïve CD4+ Ratio</td>
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<td>Memory: Naïve B-Lymphocyte Ratio</td>
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<td>Hematological Markers</td>
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<td></td>
<td>Platelets</td>
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<td></td>
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<td>Eosinophils</td>
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<tr>
<td>Cellular Ageing, Genetic, and Oxidative Stress Markers</td>
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<td></td>
<td>DNA Repair (%)</td>
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<td></td>
<td>DNA Damage: DNA Repair Ratio</td>
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<td>DNA methylation (%)</td>
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Increased rates of age related morbidities), and although there are many common co-morbidities, differences in incidence between the sexes is apparent [5]. In males specifically, cognitive decline, obesity and metabolic syndrome, cardiovascular disease (including hypertension as an underlying risk), depression, age-related androgen decline (ADAM), erectile dysfunction, testicular dysfunction with reduced sperm quantity and quality, lower urinary tract symptoms (LUTS), benign prostatic hyperplasia (BPH) and prostate carcinomas are common co-morbidities observed [14-15]. Although tests can still produce sperm throughout the lifespan, the rate and quality of production gradually declines with age [16]. Furthermore, age-related co-morbidities such as metabolic syndrome and diabetes are associated with reproductive tract inflammation and OS induced sperm damage [17-18]. The effect of ageing is also associated with increased genitourinary tract diseases in males, which directly or via their treatment options negatively influence semen quality and fertility potential [13].

In addition, an increased paternal age is also associated with increased rates of adverse birth outcomes and a negative impact on the health of the offspring via epidemiological and animal studies. Children fathered by older males have increased risk of sporadic congenital diseases (such as achondroplasia and Alport syndrome), and increased lifetime risks of cancers (including leukemia non-Hodgkin’s lymphoma, breast and prostate cancers), neuropsychiatric disorders (including autism, schizophrenia and bipolar disorder), as well as disorders with behavior and cognitive abilities [19]. These effects are postulated to be due to the accumulated DNA mutations over time, associated with reduced sperm DNA integrity as well as various epigenetic factors [19]. The accurate transmission of genotypic information is vital both prior to fertilization and following the development of the offspring during pregnancy and through life [20].

OVERVIEW OF REDOX IN CELLULAR BIOLOGY

Oxygen (O₂) molecules are essential for aerobic life on earth, driving cellular metabolism and respiration, thus primarily used for ATP-generation within the mitochondria via the electron transport chain (oxidative phosphorylation) [21]. However, the paradoxical nature of this element has become apparent in recent years as the production of associated free radicals are detrimental to life. Derived from metabolic use, O₂ is transformed into a variety of molecules termed reactive oxygen species (ROS) as by-products of cellular respiration [21-23]. The broad term ROS is used to describe oxygen-derivatives that are highly reactive and contribute to a process termed oxidative stress (OS), having important cellular regulatory roles as well as being implicated in numerous pathologies. ROS includes various sub-classes of molecules comprising of unstable oxygen ions such as superoxide (‘O₂⁻), free radicals that have one or more unpaired electrons in the outer molecular orbits, and peroxide molecules [24]. The terms free radicals and reactive oxygen species (ROS) are frequently used interchangeably, however, not all ROS are free radicals [25-26]. Hydrogen peroxide (H₂O₂), for example, is named among ROS, but is not a radical. An additional sub-class of biologically important reactive molecules comprises of nitrogen, termed reactive nitrogen species (RNS). These include nitrous oxide (NO), peroxynitrate and peroxynitrous acid [25]. For the purposes of this review, ROS will be used to encompass all endogenous highly reactive oxygen molecules.

Endogenous ROS are produced intracellularly via numerous cellular mechanisms, which may vary within different tissues. Importantly, ROS is generated by the mitochondria during oxidative phosphorylation (mtROS). This is done via a series of reduction-oxygen (redox) reactions in which there is a transfer of electrons through five mitochondrial protein complexes. However, a small leakage of electrons from protein complex I and III results in the formation of superoxide (‘O₂⁻) due to partial reductions at a rate of 0.2% - 2.0% of the oxygen consumed by the mitochondria. ‘O₂⁻ is then metabolized by superoxide dismutase (SOD) 1 and SOD2 in the mitochondria into H₂O₂, a signaling molecule in the cell [27]. As with other endogenous ROS, H₂O₂ has particularly emerged as major redox metabolite operative in redox sensing, signaling and regulation in cellular function, including the male reproductive tract and sperm function [28]. H₂O₂ may be partially reduced further to produce a hydroxyl radical (·OH). Moreover, other ROS can be generated directly (enzymatically) or indirectly (metal catalyzed) from O₂⁻ [25; 29]. Additionally, ·O₂ can undergo further metabolism by NO to produce the highly detrimental oxidant peroxynitrite that denatures proteins and damages mitochondrial membrane integrity [27].

In cells, ROS are used for numerous processes to regulate cellular function including apoptosis for the elimination of damaged cells, immune system regulation and inflammation, response to growth factors, genomic regulation, and ion transport systems. However, due to the highly reactive nature of these molecules with half-life times in the nano-second range, ROS can not only further modify other oxygen molecules, but also damage macromolecules.
such as proteins and lipids through oxidative stress (OS), defined as an imbalance between oxidants and antioxidants towards an oxidative status [24; 30]. Specifically, adaption to physiological concentrations of ROS is involved as what is termed molecular redox switches (phosphorylation/dephosphorylation) as a mechanisms of cellular signaling [9]. At physiological concentrations, the cellular adaptions are important for normal cell function. However, an excess in ROS production and/or reduction in scavenging antioxidants is creating a state of OS in which cellular adaptions lead to pathophysiological changes in a process similar to the general adaption syndrome of cellular stressors [9; 28]. The activities of OS are mediated through numerous intracellular pathways, including MAPK family members of ERK’s (ERK 1 and 2), c-Jun N-terminal kinases (JNK1, 2 and 3) and the p38 kinases (alpha, beta and gamma) [31-32]. Mediated by an unfavorable ROS-TAC mismatch, OS is known to contribute to the pathophysiology of many disease states in which antioxidants provide protection [24], many of which may also be mediated through the above signaling pathways in addition to direct toxic effects on molecules [31-32].

Due to the high cellular toxicity of OS, cellular defense mechanisms have evolved including many endogenous antioxidants that are capable of scavenging and neutralizing cellular ROS. These balances are illustrated in Fig. 1. H2O2 is quickly reduced in the mitochondria into water by catalase and glutathione peroxidase, the latter requiring glutathione as a co-factor. Three molecules of the SOD family are involved in antioxidant complexes with co-enzymes, namely copper and zinc (SOD1) or manganese (SOD2), as well as an extracellular SOD (SOD3). Other important endogenous antioxidant families include peroxiredoxin and thioredoxins [27].

It is therefore important that there is a balance between generation of ROS and their elimination by endogenous and exogenous antioxidants within the cells. Disturbances in this balance can be due to numerous factors, both internal and environmental [26]. OS can induce accumulated cellular damage, implicated in the complex pathophysiology associated with ageing and age-related chronic diseases, including the metabolic syndrome, type-2 diabetes mellitus, cardiovascular disease, malignancy and neurodegeneration [24].

Scavenging of ROS is critical, and endogenous and exogenous antioxidants are used to reduce the damaging impact of excessive OS. The endogenous system consists of enzymatic antioxidants (e.g. SOD, catalase, thiol peroxidases), and non-enzymatic antioxidants (e.g. glutathione). The exogenous antioxidant system comprises of various micronutrients (e.g. β-carotene, vitamins A, C and E; glutamine), phytonutrients, as well as trace elements such as zinc and selenium (involved in enzymatic functioning) [2].

Contrary to OS, a shift of the cellular redox status from normal physiologic levels into the reduced state is also highly problematic for cells since they will no longer be able to induce essential functions. This condition has been termed ‘reductive stress’ (RS) [33]. RS is considered as dangerous for cells as OS [34], and can be a cause for cardiac injury, neurological diseases such as Alzheimer’s disease [35-36] or dysregulations of embryogenesis [37]. This is illustrated in Figure 1.

THE FREE RADICAL THEORY OF AGEING

Currently, there is no single theory that encompasses the complexity of the cellular and molecular mechanism that would fully explain the ageing process, which occurs on the molecular, cellular and organ level. An accumulation of damaged macromolecules and cellular components is considered a hallmark of ageing and can be characterized by an accumulation of cellular damage over time, closely associated with DNA damage and proteostasis driving cellular senescence [2]. Genomic factors and OS are considered two important cellular drivers for the accumulation of the cellular and molecular damage associated with ageing [1-2; 7]. Furthermore, many of the degenerative diseases associated with ageing are also closely associated with OS and chronic inflammatory processes as part of a complex mechanism of action in these pathologies [7].

Within this context, the free radical theory of ageing has achieved prominence as an underlying mediator of age-related morbidity and mortality, in which excessive endogenous and exogenous ROS is detrimental to homeostasis [38]. This suggests that
there is an accumulation of macromolecular damage from excessive OS that is associated with age-related functional decline of organisms [39]. Cellular senescence is also preprogrammed into the genome as telomeres are unable to maintain their lengths due to the replication process. This leads to a decline in protective systems against cellular stressors, including ROS, during the ageing process [2]. Longer lived mammals, including humans, show decreased evidence of cellular oxidative damage [40]. Thus, ageing is associated as a cause and effect of an increase in the ROS: total antioxidant capacity (TAC) ratio leading to a disruption in redox signaling [9], and this OS can act as a potent inducer of cellular senescence, particularly via H$_2$O$_2$, O$_2^*$ and OH$^*$ [2].

Cellular senescence is associated with numerous phenotypic changes within cellular structure and function. This includes increased cell size and protein content (accumulation of protein aggregates), increased oxidative protein damage, giant mitochondria (produce increasing amounts of ROS with age), enlarged nucleus, increased lipofusin accumulation and reduced proteasomal and lysosomal function. In addition, numerous secretory biomarkers are produced, including pro-inflammatory cytokines, growth factors, matrix metalloproteinases and ROS [2]. The genome is then associated with a reduced capacity for DNA repair, resulting in an increased accumulation of genetic mutations and which is correlated with cellular senescence [1].

Mitochondria are considered a prominent cellular source of ROS where these highly reactive molecules are produced as a normal metabolic process in cellular respiration during normal metabolic activity. These endogenous molecules oxidize and damage lipids, proteins and DNA, causing severe OS to mammalian physiology that drives the ageing process [41]. OS is associated as a cause of an accumulation of mitochondrial DNA (mtDNA) mutation associated with cellular senescence [42]. According to this theory, O$_2^*$ production by the mitochondrial electron transport chain is readily converted to H$_2$O$_2$ by SOD2, the main defense against mitochondrial ROS [41]. The H$_2$O$_2$ can diffuse out of the mitochondria where it is converted to water via cytosolic catalase or mitochondrial glutathione peroxidase, mitochondrial peroxiredoxin and catalase [39]. If adequate scavenging of the reactive molecules is not effective, the imbalance leads to excessive macromolecular damage that accumulates over time, and is driving and accelerating the degenerative and ageing process. As pointed out before, damage to mtDNA by OS is most closely associated with accelerated ageing in animal models [43]. mtDNA damage is also accumulated by replication errors made by the mtDNA polymerase [42]. Therefore, dysfunctional mitochondria are known drivers of excessive ROS production, they create an unfavorable imbalance in the redox status, and are thus considered a major cause of cellular ageing [40]. Further evidence to support this theory in mammals using transgenic mice has shown that endogenous production of ROS due to normal metabolic process is a major limiting factor of life-span, and that transgenic mammals deficient in the antioxidant SOD2 die within the first week of life. The latter phenotypic changes, with pathophysiological evidence of oxidative damage in tissues, can be reversed with SOD2 mimics injected intraperitoneally. Catalytic antioxidant defenses can favorably modulate the life span [44]. Increasing research is focused on measures to increase life-span by reduction of accumulated mtDNA mutations [42]. It is suggested that targeting endogenous antioxidant defenses against mitochondrial oxidative damage, particularly through over-expression of mitochondrial catalase that neutralizes the leaking superoxide from mitochondria, prevents excessive OS to DNA, lipids and proteins, and this can increase the life-span as indicated in mammalian animal models [39].

In addition to the endogenous sources of ROS, various lifestyle and environmental factors can increase exogenous sources of ROS and add to the burden of OS, as well as accelerate the ageing process and age-related co-morbidity risk. This includes smoking, environmental pollution, poor nutritional choices, drugs, toxins (e.g. heavy metals, pesticides and xenobiotics) and ionizing radiation [45].

**REDOX REGULATION OF MALE FERTILITY**

ROS and antioxidants are balanced in the healthy male reproductive tract and seminal fluid, and ROS are essential to the acquisition of fertilizing ability of the male partner [46]. Although ROS was initially thought to be exclusively toxic to human spermatozoa, it is now well accepted that ROS have important physiological functions in reproduction as they are essential regulators of male fertility [47-48]. As such, low ROS concentrations play a fundamental role in various sperm processes and structure. This includes chromatin condensation, cell membrane remodeling, activation of intracellular pathways, roles in epididymal transport and maturation, hyperactivation of motility, triggering of capacitation and the acrosome reaction, sperm zona binding and oocyte fusion [46-48]. However, if there is an increase in OS for any reason, including age-associated increases [49], then the limited antioxidant defenses of the cell are overcome leading to excessive OS.

Spermatogenesis is a highly dynamic process based on several mitotic divisions of germ cells followed by two meiotic divisions to produce spermatids, which undergo chromatin condensation and membrane remodeling before being transformed into spermatozoa. Germ cells generate a significant amount of ROS as metabolic byproducts, which are important for this remodeling and chromatin condensation process in addition to other signaling and homeostatic roles [10; 49]. Yet, as compared to ROS and age associated damage to mature spermatozoa, the role of ROS in early germ cell development is still poorly studied [49].

Sperm capacitation is an essential process for fertilization and represents a complex series of molecular, physiological and morphological changes in the spermatozoal critical for acrosome reaction and oocyte binding. This is considered an oxidative event using low level ROS for redox reactions to coordinate a series of phosphorylation reactions [50-51]. Although the ROS responsible for this are still elusive, ROS-induced ROS formation has been described in sperm capacitation [50]. Spermatozoa are known to be able to generate their own ROS, and particularly H$_2$O$_2$ has been investigated, and this ROS activity is of physiological significance in tyrosine phosphorylation critical for capacitation [52]. This capacitation-associated ROS production is generated on the plasma membrane of the spermatozoan [50]. Ultimately, H$_2$O$_2$ generation, via O$_2^*$, exerts a role in intracellular protein kinase activation for sperm capacitation. Activation of the protein kinase C (PKC) pathway leads to phosphorylation of Raf to activate mitogen-activated protein kinase (MEK) proteins that are necessary to trigger late tyrosine phosphorylation. Additionally, ROS production directly activates the protein kinase A (PKA) pathway as an early event during mammalian sperm capacitation, with additional phosphorylation processes occurring at later stages. The effects of MEK, extracellular-regulated kinase (ERK), phosphoinositide-3 kinase/Akt (PI3K/Akt) pathways and tyrosine phosphorylation are essential in guaranteeing activation of the spermatozoan during capacitation [50]. However, SOD and CAT both can act as inhibitors for this process [50].

Peroxiredoxins (PRDX’s) are a class of endogenous ROS scavengers, but also act as ROS control systems, particularly involved in capacitation and oocyte binding remodeling [51]. These are well known and important antioxidant systems in seminal plasma, salvaging ROS and modulating ROS-dependent signaling pathways [53]. PRDX’s dysfunction, and related enzymes needed for their reactivation (e.g. thioredoxin/thioredoxin reductase system and glutathione-S-transferases), is known to impair capacitation, as well as sperm motility, and promotes DNA damage in spermatozoa [50]. However, it is still to be elucidated how the spermatozoan controls
the levels of the produced ROS to keep within a normal physiological range and avoid toxicity [50].

THE EFFECTS OF AGEING IN MALE REPRODUCTION AND THE ROLE OF OS

Male Fertility and Sperm Quality

Semen quality is considered an important indicator of fertility success in males, and seminal analysis guidelines and diagnostic procedures are provided by the World Health Organization (2010) [54] to routinely estimate the fertilization potential of the male partner. Age-related testicular dysfunction includes testicular atrophy with reduced volume, reduced number of Sertoli cells (and hence spermatogenesis), reduced number of Leydig cells and Leydig cell function (partly responsible for hypogonadism) and thinning of the seminiferous tubules basement membrane [16]. Increasing male age further correlates with poor sperm quality whereby different seminal parameters are affected and often even give conflicting results [16]. Moreover, paternal age is also associated with an increased risk of reduced success rates in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures as it negatively influences the number of high quality embryos [55], spontaneous abortions [56], increased pregnancy complications as well as increased risk of birth defects [57].

There are two main sources of ROS in the male reproductive tract, namely leukocytes and spermatozoa. Low concentrations of leukocytes, predominantly neutrophils, are found in most normal semen samples [25], and these cells produce ROS as part of regulatory roles during inflammation and cellular defense. ROS are also generated as a by-product of spermatozoal oxidative phosphorylation, and are therefore intrinsic, as well as important, to the male reproductive tract [47-48]. H$_2$O$_2$ is the most prevalent ROS to be synthesized by spermatozoa from reduction of O$_2^-$ [58]. In addition, apart from the epididymal contribution, ROS in seminal plasma as well as the exogenous antioxidants may originate from prostatic secretions or from the seminal vesicles. The seminal plasma is made up of a variety of lipids, proteins and amino acids, ions, cytokines and hormones, and is required to protect and nourish the ejaculated spermatozoa. Seminal plasma also contains endogenous enzymatic scavengers such as SOD, catalase and glutathione peroxidase, as well as exogenous non-enzymatic antioxidants such as vitamins C and E, zinc, co-enzyme Q10 and ubiquinol [59].

Additional ROS in the male reproductive tract derive from poor lifestyle and other exogenous sources, such as smoking, alcohol consumption, pesticides, exogenous estrogens and heavy metal toxicity, nutritional deficiencies and advanced age. Pathological conditions such as varicocele and spinal cord injuries are also mediated by OS as well as from the ageing process itself [47; 60].

Due to their extraordinary high plasma membrane content of polyunsaturated fatty acids (PUFA), spermatozoa are a sensitive target for OS-induced damage as they contain numerous carbon double-bonds which are sensitive to an oxidative assault. The relative large surface area of membrane over the sperm head and tail, the vulnerability of DNA and the lack of cytoplasm (which is mostly confined to the midpiece and contains cellular defense mechanisms) also reduce the ability for innate antioxidant defenses such as SOD1, catalase and glutathione peroxidase [61]. As such, males with idiopathic infertility have been found to be associated with a ROS-TAC mismatch and poor semen quality [62]. A ROS-TAC mismatch in seminal fluid has numerous detrimental effects on various parameters of sperm function that parallel effects of ageing on the reproductive tract. Excessive OS has been correlated with reduced sperm concentration and motility, morphological rearrangements and damage to both cellular and mitochondrial DNA, as well as mitochondrial membrane potential (MMP) [47-48; 63-65]. Furthermore, abnormal spermatozoa are postulated to undergo a form of apoptosis associated with high concentrations of H$_2$O$_2$ for-

mation that is well demonstrated to initiate the spermatozoa apoptotic pathway via caspase 3 activation and annexin-V binding (which can be prevented with pre-exposure of spermatozoa to antioxidants such as catalase and melatonin) [61]. Thus, the state between ROS and antioxidants needs to be finely balanced and maintained by various enzymatic and non-enzymatic processes [47-48; 66].

The damage to the plasma membrane is caused by a process called lipid peroxidation (LPO) whereby ROS initiate a radical chain reaction in the membrane leading to the damage and formation of toxic and mutagenic end products. LPO also leads to reduced membrane fluidity, its inability for sperm-oocyte fusion [60; 67-68], reduced activity of calcium regulation channels which negatively affects motility [69-70], and a loss of intracellular ATP production resulting in further reduction of sperm motility [71]. OS also negatively affects the structure of the axonemal flagellar during epididymal maturation, causing impaired motility following ejaculation [11]. Studies have shown that lipid soluble antioxidants, such as vitamin A, other carotenoids and especially vitamin E, are correlated with increased fertility potential due to a reduction of LPO and are thereby positively correlated with improved motility. Reduced amounts of these lipid soluble antioxidants in the reproductive tract and seminal plasma negatively affect sperm concentration, motility and morphology [72]. Furthermore, due to the nature of the compaction of DNA with proteins in sperm cells, DNA repair does not occur as readily as in other mammalian cells. Therefore, DNA damage accumulates through the lifespan of spermatozoa, a process which may be associated with impaired fertilization due to apoptosis [61]. Many of the negative effects of OS, and specifically impaired motility and oocyte binding potential, can be due to the damaging effects of ROS on spermatozoa membranes.

In addition to the cell membrane, DNA damage is a major characteristic of ROS induced defects to spermatozoa. These damages can either be due to direct oxidation of the DNA causing DNA fragmentation or due to indirect action of end products of LPO such as malondialdehyde (MDA), 4-hydroxy-2-alkenals or 2-alkenals which are mutagenic and genotoxic [73-74]. Patients with a high percentage of sperm with DNA fragmentation have an increased risk of infertility, miscarriage, and numerous disorders in the offspring [61], as this damage can be transmitted to the progeny [12]. Mechanism of damages in spermatozoa DNA are numerous, and importantly include excessive OS, aberrant spermatozoa maturation and apoptosis [12; 48; 61; 75]. DNA fragmentation is closely associated with OS base adducts evidenced by increased 8-hydroxy-2'-deoxyguanosine (8OHdG), a known marker of DNA oxidative damage [61]. Increased spermatozoa DNA damage and decreased DNA repair is observed in experimental ageing models, and oxidative DNA damage is increased in young SOD1 (SOD1+) and Catalase (Cat+) knockout mice as well as with ageing [12]. These lines of evidence further suggest a role of OS in male ageing-related fertility outcomes. SOD1 appears particularly important in this process, as it is suggested that there are compensatory responses in Wistar rats and Cat+ mice to partially alleviate OS associated with ageing [12]. In addition to DNA damage, there is extensive protein oxidation in spermatozoal and seminal plasma proteins associated with ROS. Among these are the OS markers DJ-1, PIP and lactotransferrin and PRDX’s [59].

Mitochondria are considered to be both a source of ROS and increased OS in spermatozoa, but also a target and a critical component of OS related sperm damage as mitochondrial DNA (mtDNA) is up to 100-times more susceptible to damage and mutations than nuclear DNA as it is not protected against an oxidative assault [76-78]. As a result of such mitochondrial damage, more ROS could be produced leading to a vicious cycle of further mitochondrial ROS production further mtDNA damage and loss of sperm functionality [79]. Particularly, damaged mitochondria are negatively correlated with sperm motility in a causal manner that
can be induced experimentally by ROS, and reversed with antioxidant treatments, such as vitamin E, in vitro [80]. High ROS concentrations are associated with mtDNA damage and reduced mitochondrial membrane potential (MMP) in spermatozoa, impairing ATP formation, motility and thereby fertilization capacity [78]. In a small study of oligoasthenozoospermic men, seminal MDA and ROS levels were found to be higher in infertile men than fertile men, with lower levels of antioxidants catalase and glutathione peroxidase, but no difference for SOD levels which may compensate for the excessive ROS. These seminal changes correlated with reduced sperm count and progressive motility [78].

Leukocytospermia is a marker of reproductive tract infection and inflammation, and the associated immune response increases ROS production along side down regulation of endogenous antioxidants in order to induce OS as part of the immune response. Typically, a local inflammatory response is associated with acute or chronic infections of the reproductive tract in which OS plays a significant role [60; 81-82]. Pro-inflammatory cytokines, particularly TNFα, IL1β and IL6, are known to unfavorably modulate both OS and antioxidant status, with increased concentrations correlating positively with ROS and OS in seminal fluid [83]. Furthermore, systemic inflammatory diseases are also associated with poor fertility outcomes, possibly due to concomitant reproductive tract inflammation. It is also suggested that low grade systemic inflammation associated with metabolic syndrome and type-2 diabetes mellitus is associated with local reproductive tract inflammation and poor fertility marker [18], which is likely to be associated with increased OS. Leukocytospermia is associated with increased O2·− and H2O2-positive spermatozoa associated with apoptotic markers caspase 3/7 and glutathione activation [82]. In vivo, leukocyte-induced damage can be mitigated by the addition of antioxidants to the medium, including catalase, glutathione and N-acetylcyesteine [61], with further lines of evidence suggesting that leukocyte induced OS can also be managed with Vitamin C, Vitamin E, Glutathione and CoEnzyme Q10 [60], further suggesting a central role of OS in the pathogenesis of male reproductive tract inflammation.

Spermatogenesis and Germ Cells Susceptibility

Although ageing has been thought to have limited effect on male fertility, as males continue to produce sperm into old age, germ cells from elderly males are of lower quality than younger males [49]. Furthermore, alongside ageing, OS-induced DNA damage accelerates germ cell apoptosis resulting in impaired spermatogenesis and spermatozoa [68]. Spermatogenesis occurs in the testes, primarily in association with Sertoli and germ cells [12]. In steady states, redox reactions play pivotal physiological roles in spermatogenesis [10; 49]. However, evidence shows that age-related OS compromises the development of quality germ cells. Abnormal gametes with poorly remodelled chromatin may also generate OS during spermatogenesis leading to increased abnormal spermatozoa and high percentage of DNA fragmentation. This is mediated by the increased inability to repair DNA strand breaks that occur normally during spermatogenesis. These cells are susceptible to ROS-dependent apoptotic pathway activation through caspase activation, externalization of phosphatidylserine residues, and ROS-activation from the mitochondria which further results in LPO and DNA fragmentation alongside apoptosis [61].

Age-dependent changes in spermatogenesis have previously focused on induced damage to mature spermatoocytes that have undergone compaction but are transcriptionally inactive, with fewer studies focused on germ cells in earlier phases of spermatogenesis [49]. Ageing is associated with a decreased number of Sertoli and germ cells alongside a reduction in fertility potential [12]. Furthermore, germ cells from elderly rats particularly display increased levels of ROS and associated DNA damage, when compared to younger animals [49].

In SOD1 knockout mice (SOD−/−), ageing shows a significantly increased loss of Sertoli and germ cells compared to wild type age mice, indicating a significant role of OS in detrimental spermatogenesis. This is in addition to experimental pro-oxidant exposure in these SOD−/− mice that increases ROS in spermocytes, but this increase was not observed in Catalase-Null (Cat−/−) mice which were still able to neutralize ROS [12]. In animal models, this can be prevented by overexpression of the antioxidant catalase following exogenous ROS challenge that damages spermatozoa, showing increased markers of DNA repair enzymes [84]. However, it appears that they respond and adapt to OS differently through the different developmental phases, and ageing results in redox dysfunction as aged germ cells are less able to support appropriate defenses [49].

Leydig Cell Function and Age-Related Androgen Decline

Male endocrinology, as with other systems, is complex in ageing, with multiple mediators of the hypothalamic-pituitary-testes (HPT) axis being affected. Ageing is associated with declining androgens, affecting in particular testosterone and dehydroepiandrosterone (DHEA), with corresponding increases in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG), manifesting as a male primary hypogonadism phenotype. Although dihydrotestosterone (DHT) remains relatively constant, there is a corresponding decline in the testosterone-DHT ratio negatively affecting the ageing process [4; 15].

Unlike women, androgen decline is a gradual age-related phenomenon in males, declining at an annual average rate of 1 - 2% per year (although this is highly variable, and testosterone may even increase in some men). Therefore, appropriate terms used to describe this include androgen deficiency in men (ADAM) and late-onset hypogonadism (LOH). ADAM is driven primarily by testicular dysfunction, with or without more subtle HPT-axis dysfunction and more likely secondary to the declining androgens [4; 15]. This process is closely correlated with reduced libido, sexual function and reduced fertility potential associated with hypospermatogenesis and sperm dysfunction. However, ADAM affects between 10 and 25% of the elderly, with symptomatic hypogonadism affecting 6 - 12%. Even at a very advanced age, serum testosterone levels, and associated sexual and reproductive function, may remain within normal limits for many elderly men [85].

ADAM appears to be an important mediator of age-related comorbidities in males, and this is modulated by lifestyle factors that further affect declining testosterone levels. This includes tobacco and alcohol use, increased psychological or physiological stress, obesity, type-2 diabetes mellitus, obstructive sleep apnoea and medication use. Declining testosterone is an independent risk factor for co-morbid conditions including declining cognitive function, osteoporosis, metabolic syndrome, type-2 diabetes mellitus and cardiovascular disease, and therefore can be a cause and consequence in these complex pathologies. Ultimately, men with low testosterone are more likely to die prematurely. Although some studies suggest that testosterone-replacement therapy (TRT) may offer benefit in reducing the negative impact of ageing in males, long term safety and efficacy is not yet clear. These lines of evidence suggest a significant role for testosterone and ADAM in the ageing process in males, as well as the development of comorbidities, although the mechanisms associated with this require further elucidation [4].

The free radical theory involves the damaging role of OS and various toxins on mitochondria [7]. It has been demonstrated that Leydig cell MMP, ATP synthesis and mitochondrial calcium concentrations are all required for steroidogenesis [86]. These mechanisms may be disrupted by excessive OS which is known to inhibit both ovarian and testicular steroidogenesis, most notably the initial step of cholesterol transfer into Leydig cell mitochondria and ster-
Redox Regulation of Fertility in Aging Male and the Role of Antioxidants

oxidogenic acute regulatory (StAR) protein transcription [86-89]. The result is a reduction in cAMP production, StAR mRNA and the activity of P450 cholesterol side-chain cleavage (P450_cho), and therefore the activities of Cytochrome P450 17α-hydroxylase/17, 20-lyase (CYP17) and 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3βHSD) [89-90]. H2O2 is derived from macrophages in close residence to Leydig cells in the testes, and these ROS are associated with reduced testosterone production and 3βHSD transcription alongside LPO, decreased cellular antioxidants such as SOD, catalase and glutathione transferase, and initiation of apoptosis [91]. This is then associated with a gradual decline in testosterone in ageing males that can be accelerated in chronic inflammatory diseases and other age-related co-morbidities [89]. Detailed understanding of these pathways may open the possibility of novel treatments to improve sterogenesis in ageing males and there is potential to target these pathways to modulate the consequences of ageing and improving reproductive potential and sexual function.

Prostate Ageing and OS

Benign prostatic hyperplasia (BPH) and adenocarcinoma of the prostate are common co-morbidities associated with male ageing. Risk factors, in addition to ageing major risk, include genetic inheritance and dietary factors. Prostatic hyperplasia and carcinogenesis of prostate cells, as mechanism associated with these pathologies, are considered to be driven by OS in ageing males. This may be initiated due to hormonal imbalances with age that modulates mitochondrial activity to increase ROS production and depletion of intracellular antioxidants such as glutathione peroxidase [6]. Other mechanisms common to both diseases include inflammatory mediators (e.g. chronic systemic inflammation or prostatitis) and additional hormone changes with ageing (including estrogen, progesterone and androgens changes) [92]. Prostate OS, as with other tissues, damages DNA, cell membrane and tissue proteins that trigger cellular signaling disruption, impaired homeostasis and genetic mutations [6; 92]. The structural changes associated with BPH and prostate carcinoma is associated with the syndrome of LUTS that is associated as an age-related phenomenon that has increasing OS as part of the underlying mechanisms, as well as with sexual dysfunction and poor semen quality [92]. This impacts on pathogenesis, response to therapeutics and prognosis in these conditions. Furthermore, prostatitis can increase OS in the reproductive tract and the ejaculate, impairing semen function [93].

Erectile Dysfunction

Erectile dysfunction (ED) is characterized by the inability to develop and maintain a sufficient erection of the penis for satisfactory sexual activity. Disruption of psychological, neurological, hormonal, vascular, and cavernosal factors, individually, or in combination, can induce ED [94]. Major health concerns such as atherosclerosis, hyperlipidemia, hypertension, diabetes, and metabolic syndrome have now become well integrated into the investigation of ED [95]. Age is also an important risk factor, with higher proportions of ED in men over 50 years as compared to younger men (age is also a risk factor for obesity, metabolic and hypogonadism in males) [96-97]. Alongside the epidemic of obesity, ED is a common complaint, with an age-standardized prevalence of more than 40% [97-98]. Age-related ED is associated with neurovascular dysfunction, which is closely mediated by nitric oxide (NO) suppression, is associated with vasodilation in the vascular system and increased OS in the penile tissues [99]. Antioxidants may provide some potential targets for therapeutic strategies in ED associated with OS, and in this context, various phytotherapeutic extracts have been attributed to have benefit in males with erectile dysfunction, with different levels of scientific attention, including Panax ginseng (Korean Ginseng or Chinese Ginseng), Tribulus terrestris (Devil’s Thorn or Devil’s Weed), Euonyma longifolia (Tongkat Ali), Withania somnifera (Indian Ginseng or Poison Gooseberry) and Camellia sinensis (green tea), amongst [100-101]. Although many herbs show benefit in traditional and pre-clinical settings, there is a need for the design and execution of well-controlled clinical studies to determine the efficacy and safety of plant aphrodisiacs, the design and execution of well-controlled clinical studies to determine the efficacy and safety of plant aphrodisiacs [102].

THE ROLE OF ENDOGENOUS ANTIOXIDANT DEFENSES AND AGEING

Due to the structure of the spermatozoa, these cells are significantly prone to oxidative damage particularly to the cell membrane and the DNA. The only defense is a limited system of endogenous antioxidants. These include members of the SOD family, catalase, glutathione peroxidases, PRDX’s, the nuclear factor erythroid 2-related factor 2/antioxidant response element (NRF2/ARE) pathways and nitric oxide synthase (NOS) as critical endogenous antioxidants in seminal plasma and spermatozoa [49; 61; 103].

The physiological roles of some of these antioxidants have been described previously. However, in addition to endogenous antioxidants, there are other exogenous scavengers associated with the male reproductive tract and found in seminal fluid. This includes vitamin C, taurine, tryptophane and spermine [61]. Depletion of endogenous or exogenous antioxidants is associated with OS due to an increased ROS-TAC ratio, and therefore driving cellular and tissue dysfunction in the male reproductive tract [61]. In addition to ROS being closely associated with infertility, there is also a significant percentage of infertile males that have low levels of antioxidants in the ejaculate [104].

It should be considered that genotypic mutations, known as single nucleotide polymorphisms (SNP’s), result in variations of genetic expressions. Within this context, there are numerous polymorphisms associated with the endogenous antioxidant genes in males. These include the GSTT1-null, GSTM1-null, GSTP1-IIIe105Val, SOD2-Ala16Val, eNOS-T786C, eNOS-G894T and CAT-262T/T variants [103]. These are associated with increased potential for OS, further associated with lower sperm quality, oligoasthenoteratozoospermia, oligozoospermia and reduced fertility potential in general [103]. These SNP’s should be potentially considered in the assessment of males who may benefit from antioxidant supplementation [103].

There is a loss of endogenous antioxidant production with ageing [12]. SOD1, more than catalase, is critical for maintenance of germ cells and spermatogenesis [12]. In animal models, Selvaratnam et al. [49] showed that in younger populations, the genetic transgene for SOD1, catalase and PRDX’s are upregulated in response to induced OS, but expressed at lower levels in elderly populations there is evidence of increased DNA damage, suggesting that age is related with a decreased ability to respond to increased ROS production [105]. SOD serves as a major antioxidant throughout the body, and SOD1 is particularly important in prevention of OS in the reproductive tract. Specifically, SOD1+/- mice show disrupted redox signaling, significantly advanced global ageing and age related pathologies, and reduced life-span by up to 30% [105].

PRDX’s are well known and important antioxidant systems in seminal plasma, scavanging ROS and modulating ROS-dependent signalling pathways [53]. Reduced PRDX’s in seminal plasma are associated with poor seminal quality. In a study by Gong et al. [53], reduced seminal PRDX1 and PRDX2 correlated to lower sperm concentrations, higher LPO as determined by MDA and sperm DNA damage. PRDX’s have a key role in protection of sperm from OS within the male reproductive tract. Infertile males, and ageing males, have lower levels of PRDX’s than fertile males, as these are thio-oxidized and inactive [49-51]. The dysregulation of PRDX’s and their associated reactivating systems (thioredoxin/thioredoxin reductase and glutathione S-transferases) negatively modulates sperm motility, capacitation and promotes DNA damage leading to male infertility [50-51]. This provides an interesting mechanism for decreased fertilization capacity in ageing men.
FERTILITY ASSESSMENT IN THE AGEING MALE

Infertility is defined by the WHO as ‘the inability of a couple to achieve conception or bring a pregnancy to term after 12 months or more of regular (at least three times per week), unprotected sexual intercourse’ [54]. It affects about 15% (approximately one in seven) of couples trying to conceive [64]. Of these cases, 25-50% can be attributed partially or solely to the male partner [106]. Male infertility is dramatically increasing in recent decades, with defective sperm function considered the major contributor to this phenomenon [61]. Causes of male fertility are multifactorial, including genetic diseases, organic pathologies and lifestyle and environmental factors. Well defined major causes of male factor infertility include varicoceles (±25% cases), urogenital infections (±10% cases), testicular failure characterized by endocrine failure leading to testosterone deficiency with severely impaired spermatogenesis resulting in azoospermia or severe oligozoospermia (±1.1% cases), other immunogenic causes, impotence or sexual/ejaculation inadequacy, other acquired urogenital abnormalities or strictures (e.g. structural complication following mumps orchitis), and various endocrine disorders (±1.5% cases) [85; 107]. Approximately 20 - 50% of these cases have no known etiology, termed ‘idiopathic’ [106]. Lifestyle and environmental factors negatively associated with fertility potential include consumption of alcohol and tobacco, recreational drug use (such as marijuana and cocaine), exposure to testicular heat, prolonged sitting (related to testicular heat), exposure to endocrine disruptors (e.g. pesticides; phthalates), exposure to heavy metals (e.g. lead; cadmium), stress (both physiological and psychological), ionizing radiation and even exposure to cell phone radiation [85; 107-110]. Many of these risk factors are associated with OS as an important mechanisms, and are also associated with increased rates of ageing.

A clinical assessment would include a complete case history relevant to andrology, a detailed clinical examination, relevant hormonal profile and a standard seminal analysis. Additional analysis may be warranted, including genital ultrasound imaging as required, functional sperm markers (e.g. DNA fragmentation) and genetic testing [106]. The standard seminal assessment includes routine markers such as sperm concentration, motility, vitality and morphology with seminal volume, viscosity, pH and leukocyte count, and these analyses need to be done by appropriate trained clinicians and laboratory technicians within the guidelines offered [54].

A decrease in sperm quality is considered a major reflection of the decreased ability of the male partner to contribute to fertilization [106]. In must be noted, however, that although the semen analysis remains a standard test in male fertility assessments, its clinical value is limited as 5% of fertile men and 16% of infertile men display poor semen analyses [111]. In addition to the number of spermatozoa, the functional capacity of sperm is considered a more sensitive determinant of fertility potential. This includes assessment of percentage of sperm with DNA fragmentation and damaged MMP [63]. MMP is considered a factor which most closely represents spermatozal mitochondrial function, critical for metabolic functioning and motility [112]. Of relevance, both DNA fragmentation and MMP (mitochondrial dysfunction) are mediated by increased OS from all underlying causes, as well as ageing, and this in turn results in dysfunctional reduced DNA integrity and MMP, which further increases ROS production [20; 47-48; 75]. Particularly, assessment of sperm DNA integrity is important in the male assessment, especially related to increasing paternal age and increased OS, to assess strand breaks, numerical abnormalities in sperm chromosome complement and alterations in epigenetic regulations [20].

ROS ASSESSMENT

With increasing evidence on the role of OS in male infertility and reproductive system dysfunction, there are growing indications that measurement of ROS in semen, or potentially even serum markers, should be part of standard fertility testing in males [47; 72; 113]. Accurate assessment is of importance in diagnostics, but also to guide therapeutic considerations [47; 72]. Various methods for assessment of OS exist.

Currently, there is the use of both direct and indirect measurement of OS. The major assessments currently used include the ROS, total antioxidant capacity (TAC) and the oxidation-reduction potential (ORP), MDA, thiobarbituric acid assay (TBARS) or 4-hydroxynonenal (4-HNE) as LPO markers, caspase-3 or annexin V as apoptotic markers and proteomic analysis of oxidized protein markers (e.g. protein carbonyl is a highly sensitive marker of protein oxidation in semen) that indirectly reflect increased OS [11; 20; 47; 104]. Evidence is suggesting, as with sperm DNA integrity, that diagnostic and prognostic capabilities of OS assessment may exceed the capabilities of conventional semen analysis, and may more accurately differentiate fertile from infertile men [113]. However, these methods are not suited for easy clinical diagnostics, and they are generally lengthy assays that require specialized laboratory equipment and specialized techniques [104].

These systems do not evaluate the full spectrum of OS, however, but rather different specific components that may prove to be incomplete as individual assays at a single point in the pathogenesis [104]. This ‘one-size-fits-all’ mentality pervades also into the analytics. Measuring TAC, for example, will not give useful information on the state of the organism, and should be discouraged. Rather, individual antioxidant enzyme activities and patterns of antioxidant molecules need to be assessed [9]. Therefore, a new more comprehensive and simplified measure of OS the oxidation-reduction potential (ORP) has been suggested as diagnostic marker [104]. This novel method is a direct measurement and indicator of OS in biological samples, and can be considered a more comprehensive marker of OS in biological systems, including seminal fluid. This involves the analysis of all known and unknown oxidants and antioxidants in blood, urine and seminal fluid, and reflects damaged tissues associated with OS [104]. ORP also correlates with poor semen samples and infertility risks, and this can be further developed into a routine, comprehensive and sensitive diagnostic test as it can be used as quick and cost-effective one-step screening test in clinical practice to measure OS both in semen and seminal plasma in fresh or in frozen samples [104; 114].

THERAPEUTIC CONSIDERATION IN AGE RELATED REDOX BIOLOGY AND MALE FERTILITY:

The free radical theory of ageing, particularly due to endogenous ROS, raises the potential to target these mechanisms for therapeutic approaches. The role of these antioxidants is to prevent ROS concentrations from elevating to non-physiological levels, and thus from derailing the finely balanced redox balance into oxidative stress [30]. Many of these antioxidants can be acquired through nutritional intake, with a study by Karayiannis et al. suggesting that deficiencies in dietary habits associated with increased antioxidant intake (Mediterranean Diet) is associated with poor semen quality [115]. Although there is indication that synthetic oral antioxidants have limited efficacy in disease prevention [23] and are not particularly efficient when compared to the catalytic antioxidants that mimic SOD and catalase activities [41], antioxidants have an evidence-base to be considered as a promising therapy option in the treatment and prevention of ageing and various co-morbidities, including cardiovascular disease, type-2 diabetes mellitus and cancers (as observed in diets rich in phytonutrients from fruits and vegetables) [30]. In the context of rapid increases in over-the-counter dietary supplements in recent years, a large proportion of these are antioxidant-rich items such as multivitamins, including vitamin C and vitamin E [116], and the effects on male reproduction potential required further investigation to further understand the benefits, risks and dosages of specific antioxidants or the com-
The effects of antioxidant therapy in idiopathic cases of male infertility and varicoceles has been widely studied, with conflicting data. However, antioxidants are increasingly used by urologists for sub-fertile men on the premise that OS is at least partly due to a deficiency of seminal antioxidants [104]. Although the treatment of patients with antioxidants to mitigate the effects of OS is highly debated [81], the role of OS in male factor infertility, the high incidences of OS diagnoses in males with fertility concerns and age associated changes in male reproductive system that can be associated with OS as both a cause and a consequence suggest that there is a strong indication for a role of antioxidants for therapeutics [47; 61; 68; 113]. In this context, it is essential to evaluate the impact of ROS in the patient through appropriate clinical assessment and diagnostics in order to determine which patient sub-groups may benefit from antioxidant therapy [113]. Although there is little consensus still for the type and dosages of antioxidants that should be used, evidence suggests that infertile males with excessive OS would benefit from this therapeutic approach [47] [113]. However, many studies have indicated a benefit of antioxidant therapy, or freezing of semen samples from younger age, to reduce the impact of OS and age related modifications of male fertility [46; 47; 113].

Numerous antioxidants are used orally, singularly or in combination, and include the following: Vitamins A, B complexes, C and E, CoEnzyme Q10, ubiquinol, glutathione, L-carnitine, β-carotene, lycopene, α-lipoic acid, N-acetyl-cysteine, selenium, zinc and copper [47]. Ahmadi et al. [117] and Agarwal et al. [47] provide a detailed review of these approaches in clinical trials. Oral antioxidants need to reach high concentrations in the reproductive tract and restore OS related dysfunction to improve spermatogenesis, and should reduce levels of seminal ROS [118]. Treatment should be aimed at reducing OS by lowering ROS and/or increasing antioxidants to keep only a low level necessary for biological function [68].

Lipid soluble antioxidant profiles include carotenoids, vitamin A and vitamin E as antioxidants, and MDA as a non-specific marker of lipid peroxidation. Both, serum and seminal concentrations of these markers can reflect potential reproductive tract and spermatooza dysfunction [72]. In experiments using an animal model (rabbit), high levels of vitamin E were suggested to be detrimental to fatty acid composition of spermatozoa. Vitamin E may also reduce ROS production in the male reproductive tract [117]. This vitamin E (and the fish oil separately) increased sperm production in ageing animals though, and was a strong indication for a role of antioxidants in ageing animals though, and was a strong indication for a role of antioxidants in the development and progression of BPH and cancer, and nutritional intake of vitamin E, selenium and lycopene, with evidence suggesting that micronutrient supplementation may restore ROS-TAC imbalance and improve therapeutic outcomes [133]. This problem is particularly becoming more evident as the reproductive age is increasing. However, significantly more studies are required in herbal extracts for clinical use, and research is difficult to review due to wide ranging methodologies, extracts, dosages and outcomes investigated.

Antioxidant supplementation must be used with caution, and it is recommended that any oral antioxidant treatment that is initiated in patients who have demonstrated increased seminal OS should be carefully controlled. There are concerns raised on the detrimental effect of antioxidants on the physiological roles of ROS, and there has been previous particular concern with vitamins C, E and β-carotene, in the context of a very finely balanced reduct system [134-135]. Uncontrolled use of antioxidants may lead to a reductive state known as the antioxidant paradox, as certain low level ROS is required for physiological function systemically and in the reproductive tract [81]. There are some indications that specific overconsumption of some antioxidants is associated with increased mortality risk in some populations [30]. Further areas of concern in therapeutc use is that, at least in adult male housefflies, endogenous antioxidants may also decrease endogenous antioxidant production [136]. Areas of specific concern and consideration includes lifestyle factors and (patho)physiological states associated with ROS-TAC balance. This includes smokers vs. non-smokers, dietary antioxidant intake, specific pharmacodynamics and pharmacokinetics of antioxidants, interaction with medication and other antioxidant combinations [30].

A complete and appropriate workup is required, including seminal OS assessment, before initiation of any specific therapy. The approach to treatment of male related infertility, following investigation for underlying disorders, further involves a multifactorial approach that should include the identification and avoidance where possible of harmful environmental, social and occupation risk factors, as well as identification and appropriate correction of...
nutritional imbalances associated with optimal spermatogenesis and seminal fluid production [60].

CONCLUSION
Ageing, and age-related reproductive decline in males, is closely associated with a shift from a balanced redox state to a state of OS. Genetics, lifestyle and environmental factors modulate this balance. Although a low-level ROS is important for normal physiological function including fertility, increased ROS-TAC and associated oxidative mechanisms have roles in all male age-related phenomena affecting sexual behavior and seminal quality. Important areas of research should continue to elicit the physiological role of ROS in andrology, the impact of OS on male reproduction and age-related changes, and the standardization of OS assessment in the male reproductive tract. Of further specific interest is the role of ROS in early germ cell development and spermatozoa maturation for fertilization, the mechanisms associated with downregulation of endogenous antioxidant genetic expressions with age, identification of therapeutic targets for improving endogenous antioxidant activity, and specific oral antioxidant treatment for age related OS.

The OS assessment in the male reproductive tract of ageing males is important prior to any consideration of therapeutic intervention with oral antioxidants. However, and these must be used with caution and monitoring be done for therapeutic risks (antioxidant paradox and reductive stress). Evidence suggests that antioxidants, along with appropriate lifestyle changes and avoidance of environmental risks where possible, may offer an important strategy to improve fertility outcomes in ageing males and potentially prevent the impact of excessive OS on disease development in the reproductive tract as well as globally. On the other hand, the potential detrimental effects of too high antioxidant levels and RS for male reproductive health must not be neglected as scientists and clinicians still do not know what the normal values for the redox potential in serum and semen is. Therefore, more research is required in ageing males to fully understand the physiology, pathological mechanisms and best treatment options in different ageing male sub-groups. Although it is difficult to draw broader comparisons on the efficacy of specific therapies in male infertility due to the large variability in the studies done, antioxidant treatment continues to provide increased promise in clinical practice as a therapeutic option.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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