



Review

Antioxidant Paradox in Male Infertility: 'A Blind Eye' on Inflammation

Sulagna Dutta^{1,2,†}, Pallav Sengupta^{2,3,†}, Shubhadeep Roychoudhury^{4,*}, Srikumar Chakravarthi^{1,3},
Chee Woon Wang¹ and Petr Slama^{5,*}

- ¹ Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, SP2, Bandar Saujana Putra, Jenjarom 42610, Selangor, Malaysia; sulagna_dutta1@yahoo.com (S.D.); srikumar@mahsa.edu.my (S.C.); wang.chee@mahsa.edu.my (C.W.W.)
- ² School of Medical Sciences, Bharath Institute of Higher Education and Research (BIHER), 173 Agaram Main Rd, Selaiyur, Chennai 600073, India; pallav_cu@yahoo.com
- ³ Physiology Unit, Faculty of Medicine, Bioscience and Nursing, MAHSA University, SP2, Bandar Saujana Putra, Jenjarom 42610, Selangor, Malaysia
- ⁴ Department of Life Science and Bioinformatics, Assam University, Silchar 788011, India
- ⁵ Department of Animal Morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic
- * Correspondence: shubhadeep1@gmail.com (S.R.); petr.slama@mendelu.cz (P.S.)
- † These authors have contributed equally to this work.

Abstract: The pathophysiology of male infertility involves various interlinked endogenous pathways. About 50% of the cases of infertility in men are idiopathic, and oxidative stress (OS) reportedly serves as a central mechanism in impairing male fertility parameters. The endogenous antioxidant system operates to conserve the seminal redox homeostasis required for normal male reproduction. OS strikes when a generation of seminal reactive oxygen species (ROS) overwhelms endogenous antioxidant capacity. Thus, antioxidant treatment finds remarkable relevance in the case of idiopathic male infertility or subfertility. However, due to lack of proper detection of OS in male infertility, use of antioxidant(s) in some cases may be arbitrary or lead to overuse and induction of 'reductive stress'. Moreover, inflammation is closely linked to OS and may establish a vicious loop that is capable of disruption to male reproductive tissues. The result is exaggeration of cellular damage and disruption of male reproductive tissues. Therefore, limitations of antioxidant therapy in treating male infertility are the failure in the selection of specific treatments targeting inflammation and OS simultaneously, two of the core mechanisms of male infertility. The present review aims to elucidate the antioxidant paradox in male infertility treatment, from the viewpoints of both induction of reductive stress as well as overlooking the inflammatory consequences.

Keywords: antioxidants; inflammation; male infertility; oxidative stress; reductive stress



Citation: Dutta, S.; Sengupta, P.; Roychoudhury, S.; Chakravarthi, S.; Wang, C.W.; Slama, P. Antioxidant Paradox in Male Infertility: 'A Blind Eye' on Inflammation. *Antioxidants* **2022**, *11*, 167. <https://doi.org/10.3390/antiox11010167>

Academic Editors: Paola Piomboni, Valter Travagli and Gerardo Tricarico

Received: 11 November 2021

Accepted: 14 January 2022

Published: 16 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Male factor infertility has become one of the foremost global health issues and is said to account for 20% to 30% of global infertility cases [1,2]. The actual etiology of male infertility remains enigmatic, and a large majority of male infertility cases are still classified as idiopathic, where the underlying cause(s) remains elusive [3].

Significant research has supported the notion that oxidative stress (OS) serves as the core mechanism in the majority of the idiopathic male infertility cases [4,5]. Exceedingly high concentrations of reactive oxygen species (ROS) can outweigh endogenous antioxidant capacity, reaching pathological levels and interfering with male reproductive processes. While excessive ROS levels adversely affect male fertility, a physiological concentration of ROS is vital to mediate the normal sperm functions [6].

Numerous factors can attribute to OS-mediated male infertility, but the underlying pathways for most of the factors remain elusive [7]. These include environmental, occupational, dietary, recreational as well as habitual components, each varying in intensities and duration of exposure [8–10]. Smoking is a known risk factor of male infertility. Smoking causes leukocytospermia, one of the prime sources of ROS [11]. Moreover, ROS in tobacco smoke can overwhelm endogenous antioxidant defenses. Elevated seminal ROS levels in smokers renders spermatozoa more vulnerable to OS-mediated damages, compromising sperm function and ultimately male fertility [12,13]. Studies show that impacts of alcohol consumption on male reproductive functions vary depending on the amount consumed [14,15]. However, a level of alcohol beyond which the risk of male infertility increases has yet to be established [16]. Illicit drugs that impair male fertility include cocaine, marijuana, opiates (narcotics), anabolic–androgenic steroids (AAS), and methamphetamines [17,18]. Other endogenous factors, including metabolic syndromes (such as obesity and insulin resistance), psychological stress, and dietary factors also have been widely documented as causatives of male infertility, impairing the testicular redox balance, affecting the endocrine axes that regulate male reproduction, testicular histology, leading to disrupted testicular functions, sperm membrane lipid peroxidation (LPO), sperm DNA fragmentation, genetic and epigenetic modifications of sperm, sperm dysfunctions and overall deteriorated semen quality [16,19–22].

Treatment with antioxidants is the first line of defense against OS-mediated male infertility [23–25]. Agarwal et al. (2010) had clearly described the effects of various antioxidants on male fertility, fertilization, and pregnancy rates [25]. The authors have indicated that although several antioxidants reportedly have positive impact upon fertility parameters, further randomized controlled trials are needed to demonstrate the efficacy and safety of antioxidant supplements in treating idiopathic male infertility, as well as to find the optimal dose of each antioxidant to improve fertility parameters [25]. Misuse and abuse of antioxidants, “over-the-counter” usage of antioxidant supplements, and even pauciness in antioxidant efficacy are all thought to have adverse implications by transitioning the endogenous redox status to the reductive side of the scale [26]. As a consequence, any physiological extremities of the redox spectra, OS, or reductive stress, are all detrimental to male reproductive health. The term ‘antioxidant paradox’ was coined by Halliwell in the year 2000 to describe the unexpected effects of antioxidants [27]. There is little scientific literature that has put forth the explanation of ‘antioxidant paradox’ based on the importance of maintaining a delicate cellular redox balance to support physiological levels of ROS production for robust male reproductive functions [28,29]. Henkel et al. (2018) had vividly explained how the over-usage of antioxidants may cause reductive stress, and manifest contradicting impacts of antioxidant treatment on male reproduction [30]. However, there are other mechanisms that contribute to ‘antioxidant paradox’, for which in-depth explanation in the context of male infertility is needed. This article is unique in its approach to explain the vicious cycle of OS and inflammation in addressing the often failure of antioxidant treatment for male infertility. The present article supports the fact that ‘antioxidant paradox’ in male infertility treatment may partially be explained by: (i) the failure to choose treatments that specifically target both inflammation and OS; (ii) the failure to utilize both antioxidants and anti-inflammatory drugs at the same time; and (iii) the use of nonselective agents downregulating some oxidative and/or inflammatory pathways while exaggerating others.

OS is triggered by the release of reactive oxygen and nitrogen species (ROS and RNS) by inflammatory cells (which are among the major sources of ROS/RNS) at the location of the inflammation [31]. Interestingly, a variety of ROS/RNS have been reported to activate an intracellular signaling cascade that elevates the pro-inflammatory gene expressions [19,32,33]. As a result, inflammation and OS are pathophysiological phenomena that are strongly related to one another and are intimately intertwined. Failures of antioxidant treatment for male infertility roots from the failure in selection of treatment components is specific in targeting inflammation and OS, both at the same time [34]. This notion prompted

us to consider the fundamental concepts of the interaction and interdependence of OS and inflammation in disrupting male reproductive functions, to explain the mechanism pertaining to 'antioxidant paradox' in male infertility.

2. Reactive Oxygen Species and Sperm Functions

OS represents a core mechanism causing male infertility, owing to its adverse impact on spermatogenesis, sperm maturation, and capacitation [6]. Followed by the endocrine orchestrated process of spermatogenesis in the testes, the produced spermatozoa is subjected to maturation in the epididymis, which is also crucial for sperm to attain fertilization-capacity and motility [35,36]. During this period, the maturing spermatozoa get exposed to endogenous ROS, which mediate various physiological functions, such as sperm hyperactivation, capacitation, and acrosome reactions [37]. These processes are crucial for proper fertilization, and they need immense energy that is derived from cellular metabolism, such as glycolysis or oxidative phosphorylation. Sperm capacitation occurs by a series of numerous cellular reactions allowing the fusion of sperm with zona pellucida of oocyte. This causes the acrosome reaction, which releases proteolytic enzymes [37].

While ROS are required for physiological sperm functions, excess ROS concentrations can overwhelm the antioxidant capacity. In the seminal fluid, the antioxidants, both enzymatic and non-enzymatic, resist the induction of OS and protect sperm from oxidative damage. Notable functions are played by the enzyme triad of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GP_X) [38,39]. SOD is a metalloenzyme that catalyzes superoxide anion dismutation processes and protects polyunsaturated fatty acids (PUFA) and sperm DNA integration [40–43]. Catalase converts hydrogen peroxide to molecular oxygen and water. Catalase possesses a heme structure with a central iron atom. Its enzymatic activities are observed in the cytoplasm, mitochondria, peroxisomes, as well as in endoplasmic reticulum. The sperm antioxidant system also includes GP_X, whose active site contains selenocysteine. This enzyme reduces hydrogen peroxide (H₂O₂) [41]. It has three isoforms: nuclear, mitochondrial, and cytosolic. Sperm functions are mainly aided by the mitochondrial isoform [41,44]. If the endogenous antioxidants are unable to protect cells from high ROS, cell death can occur. Thus, ROS have a dual role: they aid sperm functions, but when in excess, they also impair sperm morphology, functions, and viability [6,45].

Sperm membranes contain a high concentration of PUFA, which ensure the fluidity required for fertilization. However, PUFA is susceptible to LPO, this high level of PUFA poses a risk of oxidative damage to spermatozoa [46]. The oxidative damage that can occur is linked to membrane fluidity loss, morphological changes in the spermatozoa, mitochondrial dysfunctions, decreased vitality, and other changes that lead to fertilization failure [46]. In mature spermatozoa, the haploid and densely compressed nucleus no longer transcribes, therefore they cannot respond to stress. During maturation phases, spermatozoa lose much of their cytoplasm, the main antioxidants source. So, spermatozoa have relatively little cytoplasm, which is mostly filled with DNA which becomes vulnerable to oxidative damage causing sperm DNA fragmentation [47].

3. Antioxidants in Oxidative Stress-Induced Male Infertility

Most human diseases, including ageing, cancer, and even reproductive abnormalities, include OS as a primary central mechanism. Antioxidant therapy has recently sparked substantial research interest, owing to a rapidly ageing population. Antioxidants appeal to people because they are considered 'natural' and thus 'good' chemicals, and they are connected to a healthy diet [48]. This common belief is backed up by aggressive antioxidant product marketing campaigns in a multibillion-dollar industry. Reducing OS is thought to be a method to avoid various diseases [49,50]. Given that these components can be purchased over the counter without a prescription and that antioxidant products are used to supplement diets, it is critical that they have no negative side effects. While early research on antioxidant supplements showed that they are efficient in preventing ailments, in more

concurrent studies, efficacy of such therapies has been questioned. The US Food and Drug Administration (FDA) has made no recommendation for the antioxidant supplementation for any physiological illness. Furthermore, the European Society for Human Reproduction and Embryology (ESHRE) has issued a current consensus recommendation stating the insufficiency of evidence that supports the usage of antioxidant supplements [51]. Excessive supplementation has been found to be deleterious to health in various studies [52–54] and these concerns were supported in later studies [55]. Aside from that, it has been demonstrated that overly high quantities of antioxidants are teratogenic to embryos [56]. On the contrary, there has been research demonstrating that antioxidant therapy can help to improve male reproductive health [5,32,57–61]. In the current state of reproductive medicine research, antioxidant treatment for male infertility has received considerable attention (Table 1).

Table 1. Individual or combination antioxidant treatments with beneficial or no significant effects on semen quality.

Antioxidant Regime	Study Population	Sperm Parameters	Study
Zinc sulphate (220 mg) for 4 months	oligospermic males ($n = 14$)	21.4% (3/14) of patients achieved pregnancy	[62]
600 mg of vitamin E daily for 3 months	Infertility with high ROS	No change in SC, S_{mot} and S_{morph}	[63]
Zinc sulphate (500 mg) for 3 months	Asthenozoospermia ($n = 100$)	Improved pregnancy (22.5%) vs. placebo (4.3%)	[64]
1 g vitamin C and 800 mg vitamin E daily for 56 days	Asthenozoospermia	No change in SC, S_{mot} and S_{morph}	[65]
1 g of vitamins C and E daily for 2 months	Idiopathic infertility (38 infertile men with previous IVF/ICSI)	No change in SC, S_{mot} and S_{morph}	[66]
L-carnitine 3 g daily; L-acetyl carnitine 3 g daily; L-carnitine (2 g daily) and L-acetyl carnitine combination (1 g daily)	Idiopathic asthenozoospermia (Placebo group = 15, L-carnitine group = 15; L-acetylcarnitine group = 15; L-carnitine and L-acetylcarnitine combined group = 15)	Increase in sperm motility and normal sperm morphology	[67]
1 g Carnitine and 500 mg L-acetyl carnitine daily for 24 weeks	Asthenozoospermia	No improvement in S_{mot}	[68]
400 mg each vitamins C and E, 18 mg β -carotene, 500 μ mol zinc, 1 μ mol selenium daily for 90 days	38 infertile men with At least two failed IVF or ICSI	Increased chromatin decondensation	[69]
Menevit (lycopene, vitamins E, C, zinc, selenium, folate, garlic oil) daily for three months	60 infertile men	No significant difference in DFI	[70]
300 mg selenium daily for 48 weeks	Normozoospermia	No improvement in S_{mot} and S_{morph}	[71]
Menevit (lycopene, vitamins E, C, zinc, selenium, folate, garlic oil) daily for three months	50 infertile men with elevated OS	No change in SC, S_{mot} and S_{morph}	[72]
500 mg L-carnitine, 60 mg vitamin C, 20 mg coenzyme Q10, 10 mg vitamin E, 200 μ g vitamin B, 91 μ g vitamin B12, 10 mg zinc, 50 μ g selenium once daily for 3 months	Prospective observational study with 20 men with grade 1 varicocele and primary or secondary infertility	No change in SC, S_{mot} and S_{morph}	[73]
30 mg vitamin C, 5 mg vitamin E, 0.5 μ g vitamin B12, 750 mg L-carnitine, 10 mg coenzyme Q10, 100 μ g folic acid, 5 mg zinc, 25 μ g selenium twice daily for 6 months	7 infertile men with sperm DFI >25% Treatment group (37 patients) Placebo group (40 patients)	No change in SC, DFI	[74]

Table 1. Cont.

Antioxidant Regime	Study Population	Sperm Parameters	Study
Coenzyme Q10 = 200 mg daily	Idiopathic infertility (Placebo group = 114, Treatment group = 114)	Increase in sperm concentration, motility, and normal sperm morphology	[75]
L-carnitine = 1 g, L-acetylcarnitine = 0.5 g, fumarate = 0.725 g, fructose = 1 g, citric acid = 50 mg, zinc = 10 mg, coenzyme Q10 = 20 mg, selenium = 50 µg, Vit C = 90 mg, folic acid = 200 µg, Vit B12 = 1.5 µg daily for 3 months	Idiopathic oligoasthenozoospermia (Placebo group = 50, Treatment group = 50)	Increase in semen volume, progressive motility, and vitality in placebo group. Decrease in sperm DNA fragmentation index in treatment group.	[76]
5 mg folic acid, 30 mg zinc once daily for 6 months	1773 men planning to undergo infertility treatment with spouse	No improvement in S_{mot} and S_{morph}	[77]

SC = sperm concentration, S_{mot} = sperm motility, and S_{morph} = sperm morphology, DFI = DNA fragmentation index.

Oxygen metabolism leads to ROS generation, such as the superoxide anion ($O_2^{\bullet-}$), H_2O_2 , and the hydroxyl ($\bullet OH$) radical, which bear unpaired electrons in their outer orbits, rendering these molecules unstable and highly reactive in nature [78]. In the aerobic metabolic process, oxidative phosphorylation in mitochondria leads to ROS generation, 1–5% of which seepages out and released as metabolic cytotoxic by-products [6,37,79–83]. As a consequence of their immense reactivity and brief half-life periods (in the nanosecond range), ROS is capable of massive disruption of biological components, including lipids, proteins, and nucleic acids [84]. The overproduction of ROS thus disrupts cellular functions and leads to a variety of pathologies, including reproductive disorders [85–87]. Besides the adverse impacts of ROS on cellular processes when produced in excess, they are also essential for cell growth and maturation, intracellular signaling cascades, immune responses to infectious agents and inflammatory reactions, and pertaining to male reproductive functions, ROS aid sperm maturation, capacitation, hyperactivation, acrosome reaction as well as embryonic morphogenesis [37,40,88–90]. Superoxide scavenging by SOD catalysis, on the other hand, prevented capacitation-related tyrosine phosphorylation in human sperm, effectively rendering them infertile [91,92]. To scavenge excessive ROS, cells generally employ a variety of intrinsic and extrinsic antioxidant mechanisms. Endogenous antioxidants comprise enzymes, such as SOD, catalase, and thiol peroxidases, besides nonenzymatic antioxidants, such as glutathione. Micronutrients include L-carnitine, coenzyme Q10 (CoQ10), vitamins A, C, E, and trace elements, such as zinc and selenium [84]. Exogenous antioxidants, on the other hand, are those that must be provided to the body exogenously to maintain a healthy redox equilibrium in every live cell [93]. Obesity, which is induced by a poor lifestyle, can lead to systemic inflammation, increased inflammatory cytokine production, and OS [94–97]. Furthermore, consuming alcohol, smoking, being exposed to radiation, and being subjected to occupational or environmental pollutants all contribute to OS [98,99].

4. Induction of Reductive Stress

Uncontrolled, high antioxidant exposure disrupts cellular redox balance, resulting in ‘reductive stress’. The term ‘reductive stress’ refers to a redox balance change in the body’s reductive end of redox scale [100], a circumstance that has been compared to OS in terms of its deleterious effects [101]. A recent article has explained the redox regulation in male reproduction, analyzing the impact of both the OS and reductive stress on sperm function, and explained the “antioxidant paradox” from the viewpoint of induction of reductive stress [102]. The oxidation reduction potential (ORP) is a measure of oxidizing or reducing ability of any substance. In a pioneering study, Selvam et al. had established the physiological range of ORP (−9.76 to 1.48 mV/10⁶ sperm/mL) based on the impacts of both OS and reductive stress on sperm functions and its molecular alterations [103].

In the case of sufficient reducing equivalents, an increase in the NAD^+/NADH (oxidized and reduced forms of nicotinamide adenine dinucleotide) and/or GSH/GSSG (reduced and oxidized forms of glutathione) ratio or antioxidant enzyme overexpression can eliminate all ROS, resulting in an H_2O_2 surplus and leakage from mitochondria, urging the cells to reductive stress [104] (Figure 1). Excess NADH can disrupt mitochondrial homeostasis, cause pathological mitochondrial oxidation, and misfolding of protein in the endoplasmic reticulum [105]. Proper redox balance is essential to mediate the perfect levels of induction and restrictions to ascertain appropriate protein folding as well as the creation of disulfide bonds, that are important to determine normal structural and functional aspects of proteins [106]. The ‘unfolded protein response of the endoplasmic reticulum’ (UPR^{ER}) is triggered when reductive stress impairs disulfide bond formation [107]. Proteostasis in this compartment must be recovered by restoring correct protein folding [107]. Chronic reductive stress can potentially lead to OS, which then drives reductive stress through a feedback loop. When electron acceptors are anticipated to be greatly reduced during reductive stress, various redox proteins can transfer electrons to O_2 , enhancing ROS generation [108]. As a result, this is a two-edged sword. Excessive reducing equivalents stifle cellular growth, limit mitochondrial activity, alter protein disulfide bond formation, and impede cellular metabolism [26]. Reductive stress has previously been acknowledged in other medical domains to explain pathological conditions, such as carcinogenesis, cardiomyopathy, cerebral microvasculature, blood-brain barrier dysfunction, and neurodegenerative diseases [109–111], and the same may also apply for male infertility.

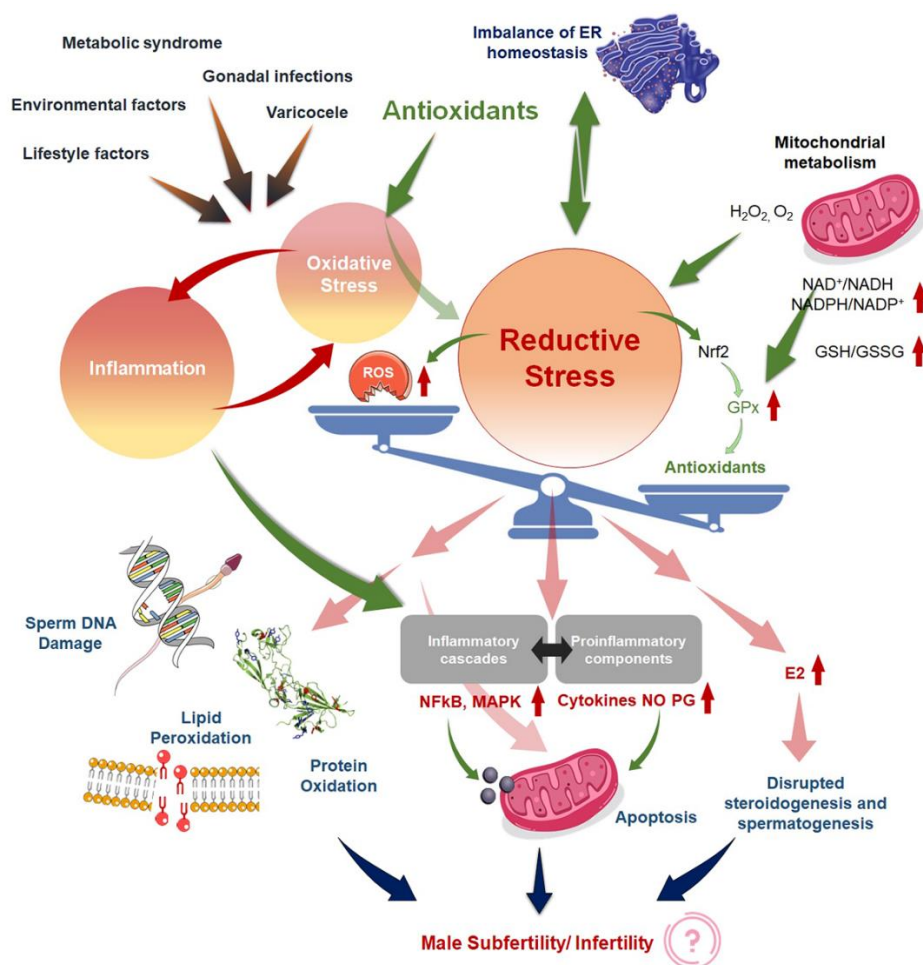


Figure 1. Mechanisms to explain ‘antioxidant paradox’ pertaining to male infertility, both by the induction of reductive stress and the failure to address the interconnected link of oxidative stress (OS)

with inflammation. Various endogenous and exogenous factors may induce OS. OS and inflammatory pathways operate in loops, each triggering the other. Antioxidants act to mitigate excess reactive oxygen species (ROS) to minimize the adverse impacts of OS on male reproductive tissues. If overused, antioxidants may shift the redox scale towards the reductive end causing reductive stress rendering insufficient ROS needed for normal physiological functions of the sperms. Moreover, reductive stress also may revert to OS conditions. Finally, the antioxidants fail to curb the inflammatory responses that may again lead to OS and OS-induced male reproductive disruptions. ER = endoplasmic reticulum; MetS = metabolic syndrome; GSH = reduced glutathione; GSSG = oxidized glutathione; ROS = reactive oxygen species; RNS = reactive nitrogen species; GPx = glutathione peroxidase; Nrf2 = nuclear factor-erythroid factor-2-related factor-2; E2 = estradiol; NFκB = nuclear factor kappa B; MAPK = mitogen activating factor kinase; NO = nitric oxide; PG = prostaglandin.

5. Antioxidants-Induced Reductive Stress

As previously discussed, antioxidant supplementation is one of the most common treatment strategies to address OS-related diseases, including male infertility. Antioxidants, in contrast to oxidants, which are electron acceptors, are chemicals that inhibit oxidation by donating electrons [112]. To sustain homeostasis, the redox potential of the body must be balanced. The equilibrium of ROS, free radical generation, and antioxidant levels must be maintained. To do this, the human body must consume antioxidants through diet, or antioxidant supplements should be used if this is insufficient due to oxidant exposure. Thus, dietary antioxidants must be distinguished from antioxidant supplements, which are well-publicized and easily available over-the-counter. Patients commonly ingest excessive amounts of over-the-counter antioxidants, which are frequently touted as health boosters because they may 'combat' pathological illnesses. Exogenous supplements typically contain large amounts of a few refined antioxidants, for instance, vitamins A, C, E, and lycopene. Furthermore, many common foods already contain a variety of vitamins and antioxidants. As a result, it is conceivable that patients will inadvertently take very high doses of antioxidants, or even excessively high doses [23]. Supplementing with antioxidants, on the other hand, has not always yielded favourable outcomes. Furthermore, several studies have shown that antioxidant consumption has detrimental implications. Vitamin E, for instance, has been reported to elevate all-cause mortality in people who take higher doses [113]. Furthermore, it is widely identified that vitamin A supplement has no anticancer effect, whereas in smokers contradictory results are reported [114]. Vitamin C deficiency have been reported to lead to increased OS and DNA fragmentation [115], while excess of vitamin C, on the other hand, may also induce OS [116]. In summary, these contradictory findings focus the significance of ROS production and antioxidant scavenging for optimal physiological processes, that include regulation of male reproduction. As a result, a delicate cellular redox balance is essential to sustain this 'homeostasis' [28,29].

Studies have demonstrated a considerably good impact of additional intakes of antioxidants, including vitamins C, E, L-carnitine, and/or CoQ10 on sperm DNA integrity and semen parameters [4,117–120]. Supplementing infertile men with antioxidants may advance pregnancy outcomes and reduce live birth rates, according to a study by Showell et al. [121]. Huang and colleagues discovered that seminal OS, which is caused by low antioxidant levels, is associated with male infertility. Antioxidant therapy is proven to be more efficient in the management of idiopathic and varicocele-mediated male infertility [122,123]. Although it is clear that antioxidants are necessary for the prevention of OS, some experts believe that such interventions have little or even adverse impact on sperm maturation and the normal physiological functions of sperm, such as sperm motility, capacitation, and acrosome reactions, which lead to deteriorated semen quality [74,124]. While ROS play an important role in the etiology of several human diseases, larger doses of antioxidant supplements have resulted in paradoxical results, prompting scientists to create the term 'antioxidant paradox' [27]. Once again, this highlights the gap in knowledge about the mechanisms by which antioxidant treatments work.

Since key sperm functions, such as capacitation and acrosome reaction, require a physiological amount of ROS, maintaining the redox balance is important for sperm. Excess antioxidants will quench these effects, preventing sperm from fertilizing oocytes [28,29]. In this regard, elevated antioxidant levels have been associated with the development of mammalian embryos [56,125]. Because these early embryos are generally exposed to an hypoxic environment in the womb, glycolytic energy generation appears to be beneficial for cellular compaction and blastulation [126]. The metabolic shifts may cause variations in the expressions of redox-sensitive transcription factors and genes. Critical embryonic development processes starting from fertilization, genome activation, and cellular differentiation, may be disrupted as a result of this. Reproductive redox biology is still a poorly understood area. Menezo and colleagues investigated whether daily supplementation with a combination of vitamins A, C, and E, selenium, and zinc enhanced sperm DNA damage [69]. Sperm nuclear decondensation, on the other hand, increased, most likely as a result of vitamin C-induced disulphide bonds decrease in protamines [127]. As a result, the chromatin becomes destabilized, resulting in failed fertilization.

As previously mentioned, several of the commonly recommended antioxidants, for instance selenium and vitamins C and E, have been linked to negative health outcomes [128]. There is yet to be any evidence of a selenium deficiency in the scholarly literature. Higher levels of seminal plasma selenium (80 ng/mL) are linked to reduced sperm motility, asthenozoospermia, and high rates of abortion in some studies, whereas 40 and 70 ng/mL of selenium have been found to be ideal for reproductive efficiency (e.g., higher pregnancy outcomes and lower rates of abortion) [129]. In fact, there is mounting indication that the antioxidant or pro-oxidant activities are ultimately dependent on their concentration, even when they are derived from natural sources [130]. Due to the synergistic activity of many herbal antioxidant compounds, when the synergistic molecule is missing, antioxidant therapy may turn from ineffective to highly deleterious [114,131].

Menezo et al. reported that antioxidants, particularly vitamins E and C (400 mg each), zinc (500 µmol), selenium (1 µmol), and carotene (18 mg) when given orally on a daily basis to male partners of couples who could not conceive even after IVF/ICSI (in vitro fertilization/intracytoplasmic sperm injection), showed reduced sperm DNA damage while showing increasing sperm DNA decondensation, potentially leading to asynchronous chromosomal condensation [69]. Capability of vitamin C to disrupt disulphide bonds in sperm DNA protamines may be generating more DNA decondensation in IVF and ICSI patients, resulting in poor pregnancy outcomes [127,132]. Reduced protamine levels will eventually cause nuclear decondensation problems and reduce fertility [69].

In a previous in vitro investigation, vitamin C was found to have both positive and negative dose-related effects on sperm membrane LPO and motility. Vitamin C given at concentrations below 1000 µM, showed improved motility in sperm and decreased LPO, but doses above 1000 µM had adverse effects and resulted in total sperm immobility when given at concentrations over 4000 µM [133]. These studies show that antioxidants have inconsistent effects on male infertility therapy, that is, the effect is dose-dependent [63,65,66,68,69,72,73,77,134]. Moreover, to appropriately address clinical issues of each patient, practitioners would need to be clear about their specific cellular redox status. The problems are (a) lack of established method for testing an individual's seminal redox state, (b) lack of knowledge about the normal redox level, and (c) as a result, there are no generally agreed cutoff values for the same [38,121]. For these reasons, patients are used to consuming antioxidants for the fact that they have been convinced that antioxidants work wonders as antiaging agents and as general health improvers, and moreover, they are also being treated for male infertility by clinicians because OS is thought to be detrimental in this case.

6. Oxidative Stress and Inflammation: Interdependence and Overlaps

Response of the body to any form of tissue damage and injury is inflammation. This process is marked by leukocytes and other immune cells getting transported via circulation to the site of injury or infection in tissues. Enhanced circulation to the inflamed area,

augmented vascular permeability (which permits larger serum substances to penetrate the tissues), and elicited leukocyte infiltration are three key alterations that occur when there is acute inflammation. The failure to eliminate the pathogens causes chronic inflammation, which is characterized by the recruitment and activation of various immune cells, such as the lymphocytes, macrophages, and others, resulting in a synchronized cytokine response. Contrary to acute inflammation, which occurs when tissue regeneration or repair occurs after the irritant is removed, chronic inflammation occurs when inflammation and healing happen at the same time rather than sequentially. Repair is usually a component of chronic inflammation because chronic inflammation is linked to irritants that degrade tissue architecture. Granulation tissue, which is made up of macrophages, fibroblasts, and new blood vessels, is commonly utilized to heal wounds [135].

One of the most pressing issues in modern medicine is the evident link between acute or chronic inflammation and the onset of infertility. With the progression of inflammatory process, there is impaired accessory gland function, sperm transport obstruction, and spermatogenesis dysregulation, all of which may lead to poor semen quality [136,137]. Proinflammatory cytokines operate locally because they are produced by locally activated cells or produced momentarily after an immune stimulus has been initiated [138]. In male gonads, cytokines are also produced physiologically and have been associated with normal functioning of the reproductive tissues [139,140], and thus, are regarded as natural seminal plasma components [141]. Testicular macrophages are the most important source of cytokines in the testes; however, it has been reported that Leydig cells and Sertoli cells also are involved in cytokines generation (interleukins 1 and 6: IL-1 and IL-6) in the male gonad [142]. The migration of leukocytes into tissues after tissue injury is regulated by cytokines, most notably tumour necrosis factor (TNF- α) [143]. TNF- α is produced primarily by macrophages and other mononuclear phagocytes and plays a function in the inflammation and activation of other leukocytes. TNF- α is a pro-inflammatory cytokine that has been linked to the activation of other leukocytes. By generating adhesion molecules and chemokines on the endothelium, TNF- α , for example, promotes the phagocytes' microbial systems. TNF- α also causes apoptosis in cells, which is a cell death. IL-1, like TNF- α , is a critical inflammatory cytokine with functions that are comparable to those of TNF- α [144].

Inflammatory stimuli to the male genital tract results in an increase in the production of ROS. Furthermore, pathogenic bacterial strains that colonize the reproductive tract may cause an increase in the free radical generation, which may result in an inflammatory reaction in the host [145–147]. As free radicals are very reactive compounds with one or more unpaired electrons, they bear the ability to oxidatively damage the biomolecules that they come in contact with. When they react almost rapidly with any chemical in their environment, they trigger a series of reactions that promotes uncontrolled cellular damage [85,148].

Excess ROS must be neutralized on a continual basis to maintain normal cellular activity. When free radicals accumulate in excess, they overwhelm the antioxidant defenses of the male reproductive tract, resulting in damage to the male reproductive system [45,149]. Excess ROS are scavenged by the endogenous antioxidant system under physiological conditions, which helps to maintain the proper redox balance in the body [25]. Antioxidants produced by the body can be either enzymatic, such as catalase (CAT), SOD, or thiol peroxidase, or they can be non-enzymatic, such as glutathione [93]. When the levels of seminal plasma ROS overwhelms the endogenous antioxidant capacity, semen parameters may get adversely affected [25,30,38].

Glutathione peroxidase (GPX) and glutathione S-transferase (GST) are antioxidant genes that are essential for normal spermatogenesis and sperm functions. Other antioxidant genes include *keap1* (Kelch-like ECH associated protein 1), *Nrf2* (nuclear factor erythroid 2 related factor 2), and *SOD* [33,150,151]. In humans, functional polymorphisms in the antioxidant genes *NRF2*, *GST*, *SOD*, *CAT*, and *GPX* have been linked to male infertility [150].

Reduced sperm concentration, motility, and functions have been linked to seminal OS in several studies [6,152–155]. It has been discovered that cytokines, which are oxidative

damage mediators, may also have an effect on the quality of sperm and male fertility at the same time. For example, the concentration of IL-12 is associated with reduced sperm density and motility [156,157]. In an earlier study conducted by the same researchers, it was discovered that the levels of IL-6 are greater in infertile men. Increased levels of certain cytokines in semen are typically related with a decrease in the overall quality of sperm [158].

7. 'Antioxidant Paradox' in the Light of the Interdependent Nature of Oxidative Stress and Inflammation

Antioxidants have failed to provide beneficial effects in human diseases that have been linked to OS for unknown reasons; however, several hypotheses have been advanced [27,159,160]. One possible explanation is that the association between OS and various human diseases is most likely not causal in many, if not all, cases, and as a result, antioxidants are rendered ineffective [159,160]. This argument is perplexing because there is a large body of literature demonstrating that OS plays a role in diseases as well as in male infertility [45,159]. According to another explanation, the type and dose of antioxidants applied in clinical trials may not have been specific enough to alleviate OS in a tissue or cell-specific manner (that is to say 'on target'), and as a result, they either showed no effect or had harmful impacts [159,160]. As a result of the complexity and interdependence of the antioxidant network, this reasoning seems to be a valid explanation of 'antioxidant paradox'. Examples include the antioxidant enzyme SOD, which can catalyze $O_2^{\bullet-}$ but also generate various other free radicals as byproducts [39]. Vitamin E acts upon the cell membrane, whereas vitamin C works both extracellularly and intracellularly in aqueous media. Vitamin C, when consumed in higher or lesser amounts than the standard daily recommendation, are associated with free radical damage to DNA [161–163]. Moreover, the inability of antioxidants to produce beneficial health effects may be because antioxidants can produce harmful effects in some situations, such as in established cancer, where a sufficient amount of ROS is required to induce apoptosis of malignant cells [164]. According to a third explanation, the lack of an appropriate method of quantifying redox status has resulted in many clinical trials being inconclusive, which does not imply that antioxidants are ineffective [106]. Although the method of quantifying redox status in humans is far from perfect, many clinical trials have found that the redox status was not measured prior to the start of and after the completion of antioxidant therapy [27,160]. It should be noted that measuring one or several pro- and antioxidant markers may not provide a comprehensive measure of the redox status of the target organ or tissue, and that the systemic redox status may not accurately reflect the status of the target tissue.

A new explanation for the antioxidant paradox may be proposed in light of the interdependent nature of OS and inflammation discussed in this review. The failures of antioxidant clinical trials may be attributed to the selection of antioxidants that do not simultaneously inhibit both OS and inflammation, or to the use of nonselective agents that block some oxidative and/or inflammatory pathways while exacerbating the effects of others. It would be necessary to quantify both the redox and the inflammatory status before, during, and after the antioxidant therapy in order to establish the validity of this explanation. However, a recent study by Djuric et al. [165] has provided experimental evidence as to why a nonselective antioxidant that acts by inhibiting ROS generation and thereby nuclear factor kappa B (NF- κ B) activation may fail. In a psychosocial stress-induced atherosclerotic animal model, selective targeting of NF- κ B subunit cRel while maintaining the activity of the p50/p65 subunits provided an antiatherosclerotic effect by limiting the proinflammatory effect of NF- κ B without impairing its anti-inflammatory and antioxidant functions [126]. Due to this finding, a nonselective antioxidant or anti-inflammatory agent that inhibits all the subunits of NF- κ B are unlikely to be effective in treating the disease, and a selective inhibitor is required to be effective. Furthermore, this finding lends support to the idea that the interdependence between OS and inflammation,

at least in some cases, may be a reasonable explanation for the antioxidant paradox, which has been previously proposed.

8. Conclusions

Seminal ROS is important to mediate normal sperm functions. However, when their concentrations overwhelm the endogenous antioxidant capacity, OS is induced causing cellular oxidative damage. Thus, antioxidant treatment finds significant relevance in the case of idiopathic male infertility or subfertility. However, often owing to inaccurate OS detection in male infertility cases, antioxidant treatment becomes arbitrary and leads to antioxidant overuse. This may induce 'reductive stress' that depletes ROS levels taking them below physiological levels critical for normal sperm functions. Moreover, reductive stress may also induce higher ROS generation, in turn causing OS. This phenomenon may partially explain the 'antioxidant paradox'. Moreover, inflammation and OS are closely linked pathologies and establish a vicious loop impairing male fertility. These raise questions regarding antioxidants therapy in treating male infertility and highlights another mechanism to address 'antioxidant paradox'. Failure in choosing treatment targeting inflammation and OS both simultaneously may be one of the main limitations in treating OS-induced male infertility. Research on combination therapies, including both antioxidant and anti-inflammatory agents simultaneously mitigating both OS and inflammatory pathways, may lead to an effective cure for OS-mediated male infertility.

Author Contributions: Conceptualization, data curation, writing—original draft preparation, S.D., P.S. (Pallav Sengupta), S.R. and S.C.; writing—review and editing, C.W.W. and P.S. (Petr Sláma). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AAS = anabolic–androgenic steroids; CoQ10 = coenzyme Q10; E2 = estradiol; ER = endoplasmic reticulum; DFI = DNA fragmentation index; ESHRE = European Society for Human Reproduction and Embryology; FDA = Food and Drug Administration; GSH = reduced glutathione; GP_x = glutathione peroxidase; GSSG = oxidized glutathione; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; keap1 = Kelch-like ECH associated protein 1; LPO = lipid peroxidation; MAPK = mitogen activating factor kinase; MetS = metabolic syndrome; NFκB = nuclear factor kappa B; NO = nitric oxide; Nrf2 = nuclear factor-erythroid factor-2-related factor-2; PG = prostaglandin; PUFA = polyunsaturated fatty acids; ORP = oxidation-reduction potential; OS = oxidative stress; RNS = reactive nitrogen species; ROS = reactive oxygen species; SOD = superoxide dismutase; UPR^{ER} = unfolded protein response of the endoplasmic reticulum.

References

1. Agarwal, A.; Mulgund, A.; Hamada, A.; Chyatte, M.R. A unique view on male infertility around the globe. *Reprod. Biol. Endocrinol.* **2015**, *13*, 37. [\[CrossRef\]](#)
2. Vander Borgh, M.; Wyns, C. Fertility and infertility: Definition and epidemiology. *Clin. Biochem.* **2018**, *62*, 2–10. [\[CrossRef\]](#)
3. Aktan, G.; Doğru-Abbasoğlu, S.; Küçükgergin, C.; Kadioğlu, A.; Özdemirler-Erata, G.; Koçak-Toker, N. Mystery of idiopathic male infertility: Is oxidative stress an actual risk? *Fertil. Steril.* **2013**, *99*, 1211–1215. [\[CrossRef\]](#)
4. Alahmar, A.T.; Calogero, A.E.; Sengupta, P.; Dutta, S. Coenzyme q10 improves sperm parameters, oxidative stress markers and sperm DNA fragmentation in infertile patients with idiopathic oligoasthenozoospermia. *World J. Men's Health* **2021**, *39*, 346–351. [\[CrossRef\]](#)
5. Alahmar, A.T.; Sengupta, P. Impact of coenzyme q10 and selenium on seminal fluid parameters and antioxidant status in men with idiopathic infertility. *Biol. Trace Elem. Res.* **2021**, *199*, 1246–1252. [\[CrossRef\]](#)
6. Agarwal, A.; Sengupta, P. Oxidative stress and its association with male infertility. In *Male Infertility*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 57–68.

7. Sengupta, P.; Dutta, S.; Krajewska-Kulak, E. The disappearing sperms: Analysis of reports published between 1980 and 2015. *Am. J. Men's Health* **2017**, *11*, 1279–1304. [[CrossRef](#)]
8. Pant, P.R. Factors affecting male infertility. *J. Inst. Med.* **2009**, *31*, 10–12. [[CrossRef](#)]
9. Lettieri, G.; D'Agostino, G.; Mele, E.; Cardito, C.; Esposito, R.; Cimmino, A.; Giarra, A.; Trifuoggi, M.; Raimondo, S.; Notari, T.; et al. Discovery of the involvement in DNA oxidative damage of human sperm nuclear basic proteins of healthy young men living in polluted areas. *Int. J. Mol. Sci.* **2020**, *21*, 4198. [[CrossRef](#)] [[PubMed](#)]
10. Lettieri, G.; Marra, F.; Moriello, C.; Prisco, M.; Notari, T.; Trifuoggi, M.; Giarra, A.; Bosco, L.; Montano, L.; Piscopo, M. Molecular alterations in spermatozoa of a family case living in the land of fires. A first look at possible transgenerational effects of pollutants. *Int. J. Mol. Sci.* **2020**, *21*, 6710. [[CrossRef](#)] [[PubMed](#)]
11. Sharma, R.; Harlev, A.; Agarwal, A.; Esteves, S.C. Cigarette smoking and semen quality: A new meta-analysis examining the effect of the 2010 world health organization laboratory methods for the examination of human semen. *Eur. Urol.* **2016**, *70*, 635–645. [[CrossRef](#)] [[PubMed](#)]
12. Bundhun, P.K.; Janoo, G.; Bhurtu, A.; Teeluck, A.R.; Soogund, M.Z.S.; Pursun, M.; Huang, F. Tobacco smoking and semen quality in infertile males: A systematic review and meta-analysis. *BMC Public Health* **2019**, *19*, 36. [[CrossRef](#)]
13. Kovac, J.R.; Khanna, A.; Lipshultz, L.I. The effects of cigarette smoking on male fertility. *Postgrad. Med.* **2015**, *127*, 338–341. [[CrossRef](#)] [[PubMed](#)]
14. Ramgir, S.S.; Abilash, V. Impact of smoking and alcohol consumption on oxidative status in male infertility and sperm quality. *Indian J. Pharm. Sci.* **2019**, *81*, 933–945. [[CrossRef](#)]
15. Muthusami, K.; Chinnaswamy, P. Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertil. Steril.* **2005**, *84*, 919–924. [[CrossRef](#)] [[PubMed](#)]
16. Durairajanayagam, D. Lifestyle causes of male infertility. *Arab J. Urol.* **2018**, *16*, 10–20. [[CrossRef](#)]
17. Fronczak, C.M.; Kim, E.D.; Barqawi, A.B. The insults of illicit drug use on male fertility. *J. Androl.* **2012**, *33*, 515–528. [[CrossRef](#)]
18. Sansone, A.; Di Dato, C.; de Angelis, C.; Menafrà, D.; Pozza, C.; Pivonello, R.; Isidori, A.; Gianfrilli, D. Smoke, alcohol and drug addiction and male fertility. *Reprod. Biol. Endocrinol.* **2018**, *16*, 3. [[CrossRef](#)] [[PubMed](#)]
19. Darbandi, M.; Darbandi, S.; Agarwal, A.; Baskaran, S.; Dutta, S.; Sengupta, P.; Khorshid, H.R.K.; Esteves, S.; Gilany, K.; Hedayati, M. Reactive oxygen species-induced alterations in h19-igf2 methylation patterns, seminal plasma metabolites, and semen quality. *J. Assist. Reprod. Genet.* **2019**, *36*, 241–253. [[CrossRef](#)]
20. Dutta, S.; Biswas, A.; Sengupta, P. Obesity, endocrine disruption and male infertility. *Asian Pac. J. Reprod.* **2019**, *8*, 195–202. [[CrossRef](#)]
21. Leisegang, K.; Dutta, S. Do lifestyle practices impede male fertility? *Andrologia* **2021**, *53*, e13595. [[CrossRef](#)]
22. Sengupta, P.; Dutta, S.; Karkada, I.; Chinni, S. Endocrinopathies and male infertility. *Life* **2022**, *12*, 10. [[CrossRef](#)]
23. Poljsak, B.; Šuput, D. Ros and antioxidants: Achieving the balance between when to use the synthetic antioxidants. *Oxidat. Med. Cell. Long.* **2013**, *2013*, 956792. [[CrossRef](#)]
24. Sheweita, S.A.; Tilmisany, A.M.; Al-Sawaf, H. Mechanisms of male infertility: Role of antioxidants. *Curr. Drug Metab.* **2005**, *6*, 495–501. [[CrossRef](#)] [[PubMed](#)]
25. Agarwal, A.; Sekhon, L.H. The role of antioxidant therapy in the treatment of male infertility. *Hum. Fertil.* **2010**, *13*, 217–225. [[CrossRef](#)] [[PubMed](#)]
26. Pérez-Torres, I.; Guarner-Lans, V.; Rubio-Ruiz, M.E. Reductive stress in inflammation-associated diseases and the pro-oxidant effect of antioxidant agents. *Int. J. Mol. Sci.* **2017**, *18*, 2098. [[CrossRef](#)] [[PubMed](#)]
27. Halliwell, B. The antioxidant paradox. *Lancet* **2000**, *355*, 1179–1180. [[CrossRef](#)]
28. De Lamirande, E.; Gagnon, C. Impact of reactive oxygen species on spermatozoa: A balancing act between beneficial and detrimental effects. *Hum. Reprod.* **1995**, *10*, 15–21. [[CrossRef](#)]
29. Kothari, S.; Thompson, A.; Agarwal, A.; du Plessis, S.S. Free radicals: Their beneficial and detrimental effects on sperm function. *Indian J. Exp. Biol.* **2010**, *48*, 425–435.
30. Henkel, R.; Sandhu, I.S.; Agarwal, A. The excessive use of antioxidant therapy: A possible cause of male infertility? *Andrologia* **2019**, *51*, e13162. [[CrossRef](#)]
31. Dutta, S.; Sengupta, P.; Slama, P.; Roychoudhury, S. Oxidative stress, testicular inflammatory pathways, and male reproduction. *Int. J. Mol. Sci.* **2021**, *22*, 10043. [[CrossRef](#)]
32. Izuka, E.; Menuba, I.; Sengupta, P.; Dutta, S.; Nwagha, U. Antioxidants, anti-inflammatory drugs and antibiotics in the treatment of reproductive tract infections and their association with male infertility. *Chem. Biol. Lett.* **2020**, *7*, 156–165.
33. Darbandi, M.; Darbandi, S.; Agarwal, A.; Baskaran, S.; Sengupta, P.; Dutta, S.; Mokarram, P.; Saliminejad, K.; Sadeghi, M.R. Oxidative stress-induced alterations in seminal plasma antioxidants: Is there any association with keap1 gene methylation in human spermatozoa? *Andrologia* **2019**, *51*, e13159. [[CrossRef](#)]
34. Biswas, S.K. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid. Med. Cell. Longev.* **2016**, *2016*, 5698931. [[CrossRef](#)] [[PubMed](#)]
35. Sengupta, P.; Dutta, S. Hormones in male reproduction and fertility. *Asian Pac. J. Reprod.* **2019**, *8*, 187–188. [[CrossRef](#)]
36. Zhou, W.; De Iulii, G.N.; Dun, M.D.; Nixon, B. Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. *Front. Endocrinol.* **2018**, *9*, 59. [[CrossRef](#)]

37. Dutta, S.; Henkel, R.; Sengupta, P.; Agarwal, A. Physiological role of ros in sperm function. In *Male Infertility*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 337–345.
38. Agarwal, A.; Prabakaran, S.A. Oxidative stress and antioxidants in male infertility: A difficult balance. *Int. J. Reprod. Med.* **2005**, *3*, 1–8.
39. Halliwell, B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* **2006**, *141*, 312–322. [[CrossRef](#)]
40. O’Flaherty, C. Redox regulation of mammalian sperm capacitation. *Asian J. Androl.* **2015**, *17*, 583. [[CrossRef](#)]
41. O’Flaherty, C. The enzymatic antioxidant system of human spermatozoa. *Adv. Androl.* **2014**, *2014*, 626374. [[CrossRef](#)]
42. Yan, L.; Liu, J.; Wu, S.; Zhang, S.; Ji, G.; Gu, A. Seminal superoxide dismutase activity and its relationship with semen quality and sod gene polymorphism. *J. Assist. Reprod. Genet.* **2014**, *31*, 549–554. [[CrossRef](#)] [[PubMed](#)]
43. Fridovich, I. Superoxide radical and superoxide dismutases. *Ann. Rev. Biochem.* **1995**, *64*, 97–112. [[CrossRef](#)] [[PubMed](#)]
44. Kolesnikova, L.; Kurashova, N.; Bairova, T.; Dolgikh, M.; Ershova, O.; Dashiev, B.; Korytov, L.; Koroleva, N. Role of glutathione-s-transferase family genes in male infertility. *Bull. Exp. Biol. Med.* **2017**, *163*, 643–645. [[CrossRef](#)]
45. Agarwal, A.; Leisegang, K.; Sengupta, P. Oxidative stress in pathologies of male reproductive disorders. In *Pathology*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 15–27.
46. Sabeti, P.; Pourmasumi, S.; Rahiminia, T.; Akyash, F.; Talebi, A.R. Etiologies of sperm oxidative stress. *Int. J. Reprod. BioMed.* **2016**, *14*, 231. [[CrossRef](#)]
47. Muratori, M.; Tamburrino, L.; Marchiani, S.; Cambi, M.; Olivito, B.; Azzari, C.; Forti, G.; Baldi, E. Investigation on the origin of sperm DNA fragmentation: Role of apoptosis, immaturity and oxidative stress. *Mol. Med.* **2015**, *21*, 109–122. [[CrossRef](#)] [[PubMed](#)]
48. Montano, L.; Ceretti, E.; Donato, F.; Bergamo, P.; Zani, C.; Viola, G.C.V.; Notari, T.; Pappalardo, S.; Zani, D.; Ubaldi, S.; et al. Effects of a lifestyle change intervention on semen quality in healthy young men living in highly polluted areas in Italy: The fast randomized controlled trial. *Eur. Urol. Focus*, 2021; *in press*. [[CrossRef](#)] [[PubMed](#)]
49. Halliwell, B. Antioxidant defence mechanisms: From the beginning to the end (of the beginning). *Free Rad. Res.* **1999**, *31*, 261–272. [[CrossRef](#)] [[PubMed](#)]
50. Willcox, J.K.; Ash, S.L.; Catignani, G.L. Antioxidants and prevention of chronic disease. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 275–295. [[CrossRef](#)]
51. Barratt, C.L.; Björndahl, L.; De Jonge, C.J.; Lamb, D.J.; Osorio Martini, F.; McLachlan, R.; Oates, R.D.; van der Poel, S.; St John, B.; Sigman, M. The diagnosis of male infertility: An analysis of the evidence to support the development of global who guidance—challenges and future research opportunities. *Hum. Reprod. Update* **2017**, *23*, 660–680. [[CrossRef](#)]
52. Bjelakovic, G.; Nikolova, D.; Gluud, L.L.; Simonetti, R.G.; Gluud, C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. *J. Am. Med. Assoc.* **2007**, *297*, 842–857. [[CrossRef](#)]
53. Bjelakovic, G.; Nikolova, D.; Simonetti, R.G.; Gluud, C. Antioxidant supplements for prevention of gastrointestinal cancers: A systematic review and meta-analysis. *Lancet* **2004**, *364*, 1219–1228. [[CrossRef](#)]
54. Stanner, S.; Hughes, J.; Kelly, C.; Buttriss, J. A review of the epidemiological evidence for the ‘antioxidant hypothesis’. *Pub. Health Nutr.* **2004**, *7*, 407–422. [[CrossRef](#)]
55. Bjelakovic, G.; Nikolova, D.; Gluud, L.L.; Simonetti, R.G.; Gluud, C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochr. Datab. Syst. Rev.* **2012**, *2012*, CD007176. [[CrossRef](#)]
56. Giussani, D.A.; Niu, Y.; Herrera, E.A.; Richter, H.G.; Camm, E.J.; Thakor, A.S.; Kane, A.D.; Hansell, J.A.; Brain, K.L.; Skeffington, K.L.; et al. Heart disease link to fetal hypoxia and oxidative stress. *Adv. Fetal Neonat. Physiol.* **2014**, *814*, 77–87.
57. Busetto, G.; Agarwal, A.; Virmani, A.; Antonini, G.; Ragonesi, G.; Del Giudice, F.; Micic, S.; Gentile, V.; De Berardinis, E. Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study. *Andrologia* **2018**, *50*, e12927. [[CrossRef](#)]
58. Sengupta, P.; Agarwal, A.; Pogrebetskaya, M.; Roychoudhury, S.; Durairajanayagam, D.; Henkel, R. Role of withania somnifera (ashwagandha) in the management of male infertility. *Reprod. Biomed. Online* **2018**, *36*, 311–326. [[CrossRef](#)]
59. Torres-Arce, E.; Vizmanos, B.; Babio, N.; Márquez-Sandoval, F.; Salas-Huetos, A. Dietary antioxidants in the treatment of male infertility: Counteracting oxidative stress. *Biology* **2021**, *10*, 241. [[CrossRef](#)] [[PubMed](#)]
60. Venditti, M.; Ben Rhouma, M.; Romano, M.Z.; Messaoudi, I.; Reiter, R.J.; Minucci, S. Evidence of melatonin ameliorative effects on the blood-testis barrier and sperm quality alterations induced by cadmium in the rat testis. *Ecotoxicol. Env. Saf.* **2021**, *226*, 112878. [[CrossRef](#)] [[PubMed](#)]
61. Venditti, M.; Ben Rhouma, M.; Romano, M.Z.; Messaoudi, I.; Reiter, R.J.; Minucci, S. Altered expression of daam1 and prep induced by cadmium toxicity is counteracted by melatonin in the rat testis. *Genes* **2021**, *12*, 1016. [[CrossRef](#)] [[PubMed](#)]
62. Tikkiwal, M.; Ajmera, R.L.; Mathur, N.K. Effect of zinc administration on seminal zinc and fertility of oligospermic males. *Indian J. Physiol. Pharmacol.* **1987**, *31*, 30–34.
63. Kessopoulou, E.; Powers, H.J.; Sharma, K.K.; Pearson, M.J.; Russell, J.M.; Cooke, I.D.; Barratt, C.L. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin e to treat reactive oxygen species associated male infertility. *Fertil. Steril.* **1995**, *64*, 825–831. [[CrossRef](#)]
64. Omu, A.E.; Dashti, H.; Al-Othman, S. Treatment of asthenozoospermia with zinc sulphate: Andrological, immunological and obstetric outcome. *Eur. J. Obs. Gynecol. Reprod. Biol.* **1998**, *79*, 179–184. [[CrossRef](#)]

65. Rolf, C.; Cooper, T.; Yeung, C.; Nieschlag, E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin c and vitamin e: A randomized, placebo-controlled, double-blind study. *Hum. Reprod.* **1999**, *14*, 1028–1033. [[CrossRef](#)] [[PubMed](#)]
66. Greco, E.; Iacobelli, M.; Rienzi, L.; Ubaldi, F.; Ferrero, S.; Tesarik, J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J. Androl.* **2005**, *26*, 349–353. [[CrossRef](#)]
67. Balercia, G.; Regoli, F.; Armeni, T.; Koverech, A.; Mantero, F.; Boscaro, M. Placebo-controlled double-blind randomized trial on the use of l-carnitine, l-acetylcarnitine, or combined l-carnitine and l-acetylcarnitine in men with idiopathic asthenozoospermia. *Fertil. Steril.* **2005**, *84*, 662–671. [[CrossRef](#)] [[PubMed](#)]
68. Sigman, M.; Glass, S.; Campagnone, J.; Pryor, J.L. Carnitine for the treatment of idiopathic asthenospermia: A randomized, double-blind, placebo-controlled trial. *Fertil. Steril.* **2006**, *85*, 1409–1414. [[CrossRef](#)] [[PubMed](#)]
69. Ménézo, Y.J.; Hazout, A.; Panteix, G.; Robert, F.; Rollet, J.; Cohen-Bacrie, P.; Chapuis, F.; Clément, P.; Benkhalifa, M. Antioxidants to reduce sperm DNA fragmentation: An unexpected adverse effect. *Reprod. Biomed. Online* **2007**, *14*, 418–421. [[CrossRef](#)]
70. Tremellen, K.; Miari, G.; Froiland, D.; Thompson, J. A randomised control trial examining the effect of an antioxidant (menevit) on pregnancy outcome during ivf-icsi treatment. *Austr. N. Z. J. Obs. Gynaecol.* **2007**, *47*, 216–221. [[CrossRef](#)] [[PubMed](#)]
71. Hawkes, W.C.; Alkan, Z.; Wong, K. Selenium supplementation does not affect testicular selenium status or semen quality in north american men. *J. Androl.* **2009**, *30*, 525–533. [[CrossRef](#)]
72. Tunc, O.; Thompson, J.; Tremellen, K. Improvement in sperm DNA quality using an oral antioxidant therapy. *Reprod. Biomed. Online* **2009**, *18*, 761–768. [[CrossRef](#)]
73. Gual-Frau, J.; Abad, C.; Amengual, M.J.; Hannaoui, N.; Checa, M.A.; Ribas-Maynou, J.; Lozano, I.; Nikolaou, A.; Benet, J.; García-Peiró, A. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade i varicocele patients. *Hum. Fertil.* **2015**, *18*, 225–229. [[CrossRef](#)]
74. Stenqvist, A.; Oleszczuk, K.; Leijonhufvud, I.; Giwercman, A. Impact of antioxidant treatment on DNA fragmentation index: A double-blind placebo-controlled randomized trial. *Andrology* **2018**, *6*, 811–816. [[CrossRef](#)]
75. Safarinejad, M.R.; Safarinejad, S.; Shafiei, N.; Safarinejad, S. Effects of the reduced form of coenzyme q10 (ubiquinol) on semen parameters in men with idiopathic infertility: A double-blind, placebo controlled, randomized study. *J. Urol.* **2012**, *188*, 526–531. [[CrossRef](#)] [[PubMed](#)]
76. Micic, S.; Lalic, N.; Djordjevic, D.; Bojanic, N.; Bogavac-Stanojevic, N.; Busetto, G.M.; Virmani, A.; Agarwal, A. Double-blind, randomised, placebo-controlled trial on the effect of l-carnitine and l-acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. *Andrologia* **2019**, *51*, e13267. [[CrossRef](#)] [[PubMed](#)]
77. Schisterman, E.F.; Sjaarda, L.A.; Clemons, T.; Carrell, D.T.; Perkins, N.J.; Johnstone, E.; Lamb, D.; Chaney, K.; Van Voorhis, B.J.; Ryan, G. Effect of folic acid and zinc supplementation in men on semen quality and live birth among couples undergoing infertility treatment: A randomized clinical trial. *J. Am. Med. Assoc.* **2020**, *323*, 35–48. [[CrossRef](#)]
78. Greabu, M.; Battino, M.; Mohora, M.; Olinescu, R.; Totan, A.; Didilescu, A. Oxygen, a paradoxical element. *Rom. J. Intern. Med.* **2008**, *46*, 125–135.
79. Bioveris, A.; Chance, B. The mitochondrial generation of hydrogen peroxide. *Biochem. J.* **1973**, *134*, 707. [[CrossRef](#)]
80. Halliwell, B. Free radicals and antioxidants—quo vadis? *Trends Pharm. Sci.* **2011**, *32*, 125–130. [[CrossRef](#)]
81. Hayyan, M.; Hashim, M.A.; AlNashef, I.M. Superoxide ion: Generation and chemical implications. *Chem. Rev.* **2016**, *116*, 3029–3085. [[CrossRef](#)] [[PubMed](#)]
82. Turrens, J.F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **2003**, *552*, 335–344. [[CrossRef](#)]
83. Raha, S.; Robinson, B.H. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem. Sci.* **2000**, *25*, 502–508. [[CrossRef](#)]
84. Halliwell, B.; Gutteridge, J.M. *Free Radicals in Biology and Medicine*; Oxford University Press: New York, NY, USA, 2015.
85. Agarwal, A.; Saleh, R.A.; Bedaiwy, M.A. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil. Steril.* **2003**, *79*, 829–843. [[CrossRef](#)]
86. John Aitken, R.; Clarkson, J.S.; Fishel, S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biol. Reprod.* **1989**, *41*, 183–197. [[CrossRef](#)] [[PubMed](#)]
87. Li, X.; Fang, P.; Mai, J.; Choi, E.T.; Wang, H.; Yang, X.-F. Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *J. Hematol. Oncol.* **2013**, *6*, 19. [[CrossRef](#)]
88. Dröge, W. Free radicals in the physiological control of cell function. *Physiol. Rev.* **2002**, *82*, 47–95. [[CrossRef](#)] [[PubMed](#)]
89. Hampton, M.B.; Kettle, A.J.; Winterbourn, C.C. Inside the neutrophil phagosome: Oxidants, myeloperoxidase, and bacterial killing. *Blood* **1998**, *92*, 3007–3017. [[CrossRef](#)]
90. Sengupta, P.; Dutta, S.; Alahmar, A.T.; D’souza, U.J.A. Reproductive tract infection, inflammation and male infertility. *Chem. Biol. Lett.* **2020**, *7*, 75–84.
91. O’Flaherty, C.N.; de Lamirande, E.; Gagnon, C. Reactive oxygen species and protein kinases modulate the level of phospho-mek-like proteins during human sperm capacitation. *Biol. Reprod.* **2005**, *73*, 94–105. [[CrossRef](#)]
92. Garratt, M.; Bathgate, R.; de Graaf, S.P.; Brooks, R.C. Copper-zinc superoxide dismutase deficiency impairs sperm motility and in vivo fertility. *Reproduction* **2013**, *146*, 297–304. [[CrossRef](#)]
93. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44–84. [[CrossRef](#)]
94. Kahn, B.E.; Brannigan, R.E. Obesity and male infertility. *Curr. Opin. Urol.* **2017**, *27*, 441–445. [[CrossRef](#)] [[PubMed](#)]

95. Tsatsanis, C.; Dermitzaki, E.; Avgoustinaki, P.; Malliaraki, N.; Mytaras, V.; Margioris, A.N. The impact of adipose tissue-derived factors on the hypothalamic-pituitary-gonadal (hpg) axis. *Hormones* **2015**, *14*, 549–562. [[CrossRef](#)]
96. Bhattacharya, K.; Sengupta, P.; Dutta, S.; Karkada, I.R. Obesity, systemic inflammation and male infertility. *Chem. Biol. Lett.* **2020**, *7*, 92–98.
97. Irez, T.; Bicer, S.; Sahin, E.; Dutta, S.; Sengupta, P. Cytokines and adipokines in the regulation of spermatogenesis and semen quality. *Chem. Biol. Lett.* **2020**, *7*, 131–139.
98. Mathur, P.P.; Huang, L.; Kashou, A.; Vaithinathan, S.; Agarwal, A. Environmental toxicants and testicular apoptosis. *Open Reprod. Sci. J.* **2011**, *3*, 114–124.
99. Sharma, R.; Biedenharn, K.R.; Fedor, J.M.; Agarwal, A. Lifestyle factors and reproductive health: Taking control of your fertility. *Reprod. Biol. Endocrinol.* **2013**, *11*, 66. [[CrossRef](#)]
100. Wendel, A. Measurement of in vivo lipid peroxidation and toxicological significance. *Free Rad. Biol. Med.* **1987**, *3*, 355–358. [[CrossRef](#)]
101. Castagne, V.; Lefevre, K.; Natero, R.; Becker, D.; Clarke, P. An optimal redox status for the survival of axotomized ganglion cells in the developing retina. *Neuroscience* **1999**, *93*, 313–320. [[CrossRef](#)]
102. Symeonidis, E.N.; Evgeni, E.; Palapelas, V.; Koumasi, D.; Pyrgidis, N.; Sokolakis, I.; Hatzichristodoulou, G.; Tsiampali, C.; Mykoniatis, I.; Zachariou, A.; et al. Redox balance in male infertility: Excellence through moderation—“μέτρον ἄριστον”. *Antioxidants* **2021**, *10*, 1534. [[CrossRef](#)] [[PubMed](#)]
103. Panner Selvam, M.K.; Agarwal, A.; Henkel, R.; Finelli, R.; Robert, K.A.; Iovine, C.; Baskaran, S. The effect of oxidative and reductive stress on semen parameters and functions of physiologically normal human spermatozoa. *Free Rad. Biol. Med.* **2020**, *152*, 375–385. [[CrossRef](#)]
104. Bjørklund, G.; Chirumbolo, S. Role of oxidative stress and antioxidants in daily nutrition and human health. *Nutrition* **2017**, *33*, 311–321. [[CrossRef](#)] [[PubMed](#)]
105. Zhang, H.; Limphong, P.; Pieper, J.; Liu, Q.; Rodesch, C.K.; Christians, E.; Benjamin, I.J. Glutathione-dependent reductive stress triggers mitochondrial oxidation and cytotoxicity. *FASEB J.* **2012**, *26*, 1442–1451. [[CrossRef](#)]
106. Murphy, M.P. How mitochondria produce reactive oxygen species. *Biochem. J.* **2009**, *417*, 1–13. [[CrossRef](#)]
107. Walter, P.; Ron, D. The unfolded protein response: From stress pathway to homeostatic regulation. *Science* **2011**, *334*, 1081–1086. [[CrossRef](#)]
108. Korge, P.; Calmettes, G.; Weiss, J.N. Increased reactive oxygen species production during reductive stress: The roles of mitochondrial glutathione and thioredoxin reductases. *Biochim. Biophys. Acta* **2015**, *1847*, 514–525. [[CrossRef](#)]
109. Klein, E.A.; Thompson, I.M.; Tangen, C.M.; Crowley, J.J.; Lucia, M.S.; Goodman, P.J.; Minasian, L.M.; Ford, L.G.; Parnes, H.L.; Gaziano, J.M. Vitamin e and the risk of prostate cancer: The selenium and vitamin e cancer prevention trial (select). *J. Am. Med. Assoc.* **2011**, *306*, 1549–1556. [[CrossRef](#)]
110. Brewer, A.C.; Mustafi, S.B.; Murray, T.V.; Rajasekaran, N.S.; Benjamin, I.J. Reductive stress linked to small hsps, g6pd, and nrf2 pathways in heart disease. *Antioxid. Redox Signal.* **2013**, *18*, 1114–1127. [[CrossRef](#)] [[PubMed](#)]
111. Mentor, S.; Fisher, D. Aggressive antioxidant reductive stress impairs brain endothelial cell angiogenesis and blood brain barrier function. *Curr. Neurovas. Res.* **2017**, *14*, 71–81. [[CrossRef](#)] [[PubMed](#)]
112. Atta, E.M.; Mohamed, N.H.; Abdelgawad, A.A. Antioxidants: An overview on the natural and synthetic types. *Eur. Chem. Bull.* **2017**, *6*, 365–375. [[CrossRef](#)]
113. Miller, E.R., III; Pastor-Barriuso, R.; Dalal, D.; Riemersma, R.A.; Appel, L.J.; Guallar, E. Meta-analysis: High-dosage vitamin e supplementation may increase all-cause mortality. *Ann. Int. Med.* **2005**, *142*, 37–46. [[CrossRef](#)]
114. Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin e and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Eng. J. Med.* **1994**, *330*, 1029–1035. [[CrossRef](#)]
115. Fraga, C.G.; Motchnik, P.A.; Shigenaga, M.K.; Helbock, H.J.; Jacob, R.A.; Ames, B.N. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Nat. Acad. Sci. USA* **1991**, *88*, 11003–11006. [[CrossRef](#)] [[PubMed](#)]
116. Aruoma, O.I.; Halliwell, B.; Gajewski, E.; Dizdaroglu, M. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. *Biochem. J.* **1991**, *273*, 601–604. [[CrossRef](#)] [[PubMed](#)]
117. Abad, C.; Amengual, M.; Gosálvez, J.; Coward, K.; Hannaoui, N.; Benet, J.; García-Peiró, A.; Prats, J. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. *Andrologia* **2013**, *45*, 211–216. [[CrossRef](#)] [[PubMed](#)]
118. Ahmadi, S.; Bashiri, R.; Ghadiri-Anari, A.; Nadjarzadeh, A. Antioxidant supplements and semen parameters: An evidence based review. *Int. J. Reprod. BioMed.* **2016**, *14*, 729. [[CrossRef](#)]
119. Alahmar, A.T.; Sengupta, P.; Dutta, S.; Calogero, A.E. Coenzyme q10, oxidative stress markers, and sperm DNA damage in men with idiopathic oligoasthenoteratospermia. *Clin. Exp. Reprod. Med.* **2021**, *48*, 150–155. [[CrossRef](#)]
120. Alahmar, A.T.; Calogero, A.E.; Singh, R.; Cannarella, R.; Sengupta, P.; Dutta, S. Coenzyme q10, oxidative stress, and male infertility: A review. *Clin. Exp. Reprod. Med.* **2021**, *48*, 97–104. [[CrossRef](#)]
121. Showell, M.G.; Mackenzie-Proctor, R.; Brown, J.; Yazdani, A.; Stankiewicz, M.T.; Hart, R.J. Antioxidants for male subfertility. *Cochr. Datab. Syst. Rev.* **2014**, *12*, CD007411. [[CrossRef](#)]
122. Garg, H.; Kumar, R. An update on the role of medical treatment including antioxidant therapy in varicocele. *Asian J. Androl.* **2016**, *18*, 222.

123. Alahmar, A.T. The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia. *Clin. Exp. Reprod. Med.* **2018**, *45*, 57. [[CrossRef](#)]
124. Silver, E.W.; Eskenazi, B.; Evenson, D.P.; Block, G.; Young, S.; Wyrobek, A.J. Effect of antioxidant intake on sperm chromatin stability in healthy nonsmoking men. *J. Androl.* **2005**, *26*, 550–556. [[CrossRef](#)]
125. Ufer, C.; Wang, C.C.; Borchert, A.; Heydeck, D.; Kuhn, H. Redox control in mammalian embryo development. *Antiox. Redox Signal.* **2010**, *13*, 833–875. [[CrossRef](#)] [[PubMed](#)]
126. Harvey, A.J.; Kind, K.L.; Thompson, J.G. Redox regulation of early embryo development. *Reproduction* **2002**, *123*, 479–486. [[CrossRef](#)]
127. Giustarini, D.; Dalle-Donne, I.; Colombo, R.; Milzani, A.; Rossi, R. Is ascorbate able to reduce disulfide bridges? A cautionary note. *Nitric Oxide* **2008**, *19*, 252–258. [[CrossRef](#)] [[PubMed](#)]
128. Ménézo, Y.; Entezami, F.; Lichtblau, I.; Belloc, S.; Cohen, M.; Dale, B. Oxidative stress and fertility: Incorrect assumptions and ineffective solutions? *Zygote* **2014**, *22*, 80. [[CrossRef](#)] [[PubMed](#)]
129. Bleau, G.; Lemarbre, J.; Faucher, G.; Roberts, K.D.; Chapdelaine, A. Semen selenium and human fertility. *Fertil. Steril.* **1984**, *42*, 890–894. [[CrossRef](#)]
130. Bouayed, J.; Bohn, T. Exogenous antioxidants—Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidat. Med. Cell. Longev.* **2010**, *3*, 228–237. [[CrossRef](#)]
131. Omenn, G.S.; Goodman, G.E.; Thornquist, M.D.; Balmes, J.; Cullen, M.R.; Glass, A.; Keogh, J.P.; Meyskens, F.L., Jr.; Valanis, B.; Williams, J.H., Jr. Risk factors for lung cancer and for intervention effects in caret, the beta-carotene and retinol efficacy trial. *J. Nat. Cancer Inst.* **1996**, *88*, 1550–1559. [[CrossRef](#)]
132. Donnelly, E.T.; McClure, N.; Lewis, S.E. The effect of ascorbate and α -tocopherol supplementation in vitro on DNA integrity and hydrogen peroxide-induced DNA damage in human spermatozoa. *Mutagenesis* **1999**, *14*, 505–512. [[CrossRef](#)]
133. Verma, A.; Kanwar, K. Human sperm motility and lipid peroxidation in different ascorbic acid concentrations: An in vitro analysis. *Andrologia* **1998**, *30*, 325–329. [[CrossRef](#)]
134. Moilanen, J.; Hovatta, O.; Lindroth, L. Vitamin e levels in seminal plasma can be elevated by oral administration of vitamin e in infertile men. *Int. J. Androl.* **1993**, *16*, 165–166. [[CrossRef](#)]
135. DePalma, A.F.; Rothman, R.H.; Lewinnek, G.E.; Canale, S.T. Anterior interbody fusion for severe cervical disc degeneration. *Surg. Gynecol. Obs.* **1972**, *134*, 755–758.
136. Purvis, K.; Christiansen, E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int. J. Androl.* **1993**, *16*, 1–13. [[CrossRef](#)]
137. Comhaire, F.H.; Mahmoud, A.M.; Depuydt, C.E.; Zalata, A.A.; Christophe, A.B. Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: The andrologist's viewpoint. *Hum. Reprod. Update* **1999**, *5*, 393–398. [[CrossRef](#)] [[PubMed](#)]
138. Dutta, S.; Sengupta, P.; Chhikara, B.S. Reproductive inflammatory mediators and male infertility. *Chem. Biol. Lett.* **2020**, *7*, 73–74.
139. Hales, D.B.; Diemer, T.; Hales, K.H. Role of cytokines in testicular function. *Endocrine* **1999**, *10*, 201–217. [[CrossRef](#)]
140. Söder, O.; Sultana, T.; Jonsson, C.; Wahlgren, A.; Petersen, C.; Holst, M. The interleukin-1 system in the testis. *Andrologia* **2000**, *32*, 52–55. [[PubMed](#)]
141. Diemer, T.; Hales, D.B.; Weidner, W. Immune-endocrine interactions and leydig cell function: The role of cytokines. *Andrologia* **2003**, *35*, 55–63. [[CrossRef](#)]
142. Maegawa, M.; Kamada, M.; Irahara, M.; Yamamoto, S.; Yoshikawa, S.; Kasai, Y.; Ohmoto, Y.; Gima, H.; Thaler, C.J.; Aono, T. A repertoire of cytokines in human seminal plasma. *J. Reprod. Immunol.* **2002**, *54*, 33–42. [[CrossRef](#)]
143. Theam, O.C.; Dutta, S.; Sengupta, P. Role of leucocytes in reproductive tract infections and male infertility. *Chem. Biol. Lett.* **2020**, *7*, 124–130.
144. Cudicini, C.; Lejeune, H.; Gomez, E.; Bosmans, E.; Ballet, F.; Saez, J.; Jégou, B. Human leydig cells and sertoli cells are producers of interleukins-1 and -6. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 1426–1433. [[CrossRef](#)]
145. Turvey, S.E.; Broide, D.H. Innate immunity. *J. Allerg. Clin. Immunol.* **2010**, *125*, S24–S32. [[CrossRef](#)]
146. Keck, C.; Gerber-Schäfer, C.; Clad, A.; Wilhelm, C.; Breckwoldt, M. Seminal tract infections: Impact on male fertility and treatment options. *Hum. Reprod. Update* **1998**, *4*, 891–903. [[CrossRef](#)]
147. Dutta, S.; Sengupta, P.; Hassan, M.F.; Biswas, A. Role of toll-like receptors in the reproductive tract inflammation and male infertility. *Chem. Biol. Lett.* **2020**, *7*, 113–123.
148. Potts, J.M.; Sharma, R.; Pasqualotto, F.; Nelson, D.; Hall, G.; Agarwal, A. Association of ureaplasma urealyticum with abnormal reactive oxygen species levels and absence of leukocytospermia. *J. Urol.* **2000**, *163*, 1775–1778. [[CrossRef](#)]
149. Agarwal, A.; Majzoub, A.; Baskaran, S.; Selvam, M.K.P.; Cho, C.L.; Henkel, R.; Finelli, R.; Leisegang, K.; Sengupta, P.; Barbarosie, C. Sperm DNA fragmentation: A new guideline for clinicians. *World J. Men's Health* **2020**, *38*, 412. [[CrossRef](#)]
150. Yu, B.; Huang, Z. Variations in antioxidant genes and male infertility. *BioMed Res. Int.* **2015**, *2015*, 513196. [[CrossRef](#)]
151. Carrell, D.T.; Aston, K.I. The search for snps, cnvs, and epigenetic variants associated with the complex disease of male infertility. *Syst. Biol. Reprod. Med.* **2011**, *57*, 17–26. [[CrossRef](#)] [[PubMed](#)]
152. Kemal Duru, N.; Morshedi, M.; Oehninger, S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertil. Steril.* **2000**, *74*, 1200–1207. [[CrossRef](#)]

153. Bisht, S.; Faiq, M.; Tolahunase, M.; Dada, R. Oxidative stress and male infertility. *Nat. Rev. Urol.* **2017**, *14*, 470–485. [[CrossRef](#)] [[PubMed](#)]
154. Ritchie, C.; Ko, E.Y. Oxidative stress in the pathophysiology of male infertility. *Andrologia* **2021**, *53*, e13581. [[CrossRef](#)] [[PubMed](#)]
155. Huang, C.; Cao, X.; Pang, D.; Li, C.; Luo, Q.; Zou, Y.; Feng, B.; Li, L.; Cheng, A.; Chen, Z. Is male infertility associated with increased oxidative stress in seminal plasma? A-meta analysis. *Oncotarget* **2018**, *9*, 24494. [[CrossRef](#)]
156. Naz, R.K.; Evans, L. Presence and modulation of interleukin-12 in seminal plasma of fertile and infertile men. *J. Androl.* **1998**, *19*, 302–307.
157. Kurkowska, W.; Bogacz, A.; Janiszewska, M.; Gabryś, E.; Tiszler, M.; Bellanti, F.; Kasperczyk, S.; Machoń-Grecka, A.; Dobrakowski, M.; Kasperczyk, A. Oxidative stress is associated with reduced sperm motility in normal semen. *Am. J. Men's Health* **2020**, *14*, 1557988320939731. [[CrossRef](#)] [[PubMed](#)]
158. Gruschwitz, M.S.; Brezinschek, R.; Brezinschek, H.P. Cytokine levels in the seminal plasma of infertile males. *J. Androl.* **1996**, *17*, 158–163. [[PubMed](#)]
159. Halliwell, B. The antioxidant paradox: Less paradoxical now? *Brit. J. Clin. Pharmacol.* **2013**, *75*, 637–644. [[CrossRef](#)] [[PubMed](#)]
160. Murphy, M.P.; Holmgren, A.; Larsson, N.G.; Halliwell, B.; Chang, C.J.; Kalyanaraman, B.; Rhee, S.G.; Thornalley, P.J.; Partridge, L.; Gems, D.; et al. Unraveling the biological roles of reactive oxygen species. *Cell. Metab.* **2011**, *13*, 361–366. [[CrossRef](#)] [[PubMed](#)]
161. Rehman, A.; Collis, C.S.; Yang, M.; Kelly, M.; Diplock, A.T.; Halliwell, B.; Rice-Evans, C. The effects of iron and vitamin c co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochem. Biophys. Res. Comm.* **1998**, *246*, 293–298. [[CrossRef](#)]
162. Beatty, E.; England, T.; Geissler, C.; Aruoma, O.; Halliwell, B. Effects of antioxidant vitamin supplementation on markers of DNA damage and plasma antioxidants. *Proc. Nutr. Soc.* **1999**, *58*, 44.
163. Podmore, I.D.; Griffiths, H.R.; Herbert, K.E.; Mistry, N.; Mistry, P.; Lunec, J. Vitamin c exhibits pro-oxidant properties. *Nature* **1998**, *392*, 559. [[CrossRef](#)]
164. Son, Y.O.; Pratheeshkumar, P.; Roy, R.V.; Hitron, J.A.; Wang, L.; Divya, S.P.; Xu, M.; Luo, J.; Chen, G.; Zhang, Z.; et al. Antioncogenic and oncogenic properties of nrf2 in arsenic-induced carcinogenesis. *J. Biol. Chem.* **2015**, *290*, 27090–27100. [[CrossRef](#)]
165. Djuric, Z.; Kashif, M.; Fleming, T.; Muhammad, S.; Piel, D.; von Bauer, R.; Bea, F.; Herzig, S.; Zeier, M.; Pizzi, M.; et al. Targeting activation of specific nf- κ b subunits prevents stress-dependent atherothrombotic gene expression. *Mol. Med.* **2012**, *18*, 1375–1386. [[CrossRef](#)] [[PubMed](#)]