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Bacterial Infection of the Male Reproductive System Causing Infertility

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Highlights

- Bacterial infections represent good candidates to explain the causes of idiopathic infertility among different populations.
- Bacterial infections can affect different sites of the male reproductive tract and different stage of Spermatozoa development, maturation and transport.
- There is ample evidence to blame different kinds of bacteria such as *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma*, *Mycoplasma*, and *Staphylococcus aureus* for male infertility.
- *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma*, *Mycoplasma*, and *Staphylococcus aureus* negatively affect sperm parameters, especially sperm integrity, motility, and morphology that would significantly decrease the fertility potential among men.
- Lack of scientific information concerning the underlying mechanism and significant role of some pathogenic bacteria such as *N. gonorrhoeae*, *E. faecalis*, and *G. vaginalis* in the reproductive system leaves more room for confusion and warrant more attention.

Abbreviations:

E. coli: *Escherichia coli*, *UU*: *Ureaplasma urealyticum*, *UP*: *Ureaplasma parvum*, *MG*: *Mycoplasma genitalium*, *MH*: *Mycoplasma hominis*, *CT*: *Chlamydia trachomatis*, *NA*: *Neisseria gonorrhoeae*, *SA*: *Staphylococcus aureus*, *GV*: *Gardnerella vaginalis*. *PA*: *Pseudomonas aeruginosa*, *E. faecalis*: *Enterococcus faecalis*, *H. pylori*: *Helicobacter pylori*, *S. agalactiae*: *Streptococcus agalactiae*, ROS: reactive oxygen species; P1: protamine 1; P2: protamine 2; IVF: in-vitro fertilization; LPS: Lipopolysaccharides; SIF: sperm immobilization factor; PMN: Polymorphonuclear leukocytes; BV: bacterial vaginosis; CASA: computer- assisted sperm analyzer; BS: bacteriospermia; LCS: leukocytospermia; PS: phosphatidyl serine; MMP: mitochondrial membrane potential; OH⁻: hydroxide anion, SGG: sulfogalactosylglycerolipid; ASA: anti-sperm antibodies; CagA: cytotoxin-associated gene A; IFA: Immunofluorescence assays; ST: serological tests.

Abstract

Bacterial infections play a disruptive and hidden role in male reproductive failure. Different kinds of bacteria are often able to interfere with reproductive function in both sexes and lead to infertility. In this study, to further evaluate the role of bacterial infections in male reproduction we provided an extensive overview of so far researches investigating the effects of bacterial infections on male fertility. We searched Medline, PubMed, Scopus and Google scholar databases to identify the potentially relevant studies on bacterial infections and their implications in male infertility. All the bacteria included in this article have negative effects on the male reproductive function; however, there is ample evidence to blame bacteria such as *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma*, *Mycoplasma* and *Staphylococcus aureus* for reduced fertility and deterioration of sperm parameters. More studies are needed to clarify the molecular mechanisms by which different bacteria exert their detrimental effects on male reproductive system. Getting more insight into probable mechanisms, would significantly facilitate the production of new, advanced, and effective remedies in the future. In view of all evidence, we strongly suggest increasing awareness among people and considering screening programs for patients seeking fertility both to avoid transmission and to improve fertility outcomes among them.

Keyword: male infertility; bacterial infection; semen abnormalities; male reproductive failure

Introduction

Infertility defines as the inability to achieve pregnancy after a year of regular intercourse [1]. Approximately 15% of couples of reproductive ages fail to achieve a wanted pregnancy within a 12-month period, despite regular unprotected sexual intercourse [2]. Reduced male fertility can be a result of congenital and acquired urogenital abnormalities, infections of the male accessory glands, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors [3]. Of all of these factors, inflammatory processes and infections are among the significant and major causes of infertility. In 40%–50% of infertile couples, abnormal semen parameters are considered to be the major male-infertility-associated factor [4]. It plays an essential role in fertility outcomes, either alone or in combination with female factors.

Semen culture is considered an important diagnostic tool in the assessment of a genitourinary tract infection. The presence of bacteria in concentrations greater than 10^3 bacteria/ml ejaculate is clinically regarded as a sign of an active infection and is called bacteriospermia [5]. Semen contamination originates from the urinary tract of patients and can be sexually transmitted from the one person to another by pathogens. It is regarded as a major health care concern among people with a significant negative impact on male fertility [6]. Approximately 15% of men with infertility have a significant number of bacterial pathogens in their semen [7]. Depending on the nature of the infection, sperm production, sperm function, and sperm transport can be threatened [8]. Despite many studies that bacteriospermia was negatively associated with fertility [9], some recent studies indicated the presence of bacteria in the semen of fertile individuals with normal sperm parameters might not be attributed to male infertility [10].

Different types of bacteria have been isolated from the semen and genital tracts of fertile and infertile men. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Helicobacter pylori*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, *Anaerococcus*, *Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa* are among the most common isolated bacteria, affecting semen quality and interfering with male fertility.

Methods

Search strategy

We used and searched Medline, PubMed, Scopus, and google scholar databases to find potentially relevant papers concerning bacterial infections in male infertility published from 1967 until August 2019. We searched these databases using keywords that were a combination of bacteria names and words associated with infertility such as male infertility, fertilization, reproductive disorder, reproductive failure, sperm abnormalities, sperm quality, sperm parameters, sperm concentration, motility, morphology, sperm viability, apoptosis, sperm integrity, and sperm damage. This procedure was carried out for every bacterium included in this study.

Types of studies

Abstracts and full articles relevant to the topic were enrolled in this study. Animal model studies and findings related to the effect of antibiotics, preventive drugs, and/or therapeutic approaches that would affect fertility potential among men were excluded. Population-based studies that investigated the correlations between bacteria and male reproductive health were included. Retrospective as well as prospective studies in cross-sectional and controlled designs were taken into consideration. *In-vitro* based experimentations that explained the effect of bacterial infections on male reproductive health have also been considered for this research. These articles were gathered and included in this research to give a deeper overview of the mechanisms by which the bacteria could affect male reproduction. Finally, we assessed information of 681 studies that were related to the area of this study. We excluded a few reports with minimal importance on the topics.

Data collection

Predesigned data extraction forms were used to collect data. The data collected from selected articles were focused on two main topics: i) prevalence of bacterial infections among men with fertility problems in different groups and their statistical associations with infertility and sperm parameters, ii) The main conclusions on *in vitro* experimentation studies concerning bacterial pathogenesis.

We tried to include the most important and updated studies to record the detrimental effects that were caused by different bacteria (*Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Helicobacter pylori*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, *Anaerococcus*, *Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa*) in the male reproductive system (human) and implicated in infertility.

Results

From 681 articles identified during the literature search, 329 articles were selected. Duplicated hits and articles that were not written in English were excluded. Of 233 reports that underwent the abstract screening, a total of 186 articles met our inclusion criteria. The whole search data flow diagram is presented in Figure 1. Data from diverse studies concerning the relationship between bacterial infection and male reproductive health are now available in this article. We particularly focused on bacterial infections and their association with reproductive failure in males. In effect, articles that met inclusion criteria were mainly focused on the effects of bacterial infections on fertility rate among infected men and the underlying mechanisms by which different bacteria caused detrimental effects on sperms and led to abnormal sperm parameters and finally infertility. Different bacteria included in this study were pooled into four different groups including, atypical, aerobic, anaerobic and a microaerophilic bacterium. A summary and the characteristics of included population-based studies are presented in Table 1 and Table 2, respectively.

Discussion

Bacterial infections are considered a substantial cause of male infertility. These microorganisms are frequently present in the genital tract and semen of both fertile and infertile men and seem to be involved in male reproductive failure in various ways. Infections of the lower genital tracts seem to have little importance; however, such infections, as well as those involving other parts of the male reproductive system, may cause microbial colonization of the semen and increase fertility problems among them. Different sites of the male reproductive tracts, such as the testis, epididymis and male accessory sex glands can be affected by bacterial infections and led to significant biological and biochemical changes in the seminal plasma. Various bacteria can influence spermatogenesis process at different levels and disrupt spermatozoa development, maturation, and transport. Presence of pathogenic bacteria in the male genital system has been mainly associated with poor sperm function, leading to infertility. These bacteria with the help of some virulent agents such as cytotoxic necrotizing factor, α -haemolysins and β -haemolysins, and from the release of soluble spermatotoxic factors such as sperm immobilisation factor (SIF) exert their detrimental effects on spermatozoa [11, 12]. Some bacterial endotoxins such as lipopolysaccharide and glycoprotein have also been detected in human semen samples and shown to deteriorate semen quality by triggering a local inflammatory reaction [13, 14]. This inflammatory response of the genitourinary tract to the invasion of microorganisms activates leukocytes and inflammatory mediators such as cytokines and ROS, which play significant roles in sperm DNA fragmentation and male infertility.

Sperm function is highly dependent on the energy that is produced in the form of ATP by the mitochondrial ATP synthase. This enzyme can also hydrolyze ATP in the absence of the proton motive force that is maintained by the mitochondrial membrane potential. The detrimental effects of reactive oxygen species on the mitochondrial membrane, sperm function, and sperm parameters have been frequently reported. Some pathogenic bacteria such as *E. coli*, *streptococci*, *staphylococci*, *Mycoplasma*, *Chlamydia*, and *Ureaplasma* produce an acute inflammatory response with a flow of leucocytes into the genital tract, resulting in an increased level of ROS production. Low level of ROS is essential for the acquisition of fertilizing ability and contribute to chromatin condensation, membrane remodeling, and activation of intracellular signaling pathways during maturation, capacitation, and acrosome reaction, whereas elevated ROS production levels have negative impacts on sperm parameters and sperm function and fertilizing capacity [7]. Oxidative stress, as a result of the self-perpetuating cascade of mitochondrial ROS production and lipid peroxidation, resulted in DNA fragmentation and sperm death caused by hydrogen peroxide (Figure. 2).

Different studies have shown that cytokines may play a significant role in the initiation of semen inflammation during bacterial infection and by negatively affecting sperm membranes interfere with sperm quality. Cytokines may lead to an increase in lipid sperm membrane peroxidation in bacterial infections that have shown to be closely associated with the accompanying leukocytospermia [15, 16]. Therefore, ROS and certain cytokines (produced by the leukocytes during infections), cooperate with each other to impose destructive effects on sperm membranes

and increase human sperm apoptosis. Tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and interleukins IL-6, IL-12, IL-17, and IL-23 are among the most important proinflammatory cytokines investigated by researchers.

Bacterial contamination in assisted reproduction

Assisted Reproductive Technology (ART) consists of all procedures that include in vitro handling of human oocytes and sperm cells or embryos with the purpose of establishing a pregnancy [17]. A number of technical factors including bacterial contamination can significantly affect the ultimate clinical outcomes in ART. The good quality of spermatozoa (motile spermatozoa with intact membrane, stable DNA, and normal morphology) is a crucial factor in assisted reproduction. Spermatozoa used in ART must function normally to be able to fertilize an oocyte and ensure a successful outcome by generating a good quality gamete. The effect of semen contamination on the outcome of assisted reproductive techniques has long been controversial. While some studies suggested that in bacterial infested cultures, the oocytes were degenerated and led to poor outcomes [18, 19], some showed the presence of bacteria in the original semen sample had no effect on fertilization, cleavage, or pregnancy rates in artificial reproduction techniques [20]. In a recent study by Fraczek and Kurpisz bacteriospermia was directly related to 15% of male infertility in a group of infertile men treated with ART [13].

The spermatozoa could serve as a transporting vector of various bacteria which may lead to iatrogenic oocyte contamination. Detrimental effects of bacterial contamination on spermatozoa may increase zygote degeneration or lead to DNA variation and increase the risk of unsuccessful fertilization or poor embryo quality and developments [21, 22]. Studies suggested that incomplete histone-to-protamine transition and abnormal sperm chromatin in infected sperm, causing decreased fertilization rate, might be due to the trimethylated H3-meK79 and hyperacetylated histone H4 which exist simultaneously with the transition protein TNP1 [22, 23]. Bacteriospermia may defect the histone-protamine exchange process, so DNA will be exposed to damage, leading to a decrease in fertilization rate and poor quality of embryos [19]. Moreover, abnormal P1/P2 ratios in infertile men who underwent ART treatment has been associated with increased sperm DNA fragmentation, lower fertilization rates, poor embryo quality and reduced pregnancy rates [24-26]. The ability of bacteria to produce inflammatory structure, toxins, and enzymes could be one reason for protamine deficiency and P1/P2 ratio alterations [27, 28].

Effect of bacterial infection on male infertility

Among the most common investigated bacterial microorganisms affecting semen quality and interfering with male fertility in different populations, there are

- A) A group of atypical bacteria including, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium*, and *Mycoplasma hominis*,
- B) A group of aerobic bacteria including, *Escherichia coli*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus agalactiae*,

C) A group of anaerobic bacteria including, *Gardnerella vaginalis* and *Anaerococcus*.

D) A microaerophilic bacterium, *Helicobacter pylori*.

A summary of the effects of some common pathogenic bacteria including, *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Neisseria gonorrhoeae* on male infertility has been shown in Figure 3.

A. Atypical bacteria

Chlamydia trachomatis

C. trachomatis, an obligate intracellular gram-negative bacterium, has been detected in the penile urethra, Leydig cells of the testis, prostate, epididymis, and seminal vesicles. Infections are often asymptomatic in up to 85%–90% of infected individuals. So, a very high proportion of *Chlamydial* infections in men remains undetected or untreated. They may present with a mucoid or watery urethral discharge, and complain of dysuria. Their discharge may be scanty and clear or may only be observed after milking the urethra (bulbar urethral massage). Recurrent infections are common with *C. trachomatis* and this is mainly because the acquired immunity to *C. trachomatis* infection is type-specific and insufficient to suppress re-infection and transmission to partners. *C. trachomatis* is considered the most common agent of non-gonococcal urethritis in men and may cause epididymitis-orchitis, prostatitis, and sperm tract obstruction [29]. *C. trachomatis* can directly or indirectly affect sperm function at different levels. *C. trachomatis* has been found frequently to increase the proportion of sperm abnormalities by affecting sperm quality and altering sperm functions; hence, impairing male fertility.

Ultrastructural studies have suggested that abnormal sperm parameters during chlamydial infection might be associated with the microorganism per se or to the host immune/inflammatory response. *In vitro* investigations revealed that incubation of *C. trachomatis* with human spermatozoa impaired sperm motility and led to premature death, perhaps as an effect of the chlamydial LPS [30]. Infection with *C. trachomatis* may also lead to a defective acrosome reaction, increase in cytotoxicity, sperm lipid peroxidation, sperm phosphatidylserine membrane externalization, and DNA damage; therefore, impact sperm functions and increase fertility problems significantly [31].

Chlamydial LPS interacted with the CD14 on the sperm surface, mainly to the head and tail region, and increased levels of sperm membrane lipid peroxidation, production of reactive oxygen species, and caspase-mediated apoptosis subsequently [32–34]. Apoptosis of spermatozoa caused by chlamydial LPS leads to persistent infection resulting in scarring of the ejaculatory ducts or loss of stereo cilia. Sperm quality can also be decreased as a result of the damage to the epithelial cells involved in spermatogenesis [35]. In different studies, sperm DNA fragmentation increased significantly in infections caused by *C. trachomatis* and was associated

with a low potential for male fecundity [36, 37]. Sellami et al. showed that DNA fragmentation increased in the sperm sample of infertile men positive for *C. trachomatis* and it was significantly associated with the loss of $\Delta\Psi_m$ and caspase3 activation that could be incriminated in apoptosis induction of spermatozoa [38].

Several electron microscopy studies in the 90s have indicated the mechanism by which *C. trachomatis* influences sperm parameters in biopsy specimens of the testis, epididymis, and semen [39, 40]. Similarly, different in vitro studies by Hosseinzadeh et al. demonstrated that *C. trachomatis* directly induced changes in the structure of sperm proteins and further premature cell death through LPS secreted by this pathogen [30]. Also, Galdiero and coworkers showed early apoptosis of human spermatozoa caused by LPS extracted from *Chlamydia* within 60 min of incubation [41]. The same result was indicated when incubating the spermatozoa from men with normozoospermic *Chlamydia* elementary bodies. It led to a significant increase in dead cells and a decline in motile sperm cells. However, after incubating sperm with dead elementary bodies the detrimental effects were eliminated, showing that these detrimental events were caused by live bacteria only [42].

In many studies, *C. trachomatis* was among the first or the most prevalent pathogen isolated from infertile males and indicated a strong association with male infertility [43] and decreased semen quality [44-47]; however, in some others no relationship was observed [48, 49].

Different investigations have reported the significantly lower percentage of sperm concentration, sperm motility, and normal morphologic forms in infertile patients with *C. trachomatis* infection [37, 45, 50-52]. Sperm motility was also lower in men with antibodies than men without antibodies in infertile couples; however, it was not associated with other semen parameters and did not affect results of in vitro fertilization [44]. Contrary to a study on 175 infertile men in Israel where the authors concluded that *C. trachomatis* might play a significant role in the reduction of sperm egg penetration ability [53]. Motamedifar et al. investigated the prevalence of bacterial contaminants of semen samples and their probable association with sperm quality among infertile men. They found that bacteriospermia was significantly higher in the infertile group than healthy controls and was associated with alterations in semen quality, which may lead to a decrease in the fertilization potential of sperm [54]. *C. trachomatis* was the most abundant bacteria in semen samples of infertile men in their study.

While these investigations showed a considerable association between *C. trachomatis* infection and male infertility, others reported no relationship between this pathogen and semen quality or male infertility [49, 55-60] Vigi et al. found no significant effect of *C. trachomatis* infection on sperm concentration, motility and/or morphology in 284 male partners of infertile couples [61]. According to their electron microscopy studies, *C. trachomatis* impacts on male fertility was not due to the alterations in sperm quality or function, but rather to the transmission of the disease to the female partners, causing inflammatory processes and promoting the generation of antisperm antibodies. It is suggested that the potential detrimental effects of *C. trachomatis* on male fertility

might not involve the semen parameters assessed routinely, but may potentially affect attachment of the sperm to the oocyte or later steps of cell division or implantation [62].

Genital ureaplasmas and mycoplasmas

Genital ureaplasmas (*Ureaplasma urealyticum* and *Ureaplasma parvum*) and mycoplasmas (*Mycoplasma genitalium* and *Mycoplasma hominis*) are natural inhabitants of male urethra. However, these microorganisms particularly *U. urealyticum* are potentially pathogenic species playing an etiologic role in both genital infections and male infertility.

Ureaplasma urealyticum

Ureaplasma urealyticum is a causative agent of non-gonococcal urethritis and with a prevalence ranging between 10 and 40%, is implicated in the pathogenesis of prostatitis, epididymitis, and infertility [63]. The exact mechanism by which *U. urealyticum* influences sperm quality has not yet been clarified but researchers have suggested that *U. urealyticum* reduced the oxidoreductive potential of the ejaculate that makes sperm more vulnerable to peroxidative damage [64]. Whilst some researchers didn't find any correlation between the presence of *U. urealyticum* and semen alterations, others have reported the presence of *U. urealyticum* in semen to be associated with a significant decrease in sperm concentration, motility, and/or in morphology.

Semen infection with *U. urealyticum* leads to an increasing proportion of sperms with high amount of residual cytoplasm around the neck. This might be the reason for higher availability of NADPH during semen infection with this atypic bacteria and why spermatozoa release high quantities of O_2^- which may cause the peroxidative effect [65]. Examination of specimens from infertile patients and fertile men showed the adhesion of *U. urealyticum* to the exfoliated germ cells and membrane of spermatozoa, mainly in the midpiece, postacrosomal or tail region [66, 67]. Adhesion of *U. urealyticum* to the sperm may lead to a looped tangling of tails and multiple sperm agglutinations and result in decreased motility. Decreased motility, integrity, and maturity of the sperm membrane might be also associated with a decreased α -glucosidase levels in the seminal fluid due to the effect of on epididymis [68, 69]. Computerized sperm analysis showed that several sperm motility parameters were significantly lower in patients with *U. urealyticum* infection. The influence of *U. urealyticum* on sperm motility was associated to reduced seminal plasma α -glucosidase levels, whereas seminal plasma acid phosphatase and fructose remained unchanged, suggesting a possible epididymal site of action [66]. Metabolic products of *U. urealyticum*, such as H_2O_2 and OH^- , are highly toxic to the sperm cells. Like mycoplasma infections, ureaplasmas may disulfate SGG, an important molecule for the sperm-egg binding; therefore, play a significant role in infertility [70].

Different investigations revealed that the prevalence of *U. urealyticum* among infertile patients was remarkably higher than in the control group [71-79], and it was significantly associated with decreased sperm concentration, morphology, motility, viability [56, 66, 71, 75, 77, 78, 80-82],

and higher fragmented DNA [64]. Reichart et al. when incubated spermatozoa with *U. urealyticum*, a significant dose- and time-dependent chromatin decondensation and DNA damage were observed. Shortly after incubation (30 min), the percentage of human spermatozoa with denatured DNA increased by almost 50% [83]. Same researchers showed that *U. urealyticum* had the ability to bind to the spermatozoa and decreased sperm motility. After four hours incubation, *U. urealyticum* caused sperm membrane alterations significantly, whereas increased sperm velocity after a short time (45 min). The authors supposed that when sperm activity depends on mitochondrial oxidative phosphorylation, these opposite effects of *U. urealyticum* happens usually at low pH, *U. urealyticum* competes with mitochondrial energy production with a consequent decline of sperm motility and viability, whereas when sperm energy metabolism depends on glycolysis (usually at higher pH), *U. urealyticum* stimulates glycolysis and, therefore, sperm activity [84]. Another research by Rose et al. indicated that *in vitro* overnight incubation with *U. urealyticum*, as well as *Mycoplasma hominis*, led to a significant reduction in sperm motility and the percentage of spermatozoa with normal form, hyperactivation, and calcium ionophore-induced acrosome reaction [85]. Shi et al. also studied *U. urealyticum* infection in 40 men and indicated that *U. urealyticum* has antigens (UreG) which cross-react with human sperm membrane proteins and in particular with the nuclear autoantigenic sperm protein that may cause infertility [86].

Some studies have reported no effect of *U. urealyticum* infection on sperm parameters [49, 60, 87-91]. Infertile patients with *U. urealyticum* infection, did not show any significant difference in seminal volume, sperm concentration, viability, motility, morphology, and leukocyte count in different studies by Gdoura and coworkers [55, 90].

Mycoplasma hominis

M. hominis is often associated with nongonococcal urethritis, bacterial vaginosis, and postbirth fever. The infections with this pathogen are usually clinically asymptomatic and their silent colonization can promote to urogenital tract infections that might influence fertility negatively [92]. Different studies have indicated that *M. hominis* play an etiologic role to male infertility by changing semen parameters such as spermatozoa density and motility [81, 90, 93]

A direct *in vitro* interaction between *M. hominis* and spermatozoa was evaluated by Rose and coworkers. This research team showed that an overnight incubation with mycoplasma species led to a considerable decrease in sperm motility, the percentage of normally shaped spermatozoa, and the proportion of acrosome-reacted spermatozoa [85]. Confocal microscopy revealed that after a short time incubation of *M. hominis* with sperm, this pathogen bounded to sperm heads, tails and, the midpiece [94]. It appears that similar to *U. urealyticum*, *M. hominis* leads to induction of nuclear decondensation and denaturation or single DNA strand break that damages spermatozoa and has no short-term consequences on viability, motility and morphology. Lower motility rate, decreased total motile sperm count [81], and abnormal sperm morphology were

observed to be significantly associated with the presence of *M. hominis* as well [90]. As opposed to a study by Diaz-Garcia et al. who found that *M. hominis* attachment towards human sperm cells did not affect their viability. They also observed that a short-term *M. hominis* interaction with human spermatozoa resulted in non-apparent or subtle damages, however, it might have implications for long-term male or couple's fertility [94]. Ahmadi et al. also evaluated the association between asymptomatic infections caused by *M. hominis* and male infertility. It was revealed that *M. hominis* in the infertile men was more than three-fold higher than in the healthy fertile individuals and was significantly related to male infertility [92].

Contrary to these studies, some investigations failed to show any effect of mycoplasmas on sperm parameters [55, 95]. Another study measured susceptibility of DNA strand breaks in sperm nuclear chromatin to in situ denaturation among 293 men and found no overall association with chlamydia, *UU*, and *MH* infection [96].

Mycoplasma genitalium

Mycoplasma genitalium has been associated with urogenital consequences such as male urethritis, balanoposthitis, prostatitis, and infertility. *M. genitalium* was the first isolated from men with non-gonococcal urethritis (NGU) more than 40 years ago. Association of *M. genitalium* with urethritis and its association to numerous female genital infections led to this hypothesis that chronic silent genital tract colonization of mycoplasmas can be related to human infertility but the evidence for its association with infertility is inconclusive.

M. genitalium infection could possibly impair male fertility potential through promoting sperm DNA damage. Early *in vitro* studies showed that *M. genitalium* binding to spermatozoa resulted in sperm agglutination and loss of motility, which can affect fertility significantly [97, 98]. Sevenstrup et al. showed that *M. genitalium* is capable of attaching to motile spermatozoa, mostly to the midpiece or neck region, and thus, the microorganism might be carried along with the spermatozoa to the female genital tract [97]. Studies on collected samples from infertile males showed that *M. genitalium* infection is common in infertile males and potentially affects the semen quality, especially sperm vitality and sperm concentration [90]; however, different studies indicated no significant association between *M. genitalium* infection and fertility [55, 71, 99, 100].

B. Aerobic bacteria

Escherichia coli

E. coli is the most common gram-negative bacilli causing different diseases in human. *E. coli* is the most common cause of urogenital infection and is implicated in the genesis of male infertility. It has been reported in several studies that *E. coli* infection caused significantly detrimental effects on male fertility and specifically sperm quality by changing sperm

motility parameters, phosphatidylserine externalization, impairment of acrosome reaction, and morphology changes of spermatozoa. Several studies have shown that *E. coli* exerted its detrimental effect on spermatozoa directly by rapidly adhering to human spermatozoa. Morphological alterations include surface structures of sperm, especially the plasma membrane of mid piece and neck as well as inner and outer acrosomal membranes of the acrosome (Figure 4) [101], suggesting that morphological defects might account for the immobilization of spermatozoa by *E. coli*. It also induced apoptosis and led to the breakdown of mitochondrial membrane [102]. The influence of *E. coli* on acrosomal morphology and function seems to be a conclusive mechanism for this pathogen to impact the fertilizing capacity of human spermatozoa [103, 104]. Early investigations have revealed the impact of *E. coli* on spermatozoa and the ability of this bacteria to attach to spermatozoa and to exert negative influences on motility parameters [105-107].

Different electron microscopy techniques have indicated the ultrastructural damages of spermatozoa induced by *E. coli*. In particular, Wolff and Diemer showed that *E. coli* attached to the surface structures of spermatozoa purportedly via type 1 adhesion molecules presenting on both bacterial pili and spermatozoa and led to significant damages to the plasma membrane of human spermatozoa and other surface structures [108, 109]. Same group of researchers further confirmed the inhibitory effect of *E. coli* on sperm motility [110]. Several studies have shown that incubation of spermatozoa with *E. coli* has led to a significant decrease in the percentage of spermatozoa with elevated MMP; which was found associated with decreased sperm motility and viability [111]. A severe breakdown in the mitochondrial membrane potential was also reported when incubating spermatozoa with the supernatant from *E. coli* culture, suggesting that the soluble factors induce apoptosis by activating several caspases and proteases responsible for mitochondrial changes [103]. Villegas et al indicated that the direct exposure of spermatozoa to *E. coli* was enough to decrease sperm quality. They found that early apoptosis was remarkably increased after incubation of spermatozoa with *E. coli* [112]. These observations were further supported by Boguen et al. who found a significant decrease in sperm motility after incubating of the spermatozoa with H strain (1:2 sperm to bacteria ratio) [113]. LPS and porins extracted from *E. coli* also have been seen to be able to bind to the plasma membrane of spermatozoa and cause cell death [114]. The exposure of *E. coli* to ejaculated spermatozoa was associated with a major decrease in both the number of cells with high mitochondrial transmembrane potential ($\Delta\Psi_m$) and the cells with normal oxidoreductive function of mitochondria, which led to severe injury of sperm membrane stability, mitochondrial activity, and finally infertility [115].

To gain further insight into the mechanism of inhibitory effects of *E. coli* on sperm motility, Prabha et al. isolated and purified the factor and named it SIF. This factor causes variable structural damage as probable morphological correlates of immobilization. SIF is a 56-kDa molecule that causes instant immobilization without agglutination of human spermatozoa at a concentration of about 1 mg/mL and death at a concentration of about 2 mg/mL. Multiple and

profound alterations were observed by electron microscopy after incubating spermatozoa with SIF, involving all the superface structures of spermatozoa [116].

In several studies, *E. coli* was among the most common pathogens having negative effects on male infertility [19, 27, 60, 82, 101, 117-120]; however, the presence of this pathogen was not found to be associated with poor semen quality and infertility [60, 117, 121].

Staphylococcus aureus

Staphylococcus aureus is an aggressive opportunistic gram-positive pathogen that colonizes the skin, anterior nares, axillae, pharynx, and urogenital tract of 20% of healthy humans. It is considered as a major cause of morbidity and mortality worldwide. Aside from that, *S. aureus* is arguably the dominant organism implicated in primary infertility, among males and females alike [122]. This bacteria express a numerous of secreted and surface proteins that promote colonization and evasion of immune responses. Surface proteins facilitate adhesion of this pathogen to tissue components and invasion into host cells. Unlike other members of the genus, *S. aureus* is well endowed with a variety of virulence factors and produces SIF protein (MW = 20 kDa). It was Prabha et al, who isolated and purified SIF from *S. aureus* and reported that SIF could cause complete immobilization of spermatozoa at a concentration of 150 mg/ml; whereas 200 mg/mL of this factor is required to kill spermatozoa [11].

An *in vitro* study showed that when spermatozoa were coincubated with *S. aureus*, a significant decrease in sperm motility and agglutination of sperms happened. Interestingly, when the bacteria were killed by heat, lysozyme, or antibiotics, no agglutination and immobilization were noticed. The exact mechanism was not clear, but the authors declared that the detrimental effects on sperm motility by live *S. aureus* might be an unrecognized component of fertility problems [123]. Esmailkhani et al. in a research assessing the prevalence of *S. aureus* in infertile male patients declared that this bacteria might be an additional negative factor worsening sperm quality and affecting male fertility [124].

S. aureus was arguably the most dominant microorganism in semen culture of infertile men [122] and was correlated with decreasing sperm density, motility [124-127], and morphology [27, 118, 119]. Also, Li et al. obtained *S. aureus* MJ015 and MJ163 from semen samples of infertile men and measured sperm motility via CASA. They reported that *S. aureus* has inhibitory impacts on the motility and morphology of sperm [128]. Enwurua et al. also reported *S. aureus* and *E. coli* as the most prevalent microorganisms in the studied group. The semen quality including volume, motility, concentration, and immobility was significantly lower in infertile men than in the fertile group [120].

Similarly, *S. aureus* was the most often isolated bacteria in a research by Zeyad and colleagues. In their investigation, bacteriospermia had a significant negative effect on sperm parameters; concentration, motility, progressive motility, chromatin condensation, and fertilization rate. As opposed to different studies that *S. aureus* was among the most pathogens isolated from semen samples, the presence of this pathogen was not correlated with abnormal semen parameters [60, 117].

Neisseria gonorrhoeae

Neisseria gonorrhoeae causes the most common infectious diseases in men and continues to be the cause of morbidity in approximately 106 million cases occurring each year [129]. As with all *Neisseria*, they carry pili on their cell surfaces, facilitating their attachment to epithelial cells [130]. *Neisseria gonorrhoeae* is a pathogen strictly limited to grow on the human host and is mostly transmitted from an infected person to another usually during sexual activities by direct contact between the mucosal membranes of the urogenital tract, anal canal, or the oropharynx. Urogenital *gonorrhoeae* may be asymptomatic in 40% of men and in adult men is often diagnosed by the observation of gram-negative diplococci within, or closely associated with PMNs on a smear from the urethral discharge [131]. This microorganism produces a wide spectrum of disease in human. In men, it causes asymptomatic mucosal colonization, mucosal inflammatory disease (urethritis), and tissue invasion that leads to orchitis, prostatitis, epididymitis in conjunction with a mucopurulent urethral discharge, which may result in testicular damage or ductal obstruction and impaired fertility. Available reports suggest a detrimental effect of *N. gonorrhoeae* infection on fertility [71, 129, 132, 133].

N. gonorrhoeae can attach to the spermatozoa by pili as T1 gonococci or by direct contact as T4 gonococci [134]. Gonococcal infection caused by *N. gonorrhoeae* triggers a flow of PMNs into the infected tissue [135]. A number of outer membrane adhesin proteins known as carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) from *N. gonorrhoeae*, encoded by the *opa* genes, engage host surface receptors [136]. Interaction between CEACAM1 and CEACAM3 with human B and T cells by *N. gonorrhoeae* *opa* proteins prevents antibody production, blocks cellular proliferation, and induces apoptosis [137]. This interaction may contribute to the transmission of *gonorrhoeae* from infected males to their sexual partners.

N. gonorrhoeae association with infertility has been poorly evaluated in men and only a few studies has analyzed its relationship with male infertility [54, 71]. In a very recent study by Khoder et al. a strong and significant association between the presence of *Neisseria spp* in semen and male infertility was revealed. In this study the risk of *Neisseria* infection was twice as high in infertile patients compared to control group [138]. In a study including 200 infertile men and 150 fertile men attending to an infertility center in Iran, *N. gonorrhoeae* was the second prevalent bacteria isolated from semen samples of infertile man which was significantly associated with sperm abnormality and decreased fertilization potential of sperm [54].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative opportunistic pathogenic bacterium and a frequent inducer of urinary tract infections in human [139]. The LPS of *P. aeruginosa* is less toxic than other gram-negative rods, which facilitates the establishment of chronic infections by eliciting a low inflammatory response [139].

This microorganism produces a quorum-sensing signaling molecule called 3-oxododecanoyl-L-homoserine lactone, which have detrimental effects on spermatozoa [140-143]. It has also been reported that exotoxin A of *P. aeruginosa* has a cytotoxic effect on cells at the chromatin level. This effect has led to various defects in spermatozoa. Because toxin target proteins concentrated mainly in tail, the detrimental effects on spermatozoa tail was more pronounced than in the other [144]. The porin from *P. aeruginosa* induces apoptosis in the epithelial cell line derived from seminal vesicles. Porins have receptors for the sperm plasma membrane, so they can impact sperm parameters directly [145]. Aside from that *P. aeruginosa* can cause epididymitis and prostatitis, and interfere with male fertility [146, 147]. In different studies *P. aeruginosa* was isolated from semen samples of infertile patients [148-150].

Weng et al. examined bacterial communities of ninety-six semen samples and *P. aeruginosa* was among the most abundant bacteria in this study. Using next-generation sequencing technology and bioinformatics analysis they showed that *P. aeruginosa* had no impact on semen quality. However, further analysis showed that *P. aeruginosa* can contribute to the deterioration of semen quality in the samples containing less *Lactobacillus* [150]. Huwe et al. analyzed the effects of different uropathogenic microorganisms including *E. coli*, *Enterococcus*, *P. aeruginosa*, and *Staphylococcus saprophyticus* on human sperm motility with a computer-assisted sperm analyzer (CASA) and reported that tests with *P. aeruginosa* resulted in a decrease of progressive motility, but not to different bacterial concentrations [110].

Enterococcus faecalis

Enterococcus faecalis is an important human pathogen, causing severe urinary and male reproductive tract infections that might be responsible for male infertility. They are part of the normal flora of the genital tracts and can cause infection when introduced to other sites. Although the influence of gram-positive uropathogenic bacteria on sperm morphology and function has been poorly investigated, it's been shown that *E. faecalis* is associated with poor semen quality and may warrant treatment. Some researchers have explained the negative influence of *E. faecalis* on membrane integrity of human sperm head, neck and midpiece [10, 151], probably mediated by a well-known virulence factor of *Enterococci* called haemolysin. Increased prevalence of genital tract infection caused by *E. faecalis* was associated with semen quality in terms of sperm motility [152, 153], concentration, and morphology [153, 154].

In different studies *E. faecalis* was among the most often microorganism isolated from semen samples of infertile patients [19, 60, 117, 155-158] and considered as a potential cause of infertility [10, 159]. A direct effect of this pathogen on semen quality was evaluated in a study in Mexico. In this study, they showed that motile spermatozoa and sperm viability were lower when the microorganisms were present in the semen and could have a direct effect on semen quality with negative consequences on fertility [155].

As opposed to these studies, in some investigations no relationship between *E. faecalis* and semen parameter were indicated. In a recent study, *E. faecalis* was the most common microorganism isolated from semen samples (30%), although, the presence of asymptomatic bacteriospermia was not correlated with abnormal semen parameters [117]. No association was also found between the presence of aerobic micro-organisms including *E. faecalis* in semen and basic semen parameters including volume, pH, concentration, total count, motility, vitality and morphology[60].

Streptococcus agalactiae

Group B *streptococcus* (GBS), also known as *Streptococcus agalactiae* is a gram-positive coccus. *Streptococcus agalactiae* was once considered as a pathogen of only domestic animals and later as a human pathogen causing severe urinary and male reproductive tract infections that may be responsible for male infertility. Apparently, *S. agalactiae* plays an impairment role in infection-induced infertility in a bacteriospermia associated with leukocytospermia (LCS/BS) state [156]. In several studies, *S. agalactiae* was among the most commonly isolated species from infertile individuals that contributed to decreased sperm motility[82], semen quality, and impaired fertilization [14, 19, 152, 160, 161].

C. Anaerobic bacteria

Gardnerella vaginalis

Gardnerella vaginalis formerly known as *Haemophilus vaginalis* and *Corynebacterium vaginale*, is consistently predominant organism isolated from women with BV which can be sexually transmitted to male partners. It has also been recovered from the semen and male urogenital tract frequently [121, 162-164]. BV is the infection of the squamous epithelium; therefore, it is probable that colonization and infection of the genital tract in males is limited to the distal urethra and is lined with squamous epithelium. Nelson et al. found *G. vaginalis* in 28% of urine samples but failed to detect *G. vaginalis* from samples of the coronal sulcus [165]. Also, it has been hypothesized that the prostate might be a potential reservoir for *G. vaginalis* [166, 167]. *Gardnerella vaginalis* appears to be a prevalent microorganism in the genital tract of male with suspected infertility and even more among their wives [168], but the role of this pathogen in the male urogenital microbiome is not well established.

In different studies, *G. vaginalis* was among the most commonly isolated species contributed to male infertility [121, 150, 159, 162, 169]. De Francesco et al. showed in 696 semen samples, *G. vaginalis* was the first abundant isolated bacterium. They realized that *G. vaginalis* and *U. urealyticum* presence negatively affected sperm concentration, motility, and morphology [82], while in another study on 108 infertile men, *G. vaginalis* in semen samples was not associated with either abnormal sperm characteristics or the inflammatory response in infected men [170]. Ison and Easmon also found no association between the isolation of *G. vaginalis* and the sperm count [171].

Anaerococcus

Anaerocci are nonmotile gram-positive cocci that are strictly anaerobic and commonly found in the human vagina and various purulent secretions but can be present in the male urinary tract infections as well [172]. Multiple statistical tests showed a significant negative association between sperm quality and the presence of *Anaerococcus* in the semen samples of infertile patients [160, 173].

Kiessling et al. found that *Anaerococcus prevoti* or *Anaerococcus vaginalis* had higher prevalence among infertile patients that might influence fertility potential [160]. Similarly, in a study consisted of 19 sperm donors and 58 infertility patients, multiple statistical tests showed a significant negative association between sperm quality and *Anaerococcus*. The presence of *Anaerococcus* in semen was negatively associated with spermatozoal physiology during sperm transition, more specifically motility and morphology. The authors declared that the presence of *Anaerococcus* might be a biomarker for low sperm quality [173].

D. Microaerophilic bacteria

Helicobacter pylori

The bacterium *Helicobacter pylori* is a gram-negative and spiral-shaped organism and is specialized to infect human gastroduodenal tract. Studies have demonstrated that *H. pylori* infection may be associated with some extra-gastrointestinal diseases [174]. Recently, increasing evidence has supported the involvement of *H. pylori* as a risk factor in both male and female infertility. Researchers have reported that *H. pylori* infection may influence the semen concentration of peptides such as ghrelin and obestatin that are involved in the regulation of testicular function, hence, affect fertility [175, 176].

Almost all cytotoxic strains of *H. pylori* express a highly immunogenic protein called CagA (cytotoxin-associated gene A). The enhanced pathogenic potential of CagA+ organisms reside in the presence of a genomic insertion called *cag* which contains genes involved in virulence. Ambrosini et al showed that the *H. pylori* infection is remarkably common in both semen samples of infertile men and the follicular fluid of women with fertility problems (100% of follicular

fluids, 50% of sperm samples) [177]. Particularly, CagA-positive patients showed a considerable reduction in sperm quality compared with the control group. Sperm motility and fertility index was significantly lower in CagA-positive patients and *H. pylori* infection increased unviable spermatozoa rate. It was mainly due to the presence of mimicry between *H. pylori* antigens and tail proteins (β -tubulin protein) of spermatozoa [178].

It has been indicated that ghrelin levels might be reduced in patients infected with the strains expressing CagA, as a result of mucosal atrophy. This mainly lies in the fact that most of the circulating ghrelin is produced in the gastric oxyntic area. In several studies, both peptides were detected in human semen [179-181]. Moretti et al. found a significantly decreased level of ghrelin in the semen specimen of patients infected by *H. pylori* strains expressing CagA, and regarded it as a possible response to the negative impacts of *H. pylori* infection on the semen quality. Sperm motility, sperm vitality, and the percentage of sperm with normal forms in the CagA-positive group remarkably declined compared to the uninfected group [176].

Figura et al. examined the relationship between anti-*H. pylori* antibodies and infertility. They found that due to a certain rate of homology between human tubulin and bacterial proteins, antibodies produced against *H. pylori* reacted with the sperm tail, centrioles, and equatorial zone of human sperm (which are rich in tubulin) without affecting sperm motility [182].

Moretti et al. recently confirmed the detrimental influence of CagA positive strains on semen quality and proposed its association with reduced reproductive potential in men. In this investigation, semen TNF- α and IL-6 concentrations were increased in *H. pylori*-positive group compared to negative patients. They found that CagA+ group showed reduced sperm motility, enhanced necrosis, and increased cytokines levels in comparison to the *H. pylori*-negative group. It was also observed that sperm motility of CagA+ group was significantly lower than CagA- group [183].

Further investigations supported the negative impact of *H. pylori* infection on semen quality. El-Garem et al. saw that the treatment of seminal *H. pylori* resulted in significantly improved sperm motility with elevated seminal *H. pylori* IgA. In their research, a significant negative association was found between *H. pylori* infection and progressive sperm motility, non-progressive sperm motility, and normal sperm morphology [174].

In a recent study, the association of *H. pylori* infection and ASA was assessed in different groups including patients with gastroduodenal diseases caused by *H. pylori*, infertile patients positive for ASA, and healthy fertile blood donors as controls. They showed that levels of both types of antibodies were significantly higher in infertile patients and sperm agglutination and immobilization tests showed a significant difference in the same group. They suggested that *H. pylori* might play a role in the induction of anti-sperm antibodies [184].

Conclusion

Bacterial infections are considered to be important etiological factors for male infertility. Different bacteria by negatively affecting spermatogenesis process, sperm functions, and/or seminal tracts, prostate, and urethra may increase fertility problems among men. This review summarizes current knowledge regarding the different sorts of atypical, anaerobic and aerobic bacteria disturbing semen quality and explains their possible role in male fertility which can be induced through multiple pathophysiological mechanisms. In this study, the significant effects of different bacteria on male infertility along with the sites of infection were summarized in simple tables and discussed. Every bacterium that affects fertility was reported to disturb spermiogram and to be involved in male reproductive failure. However, there is ample evidence to blame bacteria such as *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma*, *Mycoplasma* and *Staphylococcus aureus* for reduced fertility and deterioration of sperm parameters with more certainty. Although so far investigations have provided a great amount of information on the possible role of different bacterial infections in male infertility, there is a lack of scientific information concerning the underlying mechanism and true impacts of some pathogenic bacteria including *N. gonorrhoeae*, *E. faecalis*, and *G. vaginalis* on male reproductive system which leaves room for confusion. Asymptomatic infections, associated with recurrent and latent infections, are the major challenges to the control of infertility. Increasing awareness among populations, proper sanitation, and proper precaution are the best approaches that can be considered to prevent their transmission and inhibit their prevalence among people. Understanding the true impacts of these pathogens and getting more insight into their underlying pathophysiological mechanisms would significantly pave the way for identifying suitable biomarkers and production of new treatment strategies in the future that would minimize the risks of bacterial infections on male reproductive conception.

Conflict of Interest

The authors of the current study have no conflict of interest to disclose.

Conflict of interest

The authors report no conflict of interest.

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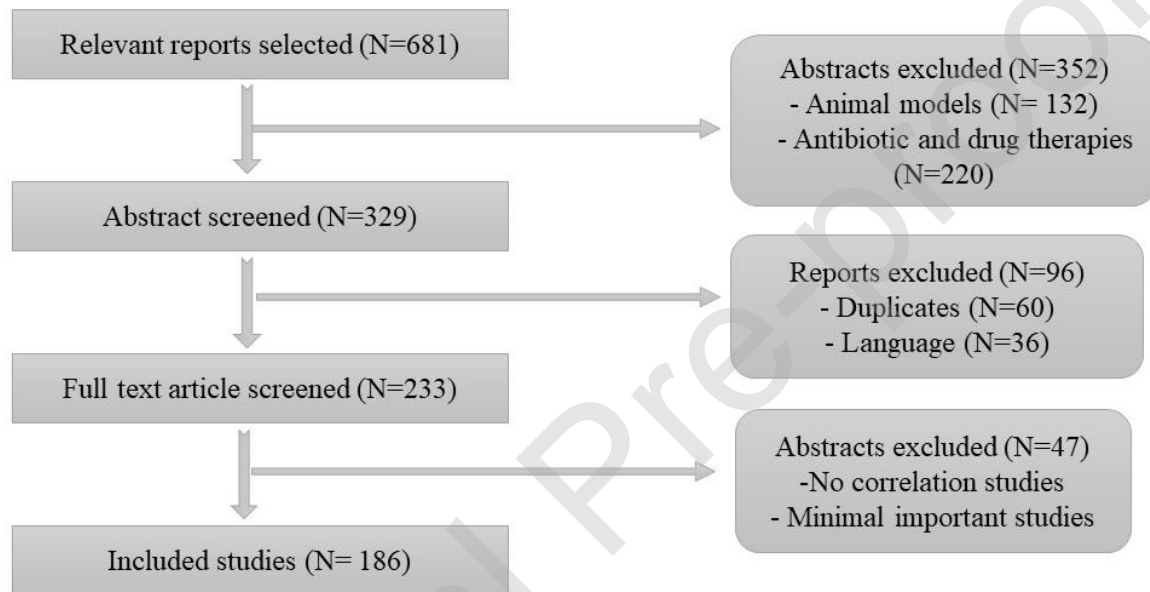
Figures

Figure 1. The flow chart for the search methodology

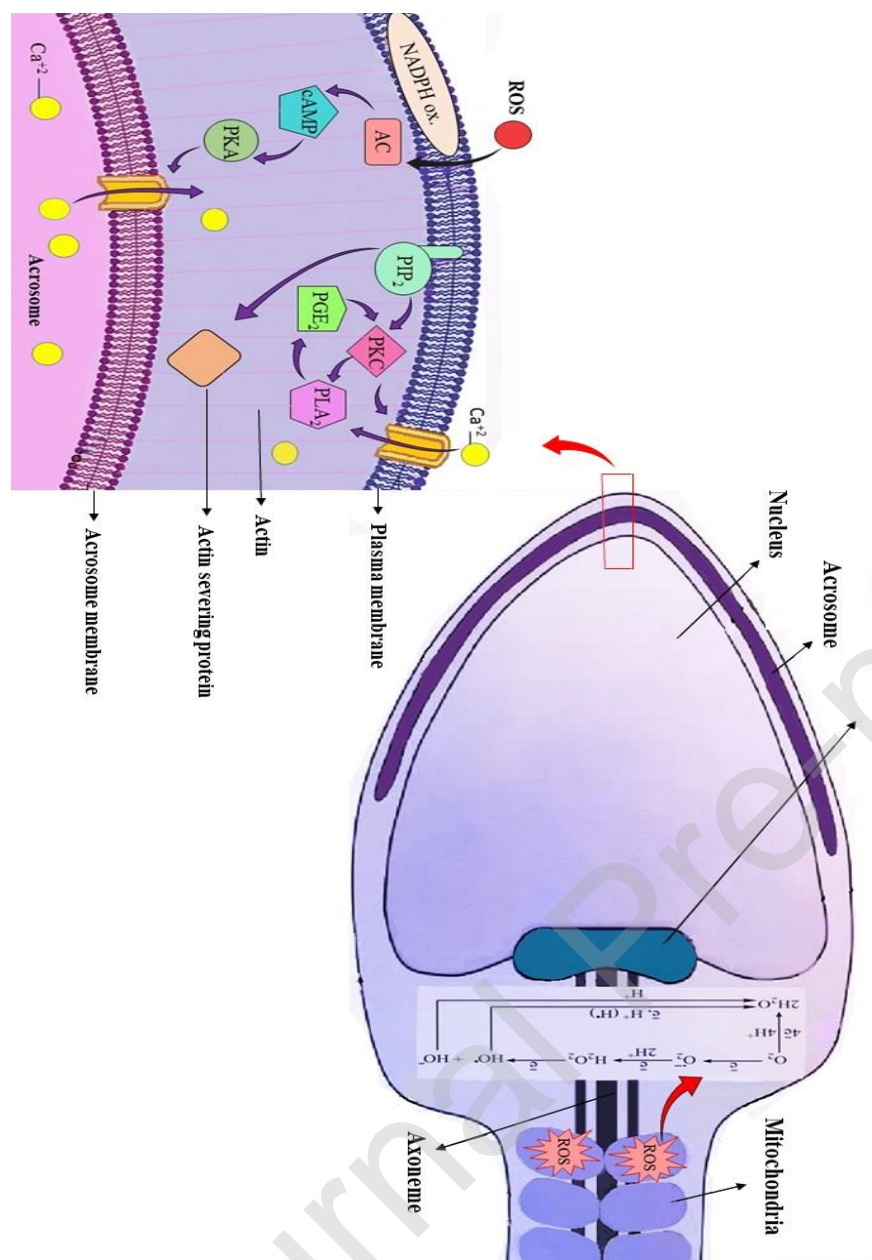


Figure 2. Mitochondrial dysregulation and ROS leakage from the inner mitochondrial membrane that would affect acrosomal reaction and sperm function negatively. If the ROS production exceeds concentrations of antioxidants in the spermatozoa, oxidative stress occurs. Increases levels of ROS damage the inner and outer mitochondrial membranes and induce premature capacitation that would affect acrosomal morphology and function; hence, impact fertilizing capacity of human spermatozoa significantly. ROS in pathogenic bacteria such as *E. coli*, *Mycoplasma*, *Chlamydia*, *streptococci*, *staphylococci*, and *Ureaplasma* leads to apoptosis and breakdown of mitochondrial membrane. Both superoxide ($O_2^{\cdot-}$) and the hydroxyl radical ($OH^{\cdot-}$) are toxic to cells and cause chromosome deletions, dicentric and sister chromatid exchanges.

Bacteria	Effects on male infertility	Locus of infection
<i>Escherichia coli</i>	Breakdown of mitochondrial membrane, impairment of acrosome reaction, sperm motility and morphology, decrease in sperm concentration, DNA damage	Prostate Epididymis Testis Seminal vesicles Urethra
<i>Chlamydia trachomatis</i>	Impairment of acrosome reaction, decrease in sperm concentration, sperm motility, viability and morphology, DNA damage	Prostate Epididymis Testis Seminal vesicles Urethra
<i>Ureaplasma urealyticum</i>	Impairment of acrosome reaction, sperm motility and morphology, decrease in sperm concentration, vitality, DNA damage	Prostate Epididymis Urethra
<i>Mycoplasma hominis</i>	Impairment of sperm, motility morphology, decreased sperm concentration and viability, DNA damage	Urethra Prostate
<i>Mycoplasma genitalium</i>	Impairment of sperm motility, decreased sperm concentration and vitality, DNA damage	Urethra Prostate
<i>Neisseria gonorrhoeae</i>	Impairment of Sperm integrity and DNA damage	Prostate Epididymis Testis Seminal vesicles Urethra

Figure 3. A summary of major causative pathogens, their effects on male infertility, and their loci of infection

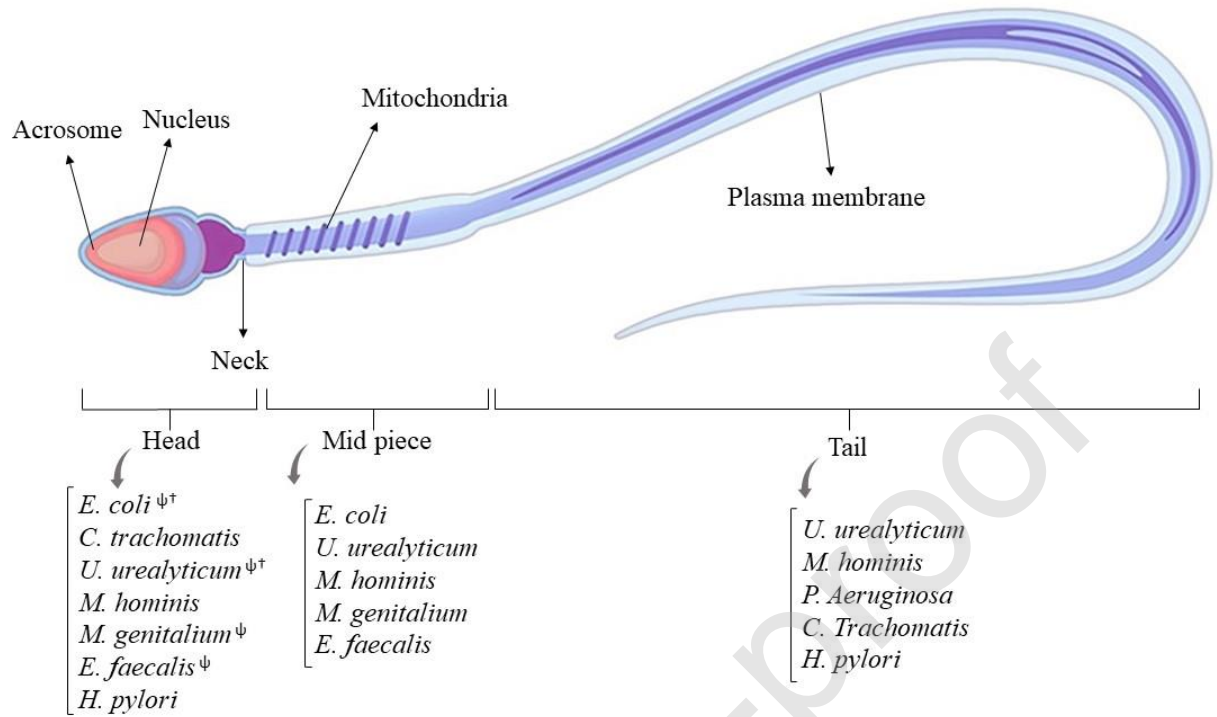


Figure 4. Different parts of sperm structure and the impact of different bacteria on these regions. ψ indicates the impact of mentioned bacteria on the neck region and \dagger shows the impact of pathogen on the acrosomal regions.

Tables

Table 1. The characteristics of included population-based studies

Study	Region	Year	Population	Infections	Method	Outcome	P value
[10]	USA	2002	299 infertile men	<i>E. faecalis</i>	Culture	Poor semen quality and decreased sperm morphology	0.005
[19]	Germany	2018	84 infertile men	<i>E. faecalis</i> , <i>E. coli</i> , <i>SA</i> , <i>S. agalactiae</i>	Culture	Decreased sperm motility, concentration, and morphology	<0.03
[27]	Nigeria	2009	500 infertile men	<i>SA</i> , <i>CT</i> , <i>E. coli</i>	Culture	Decreased sperm motility, concentration, and morphology	
[36]	Finland	2004	271 men with subfertility /367 controls	<i>CT</i>	EIA	Increased subfertility	0.012
[37]	Spain	2008	143 infertile/50 controls	<i>CT</i> , <i>Mycoplasma</i>	IFA	Decreased sperm motility, concentration, and morphology, increased DNA fragmentation	<0.0001
[38]	Tunisia	2014	85 infertile men	<i>CT</i> , <i>NG</i> , <i>UU</i> , <i>MG</i>	PCR	<i>CT</i> decreased sperm concentration and motility, increased apoptosis induction	0.02 0.04
[43]	Bulgaria	2007	60 infertile men/ 40 controls	<i>CT</i>	PCR	Increased infertility rate	
[44]	Finland	2009	90 infertile men/190 controls	<i>CT</i>	EIA	Higher prevalence, decreased sperm counts	<.001 ≤0.05
[45]	Iran	2018	40 infertile couples/20 controls	<i>CT</i>	ELISA	Decreased sperm motility and morphology	<0.05
[46]	USA	1993	52 men with idiopathic infertility/ 79 controls	<i>CT</i>	IFA	Increased infertility	
[47]	Iran	2018	165 infertile couples/165 controls	<i>CT</i>	PCR	Decreased sperm motility, concentration, and morphology	<0.001
[48]	Iran	2018	250 infertile men	<i>CT</i>	IFA	Decreased sperm motility and morphology	<0.04 <0.003
[49]	Turkey	2010	53 infertile men/ 53 controls	<i>CT</i> , <i>MH</i> , <i>UU</i>	ELISA	No effect on semen parameters	>0.05
[50]	Italy	2010	1161 patients with chronic prostatitis (707 ^P /454 ^N)	<i>CT</i>	PCR ST	Decreased sperm motility, concentration, and morphology	<0.001
[51]	Czech	2004	627infertile men (136 ^P /491 ^N)	<i>CT</i>	IFA	Decreased sperm motility and morphology	0.01 0.05
[52]	Tunisia	2001	92infertile men (33 ^P /59 ^N)	<i>CT</i>	IFA PCR	No effect on semen parameters	>0.05
[53]	Israel	1990	175 infertile men	<i>CT</i> , <i>UU</i> , <i>MH</i>	Culture	<i>CT</i> and <i>MH</i> reduced sperm egg penetration ability.	<0.02
[54]	Iran	2018	200 infertile couples/150 controls	<i>CT</i> , <i>NG</i>	PCR	Higher prevalence, lower sperm concentration and fertility potential	<0.001
[55]	Tunisia	2013	104 infertile men (84 ^P /20 ^N)	<i>UU</i> , <i>UP</i> , <i>MH</i> , <i>MG</i> , <i>CT</i>	PCR	No effect on semen parameters	
[56]	China	2014	621 infertile/615 controls	<i>UU</i> , <i>CT</i> , <i>MH</i>	PCR	<i>UU</i> lowered sperm concentration and vitality	<.05
[57]	Iran	2017	372 infertile men and 93 controls	<i>CT</i>	ELISA	No effect on sperm motility and morphology	0.213 0.401
[58]	Argentina	2005	40 chronic prostatitis men/15 controls	<i>CT</i>	PCR IFA	No detrimental effects on sperm quality	>0.05
[59]	Germany	1997	1317 men with subfertility (136 ^P /750 ^N)	<i>CT</i>	IFA	No effect on sperm quality	
[60]	Poland	2015	72 infertile men (50 ^P /22 ^N)	<i>UU</i> , <i>CT</i> , <i>SA</i> , <i>E. coli</i> , <i>E. faecalis</i>	Culture	No effect on semen parameters	> 0.05

[61]	Chile	2002	284 infertile men (110 ^P /174 ^N)	CT	IFA	No effect on sperm quality	
[66]	China	1995	1416 infertile men/375 controls	UU	Culture	Poor quality, decreased sperm motility	
[101]	Germany	1998	488 infertile men	UU, MH, E. coli	Culture	MH and E. coli decreased motility UU, MH, and E. coli increased acrosome reaction	< 0.01
[71]	Jordan	2013	93 infertile men/70 controls	UU, CT, MG, NG	PCR	Higher prevalence, NG increased infertility	0.032
[72]	Bulgaria	2015	281 infertile men/100 controls	CT, UU, MH	PCR	Higher prevalence	
[73]	China	2017	2607 infertile men	CT, MG, UU	PCR	MG and UU increased DNA damage	0.03 0.02
[74]	Greek	2017	180 infertile men (102 ^P /68 ^N)	CT, UU, MG	PCR	No effect on sperm motility and concentration	>0.05
[75]	China	2018	78 UP + and 24 UU+ infertile men	UU, UP	PCR	Decreased sperm motility	<0.046
[76]	Nigeria	2019	288 infertile men	CT, MH, UU	IFA	Higher prevalence	
[77]	Iran	2009	100 infertile/ 100 controls	UU, UP	PCR	UU decreased sperm motility and morphology	<0.001
[78]	China	2015	540 infertile men/260 controls	UU	PCR	UU decreased sperm motility and concentration	0.003
[79]	Tunisia	2008	288 infertile men (66 ^P /60 ^N)	UU, UP, MG, MH, CT, NA, SA, GV, E. coli and E faecalis.	PCR Culture	Higher prevalence of UU and MH	0.00 0.03
[80]	China	2015	19098 infertile men/3368 controls	UU, MH	Culture	Higher prevalence of UU, MH UU decreased sperm motility and morphology	<0.001
[81]	Korea	2013	50 infertile men/ 48 controls	UU, MH	Culture	Decreased sperm motility and viability	0.008 0.01
[82]	Italy	2011	696 men with subfertility	E. coli, UU, S. agalactiae, GV	Culture	GV and UU decreased sperm concentration, motility, and morphology S. agalactiae decreased sperm motility and density	<0.001 <0.01 <0.05
[87]	Greece	1987	225 infertile men	UU, CT	Culture	No effect on sperm quality	
[88]	Brazil	2003	234 infertile men	MH, UU	Culture	No effect on sperm quality	>0.05
[89]	USA	1985	96 infertile men/30 controls	UU	Culture	No effect on sperm fertility potential	>0.05
[90]	Tunisia	2007	120 infertile men	UU, UP, MH, MG	PCR	MH decreased concentration and morphology MG decreased concentration	0.007 0.03 0.05
[92]	Iran	2017	165 infertile men/ 165 controls	MH	PCR	Decreased sperm motility and morphology	<0.001
[185]	China	2014	223 infertile men/146 controls	UU	PCR	Decreased sperm motility and concentration, increased DNA fragmentation	0.01 0.04
[96]	Czech	2012	293 infertile men/ 173 fertile	CT, MG, MH, UU, UP	Culture	MG and MH decreased sperm motility, concentration, and morphology and DNA	<0.01 <0.05

						condensation, <i>UU</i> and <i>CT</i> decreased volume and viability	
[186]	Denmark	2014	145 infertile men/49 controls	<i>CT, MG, UU, NG</i>	PCR	No effect on male infertility	
[99]	Kuwait	2012	127 infertile men/ 188 fertile	<i>UU, MH, MG, CT</i>	PCR	<i>CT</i> decreased sperm motility and viability	<0.05
[100]	Israel	1994	135 infertile men /88 controls	<i>UU, MH, CT</i>	Culture ST	Higher prevalence of <i>MH</i>	< 10 ⁻⁵
[117]	India	2016	85 infertile men	<i>E. faecalis, E. coli, SA</i>	Culture	No effect on sperm parameters	>0.05
[118]	Poland	2005	53 infertile men/ 30 controls	<i>SA, UU, MH, E. coli, E. faecalis</i>	Culture	<i>UU, E. coli</i> and <i>SA</i> decreased sperm motility and morphology	< 0.05
[119]	Iran	2016	150 infertile/150 controls	<i>SA, E. Coli</i>	Culture	Decreased sperm motility and morphology	
[120]	Nigeria	2016	162 infertile/55 fertile men	<i>SA, E. Coli</i>	Culture	Decreased sperm motility, density, and morphology	
[121]	USA	1990	37infertile men	<i>E. coli, E. faecalis, GV</i>	Culture	No impact on sperm quality	
[177]	Italy	2010	30 men with idiopathic infertility (15 ^P /15 ^N)	<i>H. pylori</i>	ELISA	Decreased sperm motility	
[178]	Italy	2010	82 infertile men (38 ^P /44 ^N)	<i>H. pylori</i>	ELISA	Decreased sperm motility and volume, increased apoptosis	<0.05
[183]	Italy	2015	109 infertile men (28 ^N /81 ^P)	<i>H. pylori</i>	ELISA	Reduced sperm motility, increased apoptosis	<0.05
[122]	Nigeria	2011	124 infertile men	<i>SA, E. coli</i>	Culture	Higher prevalence	
[124]	Iran	2018	100 infertile men(10 ^P /90 ^N)	<i>SA</i>	Culture	Decreased sperm motility, concentration, and morphology	0.02 0.01
[127]	Nigeria	2005	200 infertile men	<i>SA</i>	PCR	Decreased sperm motility and volume	
[126]	Nigeria	2001	163 infertile men(78 ^P /85 ^N)	<i>SA, E. coli</i>	Culture	Poor semen quality, reduced sperm motility	
[152]	Italy	2009	264 infertile men/ 20 controls	<i>UU, E. faecalis, E. coli, SA</i>	Culture	Decreased sperm motility and fertilization rate	<0.001 <0.05
[153]	Italy	2018	273 infertile men (83 ^P /190 ^N)	<i>E. faecalis, UU, CT, SA, MH, E. coli</i>	Culture PCR	<i>E. faecalis</i> decreased sperm motility and morphology	0.026 0.003
[154]	India	2002	100 infertile men (26 ^P /74 ^N)	<i>E. faecalis</i>	Culture	Decreased sperm concentration and morphology	<0.03
[155]	Mexico	1995	119 infertile men/ 64 controls	<i>E. faecalis, E. coli, SA, GV, NG</i>	Culture	Decreased sperm motility and viability	0.0001
[159]	Italy	2016	246 infertile men	<i>E. faecalis, E. coli, SA, GV, UU, CT, S. agalactiae</i>	Culture	Higher prevalence of <i>E. faecalis, E. coli, S. agalactiae</i> and <i>UU</i>	

[162]	France	2005	543 infertile men	<i>E. coli</i> , <i>S. agalactiae</i> , <i>GV</i> , <i>UU</i>	Culture	Higher prevalence of <i>S. agalactiae</i>	
[150]	Taiwan	2014	96 infertile men (60 ^P /36 ^N)	<i>GV</i> , <i>SA</i> , <i>E. coli</i> , <i>PA</i>	PCR	Higher prevalence of <i>GV</i> and <i>PA</i> No impact on sperm quality	
[170]	Brazil	2009	192 infertile men (108 ^P /84 ^N)	<i>GV</i>	Culture	Decreased sperm motility, morphology, and viability	P<0.01
[173]	Russia	2013	58 infertile men/ 19 controls	<i>Anaerococcus</i>	PCR	Decreased sperm quality	0.0012
[149]	USA	1979	109 infertile men	<i>GV</i> , <i>UU</i> , <i>SA</i> , <i>E. coli</i> , <i>MH</i> , <i>PA</i>	Culture	Decreased sperm motility, morphology, and viability	

The information regarding male partners from couples' studies are only included.

^P indicates patients with bacterial infections

^N indicates patients with no bacterial infection

Table 2. A summary of included population-based studies

Bacteria	Affects spermiogram	Affects fertility	Study
<i>Chlamydia trachomatis</i>	Yes	Yes	[27, 36-38, 43-48, 50, 51, 54, 96, 99]
	No	No	[49, 52, 53, 55, 57-61, 74, 87, 186]
<i>Ureaplasma urealyticum</i>	Yes	Yes	[56, 66, 73, 75, 77, 78, 80-82, 96, 118, 152, 185]
	No	No	[55, 60, 74, 87-89, 186]
<i>Ureaplasma parvum</i>	Yes	Yes	[75]
	No	No	[55]
<i>Mycoplasma genitalium</i>	Yes	Yes	[37, 73, 90, 96]
	No	No	[55, 74, 186]
<i>Mycoplasma hominis</i>	Yes	Yes	[37, 53, 81, 90, 92, 96, 101]
	No	No	[55, 88]
<i>Escherichia coli</i>	Yes	Yes	[19, 27, 82, 101, 118-120, 126, 152, 155]
	No	No	[60, 117, 121, 150]
<i>Enterococcus faecalis</i>	Yes	Yes	[10, 19, 152-155]
	No	No	[60, 117, 121]
<i>Neisseria gonorrhoeae</i>	Yes	Yes	[54, 71, 155]
	No	No	[186]
<i>Staphylococcus aureus</i>	Yes	Yes	[19, 27, 118-120, 124, 126, 127, 152, 155]
	No	No	[60, 117, 150]
<i>Streptococcus agalactiae</i>	Yes	Yes	[19, 82]
<i>Gardnerella vaginalis</i>	Yes	Yes	[82, 155, 170]
	No	No	[121, 150]
<i>Pseudomonas aeruginosa</i>	No	No	[150]
<i>Helicobacter. pylori</i>	Yes	Yes	[177, 178, 183]