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The role of infections and leukocytes in male infertility

Ralf Henkel[1,2] | Ugochukwu Offor[3] | David Fisher[1]

[1]Department of Medical Bioscience, Faculty of Natural Science, University of Western Cape, Bellville, South Africa

[2]American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA

[3]Department of Pre-Clinical Sciences, Faculty of Health Sciences, University of Limpopo, Polokwane, South Africa

Correspondence
Ralf Henkel, Department of Medical Bioscience, Faculty of Natural Science, University of Western Cape, Bellville, South Africa
Email: rhenkel@uwc.ac.za

Abstract

Declining birth rates are one of the problems facing society today. Male counterparts are responsible for about half of the infertility cases, and genitourinary tract infections may play a contributing role in approximately 15% of male infertility cases. Leukocytospermia is an established indicator of infection in the male urogenital tract, although other microorganisms such as bacteria and virus may also be contributors to the etiology of male infertility. The pathophysiology of these infectious agents may be initiated by a local inflammatory reaction resulting in an increase in reactive oxygen species (ROS). This results in testicular injury, thereby affecting sperm morphology, sperm motility, sperm viability and elevation of the seminal leukocyte as a result of the genital tract infection. The infectious and inflammatory changes can result in male infertility. It is proposed that high concentrations of seminal leukocyte and infectious agents may affect sperm function resulting in clumping of motile spermatozoa, decreasing acrosomal functionality and also causing alterations in sperm morphology. However, the literature has poorly clarified the role of infection in male infertility, provoking further debate and research on this topic.

KEYWORDS

bacterial infections, leukocytospermia, male infertility, reactive oxygen species

1 | INTRODUCTION

Infertility is a challenging health issue that affects approximately 15% of couples who are at their reproductive age desiring to have children. This condition is defined by the inability to achieve a pregnancy after at least 1 year of well-timed, unprotected sexual intercourse between consenting partners who wish to have children or failure of therapeutic donor insemination (Irvine, 1998; Lackner et al., 2006). It is a highly prevalent disease worldwide, and approximately 50% of the infertility cases are attributed to the male due to numerous andrological problems that affect the male genital tract such as cryptorchidism, varicocele, hormonal disorders, ejaculatory dysfunction and infectious diseases that are categorized as male genital tract infection (MGTI) (Agarwal, Mulgund, Hamada, & Chyatte, 2015; Kumar & Singh, 2015).

The literature reports that about 15% of the male infertility cases are linked to male genital tract infections (MGTIs), which would cause an abnormal increase in leukocyte counts in the human ejaculate (Pellati et al., 2008; Sandoval, Raburn, & Muasher, 2013). The elevation of seminal leukocyte counts, a condition referred to as 'leukocytospermia', is defined by the World Health Organization (WHO) as the presence of more than 1×10^6 leukocytes/ml in the ejaculate (WHO, 2010). Leukocytospermia may indicate an obstruction or infection of the genitourinary tract, and the likely consequence can negatively affect sperm function by causing acrosomal damage, midpiece and tail defects (Aziz, Agarwal, Lewis-Jones, Sharma, & Thomas, 2004; Djordjevic, Lalic, Vukovic, Nale, & Micic, 2018; Erenpreiss, Hlevicka, Zalkalns, & Erenpreisa, 2002; Kaleli, Öçer, Irez, Budak, & Aksu, 2000).

Previous reports estimate that about 30% to 50% of male infertility patients are idiopathic (Keck, Gerber-Schäfer, Clad, Wilhelm, & Breckwoldt, 1998; Krausz, 2011). Infertile couples experience many emotions, including depression, anger and shame, and they are sometimes being mocked, embarrassed and even pressured by peers, friends and parents, particularly in societies where there are high expectations for bearing children after marriage (Wright

et al., 1991). The role of MGTI and their relevance to male infertility continues to be debated, as the mechanisms by which microorganisms and leukocytes contribute to the problem remain controversial. This review presents and considers the role of infections and leukocytes in male infertility and their implications in the development of reproductive disorders.

1.1 | Prevalence of infection-associated male infertility

In some developed countries in North America, Australia, and Central and Eastern Europe, literature reports an estimate of approximately 4.5%–6%, 8%–9% and 8%–12%, respectively, of infertile males (Agarwal et al., 2015). In developing countries, more than 25% of married men are diagnosed with primary or secondary infertility of at least 5 years' duration (Rutstein & Shah, 2004). The majority of infertility problems in the developing countries are secondary. Sub-Saharan Africa has the highest prevalence of male infertility within the developing countries (Agarwal et al., 2015). This may be due to lifestyle issues such as smoking, work-related exposure, diet or other diseases linked to sexually transmitted infections (STIs) (Lunenfeld, Van Steirteghem, & Foundation, 2004). It is suggested that the excessive cost of most infertility treatments and assisted reproductive techniques (ARTs) could represent to a significant barrier for accessing treatment in these developing countries (Lunenfeld et al., 2004).

Following idiopathic infertility (28.4%) and varicocele (18.1%), MGTIs are the third most common causes of male infertility with a prevalence of 11.6% (Nieschlag & Behre, 1997), while other studies report a prevalence of MGTI between 35% and 45% (Bayasgalan et al., 2004; Henkel et al., 2007). These infections are mainly caused by sexually transmitted pathogens such as *Chlamydia trachomatis*, *Escherichia coli and Neisseria gonorrhea*, and as a result, an excessive build-up of seminal leukocytes within the male genital tract (MGT) ensues. These pathogens and their mediators may then cause irreversible damage, especially to the testis and epididymis (Schuppe et al., 2017). On the other hand, MGTI can be seen as potentially correctable cause of male infertility as they can be treated with antibiotics and anti-inflammatories to relieve the consequences of the infection, and obstruction of the excurrent genital ducts (Weidner, Krause, & Ludwig, 1999).

1.2 | Deterioration of spermatogenesis and sperm function

Spermatogenesis is a highly specialised biological process, which depends on a precisely controlled cascade of cellular differentiation and proliferation. It begins with a cell division of diploid spermatogonial stem cells and continues with sequential cell divisions of spermatogonia and meiosis of spermatocytes to form round spermatids and finally, morphological differentiation of these cells into matures spermatozoa (Shukla, Mahdi, & Rajender, 2012). Infections

Potential areas of research

- Investigation of the effectiveness of an adjuvant therapy with antioxidants to alleviate the detrimental effect of ROS
- Investigation of the physiological role of cytokines in seminal plasma
- Investigation of the role and clinical value of leukocytospermia

or inflammatory processes in the male genital tract can alter the process of spermatogenesis, and this can lead to deterioration in the blood–testis barrier and significant formation of sperm antibodies which can be detected in serum and seminal plasma (Fijak et al., 2018). Sperm antibodies in semen may adversely affect sperm function and impair sperm motility and fertilization ability (Fijak et al., 2018). In turn, impaired spermatogenesis and poor sperm viability can result in higher leukocyte counts which then phagocytose and thereby eliminate the defective spermatozoa (Barratt, Bolton, & Cooke, 1990; Domes et al., 2012). The presence of bacteria and the recruitment of leukocytes in the male genital tract can affect male fertility through multiple mechanisms such as direct cellular interactions, agglutinations, the release of reactive oxygen species (ROS) and cytokines not only leading to deterioration of spermatogenesis, and genital tract dysfunction, but also to deteriorated sperm function and integrity (Domes et al., 2012).

ROS can cause lipid peroxidation of the sperm plasma membrane, which has an extraordinary high amount of polyunsaturated fatty acids (Parks & Lynch, 1992) and is therefore specifically prone to oxidative damage. In addition, ROS have been linked to increase in sperm DNA fragmentation (SDF) (Alahmar, 2019; Lopes, Jurisicova, Sun, & Casper, 1998; Mahfouz et al., 2010). Considering that the oxidative damage can occur in testis and epididymis as well as in the ejaculate, DNA-damaged spermatozoa can be found in all of these sites (González-Marín, Gosálvez, & Roy, 2012). Even if the male accessory sex glands are infected, sperm function including the DNA can be affected by the influence of ROS produced by activated leukocytes as these trigger apoptosis in mature human spermatozoa (Sasikumar, Dakshayani, & Sarasa, 2013). Bacteria can also directly induce apoptosis in spermatozoa (Villegas, Schulz, Soto, & Sanchez, 2005). Sperm DNA damage may affect early post-implantation embryo development and thus decrease the fertility and pregnancy rate (Borini et al., 2006). A recent meta-analysis indicated that men whose ejaculates have a percentage of more than 20% DNA-fragmented spermatozoa have a high probability of being infertile (Santi, Spaggiari, & Simoni, 2018).

An orchitis, that is an inflammatory lesion of the testis, can lead to tubular damage (Weidner et al., 2002), spermatogenic arrest and testicular atrophy, and can be the cause of intratesticular obstruction in approximately 15% of the cases (Diemer & Desjardins, 1999; Weidner et al., 2002). In a mouse model, distinct cellular and

molecular indications of testicular inflammation with disruption of spermatogenesis can be seen (Klein et al., 2020). It also appears that various immunopathological alterations in the testis play a major role leading to irreversible damage of spermatogenesis (Schuppe & Meinhardt, 2005; Schuppe et al., 2008, 2010).

2 | SEMINAL LEUKOCYTES

Most of the seminal leukocytes derive from the epididymis (Wolff, 1995). The appearance of leukocytes in the male reproductive tract and ejaculate is common (el-Demiry, 1987) as they play an important role in immunosurveillance (Kiessling, Lamparelli, Yin, Seibel, & Eyre, 1995). Yet, their role is controversially discussed. Some authors have claimed that seminal leukocytes may not be just a response to infection, but rather act to scavenge abnormal germ cells and would play some kind of positive role in surveillance and phagocytosing of abnormal and dead spermatozoa (Jung et al., 2016; Kaleli et al., 2000). Other studies, however, paint a different picture by pointing out leukocytes as major contributors of ROS production in semen (Plante, de Lamirande, & Gagnon, 1994; Pratap, Hottigoudar, Nichanahalli, Rajendran, & Bheemanathi, 2019; Sharma, Pasqualotto, Nelson, & Agarwal, 2001).

According to the WHO guidelines (WHO, 2010), the seminal leukocyte concentration should not exceed 1×10^6/ml. Leukocyte concentrations higher than this cut-off value are regarded as leukocytospermia, a poorly defined and understood condition (Brunner, Demeter, & Sindhwani, 2019). Leukocytospermia-induced sperm damage is a likely result of the high levels of leukocyte-derived ROS and inflammatory mediators (Agarwal, Mulgund, et al., 2014; Aitken & West, 1990; Henkel et al., 2005; Sharma et al., 2001). Due to their exceptionally high content of polyunsaturated fatty acids in their plasma membrane and cytoplasm (Parks & Lynch, 1992), spermatozoa are very susceptible to ROS and oxidative stress (Henkel, 2011). The excessive production of ROS has frequently been implicated as the mechanism by which pathogens and leukocytes are causing damage to male germ cells by triggering lipid peroxidation and damaging mitochondrial activity (Aitken, 2017). The sequence of events of this damage to spermatozoa involves lipid peroxidation, loss of membrane integrity, reduced motility, high incidence of DNA strand breaks and apoptosis (Henkel & Schill, 1998; Sanocka-Maciejewska, Ciupińska, & Kurpisz, 2005). Interestingly, these detrimental effects are obvious not only at the high lower reference value of 1×10^6 leukocytes/ml as recommended by the World Health Organization (WHO, 2010), but also at leukocyte concentrations of as low as 0.1×10^6 leukocytes/ml (Agarwal, Mulgund, et al., 2014; Henkel et al., 2005).

2.1 | Leukocyte subpopulations in semen

The appearance of leukocytes in an ejaculate is normal and, although infertile men generally have higher seminal leukocyte counts, apparently independent from the fertility status of a man (Wolff, 1995). The pre-dominant type of leukocyte in human ejaculates is granulocytes with 50%–60%, followed by 20%–30% macrophages and 2%–5% T lymphocytes (Wolff, 1995). Occasionally, semen samples are dominated by macrophages or T lymphocytes; the reasons for this are not clear. When correlating leukocyte subpopulation numbers with specific alterations of semen quality, high numbers of T-lymphocytes were associated with reduced sperm velocity (Wolff, 1995). This might be an effect of the T-cell cytokine interferon-γ, which has been reported to inhibit sperm motility (Fedder & Ellermann-Eriksen, 1995). In general, it is very difficult to identify effects produced by a specific leukocyte type because there is always a mixture of different leukocyte populations in semen (Wolff, 1995).

Numerous studies indicate leukocytospermia as a significant contributor to male infertility as this condition leads to decreasing sperm motility and increasing sperm DNA damage and thereby negatively affect sperm fertilizing ability (Fariello et al., 2009; Moubasher et al., 2018; Saleh et al., 2002). The incidence of pathological leukocytospermia in infertile men ranges from 2% to 35% (Barratt et al., 1990; Kung, Ho, & Wang, 1993) with usually reported averages between 10% and 20% (Lackner, Agarwal, Mahfouz, Du Plessis, & Schatzl, 2010; Zorn, Virant-Klun, & Meden-Vrtovec, 2000). Leukocytospermia causes an increase in ROS and pro-inflammatory cytokines, especially IL-8 and IL-6, for which higher seminal concentrations have been shown in men with genital tract inflammation (Aghazarian, Stancik, Pflüger, & Lackner, 2013; Lotti & Maggi, 2013; Saleh et al., 2002). Hence, elevated seminal leukocyte concentrations may reflect a genital tract infection or result from a secondary immunological response. In addition to genital tract infections, smoking, consumption of alcohol and marijuana may also increase seminal leukocyte concentrations (Close, Roberts, & Berger, 1990; Pasqualotto et al., 2008).

Leukocytospermia is associated with significantly lower progressive motility as compared to non-leukocytospermic samples (Lackner et al., 2010; Pratap et al., 2019). This deterioration of sperm motility is a likely result of the high levels of ROS and inflammatory mediators that are produced by leukocytes (Agarwal, Virk, Ong, & Du Plessis, 2014; Aitken, Smith, Jobling, Baker, & De Iuliis, 2014; Henkel et al., 2005; Saleh et al., 2002). Elevated levels of ROS can trigger apoptosis in mature human spermatozoa and thereby reduce sperm fertilizing capacity (Agarwal, Virk, et al., 2014; Aitken et al., 2014; Saleh et al., 2002). The oxidative stress caused by excessively high ROS levels can also cause lipid peroxidation of the sperm plasma membrane (Aitken, Clarkson, & Fishel, 1989; Cosci, Moretti, & Collodel, 2008; Twigg, Fulton, Gomez, Irvine, & Aitken, 1998) and has been linked to SDF and an inhibition of mitochondrial activity, which could interfere with nucleic acid synthesis, leading to chromatin damage (Pratap et al., 2019) and finally impairing tail motion (Tremellen, 2008).

On the other hand, the clinical finding of leukocytospermia is controversially discussed with different recommendations in different guidelines (Brunner et al., 2019; Sandoval et al., 2013). Despite

TABLE 1 Types of leukocytes, origin and numbers in fertile and infertile men (modified from Wolff, 1995)

Leukocyte type	Percentage in the ejaculate	Main origin	Fertile men	Infertile men
Total			170.000 (8.970–20.520.000)	1.035.000 (43.120–104.580.000)
Granulocytes	50%–60%	Prostate Seminal vesicle	100.000 (6.250–19.950.000)	537.000 (31.787–91.507.000)
Macrophages	20%–30%	Epididymis Rete testis	51.900 (2.800–997.500)	228.823 (10.395–8.123.750)
T-lymphocytes	2%–5%	Epididymis Rete testis	6.383 (ND–108.666)	31.367 (ND–5.142.250)
B-lymphocytes	Very small percentage	Epididymis Rete testis	ND (ND–25.200)	6.400 (ND–2.904.750)

the significant association between the number of seminal leukocytes and seminal ROS levels, a recent study by Homa et al. could not find higher levels of sperm DNA damage in semen samples with high leukocyte counts (Homa et al., 2019). Yet, SDF was significantly higher in semen samples with oxidative stress as measured by direct chemiluminescent measurement of ROS or with the MiOXSYS system. These results indicate that seminal oxidative stress has a multifactorial dimension and leukocytes are only one contributing factor. Therefore, the current cut-off value for leukocytospermia (≥10^6 leukocytes/ml) recommended by the World Health Organization has been criticised as being too high (Henkel et al., 2005; Sharma et al., 2001).

2.2 | Significance of seminal leukocytes

The significance of leukocytes in the semen remains highly controversial, and its association with semen quality is still a matter of considerable debate in scientific reports. It is generally believed that an increase in seminal leukocytes in ejaculated semen may indicate MGTI (Esfandiari, Saleh, Abdoos, Rouzrokh, & Nazemian, 2002; Moskovtsev, Willis, White, & Mullen, 2007; Pentyala et al., 2007; Sandoval et al., 2013). Yet, while some studies have reported no detrimental effects of leukocytospermia, other studies have correlated seminal leukocyte elevation with impaired semen parameters, especially sperm morphology, motility and viability (Aziz et al., 2004; Lackner et al., 2010).

Adding to the confusion is another study by Kaleli et al. suggesting that seminal leukocytes at concentrations between 1 and 3 × 10^6/ml would be beneficial for sperm function due to effects of scavenging of abnormal spermatozoa (Kaleli et al., 2000). Tomlinson et al. reported that leukocytes phagocytosed abnormal spermatozoa (Tomlinson, Barratt, & Cooke, 1993), whereas Kiessling et al. found an improvement in sperm motility in semen samples with a leukocyte concentration of >2 × 10^6/ml (Kiessling et al., 1995). Lackner et al. suggest that the effects of leukocytes on sperm motility and normal sperm morphology would be concentration-dependent as at concentrations lower than 0.5 × 10^6 leukocytes/ml a higher leukocyte concentration would be beneficial for motility and morphology. At higher concentrations, however, these authors reported negative effects. On the other hand, a recent meta-analysis analysing 28 case-controlled retrospective studies reported that leukocytospermia was not associated with poor male fertilizing potential in assisted reproduction programs and semen quality in asymptomatic men (Castellini et al., 2020).

3 | ORIGIN OF SEMINAL LEUKOCYTES

It is thought that a major amount of leukocytes is originating from the rete testis or epididymis (Anderson et al., 1991). Apparently, these leukocytes play a major role in immunosurveillance and phagocytic clearance of abnormal spermatozoa (Tomlinson, White, Barratt, Bolton, & Cooke, 1992). While these white blood cells are rather the macrophages, and T- and B-lymphocytes, the prostate and the seminal vesicles are rather the origin of granulocytes (Wolff, 1995). The types of leukocytes, origin and numbers in fertile and infertile men are depicted in Table 1.

3.1 | Non-inflammatory semen

Considering that for the chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) the diagnostic classification was not clear and many patients presenting without infection show pathogenic processes outside the prostate, a new classification system was introduced by the National Institutes of Health (Krieger, Nyberg, & Nickel, 1999; Nickel, 1998). In this system, category III (CP/CPPS) is subdivided into category IIIA (inflammatory CPPS) and category IIIB (non-inflammatory CPPS). Weidner, Krause, et al. (1999) and Weidner and Anderson (2008) indicate that semen parameters of patients in category IIIA (inflammatory) do not significantly differ from those in category IIIB (non-inflammatory) and fertile controls. However, a study by Krieger, Ross, Deutsch, and Riley (2003) shows that the inclusion of seminal leukocytes in the diagnostic workup resulted in the detection of 95% of the inflammatory cases. Another study indicates that the inclusion of peroxidase-positive leukocytes and polymorphonuclear elastase as parameters in the

diagnostics as levels higher than 0.113×10^6 leukocytes/ml and more than 280 ng elastase/ml are indicative of an inflammatory process (Ludwig et al., 2003).

3.2 | Inflammatory semen

Several studies reported a strong association between inflammatory semen with the presence of leukocytes and male infertility (Comhaire, Mahmoud, Depuydt, Zalata, & Christophe, 1999; La Vignera et al., 2013; Wolff et al., 1990). Semen quality can be reduced through dysregulation of spermatogenesis due to the presence of microorganisms and activation of seminal leukocytes (Comhaire et al., 1999; La Vignera et al., 2013). In turn, leukocytes release not only ROS, but also pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1α, interleukin-6 or interleukin-8 leading to an inflammatory response (Comhaire et al., 1999; Haidl, Haidl, Oltermann, & Allam, 2015; Kocak, Yenisey, Dündar, Okyay, & Serter, 2002). Evidence suggests that inflammation of the genital tract affects semen quality and can lead to deterioration of the spermatogenesis, impairment of sperm function and obstruction of the seminal tract (Azenabor, Ekun, & Akinloye, 2015). Inflammatory conditions considerably influence the secretory function of the male accessory organs. Genital tract inflammations can affect urethra, epididymis, testicles and prostate gland (Azenabor et al., 2015).

Cytokines, especially TNF-α and interleukin-8, either alone or in the presence of leukocytes, can trigger sperm lipid peroxidation (Fraczek, Sanocka, Kamieniczna, & Kurpisz, 2008; Martinez, Proverbio, & Camejo, 2007). A recent study by Chyra-Jach et al. shows that seminal interleukin-8 levels in patients with asthenozoospermia and oligoasthenozoospermia are more than 60% higher than in the controls (Chyra-Jach et al., 2018). An in vitro study by Leisegang and Henkel indicates that cytokines such as TNF-α, IL-1β and IL-6 cause a dose-dependent decline in testosterone synthesis in TM3 Leydig cells suggesting that chronic inflammation may even directly affect steroidogenesis by direct modulation of Leydig cell function, thus compromising male fertility (Leisegang & Henkel, 2018).

3.2.1 | Prostatitis

Prostatitis is an inflammation of the prostate gland with a prevalence between 4% and 11%. Prostatitis is the most common urological diagnosis in younger and middle-aged men (Nickel, Downey, Hunter, & Clark, 2001). Approximately 5%–10% of the diagnosed prostatitis are of bacterial origin (Brunner, Weidner, & Schiefer, 1983). While the Gram-negative bacterium *Escherichia coli* is the cause of bacterial prostatitis in about 80% of the cases (Lopez-Plaza & Bostwick, 1990; Weidner, Ludwig, Brähler, & Schiefer, 1999), other bacterial infections that have been isolated in prostatitis cases are Gram-positive *enterococci* (Sharp, Takacs, & Powell, 2010) *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Nesisseria gonorrhea, and Klebsiella species* (Marconi, Pilatz, Wagenlehner, Diemer, & Weidner, 2009).

Since patients present with multiple urogenital, perineal and perianal symptoms, prostatitis is characterized by very diverse clinical symptoms such as acute bacterial infection or chronic pelvic pain syndrome (prostatitis syndrome) (Roberts, Lieber, Bostwick, & Jacobsen, 1997; Roberts et al., 1998) and is recognized as one cause of male infertility with the clinical presentation varying from asymptomatic inflammation to severe urological symptoms. Literature reports have shown that prostatitis is linked with decreased prostatic excretory function and has negative impact on male fertility potential affecting sperm morphology as well as sperm motility (Alshahrani, Mcgill, & Agarwal, 2013). The National Institutes of Health proposed a classification of prostatitis syndromes in four categories (Krieger et al., 1999): acute and chronic bacterial prostatitis (categories I and II), chronic pelvic pain syndrome (CPPS) (category III) and asymptomatic inflammatory prostatitis (category IV). Men with chronic prostatitis present an episodic and relapsing condition characterized by pelvic pain, irritative voiding symptoms and effects on sexual function (McNaughton Collins, MacDonald, & Wilt, 2000).

3.2.2 | Epididymitis

Epididymitis is an inflammatory condition of the epididymis in males presenting with acute unilateral or bilateral swelling of the scrotum. In young, sexually active men, the inflammation is caused in most cases by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, whereas *E. coli* is pre-dominantly found in older men (Ludwig, 2008; Weidner, Krause, et al., 1999). In the latter group of patients, a higher risk of urethral strictures, bladder neck obstruction or benign prostatic hyperplasia (BPH) has been reported (Chan & Schlegel, 2002). In addition to the loss of sperm function, inflammatory obstruction of the epididymal duct has been considered as an underlying cause of persistent azoospermia or oligozoospermia (Schuppe & Bergmann, 2013). Histopathological results of epididymitis are characterized by massive infiltration of neutrophils in the interstitial compartment and loss of adluminal compartment and thickened lamina propria in the seminiferous tubules (Schuppe & Bergmann, 2013).

The inflammation may spread to the corresponding testes as 'epididymo-orchitis' and has consistently been associated with high rates of infertility in many clinical studies. In a report by Osegbe, many men with unilateral epididymo-orchitis had contralateral biopsies showing bilateral gonadal damage and also experienced azoospermia (Osegbe, 1991). Collective analysis indicates profound deterioration of semen quality (sperm concentration, motility, morphology) together with pronounced leukocytospermia in the acute phase of the disease (Osegbe, 1991). Due to the bacterial infection ascending to the testis, a testicular involvement with spermatogenesis being affected may occur in 60% of the cases (Ludwig, 2008). In these cases, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are etiologically responsible.

3.2.3 | Orchitis

Orchitis is an inflammatory lesion of the testes that can be caused by *Chlamydia trachomatis* and *Neisseria gonorrhea*. These pathogens are the particular cause of the disease in men younger than 35 years. In contrast, in older men, *Escherichia coli* has been found to be the predominant trigger (Schuppe et al., 2008). Furthermore, the mumps virus affects the testicles as mumps orchitis in 20%–30% of the cases (Bartak, 1973) which may lead to infertility in up to 87% of the patients (Behrman, Kliegman, & Jenson, 2004; Caseslla, Leibundgut, Lehmann, & Gasser, 1997).

Clinically, an orchitis is associated with a pre-dominantly leukocytic exudate in the seminiferous tubules resulting in tubular damage (Schuppe & Bergmann, 2013; Weidner et al., 2002). Affected seminiferous tubules show degeneration of the germinal epithelium and thickening of the lamina propria which may result in fibrosis of the tubules (Schuppe & Bergmann, 2013; Schuppe et al., 2008). As a consequence of this infection, testicular atrophy with spermatogenic arrest can occur. In turn, this would then result in poor sperm quality with low sperm counts (Schuppe et al., 2008). In about 15% of the cases of azoospermia, an orchitis is the cause of an intratesticular obstruction (Weidner et al., 2002).

3.2.4 | Urethritis

Urethritis is an inflammation of the urethra caused by sexually transmitted pathogens such as *Chlamydia trachomatis*, *Mycoplasma* or *Neisseria gonorrhoea*. *Neisseria gonorrhea* is isolated in approximately 20% of men with urethritis (Ness, Markovic, Carlson, & Coughlin, 1997), while in 30%–50% of men with non-gonococcal urethritis is caused by *C. trachomatis* and 10%–40% of infected cases of urethritis are associated with *U. urealyticum* (Ness et al., 1997). Non-sexually transmitted uropathogens such as Enterobacteriaceae and staphylococci are also triggering urethritis with an incidence between 20% and 31% (Ochsendorf, 2006). In addition, non-infectious urethritis can be caused by injuries, masturbation or certain medical treatments.

Symptoms of urethritis in men typically include urethral discharge, penile itching or tingling, and dysuria (Brill, 2010). In a prospective study by Osegbe, 45 men with gonococcal urethritis showed extensive seminiferous tubular necrosis and inflammatory cell infiltration. After a 2-year follow-up period, 27% of the patients were found to have persistent azoospermia, and 33% had no significant improvement in sperm density (Osegbe, 1991).

3.2.5 | Male accessory gland infections (MAGI)

Originally, the term 'male accessory gland infection' (MAGI) was established to attribute ejaculatory inflammatory signs to the originally affected organ which may explain why this specific term was chosen (Comhaire, Verschraegen, & Vermeulen, 1980). It comprises the infection/inflammation of the prostate, seminal vesicles and the Cowper's glands. According to Dohle et al. (2005), however, the definition would be wider and includes epididymitis and inflammations of the excurrent duct system, thus anatomical parts of the male reproductive system that are not deemed accessory glands. However, in the light of all these organs being commonly affected and the notion by Fijak et al. (2018) that the term MAGI ignores important diagnostic aspects of infectious/non-infectious or autoimmune inflammatory conditions. Since a clear distinction between specific localized infections cannot be made (Krause, 2008), one should rather use the term 'male genital tract inflammation' (Haidl, Haidl, Allam, & Schuppe, 2019).

Previously, the presence of one or more abnormal sperm parameters associated with general symptoms of male genital tract inflammations such as leukocytospermia, presence of bacteria and a history of urogenital tract infections/inflammations was widely accepted as parameters having diagnostic significance (Rowe, Comhaire, Hargreave, & Mellows, 1993). However, more recently, elevated levels of polymorphonuclear granulocyte elastase (≥230 ng/ml) and pro-inflammatory cytokines such as IL-6 and IL-8 have emerged as more promising parameters in the diagnosis and management of MAGI (Depuydt, Bosmans, Zalata, Schoonjans, & Comhaire, 1996; Grande, Milardi, Baroni, Luca, & Pontecorvi, 2018; Kocak et al., 2002; Schiefer & von Graevenitz, 2006) as these parameters may significantly compromise sperm functions by directly affecting sperm function and increasing seminal ROS levels (Aitken et al., 1998; Fraczek et al., 2008; Henkel et al., 2005). Considering that the infection/inflammation is also affecting the secretory functions of the accessory glands, determination of their secretions such as citric acid, fructose, α-glucosidase, phosphatase or zinc (Krause, 2008; Wolff, Bezold, Zebhauser, & Meurer, 1991) can also be done to provide additional information (La Vignera et al., 2013).

4 | UROGENITAL TRACT INFECTION AND LEUKOCYTOSPERMIA

Infections in the male genital tract are discussed as a leading cause of male infertility as infections adversely affect sperm parameters (low sperm count, sperm motility, sperm morphology, etc.) and may lead to activation and accumulation of seminal leukocytes. Generally, male genital tract infections are characterized by leukocytospermia (≥10^6 peroxidase-positive leukocytes/ml), elevated levels of polymorphonuclear granulocyte elastase (≥230 ng/ml), ROS and cytokines (Depuydt et al., 1996; Kocak et al., 2002; Schiefer & von Graevenitz, 2006). A study by Punab et al. suggests that seminal leukocyte counts correlate significantly with the number of different microbes as well as the total microbial count (Punab, Lõivukene, Kermes, & Mändar, 2003). In addition, the increase in the leukocyte numbers is associated with the initiation of phagocytosis.

Leukocytes release pro-inflammatory cytokines IL-6 and IL-8 (Aghazarian et al., 2013; Arata de Bellabarba et al., 2000; Lackner et al., 2010; Saxena, Soni, Randhawa, & Singh, 2019), which, in turn,

modulate the pro- and antioxidative system activation and promote an oxidative burst with the production of ROS (La Vignera et al., 2014; Saleh et al., 2002). Yet, the role of leukocytes in semen is controversially discussed. While Kiessling et al. indicated that seminal leukocytes have rather positive effects by surveilling spermatozoa for their normal function (Kiessling et al., 1995), Lackner et al. see a rather differentiated role with lower concentrations than 0.5×10^6/ml having positive effects and higher concentrations negatively affecting sperm motility and morphology (Lackner et al., 2010). Contrary, Trum et al. (1998) report that leukocytospermia (>10^6/ml) is of no diagnostic value to differentiate men with genital tract infections. Arata de Bellabarba et al. (2000) point out that leukocytospermia is clearly associated with poor semen quality. On the other hand, the presence of leukocytospermia has apparently no negative effect on fertilization and pregnancy after ICSI and IMSI (Cavagna et al., 2012). Fraczek et al. (2014) concluded that seminal bacteria are negatively associated with sperm fertilization potential, thus emphasising the negative impact of bacteria and leukocytes on sperm motility and sperm membrane lipid bilayers.

The kinetics of urogenital tract infections may be characterised by different mechanisms (Fraczek & Kurpisz, 2007). The initial steps begin with the direct effects of bacteria and leukocytes on spermatozoa followed by indirect effects of the bacteria and leukocytes on spermatozoa. Several pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Ureaplasma urealyticum* or *Mycoplasma hominis* have been show to decrease various sperm functions such as count, motility or mitochondrial membrane potential (Fraczek et al., 2012; Isaiah, Nche, Nwagu, & Nnanna, 2011; Köhn et al., 1998; Mazzoli et al., 2010; Mehta, Sridhar, Kumar, & Kumar, 2002; Rybar et al., 2012; Sanocka-Maciejewska et al., 2005). This direct interaction of bacteria with spermatozoa appears to be a consequence of adhesion of the pathogens to the sperm membrane, which contains a high amount of glycoproteins, and is therefore susceptible to bacteria binding in receptor-specific interactions resulting in agglutinations (Kaur & Prabha, 2014; Monga & Roberts, 1994).

Indirect effects of bacteria and leukocytes on spermatozoa include the actions of ROS, the induction of apoptosis or necrosis and immune reactions, which include the effects of pro-inflammatory cytokines. Several studies show a significant positive relationship between the number of leukocytes and the seminal ROS levels (Esfandiari, Sharma, Saleh, Thomas, & Agarwal, 2003; Henkel et al., 2005; Henkel & Schill, 1998; Homa et al., 2019; Lobascio et al., 2015). Excessive amounts of ROS are causing oxidative stress which is known to have detrimental effects on spermatozoa as they trigger lipid peroxidation of the extremely susceptible sperm plasma membrane or directly cause sperm DNA damage (Aitken et al., 2014; Lopes et al., 1998; Mahfouz et al., 2010; Parks & Lynch, 1992). Finally, the end products of lipid peroxidation, which are not only mutagenic and genotoxic (Luczaj & Skrzydlewska, 2003), but also cytotoxic, ultimately cause DNA damage by formation of DNA adducts (Esterbauer, 1993). Furthermore, these end products such as malondialdehyde or 4-hydroxy-nonenal may persist in the semen for a prolonged period of time after the infectious agent has been eradicated, and may therefore further damage the male germ cells (La Vignera et al., 2014). According to Fraczek et al. (2016), bacteria mainly cause mitochondrial-dependent apoptotic cell death of spermatozoa, whereas leukocytospermia is the driving force for oxidative damage of the spermatozoa.

Another indirect effect of an infection is the induction of apoptosis. Although apoptosis in ejaculated human spermatozoa is controversially discussed, a number of reports suggest this process as one cause for male infertility (Roessner, Paasch, Kratzsch, Glander, & Grunewald, 2012; Said et al., 2006; Zorn, Golob, Ihan, Kopitar, & Kolbezen, 2012). During this process, mature spermatozoa externalise phosphatidylserine from the inner membrane leaflet to the outer leaflet and lose mitochondrial membrane potential (Koppers, Mitchell, Wang, Lin, & Aitken, 2011). Consequently, the mitochondrial electron transport chain is uncoupled resulting in intracellular ROS production, which then exerts their deleterious effects on the spermatozoa (Aitken et al., 2012; Koppers et al., 2011; Treulen, Uribe, Boguen, & Villegas, 2015). In patients with urogenital tract infections and inflammations, higher percentages of apoptotic spermatozoa have been reported (Allam et al., 2008; La Vignera, Condorelli, D'Agata, Vicari, & Calogero, 2012).

4.1 | WHO definition

There is controversy whether the WHO definition of leukocytospermia as more than 1×10^6 leukocytes/ml in semen is a valid threshold (WHO, 2010). However, on the basis of the outcomes of semen quality and results of in vitro fertilization, De Geyter, De Geyter, Behre, Schneider, and Nieschlag (1994) indicated no association between leukocytospermia and fertilization in vitro. Only at leukocyte concentrations higher than 6×10^6/ml IVF success rates dropped. Wolff indicated that a cut-off value for leukocytes in fertile men varies from 1×10^6 to 2×10^6/ml in semen, which would indicate that the clinically relevant level for leukocytospermia could be higher than 10^6/ml (Wolff, 1995). On the other hand, others consider it as a very high cut-off (Henkel et al., 2005; Punab et al., 2003; Sharma et al., 2001). In these studies, significant differences in motility, SDF, etc., were found with cut-off values as low as 0.1×10^6/ml. Aitken and co-workers even reported significant effects in motility if Percoll-separated spermatozoa were exposed to more than 2×10^4/ml (Aitken et al., 1995).

4.2 | Pathogens causing urogenital infections

The most prevalent pathogens in the male reproductive tract *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhea*, *Mycoplasma hominis*, *Mycoplasma genitalium* or *Escherichia coli*. Except for *Escherichia coli*, which is particularly responsible for epididymo-orchitis or prostatitis in 65%–80% of the cases (Pellati et al., 2008), the other listed uropathogens are sexually transmitted. These microorganisms have been shown to greatly affect the

fertilizing capacity of males due to their effects on human spermatozoa (Huwe et al., 1998; Sanocka-Maciejewska et al., 2005; Villegas, Schulz, Soto, & Sanchez, 2005). They can cause an asymptomatic inflammation in the male urogenital tract (Sanocka-Maciejewska et al., 2005; Villegas, Schulz, Soto, & Sanchez, 2005) or an acute inflammatory response with a flow of leukocytes into the genital tract leading to deterioration of spermatogenesis, impairment of sperm function (Skau & Folstad, 2003) and increase the production of ROS (Villegas, Schulz, Soto, Iglesias, et al., 2005).

4.2.1 | *Chlamydia trachomatis*

According to the World Health Organization (2011), chlamydial infections are the most common sexually transmitted disease with about 101 million infections being detected annually. Since this infection is rather asymptomatic in about 50% of men and up to 80% in women, this number is rather underestimated (Gonzales et al., 2004) and even newborns are infected during delivery accounting for 25%–50% of conjunctivitis and 10%–20% of pneumonia in babies. Villegas, Pinon, Shor, and Karchmer (1991) demonstrated elementary bodies of *Chlamydia trachomatis* in the connective tissue in the testis and in Leydig cells. Other organs of the male reproductive tract such as the prostate (Corradi et al., 1996), epididymis and seminal vesicles (Bornman et al., 1998) were also positive for Chlamydia. Other studies showed that the lipopolysaccharides secreted by Chlamydiae can directly damage spermatozoa leading to cell death, induction of apoptosis and protein alterations (Eley, Hosseinzadeh, Hakimi, Geary, & Pacey, 2005; Hosseinzadeh, Brewis, Pacey, Moore, & Eley, 2000; Hosseinzadeh, Pacey, & Eley, 2003).

This pathogen is the most important etiologic cause of non-gonococcal urethritis and acute epididymitis in men younger than 35 years (Bar-Chama & Fisch, 1993). Kokab et al. established a significant relationship between infections with *Chlamydia trachomatis* and increased IL-8 levels as well as seminal leukocyte concentrations (Kokab et al., 2010). The percentage of progressively motile spermatozoa decreased in patients with *Chlamydia trachomatis* infections. Infection with these bacteria has significant detrimental effects, not only on sperm parameters in general, but also specifically on DNA integrity (Zeyad, Amor, & Hammadeh, 2017).

4.2.2 | Mycoplasmataceae

Mycoplasmataceae is a family of bacteria which comprises *Mycoplasma* and *Ureaplasma*. Mycoplasmas are sexually transmitted bacteria causing clinical presentation such as cervicitis, urethritis, endometritis (Cohen et al., 2002; Sethi, Singh, Samanta, & Sharma, 2012), damages to the acrosomal membrane (Köhn et al., 1998) or can damage the sperm DNA via the secondary effects of the infection (Gallegos et al., 2008).

Ureaplasma urealyticum

Ureaplasma is one genus of the bacterial family of Mycoplasmataceae. In this genus, *Ureaplasma urealyticum* and *Ureaplasma parvum* are the most important pathogens of the Ureaplasma genus with andrological relevance. *Ureaplasma urealyticum* is frequently found in the urethra of sexually active men (Purvis & Christiansen, 1993) and causes symptomatic and asymptomatic non-gonococcal urethritis in up to 25% of the cases (Moskowitz & Mellinger, 1992), as well as pelvic inflammatory disease or infertility (Schiefer, 1998; Weidner, Ludwig, et al., 1999). Apart from causing higher seminal viscosity (Wang et al., 2006), an infection with *Ureaplasma urealyticum* has also effects on the male germ cells by negatively affecting sperm morphology (Zhang et al., 2011) as well as concentration, motility and vitality (Calogero, La Vignera, Condorelli, D'Agata, & Vicari, 2011; Köhn et al., 1998).

Although the role of *Ureaplasma* species was controversially discussed in the past (Kjaergaard et al., 1997; Potts, Sharma, et al., 2000), there is mounting evidence that these pathogens, particularly *Ureaplasma urealyticum*, play a significant role in genital tract infections under specific conditions (Beeton, Payne, & Jones, 2019). It appears that in infertile men, *U. urealyticum* is more prevalent than *U. parvum* (Zeighami, Peerayeh, Yazdi, & Sorouri, 2009; Zhang et al., 2014; Zhou, Ma, Shi, & Liu, 2018) indicating that *U. urealyticum* has a clearly higher causative role in infertile men. Nevertheless, *U. urealyticum* is also increasingly identified to cause adverse pregnancy outcomes (Capoccia, Greub, & Baud, 2013) and both *U. urealyticum* and *U. parvum* are implicated in chorioamnionitis (Sweeney, Dando, Kallapur, & Knox, 2017).

Mycoplasma hominis *and* Mycoplasma genitalium

Mycoplasma is another genus of the bacterial family of Mycoplasmataceae. These bacteria lack a cell wall. Andrologically important mycoplasmas are *Mycoplasma hominis* and *Mycoplasma genitalium*, both of which are causing urogenital infections (Andrade-Rocha, 2003; Deguchi & Maeda, 2002) and have been recognised as a common sexually transmitted disease (Yoshida, Maeda, Deguchi, & Ishiko, 2002). Infection incidences for *Mycoplasma hominis* and *Mycoplasma genitalium* are reported with 10.8% and 5%, respectively (Gdoura et al., 2007). Both pathogens are reported to affect the onset of pregnancy as they can attach and penetrate the sperm plasma membrane (Diaz-Garcia, Herrera-Mendoza, Giono-Cerezo, & Guerra-Infante, 2006; Taylor-Robinson, 2002; Vallely et al., 2018). Spermatozoa from samples tested positive for *Mycoplasma* DNA show a lower penetration rate into zona-free hamster oocytes as compared with controls (Kalugdan, Chan, Seraj, & King, 1996). A study by Ahmadi, Mirsalehian, Sadighi Gilani, Bahador, and Talebi (2017) shows that even an asymptomatic infection with *Mycoplasma hominis* significantly affects semen parameters including sperm count, motility, morphology, seminal ROS production and total antioxidant capacity. In contrast, the ejaculatory volume and pH were not negatively affected. After antibiotic treatment with doxycycline, all seminal parameters improved significantly.

4.2.3 | *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a Gram-negative diplococcus bacterium, which equally infects men and women at reproductive age, causing gonorrhoea (Edwards & Apicella, 2004) and is manifesting with urethritis, cervicitis and/or salpingitis (Da Ros & da Silva Schmitt, 2008). In men, it can also lead to prostatitis, epididymo-orchitis and infertility due to testicular damage or ductal obstruction (Bar-Chama & Fisch, 1993). The attachment of these bacteria to other cells including spermatozoa, where they may bind to an asialoglycoprotein receptor that binds lipopolysaccharides (Harvey et al., 2000), is mediated by pili as T1 gonococci to the cell membrane (Gomez, Stenback, James, Criswell, & Williams, 1979; Krause, 2008). In addition, T4 gonococci may also directly attach to spermatozoa (Stohl et al., 2012). In turn, this will attract leukocytes to the infection site and consequently increase seminal ROS levels, thereby damaging the male germ cells (Ochsendorf, 1999; Potts, Notarianni, & Jefferies, 2000).

Usually, the infection is symptomatic with severe dysuria and a purulent urethral discharge. Gonorrhoea is the second most frequently reported sexually transmitted disease in the United States with 583,405 reported cases in 2018 (Centers for Disease Control and Prevention, 2019). A recent meta-analysis of 147 studies from 56 countries indicates a global prevalence of 2.2% with Africa having the highest prevalence of 5.0% and the South-East Asia region with one out of 843 patients in 8 studies tested positive (0.1%) lowest (Chemaitelly et al., 2020).

4.2.4 | *Escherichia coli*

Escherichia coli is a Gram-negative bacterium causing most infections in the urogenital tract and male accessory gland (Weidner, Ludwig, et al., 1999). Several studies have described direct detrimental effects of *Escherichia coli* on sperm motility (Diemer, Huwe, et al., 2000), acrosome reaction (Köhn et al., 1998) and fertilization potential (Diemer et al., 2003; Huwe et al., 1998; Villegas, Boguen, & Uribe, 2017). The detrimental effects on motility and acrosome reaction might be due to the direct interaction of the pathogen by means of pili (Diemer et al., 2003; Sanchez, Villagran, Concha, & Cornejo, 1989) with the sperm plasma membrane leading to morphological alterations (Diemer, Huwe, et al., 2000). This decrease in sperm motility has been attributed to an agglutinating effect on spermatozoa (Kaur & Prabha, 2014; Liu et al., 2002). Sperm agglutination can be caused by bacterial type 1 and P fimbriae; particularly, the type 1 fimbriae of *Escherichia coli* cause a pattern of head–head type agglutination because they bind mannose residues in the head region of spermatozoa (Monga & Roberts, 1994).

According to Villegas et al., *Escherichia coli* is even able to induce early apoptotic events by activating several caspases and proteases responsible for mitochondrial changes and SDF (Fraczek & Kurpisz, 2015; Villegas, Schulz, Soto, Iglesias, et al., 2005). This leads to a breakdown of the sperm mitochondrial membrane potential, thus causing degeneration of ATP production. Moreover, soluble products of *Escherichia coli* reportedly decrease sperm motility by causing defects in the sperm cell's mitochondrial function (Barbonetti et al., 2013; Schulz, Sánchez, Soto, Risopatrón, & Villegas, 2010).

There are several mechanisms by which *Escherichia coli* is detrimental to spermatozoa. One of them is by mediating seminal leukocyte activation (Villegas et al., 2017). Leukocytes release pro-inflammatory cytokines (IL-6 and IL-8), which trigger a decrease in sperm motility (Diemer, Ludwig, Huwe, Hales, & Weidner, 2000; Villegas et al., 2017). This damaging effect seems to be mediated by polymorphonuclear (PMN) leukocytes. In addition, ROS released after bacteria-induced activation also play a major role in the deterioration of sperm quality. Specifically, *Escherichia coli* is able to induce a higher ROS production than other bacteria (Moretti et al., 2009; Villegas et al., 2017).

4.2.5 | Viruses

A growing body of evidence indicates that a number of viruses can infect all parts of the male genital tract and thereby negatively affect the male fertility potential. Among these viruses are the mumps virus (orchitis), human immunodeficiency virus-1 (orchitis and prostatitis), Coxsackie virus (epididymitis), cytomegalovirus (vesiculitis), human papillomavirus and herpes simplex virus (prostatitis) (Dejucq & Jegou, 2001). These infections lead to male infertility by increasing seminal leukocyte concentrations (Karamolahi et al., 2019; Umapathy, Simbini, Chipata, & Mbizvo, 2001; Weinberg, Sar-Shalom Nahshon, Feferkorn, & Bornstein, 2020; Wu et al., 2019). The latter virus can infect the testes and male sex accessory glands (Le Tortorec & Dejucq-Rainsford, 2010). Sexually transmitted viruses, among them human papillomavirus, hepatitis B virus, herpes simplex virus or Epstein–Barr virus, have been detected in semen (Dejucq & Jegou, 2001; Kapranos, Petrakou, Anastasiadou, & Kotronias, 2003).

There are two different modes by which viral infections can enter the male urogenital tract system: ascent through the urethra or hematogenously (Keck et al., 1998). These effects can be exerted either by direct toxic effects on the cells or indirectly via local inflammatory or immunological reactions (Keck et al., 1998). Due to the highly restrictive nature of the blood–testis barrier, the pathogens can possibly survive for extended periods by escaping immunosurveillance if they penetrate into the seminiferous tubules (Anderson & Politch, 1996). This is an aspect that can be of particular importance for assisted reproduction as this requires appropriate andrological diagnosis and management of the patient as well as proper handling of semen samples by the embryologist, because semen is a vector to propagate viruses. In addition, HIV viruses can bind to and penetrate into spermatozoa via a CD-4-independent receptor and/or the HIV co-receptor CCR5 (Bandivdekar, Velhal, & Raghavan, 2003; Muciaccia, Padula, Gandini, Lenzi, & Stefanini, 2005). Viruses originating from the testes or the epididymides can also attach to the male germ cells (Le Tortorec & Dejucq-Rainsford, 2010; Muciaccia et al., 2007).

Since angiotensin-converting enzyme-2 is present on Leydig and Sertoli cells and the fact that SARS-CoV-2, the virus that causes COVID-19, utilises this enzyme as a receptor to enter human cells, it is plausible that COVID-19 could affect testicular function and therefore male fertility (Illiano, Trama, & Costantini, 2020).

5 | MEDIATORS AND MECHANISMS OF INFLAMMATORY DAMAGE

5.1 | ROS

In urogenital tract infections, bacteria can be observed in the semen shortly after their entry into the male genital tract. In the second phase of the infection, leukocytes are attracted to combat the infection. While bacteria can directly interact with the spermatozoa and trigger the mitochondrial pathway of apoptosis (Barbonetti et al., 2013; Fraczek et al., 2012; Schulz et al., 2010), macrophages release cytokines and ROS as pro-inflammatory mediators of the infection (Fraczek & Kurpisz, 2015; Shahed & Shoskes, 2000; Zhou, Xiao, Zheng, Dong, & Zhang, 2006). Thereby, the pathogens are triggering the ROS release by stimulating glucose-6-phosphate dehydrogenase which is leading to the production of large amounts of NADPH providing the electrons to reduce oxygen to superoxide anion and other ROS. Consequently, these ROS trigger the activation of chemokines CXCL5, CXCL8, IL-6 and IL-8 (Comhaire, Bosmans, Ombelet, Punjabi, & Schoonjans, 1994), which will trigger further ROS production resulting in seminal oxidative stress and infertility (Agarwal, Saleh, & Bedaiwy, 2003).

5.2 | Cytokines

Since the testis, specifically the adluminal compartment, is an immune-privileged organ (Head, Neaves, & Billingham, 1983), Sertoli, Leydig and peritubular cells as well as testicular macrophages and testicular somatic cells are secreting immunoregulatory and immunosuppressive factors (Gao et al., 2016; Kaur, Thompson, & Dufour, 2014). An immunosuppressive phenotype of macrophages populating the testes produces CD163 and the anti-inflammatory cytokine IL-10 (Wang et al., 2017). In case of a testicular infection, secretion of pro-inflammatory cytokines such as TNF-α, IL-1 or IL-6 is reduced, while secretion of anti-inflammatory IL-10 is increased (Bhushan et al., 2011, 2015; Zhao, Guo, Wu, Xiong, & Zhou, 2008). In addition, Sertoli cells also secrete anti-inflammatory factors such as activin A, which appear necessary to compensate an excessive immune response (Hedger & Winnall, 2012). In contrast to the testis, the epididymis has not been shown to be immune-privileged as an immune response in the epididymis or other parts of the male reproductive tract. This can be seen in the fact that the incidence of epididymitis or other genital tract infections is much higher than that of an orchitis (Hedger, 2011).

Following a bacterial infection, leukocytes infiltrate the epididymis and the interstitial space after 1 day and 3 days, respectively (Lang et al., 2013; Tanaka, Fujisawa, Arakawa, & Kamidono, 1995). Alongside the leukocyte infiltration, an increase in pro-inflammatory cytokines can be seen (Turner, Mammen, Kavoussi, Lysiak, & Costabile, 2011), which, in turn, triggers lipid peroxidation and further ROS production by leukocytes.

5.3 | Proteases

After stimulation by pathogens, leukocytes do not only release ROS and cytokines, but also release proteases such as cathepsin G, collagenase and elastase. The latter can be distinguished in a serine proteinase coming from polymorphonuclear granulocytes and a metalloproteinase from macrophages (Zorn, Vidmar, & Meden-Vrtovec, 2003). Since free elastase is causing cell (Hautamaki, Kobayash, Senior, & Shapiro, 1997) and DNA damage (Yang, Kettritz, Falk, Jennette, & Gaido, 1996) and therefore negatively affects sperm functionality. On the other hand, Maegawa et al. (2001) and Henkel et al. (2003) did not find any negative effect of elastase on sperm motility. In seminal plasma, the enzyme is inhibited under normal conditions by α_1-protease inhibitor and α_2-macroglobulin (Kramer et al., 1990, 1992) and secretory leukocyte protease inhibitor is able to restore sperm motility that was affected by elastase (Moriyama et al., 1998) and could possibly be the reason why no association between the seminal elastase concentration and motility was found.

6 | INDUCTION OF ANTISPERM ANTIBODIES

Since the first reports on antisperm antibody-related male infertility by Rumke (1954) and Wilson (1954), this aspect has been investigated and controversially discussed (Francavilla & Barbonetti, 2017). In respect to male genital tract infections, an association between genital tract infections, infertility and antisperm antibodies has repeatedly been reported (Fjällbrant & Obrant, 1968; Witkin & Toth, 1983). Incidences of antisperm antibodies vary between 2% (Barbonetti et al., 2019) and 15% (Witkin, 1988). Higher incidences are reported in patients with genital tract infections and obstructions as well as after vasectomy and cystic fibrosis (Marconi & Weidner, 2017). It has also been shown that antisperm antibodies occur significantly more often in patients with a history of epididymitis than with testicular abnormalities (Lotti et al., 2018). This is probably due to the highly restrictive nature of the blood–testis barrier, which is only damaged in acute or chronic testicular infections or inflammations with testicular immune dysregulation. In cases of antisperm antibodies occurring naturally, the cause remains idiopathic.

The prognostic value of the presence of antisperm antibodies is controversially discussed. Leushuis et al. (2009) report in a large study that included 1,794 couples no prognostic value of the MAR test for the chances of achieving a pregnancy. In contrast, Barbonetti et al.

(2020) in a smaller study of 108 couples observed a significantly lower chance of live births after natural conception and intrauterine insemination if the MAR test was 100% positive. The authors therefore recommend testing for antisperm antibodies in the basic fertility workup.

7 | LEUKOCYTE INFILTRATION AND ART

Considering that leukocytes have a significant negative influence on sperm functions, the question arises whether leukocytospermic semen samples can be used for assisted reproduction. Surprisingly, the general presence of leukocytes in semen does not correlate with sperm concentration, motile sperm count and normal sperm morphology (Henkel et al., 2005). Aitken, West, and Buckingham (1994) indicated that the fertilizing potential of washed spermatozoa as assessed by acrosome reaction and the sperm–oocyte penetration test is not associated with the seminal leukocyte count. However, if leukocytes contaminated the washed sperm preparations, sperm functionality was significantly compromised. On the other hand, several studies show various techniques of assisted reproduction, namely IUI, IVF, ICSI and IMSI, and the leukocyte count does not have an influence on fertilization, implantation, pregnancy and live birth rates (Barraud-Lange, Pont, Pocate, et al., 2011; Barraud-Lange, Pont, Ziyyat, et al., 2011; Castellini et al., 2020; Cavagna et al., 2012; Ricci et al., 2015). It only appears that seminal leukocyte counts higher than 10^6/ml lead to lower pregnancy outcome with an elevated rate of early pregnancy loss (Barraud-Lange, Pont, Pocate, et al., 2011; Barraud-Lange, Pont, Ziyyat, et al., 2011). The meta-analysis by Castellini et al. (2020) further indicates that these recommendations are restricted to patients asymptomatic for genital tract infections.

These studies indicate that seminal leukocytes are only detrimental to spermatozoa and the fertilization process if they are in close vicinity of the male germ cells and if the seminal antioxidant system is not overwhelmed by the respiratory burst of activated leukocytes leading to the release of high amounts of ROS and cytokines. If leukocytes are removed by suitable sperm separation techniques prior to assisted reproduction, the yielded spermatozoa are fully functional and competent to fertilize oocytes.

8 | TREATMENT OF INFECTIONS

Considering that male genital tract infections are in their majority caused by bacterial pathogens, such infections can be treated with antibiotics and anti-inflammatories and are therefore potentially correctable causes of male infertility (Weidner, Ludwig, et al., 1999). Yet, it is to be noticed that many of these pathogens are sexually transmitted. Therefore, both partners have to be treated after proper diagnosis and administration of a suitable antibiotic after semen culture (Solomon & Henkel, 2017). Whereas guidelines for the treatment of acute bacterial epididymitis, epididymo-orchitis and specific granulomatous orchitis have been published (Weidner et al., 2002; Centers for Disease Control and Prevention, 2015), this is not the case for chronic infections and inflammations. Hence, treatment of these diseases rather relies on empirical and a small number of uncontrolled studies (Ludwig, 2008; Weidner et al., 2002). On the other hand, for viral infections such as mumps orchitis, a systemic treatment with 2β-interferon (Pal, 2013; Yeniyol, Sorguc, Minareci, & Ayder, 2000) was used to prevent testicular atrophy.

On the other hand, lesions due to the inflammatory processes can be alleviated with an antiphlogistic therapy, with both corticosteroids and non-steroids, and have shown significant positive effects on various semen parameters (Haidl, 2002; Hendry, Stedronska, Hughes, Cameron, & Pugh, 1979; Montag, van der Ven, & Haidl, 1999). In addition, antioxidant therapies to counteract the oxidative stress may be considered, but are still debated (Arafa et al., 2020; Haidl et al., 2019; Steiner et al., 2020; Tremellen, 2008).

9 | CONCLUDING REMARKS

Male genital tract infections are the third most common cause of male infertility. Despite the deleterious effects male genital tract infections can cause to the overall health of the patients in general, the male genital tract and spermatozoa in particular, hence on male fertility, these conditions can be rationally treated as most of these infections are due to bacterial pathogens. However, it is required that a proper andrological diagnostic with semen culture is carried out in order to avoid bacterial drug resistance. In addition, the treatment must be long enough to eradicate the infection. Moreover, it is mandatory that both partners have to be treated as otherwise continuous reinfections would occur. Since many patients suffer from asymptomatic, 'silent' infections, it is also essential that clinicians properly identify these conditions. Conversely, for patients trying to achieve pregnancy it is important to know that moderately high seminal leukocyte counts do not have a negative impact on assisted reproductive techniques, because the leukocytes are eliminated during sperm preparations. Therefore, this review aimed at providing an update on the current knowledge of pathophysiology of the most common male genital tract infections.

Key points

- Bacteria and viruses can directly interact with spermatozoa and compromise sperm functions
- As consequence of the infection, leukocytes are infiltrating the male genital tract and produce high amounts of ROS and cytokines
- Unphysiologically high levels of ROS are highly detrimental to spermatozoa
- Damages to the blood–testis barrier and much more commonly to the blood–epididymis barrier may lead to the production of antisperm antibodies
- Infections have to be treated properly
- Silent genital tract inflammations do not necessarily impact negatively on assisted reproduction

ORCID

Ralf Henkel https://orcid.org/0000-0003-1128-2982

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