INTRODUCTION

Reactive oxygen species (ROS) production by spermatozoa was initially discovered by MacLeod (1943). Subsequently, Aitken and Clarkson (1987) observed that spermatozoa also produce ROS when exposed to a calcium cascade induced by ionophore A12187. The influx of calcium and ROS allows capacitation to occur, which is the process by where the spermatozoa’s plasma membrane increases fluidity and gains the ability to fuse with the oocyte (de Lamirande & Gagnon, 1993). By adding catalase, a ROS scavenger, researchers found that the amount of capacitated spermatozoa decreased by 47% (Griveau, Renard, & Lannou, 1994). This proved that ROS play a vital role in sperm maturation and fertilisation capability.

Reactive oxygen species can either inhibit or activate several enzymes to facilitate capacitation. The activation of kinases occurs either directly or via a secondary messenger to facilitate the necessary physiological response. These highly reactive, mainly radical-based oxygen derivatives bind directly to phosphokinase C (PKC) which, in turn, activates PKC and allows it to move to the phosphotyrosine binding domains on the plasma membrane (De Lamirande & O’Flaherty, 2008; Signorelli, Diaz, & Morales, 2012; Visconti et al, 1995). On the other hand, ROS can prevent tyrosine kinase inhibition by oxidising phosphatase, subsequently allowing tyrosine kinases to move to the phosphotyrosine binding domains on the plasma membrane (Hecht & Zick, 1992). However, the intermediate steps from tyrosine kinase activation to the end of capacitation remain to be fully understood; yet, the end result is an efflux of cholesterol and greater membrane fluidity (Cross, 1998; Davis, 1981) (Figure 1).

The plasma membrane of spermatozoa contains extraordinary high amounts of polyunsaturated fatty acids (PUFAs) which contribute to its membrane fluidity. In turn, these PUFAs render spermatozoa highly susceptible to oxidative damage (Aitken, Harkiss, & Buckingham, 1993). Reactive oxygen species attack of PUFAs generates a cyclic, propagating cycle called a radical chain reaction whereby the unconjugated double-bond groups of the PUFAs will undergo an initial electrophilic attack by ROS (Aitken, 2017), which will eventually result in the formation of malondialdehyde (MDA),...
4-hydroxynonenal (4-HNE) and acrolein (Agarwal, Saleh, & Bedaiwy, 2003; Aitken, 2017). These reactive aldehydes are markers for oxidative stress, as they will subsequently cause damage by reacting with positive, hydrophilic amino acids in proteins, which will not only yield further ROS production, but also cause mitochondrial dysregulation and ROS leakage from the inner mitochondrial membrane. If the ROS production exceeds concentrations of antioxidants in the spermatozoa, oxidative stress (OS) ensues. OS affects the plasma membrane’s integrity and induces premature capacitation, which renders spermatozoa less suitable for fertilisation (Velando, Torres, & Alonso-Alvarez, 2008; Villegas et al., 2003). When the concentration of ROS exceeds the physiological needs, spermatozoa experience OS, which results in infertility (Agarwal et al., 2003; Sanocka, Miesel, Jedrzejczak, & Kurpisz, 1996).

2 | SOURCES OF OS

Although numerous factors (Figure 2) contribute to oxidative stress-related male infertility, the majority of seminal OS is attributed to leucocytes and immature spermatozoa. During normal spermatogenesis, spermatozoa will reduce the size of their cytoplasm as Sertoli cells will phagocytise it during spermiogenesis (Gomez et al., 1996). However, an arrest in spermiogenesis will result in excess cytoplasm around the midpiece (Hampl, Drábková, Kandár, & Stépán, 2012) with the resulting immature spermatozoa producing excessive amounts of ROS, which, in turn, leads to OS (Aitken & Baker, 1995). The retained cytoplasm allows the formation of nicotinamide adenine dinucleotide phosphate (NADPH) from retained glucose-6-phosphate content (G6PDH) via the hexose monophosphate shunt (Frederiks & Vreeling-Sindelárová, 2001).

NADPH leads to the generation of ROS by means of two pathways. The first pathway includes a membrane-bound NADPH oxidase enzyme that uses oxygen as a source to produce the superoxide anion, which can further produce other highly reactive molecules such as hydrogen peroxide (Frederiks & Vreeling-Sindelárová, 2001). The second pathway consists of NADPH dehydrogenase, also known as diaphorase, which is responsible for oxidation–reduction (redox) reactions in the mitochondria and the generation of ROS (Gavella & Lipovac, 1992; Siegel, Gibson, Preusch, & Ross, 1990) (Figure 3).

2.1 | Varicocele

Varicocele is defined as an atypical dilatation and tortuosity of the Pampiniform venous plexus inside the spermatic cord (Agarwal et al., 2009; Will et al., 2011). Globally, 15% to 20% of men with fertility problems are diagnosed with varicocele (French, Desai, & Agarwal, 2008), resulting in elevated seminal ROS levels and lower total antioxidant capacity (Pasqualotto et al., 2008). Varicocele promotes ischaemia in the spermatic veins and results in increasing levels of inflammatory cytokines and nitric oxide (NO; Ozbek, Turkoz, Gokdeniz, Davarci, & Ozugurlu, 2000).

Moderate concentrations of specific cytokines play a critical role in maintaining physiological functions of cells inside the testes. Specifically, interleukin (IL)-1 regulates the functions of testicular cells including Sertoli and Leydig cells (Sultana, Svechnikov, Weber, &
Moreover, certain cytokines, including IL-37 and IL-18, are upregulated in the seminal plasma of varicocele patients (Zeinali, Hadian Amree, Khorramdelazad, Karami, & Abedinzadeh, 2017). These elevations lead to inflammatory response activation, leukocyte recruitment and ROS production, which are detrimental to normal testicular functions (Allen & Gow, 2009). Consequently, ROS can disrupt the blood-testis barrier, the sperm plasma membrane and DNA integrity (Ha, Park, & Park, 2011; Sabeti, Pourmasumi, Rahiminia, Akyash, & Talebi, 2016).

Furthermore, endothelial nitric oxide (NO) synthase is upregulated in varicocele testes to increase blood flow into testicular tissues and compensate for the hypoxia caused by venous stasis (Agarwal et al., 2009). In this case, high NO concentrations can be detrimental as NO can react with superoxide free radicals to form highly reactive nitrogen species such as peroxynitrite and peroxynitrous acid, which could lead to infertility (Romeo et al., 2003). Other potential causes of OS in varicocele patients include leptin receptors, glial cell line-derived neurotrophic factor receptor-α1 (GDNF-α1), HO-isoenzyme 1 and voltage-dependent calcium channels (Benoff et al., 2005; Konukoglu, Serin, & Turhan, 2006; Shiraishi & Naito, 2005).

2.2 Tobacco usage

Tobacco smokers exhibit higher levels of seminal ROS and OS markers than nonsmokers (La Maestra, Flora, & Micale, 2015). Smoking not only increases leukocyte levels by about 48% and ROS level by 107%, but also decreases reactive oxygen species-total antioxidant capacity (ROS-TAC) by 10 points in the seminal plasma (Close, Roberts, & Berger, 1990; Saleh et al., 2002; Zhang et al., 2013). In smokers, proinflammatory leukocytes are recruited, which eventually increases seminal ROS levels affecting spermatogenesis and sperm quality (Zhang et al., 2013). Thus, tobacco smoking affects the sperm chromatin integrity through the induction of OS and generating oxidised DNA base adducts such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Aitken, Gibb, Baker, Drevet, & Ghargozloo, 2016). Spermatozoa only possess one enzyme, 8-oxoguanine glycosylase (OGG1), for the base excision repair mechanism and do not possess any downstream components of the base excision repair (BER) pathway such as apurinic endonuclease 1 (APE1) and DNA repair proteins such as X-ray repair complementing defective repair (XRCC1); this unique feature makes spermatozoa susceptible to DNA damage (Aitken et al, 2016).

The consequences of tobacco usage include decrease in sperm concentration, motility, viability and morphological abnormalities (Said, Ranga, & Agarwal, 2005). Through the processes of hydrolysis, pyrolysis, oxidation, decarboxylation and dehydration, tobacco releases mutagens and carcinogens such as radioactive polonium, benzopyrene, dimethylbenzanthracene and polycyclic aromatic hydrocarbons (Colagar, Jorsaraee, & Marzony, 2007; Richthoff, Elzanaty, Rylander, Hagmar, & Giwercman, 2008). Many of these toxic compounds are also found to induce chromosomal aberrations in spermatozoa, leading to a reduced likelihood of success in assisted reproductive therapies (ART) (Anderson, Nisenblat, & Norman, 2010; Frey, Navarro, Kotelchuck, & Lu, 2008; Ozgur, Isikoglu, Seleker, & Donmez, 2005; Rubes et al, 1998; Soares & Melo, 2008; Vine, 1996). Smoking may also have direct and indirect effects on various male urogenital organs by causing conditions such as epididymitis, varicocele, erectile dysfunction, reduced accessory gland function and alternations of the hypothalamic-pituitary-gonadal axis (Harlev, Agarwal, Gunes, Shetty, & Plessis, 2015). For example, smoking could also inhibit adequate sperm maturation in epididymitis, increase the likelihood of oligozoospermia for tobacco abusers and varicocele patients and reduce various vesicular and prostatic parameters (Harlev et al, 2015).
2.3 | Alcohol

Alcoholic men of reproductive age have been shown to have significantly decreased antioxidant levels and a significant increase in serum lipid peroxide (LPO) compared to those of nondrinkers (Saalu, 2010). Alcohol interferes with the body’s antioxidant defence mechanism (Agarwal & Majzoub, 2017) by impairing the metabolic pathway and generating highly reactive and toxic free radicals as by-products (Das & Vasudevan, 2007) suggesting a link between OS and alcohol consumption. Studies have shown a reduction in sperm concentration, percentage of normal sperm morphology, sperm count, total progressive motility and testicular integrity in oligozoospermic men (Anderson et al, 2010; Muthusami & Chinnaswamy, 2005; Vine, 1996). Alcohol usage also reduces in vitro fertilisation (IVF) success and increases miscarriage rates (Klonoff-Cohen Lamirande, & Gagnon, 1994). Globally, about 10%-20% of infertile men have elevated seminal leucocyte concentrations caused by infections or inflammatory responses and other factors (Henkel et al., 2007). The most common leucocytes in the ejaculate are polymorphonuclear granulocytes (50%-60% of all leucocytes in ejaculate) and macrophages (20%-30% WBCs that destroy foreign material and secrete cytokines) (Wolff, 1995). Macrophages are located in the tissues. Upon activation, macrophages will release proteases, neutrophil chemotactic factors and ROS that will signal to the endothelial cells lining the blood vessels to present certain chemicals on the inner walls of the blood vessels (Mosser & Edwards, 2008). Each of the WBCs produces a large amount of ROS to fight infections and eliminate pathogens by stimulating the activity of G6PDH causing the production of high amounts of NADPH. Furthermore, NADPH oxidase takes an electron from NADPH to convert oxygen into a superoxide anion. In turn, excessive OS will activate chemokines CXCL5, CXCL8, IL-6 and IL-8 (Comhaire, Bosmans, Ombelet, Punjabi, & Schoonjans, 1994; Sandoval, Raburn, & Muasher, 2013). Consequently, an imbalance between antioxidants and seminal ROS levels occurs, which leads to OS-induced infertility (Agarwal et al., 2003). High concentrations of seminal leucocytes reduce sperm concentration/motility and cause abnormal sperm morphology (Aziz, Agarwal, Lewis-Jones, Sharma, & Thomas, 2004; Saleh et al, 2002). Early detection of novel biomarkers such as TLR2, TLR4, COX-2 and Nrf-2 can perhaps help facilitate better therapeutic interventions to alleviate OS-induced infertility (Hagan et al, 2015).

2.4 | Obesity/metabolic syndrome

Obesity is characterised by excessive accumulation of adipose tissues, particularly visceral adipose tissue. The hormonal basis of male infertility is adipose tissues’ formation of oestriadiol (Nelson & Bulun, 2001). Adipose fibroblasts contain aromatase which converts testosterone into oestradiol thereby not only decreasing serum testosterone levels, but by increasing serum oestradiol concentrations causing a decrease in inhibin B levels (Cabler, Agarwal, Flint, & Plessis, 2010). Obesity results in male infertility by dysregulating these endocrine pathways, and increased seminal ROS levels, and is inducing physical manifestations such as sleep apnoea, and erectile dysfunction (Cashou, Plessis, & Agarwal, 2012).

Obesity also causes a systemic inflammatory response which impacts semen parameters and semen quality (Trayhurn, 2013). The increased visceral abdominal fat can lead to alterations in adipokine secretion and the recruitment of proinflammatory white blood cells, and increased NADPH oxidase activity which, in turn, generates additional ROS and decreases sperm DNA integrity (Esposito et al., 2006; Palmer, Bakos, Fullston, & Lane, 2012). In addition, those with a high-fat diet will produce more reducing agents (NADH and FADH₂) to create the electron gradient needed to form ATP (Tiganis, 2011). Given the lack of ATP demand, the reducing agents will promote electron leakage and formation of superoxide radicals. Superoxide radicals are quickly removed by superoxide dismutase and generate hydrogen peroxide, which can be excessively produced by the skeletal muscle, promoting systemic ROS production (Patti et al., 2003). This systemic ROS production has a detrimental effect on spermatogenesis and causes DNA damage (Bachir & Jarvi, 2014; Kashou et al., 2012).

Metabolic syndrome is a combination of three of the following five criteria: (a) excess waistline adiposity; (b) high fasting blood glucose level; (c) decreased HDL levels; (d) high triglyceride; and (e) hypertension (Morrison & Brannigan, 2015). Men with metabolic syndrome have increased adipokine secretion for tumour necrosis factor-alpha (TNF-α) and inflammatory interleukins (ILs) which results in the recruitment of macrophages in the seminal plasma (Leisegang, Bouic, & Henkel, 2016; Morrison & Brannigan, 2015). However, in combination with diabetes mellitus type 2, obesity-related infertility is exacerbated by IL-6 and TNF-α disruption of the hypothalamic-pituitary-gonadal axis (Bhasin et al, 2010). Disruption of the axis results in decreased testosterone levels and, subsequently, in hypogonadism.

2.5 | Leukocytospermia

Leucocytes are the main source of ROS in semen, producing up to 1,000 times more ROS than normal spermatozoa (Plante, Lamirande, & Gagnon, 1994). Globally, about 10%-20% of fertile men have elevated seminal leucocyte concentrations caused by infections or inflammatory responses and other factors (Henkel et al., 2007). The most common leucocytes in the ejaculate are polymorphonuclear granulocytes (50%-60% of all leucocytes in ejaculate) and macrophages (20%-30% WBCs that destroy foreign material and secrete cytokines) (Wolff, 1995). Macrophages are located in the tissues. Upon activation, macrophages will release proteases, neutrophil chemotactic factors and ROS that will signal to the endothelial cells lining the blood vessels to present certain chemicals on the inner walls of the blood vessels (Mosser & Edwards, 2008). Each of the WBCs produces a large amount of ROS to fight infections and eliminate pathogens by stimulating the activity of G6PDH causing the production of high amounts of NADPH. Furthermore, NADPH oxidase takes an electron from NADPH to convert oxygen into a superoxide anion. In turn, excessive OS will activate chemokines CXCL5, CXCL8, IL-6 and IL-8 (Comhaire, Bosmans, Ombelet, Punjabi, & Schoonjans, 1994; Sandoval, Raburn, & Muasher, 2013). Consequently, an imbalance between antioxidants and seminal ROS levels occurs, which leads to OS-induced infertility (Agarwal et al., 2003). High concentrations of seminal leucocytes reduce sperm concentration/motility and cause abnormal sperm morphology (Aziz, Agarwal, Lewis-Jones, Sharma, & Thomas, 2004; Saleh et al, 2002). Early detection of novel biomarkers such as TLR2, TLR4, COX-2 and Nrf-2 can perhaps help facilitate better therapeutic interventions to alleviate OS-induced infertility (Hagan et al, 2015).

2.6 | Infections

The body has three main defence mechanisms protecting against bacterial invasions: tight junctions between skin epithelium, the innate immune response and the adaptive immune response. Potential pathogens infect individuals through tears in membranes during sexual intercourse. In addition, different bacteria usually have predilections for infecting specific tissue types. For example, Neisseria species live on mucous membranes in the genital tract.

Effector B cells are the subtype of B cells that can create antibodies. In order for effector B cells to be activated, MHC IIs are presented on professional antigen-presenting cells (APCs) such as macrophages and B cells. They signal to CD4 T-helper cells and release cytokines to recruit more macrophages. Neutrophilic...
chemotactic factors released by macrophage allow neutrophils to navigate between the endothelial cells and enter the tissues. Activated macrophages, neutrophils and eosinophils increase oxygen uptake to undergo a series of respiratory burst.

Antibiotics tend to be highly effective in neutralising certain bacteria by enhancing phagocytosis. With aggressive antibiotic administration, oral antibiotics reach the colon in active form and thus suppress susceptible microorganisms; and normal microflora; these events could lead to reduced colonisation resistance (Edlund & Nord, 2000). A recent murine study indicates that antibiotics may also reduce the ability of immune cells such as macrophages to fight off infection as the antibiotics block off macrophages’ respiration (Yang, Bening, & Collins, 2017).

### 2.7 | Sexually transmitted disease

Infections of the male genital tract with bacteria, viruses and protozoa have negative impacts on male fertility. With annually 325,900 new cases for gonorrhoea, 500,000 for chlamydia and 70,000 for syphilis, these three sexually transmitted diseases (STDs) are the most prominent in the United States (Cates, 1999). Bacteria interact with the host’s inflammatory and immune response differently through their unique structures, special enzymes and toxins, to evade the host’s response. Regardless of which specific organ has the resultant inflammation, there will be an elevation of polymorphonuclear leucocytes and granulocytes which can lead to an increase in OS and impaired spermatogenesis (Ahmad, Plessis, & Agarwal, 2017). Interestingly, the specific types of cytokines, chemokines, cell adhesion molecules and C-reactive proteins released at the site of infection depend on the respective pathogen and individual host response (Newton & Dixit, 2012).

IL-1 increases vascular endothelium activity and permeability and activates lymphocytes; IL-6 regulates temperature during inflammation; IL-8 acts as a neutrophil chemotactic factor; IL-10 downregulates inflammatory response by blocking NF-kB activation and Th1 cell expression; TNF-α increases vascular endothelial activity and permeability; and interferon gamma (IFN-γ) activates macrophages (Singer & Ouburg, 2016). Differences in cytokine expression can alter disease progressions such as tubal pathology and related fertility problems (Singer & Ouburg, 2016; den Hartog et al, 2009; Karimi et al, 2009).

As responses to infections such as STDs, cytokine levels also increase in larger concentrations (Azenabor, Ekon, & Akinloye, 2015). There are certain types of microorganisms that can survive for a long period of time without causing overt signs of infections during the latent period. For pathogens that have penetrated the structural barriers, normal inflammatory responses include pain, redness, heat and vasodilatation allowing leucocytes to exit blood vessels and interact with vascular endothelium. The increase in vessel diameters, immunoglobulins, complements and other semen proteins depends on the adhesive interactions activated by cytokines (Azenabor et al, 2015). Whereas the intention of the inflammatory response is to protect the host, the response itself could cause harm, such as an elevated level in OS and the unintentional release of granulocytes from the phagocytes (Azenabor et al., 2015).

### 2.8 | Neisseria gonorrhoeae

Upon infection, the male urogenital tract reflects two types of changes—an increase in diameter and a reduction in the velocity of seminal flow (Azenabor et al, 2015). As a result, there will be elevated levels of ROS. The causative agent of gonorrhoea, N. gonorrhoeae, colonises the genital, rectal and nasopharyngeal mucosa during the infection (Château and Seifert, 2016). Among Gram-negative bacteria, N. gonorrhoeae is unique because they turn over large amounts of peptidoglycan during growth (Mavrogiorgos, Meekasa, Yang, Kelliher, & Ingalls, 2014). N. gonorrhoeae is capable of activating TLR2, TLR4 and cytosolic receptor nucleotide-binding oligomerisation domain (NOD)-1 and NOD2 in regulating the immune response to the bacterial antigen in the innate immune response. This process further activates transcription factor NF-kB and polyubiquitination of the adaptor receptor-interacting serine-threonine kinase 2 (RIPK2) (Mavrogiorgos et al., 2014). The activation of the NOD receptor implies that the immune response can induce not only cytokines (IL-16) and chemokines (CCL17 and CCL22), but also growth factors (CSF1), complement proteins and other cytosolic pattern recognition receptors on cell surfaces, such as TLR2 and TLR4 (Mavrogiorgos et al., 2014).

Furthermore, IL-1 induces apoptosis in semen via proliferation and differentiation of β-cells to induce neutrophil and monocyte generations with the assistance of chemoattractors such as IL-8 (Azenabor et al, 2015). For N. gonorrhoeae, IL-1 and IL-8 expression affects the clinical outcome significantly (Singer & Ouburg, 2016). Duru, Morshed, and Oehninger (2000) showed that there is a negative correlation between seminal oxidative stress and sperm concentration, function and motility. It is also suggested that cytokines could be the mediator of semen quality and male infertility (Azenabor et al, 2015). In addition, a study indicates that increased levels of IL-1β were associated with a decrease in sperm motility and an increase in seminal ROS and malondialdehyde (MDA), a by-product of lipid peroxidation (Koçak, Yenisey, Dündar, Okyay, & Serter, 2002).

### 2.9 | Chlamydia trachomatis

Chlamydia trachomatis has the highest prevalence rate globally with 4.2% for women and 2.7% for men being infected (Newman et al, 2015). Chlamydia presents men with a clinical challenge as 50% of men infected with chlamydia will not receive empirical treatment for it (Rietmeijer, Van Bemmelen, Judson, & Douglas, 2002). The mechanism behind Chlamydia trachomatis-induced male infertility remains controversial. Currently, there are three main theories—(a) high level of WBC through cytokine stimulation; (b) interaction with CD 14 receptor; and (c) development of antisperm antibodies. The first proposed theory is about the interactions among high level of WBCs through cytokine stimulations, the presence of lipopolysaccharides
(LPS) and the development of antisperrm antibodies (Ahmad et al., 2017). More specifically, this theory indicates that when Chlamydia trachomatis reaches the epithelial cell, infection often results in tissue damage and thus stimulates IL-1 (Arend & Dayer, 1994). IL-1 (which consists of \( \alpha \) and \( \beta \) subunits) stimulates polymorphonuclear WBCs and macrophages and then subsequently induces IL-6, IL-8, IL-10, TNF-\( \alpha \) and IFN-\( \gamma \) (Lavranos, Balla, Toztorzopoulou, Syriou, & Angelopoulou, 2012; Nandipati, Pasqualotto, Thomas, & Agarwal, 2005; Singer & Ouburg, 2016). The IL-6 level in the seminal plasma is higher in infertile men (Koçak et al., 2002; Naz & Kaplan, 1994). Similarly, MDA levels were higher in the semen of infertile men, which suggests that IL-6 is involved in lipid peroxidation (Camejo, Segnini, & Proverbio, 2001). This series of cascade reactions indirectly activates OS, which contributes to male infertility.

The second proposed mechanism is through the interaction between CD14 receptor and high concentration of LPO present on the sperm membrane to induce excessive ROS (Ahmad et al., 2017), whereas the third proposed mechanism points to the development of the antisperrm antibodies from invasion of lymphocytes, macrophages, plasma cells and eosinophils. The activation cascade of ILs invokes more secretory IgA, IgM and IgG antibodies, in which IgA and IgG are generally associated with decreased levels in semen parameters and pregnancy outcomes (Idahl et al., 2010). A meta-analysis carried out on cytokine-level variations on the progression and outcome indicates that IL-1, IL-6, IL-8, IL-10, TNF-\( \alpha \) and IFN-\( \gamma \) variations in hosts could potentially affect the clinical outcome for the patient (Singer & Ouburg, 2016). Given that there are several theories, early diagnostic testing and antibiotic administration (i.e., macrolides) are essential for STD eradication.

2.10 | Treponema pallidum

The primary lesion of syphilis, originating from T. pallidum, takes about three weeks to develop. Even though the infection contains many spirochaetes and is highly contagious, the serologic test could be negative for up to 12 weeks. Despite the administration of inexpensive and effective antibiotics, there are still more than 10.5 million cases around the world and potential infection relapses (Schmid, Rowley, Samuelson, Tun, & Guraib, 2009; Sellati et al., 1998) with about 30% of the patients resulting in the development of tertiary syphilis (Kampmeier, 1964). Yet, the circumstances of this multifaceted shifting balance between persistent bacteria trying to evade the host’s immune responses are not well understood (Cruz et al., 2012).

For the innate immune response, T. pallidum contains abundant lipoproteins capable of activating macrophages and dendritic cells (DC) via CD14 and Toll-like receptor (TLR)-1- and TLR2-dependent pathways (Alexopoulou et al., 2002; Brightbill et al., 1999; Lien et al., 1999; Sellati et al., 1998; Wooten et al., 2002). Due to the special bacterial structure without surface-exposed lipoproteins, pathogen-associated molecular patterns (PAMPs) are not readily available to TLRs (Cruz et al., 2012). Afterwards, tissue-based DC travel to lymph nodes to present the antigens to naive B and T cells to further elicit the adaptive immune response (Cruz et al., 2012). The limited exposure to innate and adaptive immune response, as a result of the lack of surface-exposed lipoproteins, is not sufficient to control the bacteria; this leads to a substantial number of spirochaetes avoiding phagocytosis (Cruz et al., 2012).

Tissue lesions are one of the clinical manifestations for T. pallidum, as a result of provoking an intense cellular immune response (Cruz et al., 2012). The study by Voorhis, Barrett, Nasio, Plummer, and Lukehart (1996) shows that expressions of Th1 cytokines, such as IL-1, IL-12 and IFN-\( \gamma \), play a role in bacterial clearance. When these cytokines are abnormally elevated, inflammation becomes harmful because of OS-induced sperm DNA damage and apoptosis (Azenabor et al., 2015).

2.11 | Mycoplasma

There are two species of mycoplasmata, which are clinically relevant for male infertility: M. hominis and M. genitalium (Andrade-Rocha, 2003; Gimenes et al., 2014). The prevalence of mycoplasma infections is 4.8% for M. genitalium and 9.6% for M. hominis (Gdoura et al., 2008). 41% of men with recurrent urethritis are positive for M. genitalium (Wikström & Jensen, 2006). Infections with M. hominis and M. genitalium decrease sperm motility and normal morphology and increase DNA damage (Andrade-Rocha, 2003; Zeighami, Peeryayeh, Yazdi, & Sorouri, 2009; Zinzendorf et al., 2008). Gallegos et al. (2008) reported that inflammation-induced ROS production is the source of DNA damage in mycoplasma-mediated infections. After a course of antibiotics and antiinflammatory steroids, sperm DNA damage significantly decreased (\( p < 0.001 \); Gallegos et al., 2008).

2.12 | Ureaplasma

Ureaplasma urealyticum and Ureaplasma parvum are the two clinically relevant species of ureaplasma responsible for male infertility with a prevalence of 15.6% and 2.9% respectively (Gdoura et al., 2008). Infection rates for ureaplasma in male infertility patients vary between 5% and 42% (Abusarah, Awwad, Charvalos, & Shehabi, 2013; Zhou, Ma, Shi, & Liu, 2017). Whereas sperm motility and normal sperm morphology decrease, sperm DNA damage increases. As ureaplasma does not trigger an inflammatory process which would degrade semen quality (Zhou et al., 2017), the rationale as to why these semen parameters deteriorate remains unknown (Andrade-Rocha, 2003; Nunez-Calonge et al., 1998; Zhou et al., 2017; Zinzendorf et al., 2008). These pathogens accumulate in the urethra and likely bind directly to spermatozoa upon ejaculation (Nunez-Calonge et al., 1998; Zeighami et al., 2009), a process which is thought to cause the increased DNA damage and the loss in membrane integrity by formation of ROS (Gallegos et al., 2008; Reichart, Kahané, & Bartooov, 2000).

2.13 | Escherichia coli

Escherichia coli is considered the most significant bacterium in infection-mediated male infertility (Comhaire, Mahmoud, Depuydt, Zalata, & Christophe, 1999). E. coli decreases sperm motility, decreases vitality and
increases DNA damage (Dahlberg, 1976; Diemer et al., 1996; Domes et al., 2012; Moretti et al., 2009; Sanocka-Maciejewska, Ciupińska, & Kurpisz, 2005). E. coli-mediated infertility has many modalities as to how it can cause male infertility. First, E. coli causes recruitment of leucocytes and thereby promotes neutrophilic production of ROS (Diemer et al., 2003; Moretti et al., 2009). Proinflammatory cytokines, such as IL-6, can directly rupture cellular membranes, which decreases sperm motility (Ramirez, Carreras, & Mendoza, 1992; Yamauchi-Takahara et al., 1995). In addition, E. coli diminishes sperm membrane integrity by binding directly to their membrane and introducing porins to the spermatozoa's membrane (Galdiero et al., 1988). In turn, porin formation causes the release of cytoplasmic content and causes a significant reduction in viability ranging from 80% to 100%. Finally, bound haemolytic subspecies of E. coli cause a decline in sperm vitality by decreasing the sperm plasma membrane potential, which mediates intracellular ROS production (Boguen, Treulen, Uribe, & Villegas, 2015).

2.14 | Prostatitis

Among male urogenital diseases, prostatitis remains to be the most in-depth studied inflammatory diseases. In acute and chronic bacterial prostatitis, pathogens can affect spermatozoa either directly (depending on the strain of the pathogen) or indirectly by inducing an inflammatory response in the seminal tract via the activation of cytokines such as IL-6, IL-8 or TNF-α (Alshahrani, McGill, & Agarwal, 2013; Martínez-Prado & Camejo Bermúdez, 2010). Increased cytokine levels lead to OS, which not only affect the spermatozoa (Dobrakowski et al., 2017), but can also potentially cause a systematic response by decreasing hormone levels of testosterone (Binl et al., 2015; Leisegang & Henkel, 2018). High levels of IL-6, IL-8 and TNF-α may also affect sperm transit during ejaculation if the infection spreads to the testis (Camejo et al., 2001; Nandipati et al., 2005; Rajasekaran, Hellstrom, Naz, & Sikka, 1995; Martínez, Proverbio, & Camejo, 2007; Sanocka, Jędrzejczak, Szymała-Kaękol, Fraćzek, & Kurpisz, 2003). Increased oxidative stress also directly damages sperm DNA, thus compromising the paternal genomic contribution to the embryo (Tremellen, 2008).

Infection and inflammation in the prostate activate leucocytes, which could increase ROS. For this reason, infections require immediate attention because an elevated amount of ROS can affect up to 35% of men consulting for infertility (Henkel, 2012). This is primarily due to the fact that untreated prostatitis can lead to oligozoospermia, asthenozoospermia or azoospermia (Schuppe et al., 2008; Weidner, Colpi, Hargrave, Papp, & Pomerol, 2002). Untreated/undertreated infections can also lead to chronic disease, which is more difficult to treat than acute disease. In the example of bacterial prostatitis, four different antibiotics—fluoroquinolones, tetracyclines, macrolides and trimethoprim (alone or combined with sulfamethoxazole)—were introduced as an option for treatment (Lipsky, Byren, & Hoey, 2010). Antibiotic administration remains to be the cornerstone for the treatment of bacterial prostatitis, and numerous studies have indicated that antibiotics can significantly improve semen parameters and pregnancy rates. Yet, antibiotic treatment needs to be performed carefully and after proper testing for drug resistance as recent attention has also been brought upon multidrug resistance (Krupp & Madhivanan, 2015).

Combination treatment is generally recommended for patients if first-line treatment is ineffective (Magri et al., 2007). Although most combinations are indifferent or additive, ciprofloxacin and rifampin appear to be effective against Staphylococcus aureus (Oliphant & Green, 2002) whereas fluoroquinolones chelate with cations such as aluminium, magnesium, calcium, iron and zinc. This drug interaction significantly lowers serum drug concentrations available to penetrate target tissues (Oliphant & Green, 2002). Prostate epithelial cells are capable to accumulate cellular zinc that is severalfold higher than most other mammalian cells (Costello, Feng, Milton, Tan, & Franklin, 2004). Given the above explanation, the accumulation of zinc in prostate may prevent fluoroquinolones from reaching their optimal efficacy.

2.15 | Microorganism mutations leading to more OS

Antibiotic overuse can cause pathogens to genetically mutate, leading to antibiotic resistance. This is especially true with bactericidal antibiotics. The use of such antibiotics can lead to changes in cell’s metabolism to promote increased levels of reactive oxygen species (Dwyer, Kohanski, & Collins, 2009). For example, fluoroquinolones bind with DNA gyrase (topoisomerase II, product of gyrA and gyrB) and/or topoisomerase IV (product of parC and parE) to induce cell death (Dwyer et al., 2009). Through the process of phagocytosis (macrophages and neutrophils engulf microorganisms by a combination of ROS, RNS and enzymatic digestion), there is a sudden production of oxidative burst through both macrophages and neutrophils (Vatansever et al., 2013). An elevated level of OS will be produced when the pathogen attempts to escape from the antibiotic treatment through mutation. To eliminate mutated bacteria, a higher amount of antibiotic-induced ROS will be needed to elicit the same effect (Dwyer et al., 2009), a scenario that could occur if a patient has received multiple prior administrations of antibiotics. Antibiotic resistance does not allow for effective elimination of the causative pathogen.

If the pathogen is not entirely eradicated, the dosage of the antibiotic is usually increased or combined with another antibiotic to maximise efficacy. However, multidrug resistance could also lead to oxidative stress as the microorganisms attempt to defend the body against the drug; a study by Dwyer et al. (2014) showed that antibiotics induce complex redox alterations, which contribute to cellular damage and death. More specifically, alterations in central metabolism, cellular respiration and iron metabolism occur during drug interactions of target-specific processes (Dwyer et al., 2014). The addition of ROS and other damaging substances such as cytokines makes it more difficult for the bacteria to be eradicated, which could rather prolong acute prostatitis (Dwyer et al., 2014).

3 | VIRAL INFECTIONS

3.1 | Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) causes a decrease in semen motility, vitality and forward progression when CD4 cell counts are
below 350 cells/microlitre (Wang et al., 2014). Otherwise, HIV-seropositive men who are asymptomatic do not show a decrease in fertility (Garolla et al., 2013; Lorusso et al., 2010; Wang et al., 2014). In men with symptomatic HIV infections, recruitment of monocytes, macrophages and leucocytes is found in semen (Garolla et al., 2013).

With current highly active antiretroviral therapy (HAART) regimens increasing life expectancy for HIV-infected men, investigations on how HAART affects male fertility show a negative impact on semen quality (Frapsauce et al., 2015; Garolla et al., 2013; Kehl et al., 2011; Wang et al., 2014). The use of nonnucleoside reverse transcriptase inhibitors may induce oxidative stress as they are mitochondrial toxic (Kehl et al., 2011; Pavili et al., 2010) and disrupt the mitochondrial potential membrane (Frapsauce et al., 2015). The disruption in mitochondria results in decreased motility and forward velocity for men on nonnucleoside reverse transcriptase inhibitors, particularly efavirenz (Frapsauce et al., 2015).

### 3.2 Hepatitis

Hepatitis B and hepatitis C are both viruses found to cause male infertility. Hepatitis B is considered to worsen fertility parameters given its ability to pass through the blood–testis barrier (Garolla et al., 2013; Vicari et al., 2006). Given the hepatitis B virus is able to pass through the blood–testis barrier, it can directly transfer its genome in spermatozoa which results in defective spermiogenesis and decreased fertilisation rates (Zhou et al., 2017). Spermatozoa exposed to hepatitis B were found with increased phosphatidylserine externalisation and lipid peroxidation measured by MDA formation (Kang et al., 2012; Qian, Li, & Li, 2016). Men with chronic hepatitis B had high seminal concentrations of IL-18, which causes natural killer cells to secret proinflammatory cytokine IL-18, which causes natural killer cells to secret proinflammatory cytokine IL-18, which causes natural killer cells to secret proinflammatory cytokine INF-γ (Qian et al., 2016). These authors found a positive correlation between inner MDA formation and IL-18 concentration, suggesting MDA formation is caused by an inflammatory process. However, there is currently no study directly relating IL-18 and leucocyte activation within the male reproductive tract from hepatitis B infection.

Hepatitis C transmission is primarily through intravenous drug abuse and mother-to-child transmission (Sacks-Davis, McBravine, Grebely, Hellard, & Vickerman, 2015; Benova, Mohamoud, Calvert, & Abu-Raddad, 2014). Regarding the current opiate crisis in the United States, primary opiate abusers are of reproductive age (mean age of 22.9 years) and significantly contribute to the increased transmission of hepatitis C (Davis et al 2015; Cicero, Ellis, Surratt, & Kurtz, 2014). In contrast to hepatitis B, the hepatitis C virus does not pass through the blood–testis barrier and cannot cause direct oxidative stress for spermatozoa (Garolla et al., 2013). Chronic hepatitis C infections cause systemic elevations in TNF-α and NO (Machida et al., 2006). As a result, chronic inflammation and activation of lymphocytes and polymorphonuclear leucocytes appear (La Vignera, Condorelli, Vicari, D’Agata, & Calogero, 2012). Polymorphonuclear leucocytes produce ROS, via NOX2, which causes a loss in mitochondrial membrane potential in the spermatozoa thus causing further ROS propagation and OS (La Vignera et al, 2012, Machida et al, 2006, Lorusso et al, 2010). Hepatitis C-induced OS causes a decrease in sperm motility but unchanged ejaculatory volume, apoptosis and increased DNA damage (Machida et al., 2006; La Vignera et al, 2012, Hofny et al 2011; La Vignera et al, 2012; Lorusso et al, 2010).

### 4 CONCLUSIONS

There are many specific inflammatory/infectious processes that cause male infertility. Therefore, it is important to correctly identify the causative agent as each possesses a unique mechanism of damage as well as specific susceptibilities to antibiotics when considering treatment. Many factors contribute to the decision to treat OS in male infertility. Modifiable lifestyle changes such as tobacco/alcohol cessation, healthy diet or antioxidant supplementation with different chances of success are recommended. On the other hand, antibiotic treatment remains to be the cornerstone of treating bacterial-mediated infections where certain antibiotics can also cause OS-related damages. Yet, an increasing risk of infections with multidrug-resistant bacterial strains has to be taken into consideration. Whereas the treatment of choice for viral infection is antiretrovirals, nonnucleoside reverse transcriptase inhibitors pose a potential decrease in fertility. Thus, more efforts need to be made to better understand the causes and effects of oxidative stress on male fertility, so that better and more sustainable treatment regimens can be developed.

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