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Review article

Effects of bacteria on male fertility: Spermatogenesis and sperm function

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ABSTRACT

Bacterial infection can negatively affect different parts of the male genital tract and subsequently cause impaired spermatogenesis and male fertility. However, most of the previous studies have focused on the infected organs of the male genital tract and there are not many studies that investigated the direct effect of bacteria on sperm and their mechanism of action. Interestingly, bacteria can induce different damages on sperm cells such as DNA fragmentation, cell membrane peroxidation, and acrosome impairment. Such negative effects can be mediated by bacteria-secreted toxins and metabolites or by direct attachment of bacteria on the sperm cells and subsequent activation of signaling pathways related to oxidative stress, apoptosis, and inflammation. These bacteria-induced changes can impair semen parameters and subsequently cause infertility. Given the significant destructive effect of some bacteria on sperm function and male fertility, in this study, we reviewed the impact of male urogenital bacteria on spermatogenesis and sperm functions as well as the underlying mechanisms by which the bacteria can damage sperm.

1. Introduction

Infertility, which affects > 20% of couples, has increased as a significant problem over the last thirty years. Studies showed that up to 50% of infertility cases are attributed to male factors and 40% are because of female factors; the remaining 10% are due to both. Recent statistics show that the percentage of male infertility has elevated from 40% to 60% since the 1980s [1–3]. Despite the massive progress in the understanding of human reproductive physiology, the causes of approximately 50% of infertility cases remain unknown, which are considered as idiopathic infertility. Multiple factors including endocrinal disorders, lifestyle, varicocele, ejaculatory disorders, hypogonadism, heavy alcohol consumption, genetic defects, sperm dysfunction, and urogenital tract infections are classic causes of male infertility [4].

Bacterial infections could damage different cells and affect their functions [5]. A variety of microorganisms, in particular, viruses and bacteria have been identified as the urogenital tract pathogens which can cause subfertility [6]. Infections caused by different gram-negative and positive bacteria are responsible for 15% of male primary infertilities [7]. In this respect, it has been documented that bacterial and viral infections caused by *Mycoplasma genitalium* (*M. genitalium*), *Human papillomavirus* (HPV), *Chlamydia trachomatis* (CT), hepatitis B virus (HBV), *Streptococcus faecalis* (*S. faecalis*), mumps, and tuberculosis are associated with male infertility [8–11]. Although the clinical importance of the genital bacterial infections in male fertility is commonly known, the harmful effects of different microbial species on male gametes are still being debated.

It has been reported that some of the bacterial infections are

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associated with prostatitis or epididymitis, urethritis, and orchitis or impairment of male sexual accessory gland function which can impair sperm vitality and motility [12–15]. Furthermore, bacteria of semen can contaminate the female genital tract during ejaculation; as a consequence, the sexually transmitted infections might cause gynecological disorders such as endometritis, cervicitis, ectopic pregnancy, and embryonic or fetal death [16,17].

Most studies have reported that the invasion of different microorganisms into the male reproductive tract could be detrimental to the semen quality and sperm function. Since the bacteria-induced male subfertility has not been comprehensively studied yet and on the other hand, impaired semen profile due to bacterial contamination is less considered in the clinical setting, in this review, we focused on the effects of male urogenital bacteria on spermatogenesis and sperm functions as well as the mechanism of action by which the bacteria can negatively affect sperm. This study can shed more light on the role of bacteria in male infertility and also encourage clinicians to more consider the bacteria contaminations.

2. Male reproductive system and bacterial infections

Urogenital tract infection is one of the significant infections and its early diagnosis and treatment are essential. Several gram-negative or gram-positive bacteria can colonize in the male genital tract, and so different gram-positive bacteria including *Staphylococcus aureus* (*S. aureus*), *S. faecalis*, *Streptococcus agalactia* (*S. agalactia*), and *Staphylococcus saprophyticus* (*S. saprophyticus*), as well as Gram-negative bacteria such as *Escherichia coli* (*E. coli*), *Ureaplasma urealyticum* (*U. urealyticum*), *Bacteroides* spp., CT, and *N. gonorrhoeae* have been isolated from the male genitourinary tract. Among bacterial infections, ureaplasmosis caused by *U. urealyticum*, chlamydiosis caused by CT, gonorrhoea caused by *N. gonorrhea*, syphilis caused by *Treponema pallidum* (*T. pallidum*), and nosocomial infections caused by enterococci (*e.g. Enterococcus faecalis*) are more common bacterial diseases causing urogenital infections [18,19]. Among these bacteria both CT and *E. coli* are the most widespread ones. Interestingly, it has been shown that human intestine floras such as *S. faecalis*, as well as many intestinal and extra-intestinal pathogenic *E. coli* strains, are the prominent uropathogenic factors [20,21]. In this regard, Pal et al. have reported a male C3H/HeN mice model of chlamydial infection by the mouse-adapted *Chlamydia muridarum* (*C. muridarum*) *via* the meatus urethrae caused the ascending infection, involving bladder, urethra, epididymides, and testes [22]. Ascending infections originating from sexually transmitted pathogens such as CT, as well as the hematogenous spread of systemic bacteria are the other most common reasons for urogenital infections [23].

Previous findings supported the potential role of various bacterial species in affecting different parts of the male genital tract which can cause chronic prostatitis/chronic pelvic pain syndrome, epididymitis, urethritis, and orchitis [24]. In this regard, an association has been reported between the chlamydial infection with prostatitis and epididymitis, which later can lead to stenosis of the duct structure, orchitis, and male sexual accessory gland impairment [25]. Moreover, the chlamydial infection has been identified as an etiological agent of approximately 50% of non-gonococcal urethritis as well as of the vast majority of post-gonococcal urethritis [26]. *Escherichia coli* has also been associated with male accessory gland infection and it has been isolated from the prostatic secretion in about 65–80% of men with chronic bacterial prostatitis problems [27]. Many authors have emphasized that the infections in the male genitourinary tract may affect fertility through testis damage and/or affecting the biologic function of mature gametes [28,29]. For example, Sobinoff et al., have shown that infection of male mice by *C. muridarum via* intrapenile inoculation could lead to ascending the infection to the testis and changing Sertoli cell function [30]. Such a significant effect of bacteria on the male genital tract might affect spermatogenesis and sperm function which are discussed in the next sections.

3. Bacteria and spermatogenesis

Spermatogenesis is a continuous and complex process of development, during which spermatogonia differentiate into spermatozoa [31]. During this process spermatogonia differentiate into spermatocytes; spermatocytes produce spermatids by a meiotic division and after the production of round spermatids, they can be matured into spermatozoa and be released into the testicular tubule lumen. The spermatogenesis takes place in a stepwise manner and is regulated by the interaction of different endocrine, autocrine, paracrine, and hormonal stimuli [32,33]. Several factors such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and androgen binding protein (ABP) are involved in the regulation of spermatogenesis. Luteinizing hormone stimulates testosterone production from Leydig cells, and FSH in accompany with testosterone stimulates Sertoli cells for spermatogenesis [34]. Sertoli cells also send nutrients and paracrine factors to the germ cells. It has been shown that different factors such as obesity, diabetes, hyper- and hypothyroidism, exogenous testosterone, environmental chemicals, varicocele, genetic disorders, and bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *E. coli* can impair the spermatogenesis and cause changes in sperm quantity and quality [35–39].

Many studies have mentioned that the bacterial infections in the male genitourinary tract might impair fertility *via* affecting testis and spermatogenesis. For example, it has been reported that infection of mice with *P. aeruginosa* could obliterate the ability to produce germ cells in the seminiferous epithelium and so impair the spermatogenesis *in vivo* and *in vitro* [40]. Sobinoff et al. have also shown that *C. muridarum* infection in the mice testis increased Sertoli cell apoptosis and decreased the number of germ cells. They also reported abnormal testis morphology and a reduction in seminiferous tubule diameter [30]. It has been revealed that Sertoli cells were more susceptible to chlamydial infection and had a significant reduction in cellular metabolic activity compared to Leydig and epididymal cells [30]. In a unilateral *E. coli* epididymitis rat model, a progressive loss of testicular integrity along with impaired spermatogenesis on the inoculated side has been observed [41]. It has also been generally accepted that *S. aureus* is a pathogenic agent that can damage the germ cells. However, the effect of some bacteria on spermatogenesis is still debatable; for example, both neutral and negative effects of chlamydia on spermatogenesis have been previously reported. Sobinoff et al. have demonstrated that infection of mice with *C. muridarum* led to ascending infection to the testes and impaired spermatogenesis especially at the early stages [30]. Several studies have also shown that a CT infection could reduce the number of normal sperm [42,43]. In contrast, several studies have not found any relationship between impaired spermatogenesis and a CT infection [44,45]. In addition to the bacterial species, the bacterial strain is a determinative factor for the bacterial effect on spermatogenesis. In this regard, Baud et al. reported that although both E and D serovars of CT are associated with urogenital infections, only serovar E could significantly decrease both spermatozoa motility and viability [46].

Previous studies have indicated that apoptotic signaling pathways, proinflammatory cytokines, and oxidative stress play important roles in bacteria-induced spermatogenesis dysfunction [47–49]. Most probably, the activated leukocytes following infection are the main source of reactive oxygen intermediate overproduction; it has been well documented that there is a significant association between oxidative stress and functional deficiency of germ cells [50,51].

Bacteria and their associated infections can seriously affect spermatogenesis and consequently semen parameters. For example, Rana and colleagues recently showed that incision of *P. aeruginosa* into the right vas deferens in mice could lead to severe damages of spermatogenesis [40]. In a similar study conducted by Yongning et al. it has been

demonstrated that uropathogenic *E. coli* could cause hypo-spermatogenesis and germ cell loss. This group suggested that necrotic changes in Sertoli cells and activation of cell-death pathways in the seminiferous tubules could contribute to impaired spermatogenesis [52]. While it is clear that bacteria may influence male fertility through the deterioration of spermatogenesis, the underlying mechanisms for various bacterial infections have largely remained unknown. It has been presented that toxic factors secreted by bacteria can also have negative effects on spermatogenesis. For example, some bacterial species generate lipopolysaccharides (LPS), an active component of the gram-negative bacteria cell wall, which can disrupt testicular steroidogenesis and spermatogenesis, possibly *via* activation of acute inflammatory response; a strong association between inflammation of the reproductive system and spermatogenesis impairment has been well-documented [53,54]. On the other hand, the inflammatory mediators such as interleukin (IL)-2 and IL-8 increase the levels of reactive oxygen species (ROS) and cause oxidative stress which can consequently affect spermatogenesis [55].

4. Bacteria and semen parameters

Although semen analyses have a limited role in the assessment of infertility, it is still used to predict the fertility status in men [56].Studies showed that bacterial infection exerts unfavorable effects on spermatozoa in both animals and humans. Jungwirth et al. have demonstrated that approximately 20% of infertile individuals with azoospermia have a history of an infection or inflammation in the urogenital tract [57]. The presence of *S. faecalis* in semen is associated with a high prevalence of oligozoospermia and teratozoospermia [21]. *S. aureus* as the causative organism of seminal infections can cause oligozoospermia, asthenozoospermia, teratozoospermia, and azoospermia [58]. Moreover, Idahl et al. reported an increase in the teratozoospermia index in infertile couples infected with CT [59]. It has also been reported that the azoospermia is correlated with the presence of *Pseudomonas aeruginosa*, and *E. coli* has a high occurrence among the oligozoospermic patients [60]. The bacteria involved in semen contaminations ordinarily originate from the urinary tract of subjects or might be transmitted by the partner through sexual intercourse.

Most studies have reported that the invasion of bacteria into the reproductive system may have detrimental effects on sperm parameters [61,62]. However, several researchers demonstrated that there was no significant difference in the sperm quality and quantity between infected and non-infected patients [63,64]. It seems that the effect of bacteria on sperm function depends on the species, type, and concentration of microorganisms [51]. Enwurua and colleagues reported that the gram-negative organisms have a more negative effect on the quality of semen compared to gram-positive organisms [18]. It has been revealed that gram-negative bacteria such as *E. coli*, CT, and *Pseudomonas aeruginosa*, as well as gram-positive bacteria such as streptococci, staphylococci, and enterococci, could negatively affect the quality of sperm. The effects of bacteria on sperm parameters and the underlying mechanisms are listed in Table 1.

4.1. Concentration

A low concentration of sperm can be a result of genital tract infection. For example, studies showed that the presence of *P. aeruginosa* or *S. faecalis* in the seminal fluid could lead to a reduction in sperm concentration [21,65]. It also has been reported that the genital tract infection by *Enterococcus faecalis* (*E. faecalis*) or CT is associated with compromised sperm concentration [67,68]. Moreover, Moretti et al. have illustrated that among 70 patients with *E. faecalis* contamination, 54 patients were infertile along with a significant reduction in sperm count [66]. In contrast, Motrich and colleagues showed that chlamydial infection had no significant unfavorable effect on sperm concentration [45]. Moreover, Gdoura et al. found no significant association between CT infection and semen volume as well as sperm count [80]. The underlying mechanism that can explain the negative effect of bacterial infection on sperm count is not clear, but as we already explained in Section 3, it can be due to the reduction of spermatogenesis (possible molecular mechanism are discussed in Section 3 Bacteria and spermatogenesis).

4.2. Motility

Sperm motility is the most apparent and most essential sperm function necessary for successful fertilization [56]. Interestingly, *in vivo* and *in vitro* studies showed a negative effect of bacteria such as CagA-positive *Helicobacter pylori*, mycoplasmas, staphylococci, streptococci, and different strains of *E. coli* on the motility of sperm [69–71,73]. The severity of bacterial damage on sperm motility depends on the concentration and serotype of the bacteria. For example, it has been observed that the H strain of *E. coli* could negatively affect sperm motility at different ratios (1:2, 1:16, and 1:128), while the NH-ATCC strain had a similar effect just at high concentrations (ratio of 1:128 sperm/bacteria) [81]. Moreover, Boguen et al. reported that two O6 uropathogenic *E. coli* (UPEC) strains did not cause sperm motility reduction [82]. Nevertheless, it has been demonstrated that sperm agglutinating factor (SAF) from *E. coli* could decrease the motility of mouse sperm [72]. Several epidemiological reports also presented that a CT infection of the male genital tract was associated with a reduction in sperm motility [42,67]. Al-Janabi et al. have found an association between the presence of *P. aeruginosa* in seminal fluid and changes in sperm motility [65]. Improvements in sperm speed and motility patterns have been also seen following the elimination of mycoplasma infections [83]. It has been found that chlamydial LPS from both E and LGV CT serovars, as well as chlamydial elementary bodies (EBs) from serovar E were able to reduce sperm motility [25]. However, an *in vitro* study showed that CT (serovar LGV or E) could not significantly affect the motility of highly motile spermatozoa from healthy donors or C57BL/6 mice [84]. Similarly, Gdoura et al. reported that sperm motility was not associated with the detection of CT DNA in the semen of sterile men [80]. Jacques et al. [85] and Huwe et al. [86] also reported no significant destructive effect of enterococci strains on sperm motility.

Different mechanisms have been suggested for the negative effect of bacteria on sperm motility, according to the type of bacterium. For example, it has been revealed that *E. coli* inhibits sperm motility through the attachment of bacterial fimbria to the receptors on the acrosome or flagellum of spermatozoa [87]. Another possible underlying mechanism is secreting toxic factors, such as LPS endotoxin [84]. Dwindling in sperm mitochondrial membrane potential ($D\Psi m$) and acrosomal membrane disruption can be further reasons for sperm motility reduction following bacterial infection [78,88]. It has been revealed that a decrease in sperm motility was associated with the presence of IgG and IgM antibodies against CT in the serum of men in infertile couples [78]. The 3-oxododecanoyl-L-homoserine lactone produced by *P. aeruginosa* represents the factor responsible for the reduction of sperm motility [89]. Moreover, previous studies demonstrated that hemolysin, a recognized virulence factor of enterococci, could damage the membrane integrity of human sperm and so cause abnormal motility [68].

4.3. Morphology (both observational and molecular evaluations)

Several documents showed a negative association between the presence of different strains of *E. coli*, CT, *P. aeruginosa*, *E. faecalis*, *Bacteroides ureolyticus* (*B. ureolyticus*), mycoplasma species, and *S. faecalis* in seminal fluid or male genital tract with sperm morphology [43,65,74,76]. The destructive effect of *B. ureolyticus* or its toxins on normal sperm morphology possibly due to membrane peroxidation has also been mentioned [74]. *Ureaplasma urealyticum* can bind to the human sperm membrane and consequently influence sperm

Table 1
Effect of bacteria on sperm parameters.

Bacteria	Sperm parameter	Mechanism(s)	Ref.
Pseudomonas aeruginosa, *Streptococcus faecalis*, *Enterococcus faecalis*, *Chlamydia trachomatis*	Concentration	• Impairing spermatogenesis • Causing leukocytes-induced oxidative stress • Inducing Sertoli cells apoptosis • Reducing the number of germ cells • Lipopolysaccharides-induced steroidogenesis disruption	[21,65–68]
Helicobacter pylori, *Chlamydia trachomatis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycoplasma* spp., *Staphylococcus* spp., *Streptococcus* spp.	Motility	• Agglutination of sperm by bacterial toxins (*e.g.* sperm agglutinating factor) • Damaging sperm membrane integrity by bacterial toxins (*e.g.* 3-oxododecanoyl-Lhomoserine lactone) • Attachment of bacterial fimbria on the acrosome or flagellum of spermatozoa • Reducing sperm mitochondrial membrane potential (DΨm)	[42,65,67,69,70–73]
Chlamydia trachomatis, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus faecalis*, *Bacteroides ureolyticus*, *Ureaplasma urealyticum*, *Mycoplasma* spp.	Morphology	• Impairing spermatogenesis and sperm maturation • Vacuolization of spermatozoa	[43,65,74,75,76]
Escherichia coli, *Chlamydia trachomatis*, *Pseudomonas aeruginosa*, *Staphylococcus viridians*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus faecalis*	Viability	• Damaging sperm membrane integrity • Causing oxidative stress • Inducing sperm apoptosis • Reducing sperm mitochondrial membrane potential (DΨm) • Damaging sperm DNA integrity and chromatin structure	[25,40,77,78]
Escherichia coli, β-hemolytic enterococci, *Ureaplasma urealyticum*, *Bacteroides ureolyticus*, *Pseudomonas aeruginosa*	Fertilization capacity	• Reduced inducibility of acrosome reaction • Damaging sperm membrane integrity • Damaging sperm acrosomal cap	[68,79]

Ref., references; spp., species.

morphology [75]. However, some studies reported the lack of negative effects of bacteria on sperm morphology. In this regard, it has been reported that the detection of chlamydial infection in semen had no relation with sperm morphology [43]. The underlying mechanism which can affect sperm morphology following bacterial infection is not determined yet. However, studies have suggested that the most important mechanism can be associated with the production of reactive oxygen species (ROS) by bacteria or bacteria-induced inflammatory cascades [90,91]. Oxidative stress following excessive ROS production can negatively affect the sperm cell membrane as well as its acrosomal region, which can subsequently result in morphologically abnormal sperm [92–94].

4.4. Viability

Reduction in sperm viability has been reported following contamination with different strains of *E. coli*, CT, and *P. aeruginosa in vivo* and *in vitro* [25,40,78]. Moreover, *in vitro* infections of human sperm with *Staphylococcus viridians* (*S. viridians*), *Staphylococcus epidermidis* (*S. epidermidis*), *S. aureus*, and *S. faecalis* have shown markedly lower viability [77]. It has been also demonstrated that *in vitro* co-incubation of sperm with CT could result in premature sperm death. The SAF and 3-oxododecanoyl-L-homoserine lactone produced respectively by *E. coli* and *P. aeruginosa* represents the responsible factors for the reduction of sperm viability [72,89]. According to Boguen et al. findings, although sperm vitality was reduced with the H strain of *E. coli* in a 1:2 sperm/bacteria ratio, while the non-hemolytic strains of *E. coli* could not negatively affect vitality, even in a 1:128 bacteria/spermatozoa ratio [81]. Motrich and colleagues also showed that chlamydial infection had no significant unfavorable effect on sperm viability [45]. Furthermore, exposure of highly motile spermatozoa from healthy donors or C57BL/6 mice to CT (serovar LGV or E) did not show a significant reduction in sperm viability [95]. So, it seems the viability of sperm can be affected by just some bacterial strains and it also depends on the dose of bacteria. The reduced viability of sperm following bacteria contamination could be due to at least two mechanisms; 1) some bacteria release soluble spermatotoxic factors such as sperm immobilization factor (SIF) which can reduce sperm viability by decreasing mitochondrial ATPase activity [96], and 2) bacteria-induced inflammation can result in excessive ROS production that consequently causes sperm DNA damage and apoptosis [97,98].

4.5. Fertilization capacity

The acrosome reaction (AR) is a crucial step in the sperm fertilization process and is required for sperm penetration in the oocyte zona pellucida. So, any disruption in AR following bacterial infection can reduce sperm fertilization capacity. In this regard, it has been indicated that *in vitro* exposure of human sperm to *E. coli* could decrease the inducibility of AR in a bacterium/sperm ratio-dependent manner. Reduced inducibility of AR has been observed when the *E. coli*/spermatozoa ratio was at least 1:5 [79]. Human sperm treated with β-hemolytic enterococci also presented lower hypoosmotic swelling test scores, demonstrating an impaired membrane function and fertilizing capacity of sperm [68]. The infection of the male urogenital tract with *U. urealyticum* has been also reported as a cause of sperm fertilization reduction [51]. Furthermore, Fraczek et al. analyzed the penetration ability of *Bacteroides ureolyticus*-treated sperm in hamster oocytes and indicated a significant reduction in the penetration and fertilization capacity [99]. Furthermore, it has been demonstrated that *P. aeruginosa* could induce premature acrosome loss of sperm [40]. However, two studies found no significant difference in the fertilization rate of spermatozoa from mycoplasma-infected patients compared to non-infected couples [100].

The possible mechanisms by which bacteria might influence the fertilizing potential of spermatozoa are negatively affecting sperm membrane, acrosomal cap, and mitochondria; the underlying mechanisms are discussed in detail in Section 5.

5. Bacteria effects on spermatozoa

The WHO parameters only explain a few aspects of sperm function and quality; their predictive power concerning fertility is quite low. Therefore, over the past decade, an attempt for finding better predictors

of male fecundity has resulted in a raised focus on sperm DNA integrity, acrosome status, and integrity of the cell membrane. In the following we discuss the effect of bacteria on these factors.

5.1. DNA

Sperm DNA and chromatin assessment have been recommended as predictors of fertility potential [101]. Successful reproduction partially depends on the integrity of the sperm DNA, and its effect on fertility goes beyond fertility and pregnancy outcomes. Clinical evidence has shown that damage to human sperm DNA may negatively influence reproductive outcomes [102]. In this regard, it has been demonstrated that the sperm of men with a high percentage of DNA damage have a shallow potential for both *in vitro* and *in vivo* fertilization [103].

It has been revealed that bacterial contamination can negatively influence sperm DNA integrity. *Escherichia coli*, CT, *U. urealyticum*, *S. aureus*, *P. aeruginosa*, and *Mycoplasma* spp. are the bacterial species leading to sperm DNA fragmentation [104]. Gallegos et al. have found that subjects infected with mycoplasma and CT had notably higher sperm DNA fragmentation compared with fertile control men [105]. In line with their study, Sellami and colleagues showed a slight increase in sperm DNA damage in male partners of infertile couples infected with CT [106]. *Chlamydia trachomatis* also causes sperm phosphatidylserine externalization in addition to DNA fragmentation [107]. The effect of bacteria on sperm DNA fragmentation associates with the bacterial load, bacterial growth rate, and incubation. For example, bacterial presence in the bull seminal fluid increased the rate of sperm DNA damage by approximately 20-fold in the first 48 h [77].

One of the possible reasons for sperm DNA fragmentation following bacteria contamination is that some bacteria induce the apoptotic process in spermatozoa which accompanies DNA fragmentation [108]. In this regard, it has been demonstrated that bacteria released Porins and LPS that can induce apoptosis in the spermatozoa [109]. Furthermore, it has been indicated that inflammatory processes are highly activated in the reproductive tract of males with a bacterial infection. On the other hand, inflammatory mediators such as tumor necrosis factor (TNF)-α, IL-6, and IL-8, have been found to increase the DNA fragmentation of ejaculated human spermatozoa [110,111]. Moreover, inflammation-induced oxidative stress can lead to numerous forms of DNA damages, such as chromatin cross-linking, DNA strand breaks, base oxidation, and chromosome deletion [112,113].

5.2. Cell membrane

A range of previous studies has reported the strong negative impact of various bacterial strains on spermatozoa plasma membrane integrity, including phosphatidylserine externalization or lipid peroxidation. Peroxidation of the sperm membrane architectures is one of the significant effects related to increased production of ROS [51,114]. Bacteria can induce oxidative stress in seminal fluid that consequently can cause sperm membrane damage and infertility [51]. Fraczek and colleagues have shown that the percentage of M540-negative spermatozoa (representing the scrambling level of the phospholipids in the plasma membrane) in human semen samples infected with *E. coli*, *B. ureolyticus*, and *S. haemolyticus* was significantly lower than the uninfected samples [115]. They also have reported that *E. coli*, *Streptococcus oralis* (*S. oralis*), *Staphylococcus haemolyticus* (*S. haemolyticus*), *U. urealyticum*, and *B. ureolyticus* increased the malondialdehyde (MDA) level in sperm membranes [51]. It has been demonstrated that membrane alterations following *U. urealyticum* contamination may result in the exposure of sperm antigens to the immune system, and consequently, production of the anti-sperm antibodies, which have been associated with infertility [116]. Barbonetti et al. previously reported that *E. coli* could increase the production of ROS and membrane lipid peroxidation in sperm cells [47]. Conversely, Suarez et al. have demonstrated that CT neither causes spermatozoa ROS overproduction nor membrane lipid peroxidation; possibly due to the fact that CT inhibits nicotinamide adenine dinucleotide phosphate (NADPH) activity and hence reduces ROS production [95]. The harmful effect of bacteria on the plasma membrane lipid of spermatozoa may be mediated by direct contact between spermatozoa and bacterium or indirectly by bacterium-released toxins. For example, hemolysin released from *E. coli* and enterococci can impair plasma membrane integrity [68]. Studies have shown that the levels of sperm membrane damage are associated with white blood cells (WBC) in semen and can be more enhanced in the presence of bacteria [51]. So, it can be postulated that the additional presence of WBC in bacteria-contaminated samples could increase the risk of sperm membrane peroxidation.

5.3. Acrosome

The acrosome is a specific secretory structure on the head of spermatozoa. Studies reported impairment of acrosome following different bacterial contaminations such as gram-negative *E. coli.* The presence of bacteria in the seminal fluid could lead to changes in the inner and outer membranes of the acrosome [117]. It has also been observed that the co-culture of spermatozoa with supernatant of *S. aureus* resulted in a swollen and partly disintegrated acrosome, as well as enlarged subacrosomal space between the inner acrosomal membrane and the nuclear membrane [118]. Furthermore, it has been reported that *Campylobacter* could damage the acrosome of ram spermatozoa [119]. Reactive oxygen species are one of the possible factors which are involved in bacteria-induced sperm acrosomal membrane peroxidation and so acrosome reaction impairment. Since the post acrosomal and equatorial regions of the sperm play a vital role in fertilization of the oocyte, impairment of the acrosomal membrane by bacteria can negatively influence the conception rate [120]. However, Wolff et al. did not find any significant change in the spontaneous acrosome reaction of spermatozoa incubated with *E. coli* [121]. So, it seems that the type of bacteria can be an important factor in affecting sperm acrosome.

5.4. Vacuoles

Previous studies reported that infertile men possess a higher percentage of vacuoles in the sperm head. It has been reported that spermatozoa with large head vacuoles (> 13% of the head surface) had a higher percentage of DNA fragmentation compared with those with small vacuoles [122]. Unlike these findings, some studies did not find any connection between sperm head vacuoles and chromosome abnormalities or DNA damage [123,124]. There are limited studies about the effects of bacteria on vacuoles in the spermatozoa. Electron microscopic assessment revealed profound and multiple changes in the ultrastructure of spermatozoa such as cytoplasmic vacuoles following *E. coli* infection [88]. Moreover, vacuoles within the nuclear chromatin and spermatozoa with a pattern of a loose fibrillar–microgranular chromatin network have been reported in semen samples of subfertile or infertile men infected with CT and mycoplasmas [125].

5.5. Mitochondria

Deficiencies in mitochondrial sheath located in the midpiece of spermatozoa could be a reason of impaired motility of sperm and can also interfere with vital energy-dependent processes including acrosome reaction, sperm capacitation, and hyperactivation, the interaction of spermatozoa with zona pellucida, transport of metabolites and ions, and fusion of spermatozoa with the oocyte [126–130]. Evidence showed that bacterial infection could negatively affect sperm mitochondria possibly through increasing ROS levels. Studies showed that co-incubation of human spermatozoa with *B. ureolyticus* and *S. haemolyticus* resulted in impaired mitochondrial activity [115]. Most probably, the bacteria-induced sperm mitochondrial defects can lead to male infertility. Spermatozoa DΨm is an early apoptotic marker in

human spermatozoa [131,132]. Several studies have shown correlations between the level of DΨm and semen parameters, proposing the importance of DΨm levels in spermatozoa for the assessment of male fertility potential. A reduction in DΨm could increase plasma membrane permeability and DNA fragmentation [133]. Interestingly, a decrease in the proportion of spermatozoa with high DΨm and a decrease in oxidoreductive capability of sperm mitochondria have been reported following incubation with *E. coli* [115]. Moreover, the negative effects of *P. aeruginosa* and *E. coli* on the integrity of DΨm have been described [129,134]. These studies confirm that bacteria can directly or indirectly negatively affect sperm mitochondria and so decrease sperm fertilization capacity.

6. Mechanism of the effect of bacteria

6.1. Attachment of bacteria to the sperm

One of the possible mechanisms by which bacteria can affect sperm function is the direct attachment of the bacteria to the sperm and production of toxins and metabolites. In this regard, *Waddlia chondrophila* (*W. chondrophila*), *Ureaplasma diversum* (*U. diversum*), and CT can bind and enter into spermatozoa and so affect the spermatozoa fertilization potential [76,135,136]. Many bacteria, including *M. genitalium*, *N. gonorrhoeae*, CT, *S. haemolyticus* and *B. ureolyticus*, *E. coli*, *Mycoplasma hominis* (*M. hominis*), *Klebsiella pneumonia* (*K. pneumonia*), *U. urealyticum*, and *U. diversum* have also the ability to attach to human spermatozoa [137–139]. More interestingly, some of these bacteria can be carried by sperm into the female genital tract during mating [73,140].

Studies have demonstrated that attachment of some bacteria could negatively affect sperm functions. For example, it has been seen that *Ureaplasma* could attach to the spermatozoa membrane and initiate some enzymatic activities which could negatively affect the sperm membrane and lead to a reduction in hypo-osmotic swelling test score [116]. *Escherichia coli* attachment to the sperm can decrease its motility and increase agglutination [121]. Furthermore, transmission electron microscopy has revealed that all CT serovars I, D and H can attach to human spermatozoa and induce sperm DNA damage [136,141]. It has been indicated that spermatozoa-attached *U. urealyticum* produces metabolites such as superoxide and hydrogen peroxide which could negatively influence sperm acrosomal integrity by decomposing the lipid of the acrosomal membrane [142].

Different mechanisms and receptors mediate the bacteria-sperm adhesion. Studies reported that pili and fimbriae are bacterial attachment structures [121]. Moretti and colleagues have also reported that these components are the key factors in the pathogenicity of *Morganella morganni* (*M. morganni*) and *E. coli* [66]. On the other hand, sialoglycoproteins at the sperm surface can act as receptors for *M. genitalium* [143]. Moreover, mannose receptors on human spermatozoa have been introduced as *E. coli* adherence mediators [121]. Scanning electron microscope findings also showed the presence of a thin fibril between the damaged spermatozoa and *E. coli* in the acrosome, neck, and tail [115]. It has been also indicated that attachment of *Mycoplasma* spp. and *U. diversum* to spermatozoa is associated with the high fraction of coiled tails and the lumps on the surface of spermatozoa, respectively [120,144].

6.2. Bacteria-produced toxins and metabolites

Some bacteria can release different factors such as LPS, EBs, hemolysin, Shiga-like toxin, and peptidoglycan fragments that can potentially affect spermatozoa functions. In this regard, porins, low-molecular sperm immobilization factor, and LPS extracted from some bacteria such as *E. coli*, have been demonstrated to induce immobilization or even cell death in spermatozoa [145]. Lipopolysaccharide is a toxic component of the outer wall of all gram-negative bacteria such as chlamydiae which can bind to the sperm and suppress its motility [146]. It has been reported that incubation of sperm with LPS could increase reactive oxygen intermediate (ROI) levels and decrease sperm motility; however, this process could be reversed by the administration of antioxidants into the medium [147]. Furthermore, an apoptotic effect of LPS or porins from *Pasteurella multocida* (*P. multocida*) and *Salmonella enterica* (*S. enterica*) on sperm has been observed [109]. The apoptotic effect of LPS possibly is mediated through binding to CD14 on the spermatozoa surface and triggering overproduction of ROS as well as caspase-mediated apoptosis [84]. On the contrary, Sikka et al. have shown that LPS could not affect sperm cells unless in the presence of interferon-ϒ [148]. It should be mentioned that LPS-induced inflammation can also disrupt spermatogenesis and inhibit testicular steroidogenesis [54,149].

The spermicidal potential of LPS depends on the type of bacterium. For example, LPS purified from chlamydia is about 500 times more potent than *E. coli*-derived LPS; possibly because of LPS structural differences among the various bacteria [84]. Another previous study showed that LPS at a concentration of 50 μg/mL extracted from *E. coli*, *Proteus mirabilis* (*P. mirabilis*), and *Salmonella typhimurium* (*S. typhimurium*) could kill approximately 80%, 65%, and 90% of the sperm, respectively [145].

A negative effect of EBs extracted from CT serovar E on sperm motility has previously been reported [150]. Moreover, it has been seen that the incubation of EB with normozoospermic human sperm could induce their apoptosis [107]. Initially, EBs attach to the spermatozoa surface and penetrate the cell. Following a latent phase of about eight hours, EBs change into metabolically active reticulate bodies (RBs). The reticulate bodies begin replicating inside the vacuole *via* binary fission. When the replication cycle ends, RBs again convert into EBs, which are released in the extracellular space following the sperm lysis [151].

The hemolysin is a calcium-dependent toxin that forms pores by inserting it into the host cell membrane. The pores lead to releasing of potassium and influx of sucrose, mannitol, and calcium, which consequently cause osmotic lysis and cell death [152]. It has been reported that the hemolysin of Beta-hemolytic enterococci led to human spermatozoa membrane damage [68]. It seems one of the mechanisms of the effect of the alpha-hemolysin is inactivation of the phosphatidylinositol 3′-kinase–protein kinase B (PI3k/Akt) pathway [153].

Shiga-like toxin one of the *E. coli* toxins, can also negatively affect sperm function. It has been reported that this toxin could induce chromatin condensation, DNA fragmentation, reduction in cell size, cell membrane vesiculation, and the production of apoptotic bodies in Hep-2 cells [154]. SIF as a ~20 kDa protein extracted from *S. aureus* is another bacteria-released factor that can decrease the motility of spermatozoa [155]. The 3-oxododecanoyl-L-homoserine lactone secreted by *P. aeruginosa* has also the potential to decrease sperm motility and viability [89]. Nitric oxide (NO) is possibly the other factor secreted by some bacteria that can negatively influence sperm fertility. The possible mechanism is that high NO levels increase the sperm apoptotic rate [156].

Outer membrane vesicles (OMVs) are the main non-cellular ingredients extracted from gram-negative bacteria. The OMVs may bind to the spermatozoa membrane and influence the capacity of sperm–oocyte fusion. It has been demonstrated that LPS present on the OMV surface plays the main role in inducing apoptosis and autophagy of cells; possibly *via* increasing ROS accumulation and the DΨm. Moreover, OMVs can increase the expression of LC3 (a central protein in the autophagy) and caspase 3 (an apoptosis-related protein), as well as reduce the expression of Bcl2 (an anti-apoptosis-related factor) in sperm.

6.3. Bacteria-induced inflammation and oxidative stress

One of the main consequences of bacterial urogenital infections is the overproduction of proinflammatory cytokines and reactive oxygen.

A strong association between the presence of inflammation in the male reproductive tract and infertility has been reported. For example, *Chlamydia trachomatis* could indirectly damage the sperm by releasing inflammatory mediators such as cytokines and ROS [157]. It has been reported that men with genital *U. urealyticum* infection had an abnormal expression of IL-17 and IL-18 which was negatively associated with seminal fluid volume, spermatozoa density, survival rate, activity, forward movement percentage, and standard morphological rate [156]. It can be assumed that ROS mediated spermatozoa damages resulted from *U. urealyticum*-induced inflammation. Although the low concentration of ROS is required for sperm motility, capacitation, maturation, AR, and fertilization, a higher level of ROS can be deteriorating [158]. The increase in sperm intracellular reactive oxygen species (iROS) induced by some, but not all, strains of *E. coli* such as NH-ATCC and H seems to be associated with the loss of sperm DΨm and a subsequent decrease in fertility [81]. Interestingly, neither extra ROS production nor membrane lipid peroxidation has been observed in sperm incubated with serovar E and LGV of CT [95]. It has been suggested that the presence of *U. urealyticum* in semen can cause overproduction of ROS and so spermatozoa membrane damage [159]. A reduction in levels of some microelements such as selenium or zinc in semen infected with *U. urealyticum* may lead to the impairment of antioxidant defense [61,160]. Furthermore, the chemiluminometric observations showed an enhancement in ROI produced by white blood cells after incubation with *S. haemolyticus* and *E. coli* [51]. There are reports showing high ROI production in the seminal fluid of men with leukocytospermia ($\geq 1 \times 10^6$ WBC/mL semen) and bacterial infections [161,162]. It is well known that bacteria themselves or their endotoxins can induce inflammation and so ROI production in leukocytes [163]. The attack of ROS to the membrane lipids of spermatozoa can lead to an irreversible alteration in membrane fluidity and its nonspecific permeability, and consecutively disrupt sperm acrosomal reaction and capacitation [164,165]. High levels of ROS also induce DNA fragmentation of the spermatozoa [166].

6.4. Bacteria-induced autoimmune responses

Cross-reactivity between carbohydrate antigens on bacterial walls and sperm antigens has been reported by Kurpisz and Alexander [166]. Moreover, several studies reported an association between chronic infection of the male genital tract with the production of anti-sperm antibodies (ASA) [167,168]. The main ASA isotype in infected men is IgA, although IgM or IgG are sometimes existing [169]. It is well-known that ASA can bind to the surface antigens of sperm and consequently damage them. The chronic inflammatory responses are believed to play a pivotal role in ASA production [170,171]. In this regard, elevated ASA production has been reported in patients with gastroduodenal disease infected with *H. pylori* [172]. Moreover, it has been documented that CT infection could stimulate ASA production in both male and female patients [170]. The humoral immune responses to CT contamination are also correlated with the increase of autoimmune responses to spermatozoa. ASA is produced during the chlamydial genital infection leading to the release of pro-inflammatory cytokines from activated T cells, which can subsequently cause activation of macrophages to phagocyte both CT microorganisms and spermatozoa [157].

The production of antibodies against spermatozoa in bacterial infections is probably due to the cross-reactivity between antigens of spermatozoa and various exogenous antigens of bacteria [166]. In supporting this hypothesis, similar antigenicity has been reported between spermatozoa with *E. coli*, *CT*, *Staphylococcus aureus*, *Streptococcus viridans*, *Ureaplasma urealyticum*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans*, and *Salmonella typhi* [173,174]. Therefore, it can be postulated that the chronicity of bacterial infections can cause anti-sperm immunity and immunologic infertility.

7. Conclusion

Various species of bacteria can colonize in different parts of the male genital tract. Testicular damage, prostatitis, epididymitis, urethritis, orchitis, and impaired spermatogenesis following the bacterial infection can be causes for male infertility. Most of the previous studies have focused on the male genital tract infections and the infected organs and there are not many studies that have investigated the direct effect of bacteria on sperm and their mechanism of action. Interestingly, bacterial contamination of semen, which ordinarily originates from the urinary tract or by the partner through sexual intercourse, can induce DNA fragmentation, cell membrane peroxidation, acrosome impairment, vacuolization, and mitochondrial damage in sperm cells. Bacteria can exert these effects by toxins or by direct attachment to the sperm cells and subsequent activation of signaling pathways related to oxidative stress, apoptosis, and inflammation. These bacteria-induced changes in the sperm can impair semen parameters including concentration, motility, morphology, viability, and fertilization capacity, and subsequently cause infertility. Unfortunately, sometimes mild bacterial contamination is neglected in men suffering from subfertility. Given the significant destructive effect of some bacteria on sperm cells, the type of bacterial contamination in the patient's genital tract should be diagnosed and its potential negative effects on male fertility and the underlying mechanisms should be taken into account in the treatment of bacteria-induced subfertile men. Furthermore, future studies are recommended to investigate possible therapeutic strategies to inhibit bacteria-induced sperm damage based on the type of bacterium and its potential damages on sperm cells. Moreover, optimized protocols for sperm incubation/preparation and freezing/thawing are required in assisted reproductive technologies (ART) to inhibit further bacteria-induced damages or improve damaged sperm cells in the cases with contamination; for example, using antioxidants or antibiotics during sperm processing.

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Declaration of competing interest

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