The Impact of Bacterial Infections on Human Spermatozoa

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Abstract
Male urogenital tract infection (UTI) is one of the most important causes of male infertility, being associated with 8%-35% of male infertility. Pathogenic bacteria may interfere with infertility treatment involving the application of in vitro fertilization. Microorganisms might affect the spermatozoa function in different ways: (a) By direct contact on sperm cells; by the help of some organelles such as pili; causing agglutination of motile sperm, reducing ability of the acrosome reaction, and also causing alterations in cell morphology. (b) Trigger a local inflammatory reaction leading to increase in reactive oxygen species (ROS). (c) Induction of sperm autoantibodies. (d) Production of cytotoxic factors. (e) Infection treatment with antibiotics for long time may lead to defect in the sperm. The most frequently isolated bacteria from semen samples include Staphylococcus aureus, Escherichia coli, Streptococci, Klebsiella sp, Mycoplasma hominis, Chlamydia trachomatis and Enterococcus faecalis. The infection with these bacteria has significantly negative effect on sperm parameters and DNA integrity. DNA fragmentation may cause infertility, miscarriage, and birth defects in offspring. Therefore it may be a more objective marker of sperm function. The exact molecular mechanism of how bacteria affect chromatin and sperm nuclear protein still unknown. The bacterial infections lead to premature emergence of histone H3 methylation at lysine 79 (trimethylated H3K79) and hyperacetylated H4 which simultaneously occurred with transition protein TNP1. In mammals, reduced levels of histone H4 hyperacetylation correlates with impaired fertility. Further researches on this topic are necessary.

Keywords: Bacterial Infection, Sperm proteins, PRM1/PRM2 ratios

Introduction

The infertility is common clinical problem. Sixty percent of patients treated with assisted reproductive technology (ART) had suffered inflammation or infection. There is direct relation between male infertility and genital tract infection, this relation represents important problem in contemporary andrology (1).

The bacteria responsible for semen infection may originate from the urinary tract or be sexually transmitted (1). The unhygienic sex represents important way for permeation of such these bacteria to genital tract.

The male reproductive system includes 2 testicles, accessory reproductive ducts and secretory glands (semenal vesicles and prostate gland). Testicles are components of reproductive and the endocrine system. Therefore, the 2 main functions of the testicles are: producing sperm (approximately 1 million per hour) and male sex hormones (e.g. testosterone) (2).

Spermatogenesis occurs within the seminiferous epithelium on the surface of the sertoli cells. Sperm pass in different stages of maturation before a gamete can leave the testis. These processes include mitotic multiplication and propagation of the spermatogonial stem cells (SSCs), meiotic division of genetic material and maturation of spermatozoa (3).

Human normal semen consists of sperm and fluids from each of the vas deferens, the seminal vesicles, the prostate gland and the mucous glands, especially the bulb urethral glands. Seminal vesicle fluid represents 60% of the semen where serves to wash the sperm out of the ejaculatory duct and urethra (4).

According to the fifth edition of manual for semen analysis of WHO, 2010 (5), the normal values of semen determined as follow:

- sperm concentration is ≥15 × 10⁶/mL and the total sperm count is ≥39 × 10⁹/ejaculate, ≥40% motility, 32% progressive motility, ≥4% normal forms and white cell count, <10⁴/ml of ejaculate.

Sperm Chromatin and Nuclear Protein

Normally, the sperm chromatin is a highly compact structure consisting of DNA and heterogeneous nucleoproteins. It is compacted and insoluble in order to protect DNA and facilitate transport of the paternal genome through the male and female reproductive tracts (6).

Sperm chromatin differs from somatic cells in both constituents and arrangement. Human sperm nuclei contain approximately 85% protamines and 15% histones in their nucleoprotein component, (7). Protamines, which
are half the size of histones, replace the most of histone during maturation process and the chromatin becomes condensed unique supercoiled structure named toroids (8). Protamine 1 and 2 (P1&P2) are the most nuclear proteins in human sperm nucleus packaging the sperm DNA, where P1 is produced as a mature protein while P2 is produced as a precursor protein (9).

The retained histones may be associated with telomeric sequences and these are the first structures in the sperm nucleus which act as a trigger to oocyte for pronucleus formation to achieve the process of fertilization and early embryo development (10,11).

Protamines are small size proteins contain a lot of positively charged amino acids, especially arginine. This positive charge allows the formation of a highly condensed complex with the paternal genomic DNA, which has a strong negative charge (12).

Protamines size ranging between 4000-12000 Da, are composed of more than 50% arginine (13). There are 2 types of protamines, namely protamine 1 (P1) and protamine 2 (P2). The incorporation of these 2 proteins into the sperm chromatin is strictly regulated, resulting in specific P1/P2 ratio (14).

In human sperm, P1/P2 ratio is approximately 1 in fertile men (15,16). The P1 of the mammalian placenta is exactly 49 or 50 amino acids long (17). P2 is slightly larger than P1, where contains about 63 amino acids, and is the predominant form of P2 in the mature sperm head. In human sperm, there are 2 differently processed forms of protamine 2; P2 and P3. The 2 forms of the P2 protein differ only in their three amino-terminal amino acids - P3 is 3 amino acids shorter (at 54 amino-acid residues) than P2 (57 amino acids), and they encode by the same gene (PRM2 gene) (17). P2 also differs from P1 in that P2 binds zinc, in human and other mammalian species coordinate one zinc atom per molecule of P2 (18).

Disulfide cross-links between the cysteine-rich protamines are responsible for further compaction and stabilization of the sperm nucleus (19,20).

During spermiogenesis, the majority of nucleosomal histones are replaced by protamines in a multi-step process resulting in intensive chromatin (21). First, the somatic histones are replaced by testis-specific histone variants, followed by the replacement of these by transition proteins (transition protein 1 and 2). Finally, transition proteins are replaced by protamines during the spermatid elongation process (15,16).

The deficiency of protamine replacement may not only be a marker of abnormal spermiogenesis, but may also affect the function of the paternal genome contribution during embryogenesis. It may result in suboptimal embryogenesis and/or increased risk of mutations to the offspring (21).

The exact mechanism by which DNA damage arises in human spermatozoa is not clearly understood and three mechanisms have been proposed: defective sperm chromatin packaging, apoptosis, and oxidative stress (22).

**Sperm DNA Fragmentation**

Fertilization is the process of penetration of sperm into oocyte. The achievement of this process as well as embryo development depends on the DNA integrity of the sperm (23).

Male infertility has classically been diagnosed by microscopic assessment of concentration, motility and morphology of the sperm in the ejaculate. These tests are essential to provide the basic information of the sperm quality. Sperm DNA fragmentation (SDF) tests can differentiate fertile from infertile males, high levels of SDF are positively correlated with lower fertilization rates in IVF (in vitro fertilization), impaired the implantation rates and an increased abortion incidence (24).

Sperm DNA fragmentation may cause infertility, miscarriage, and birth defects in offspring (25). There are 2 types of factors may cause sperm DNA damage:

1) Intrinsic factors including in the ejaculates such as oxidative stress, apoptosis and failure in the histone-protamine replacement (26,27). Sperm DNA becomes exposure to damage if chromatin packing is not completed during sperm maturation (24).

DNA fragmentation may also occur during spermiogenesis by endonucleases (topoisomerases), this enzyme act to relieve the increased DNA torsional stress during the DNA condensing and packaging into the differentiating sperm head (28).

2) Extrinsic factors such as storage temperatures, handling conditions, lapse of time after ejaculation, infections, reaction to medicines, or post-testicular oxidative stress (24).

Sperm DNA damage may affect the early post implantation embryo development in ART and thus decrease the fertility and pregnancy rate (29).

Some reports have indicated that when >30% of sperm DNA is damaged, natural pregnancy is not possible (30). Also, it has been proposed that the sperm DNA integrity may be a more objective marker of sperm function as opposed to the standard semen analysis (30).

Hofmann and Hilscher (31) mentioned that various nuclear alterations including an abnormal chromatin structure, chromosomes with microdeletions, aneuploidies and DNA strand breaks can be detected in infertile men. Damaged DNA has been observed in testicular, epididymal and ejaculated sperm (24).

DNA repair process occurs in developing sperm but it is terminated as transcription and translation stops post-spermiogenesis. So that mature sperms do not have mechanism to repair DNA abnormality that occurs during their transit and storage in the epididymis or post-ejaculation. However, oocytes and early embryos have been shown to repair some types of sperm DNA breakage. Consequently, the biological effect of damaged sperm chromatin structure depends on the combined effects of level and type of sperm chromatin damage and the ability of the oocyte to repair it (24).

In mammalian sperms, DNA fragmentations can occur in 2 forms: single (SSB) and double DNA strand breaks.
(DSBs), and it is particularly frequent in the ejaculates of subfertile males (32).

The sperm DNA fragmentation could be induced by oxidative attacks like the hydroxyl radical and ionizing radiation results in the formation of 8-OH-guanine and 8-OH-20-deoxyguanosine (8-OHdG) at a first stage and single-stranded DNA fragmentation. Hydroxyl radical formation may result in the indirect induction of double-stranded sperm DNA damage through the activation of sperm caspases and endonucleases (33).

DNA double-strand breaks are extremely harmful lesions that can lead to genomic instability and cell death. There are several possibilities for a cell that is facing DNA damage: despite DNA damage it may be repaired, fertilization of an oocyte by a spermatozoon with double-stranded DNA fragmentation could happen without repairing the DNA and result in abnormal embryo and abnormal fetal development (34).

 Genome integrity controlled by means of complicated cellular network. Nevertheless, during initiation of DNA damage by genotoxic stress, series of proteins, in response, are immobilized. There are some proteins complexes act as sensors, transducers and effectors of DNA damage which induced by Double strand breaks DSBs (35). However if un-repaired DSBs persist, cells can undergo apoptosis to prevent the accumulation of potentially tumorigenic mutations. If all the damage responses fail, de novo mutations will appear (36).

**Male Genital Tract Infection and Bacteriospermia**

**Male Urogenital Tract Infection**

Male genital tract infection is one of the most important causes of male infertility worldwide. Invasion of bacteria into the male genital tract has been frequently shown to be associated with impaired sperm function, leading to infertility (37). Male urogenital tract infections (UTIs) play an important role in male infertility, being associated with 8%-35% of male infertility. Asymptomatic bacteriospermia play a major role (37,38). A recent study mentioned that UTIs are associated with about 15% of male infertility (1).

It has been observed that the presence of pathogenic organisms may interfere with infertility treatment involving the application of IVF and intra-uterine insemination (39). Pathogenic bacteria such as streptococci, staphylococci, Mycoplasma, Chlamydia and Ureaplasma produce an acute inflammatory response with a flow of leucocytes into the genital tract leading to increase the level of reactive oxygen species (ROS) production (29,40-42). Excessive like these substances have negative effects on sperm parameters (43). Hammadeh et al (44) reported that the increase of ROS concentration in seminal plasma has negative effects on sperm vitality, membrane integrity, sperm density, chromatin condensation, and DNA single stand breaks.

Cunningham and Beagley (45) referred to some pathogenic bacterial species that well-known as causative pathogens of genitourinary infections and can interact with spermatozoa such as *Escherichia coli*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis*. Mehta et al (46) isolated some pathogenic bacteria from semen samples of male partners in infertile couples, including *Enterococcus faecalis*, micrococci, and alpha-haemolytic streptococci. Other studies mentioned that the contamination and colonization of some bacteria in the male urogenital tract, rather than infection, could also contribute to the decrease in sperm quality (47,48).

**Asymptomatic Bacteriospermia**

Asymptomatic bacteriospermia (ABS) is an invisible infection in the male genital tract and considered as a major cause of male infertility (49).

The passive or active invasion of these bacterial strains induce a generalized or local reaction in the urogenital tract and is often observed as an asymptomatic subclinical inflammation caused by pathogens (50,51).

Khalili and Sharifi-Yazdi (52) isolated different bacterial species from 34.4% of semen samples, like *Streptococcus pyogenes*, *Enterococci*, *E. coli* and staphylococci. These bacteria had negative effects on the morphology and the motility of sperm.

Fraczek et al (53) concluded that the incubation of sperm with bacteria and/or leukocytes was associated with reduction of their fertilization potentials resulting in the negative impact of bacteria and white blood cells (WBCs) on the sperm motility and sperm membrane lipid bilayers.

In Iran, Golshani et al (49) found that 35.22% of infertile men showed at least 1 pathogen. *E. coli*, Coagulase-negative staphylococci (*saprophyticus*), group B streptococci, 5.88% enterococci, *Candida* sp., gonococci, *Staphylococcus aureus*, *Klebsiella* sp. and *Providencia* sp. were isolated. Also, there was a significant (P<0.001) positive relation between the bacteriospermia and immotile sperm rat and abnormality of sperm morphology.

**Leukocytospermia**

A high concentration of WBCs (≥1×10⁶/mL) in semen samples is a marker of microbial inflammation (54). Male accessory gland infections which produced leukocytes was a condition frequently detected in infertile patients (55,56). Leukocytes appear in semen as the addition to bacteriospermia at the second stage of the UTI, and remain present in semen for some period of time following the elimination of the bacteria in the third stage (1).

In vitro studies have shown significant positive correlations between WBCs in semen and deterioration in total sperm count (50), motility (53,57), morphology (50,57,58) and sperm viability (57).

Many authors have concluded that the leukocytospermia has a negative impact on semen quality due to the production of reactive oxygen species (ROS) (59-61). The ROS produced by leukocytes increase the apoptosis in mature human spermatozoa (29). Other authors reported that semen samples with leukocytospermia are more likely to evidence sperm with DNA fragmentation (58,62).
On the other hand, some investigators reported that the final effects of the cells of the immune system on spermatozoa may depend on their activity, regardless of the number of leukocytes in the semen (63-65). However, Golshani (49) mentioned that the presence of bacteriospermia and leukocytospermia did not correlate with each other. It seems that leukocytospermia is a poor marker to predict bacteriospermia.

**The Effects of Bacterial Infection on Sperm Parameters**

The presence of bacteria might alter the sperm quality (48). Microbial infections have been reported to reduce sperm viability (66). Microorganisms might affect the male reproductive function in different ways:

1) Some pathogenic bacterial strains present in semen may act directly on sperm cells causing the agglutination of motile sperm, reducing the ability for the acrosome reaction, and also causing alterations in cell morphology (67). For example *E. coli* strains are known for their ability to immobilize and damage the morphology of spermatozoa by direct contact, mediated by attachment organelles such as pili or type-1 fimbriae (projections) and mannose receptor-dependent interactions (68). Also, the sperm surface is rich in glycoproteins and is therefore susceptible to the nitrification of bacteria such as *E. coli*, *C. trachomatis*, *U. urealyticum*, *Staphylococcus haemolyticus* and *Bacteroides ureolyticus* with spermatozoa leading to the loss of sperm motility and normal morphology (1).

Some researchers were isolated the spermagglutination factor from *S. aureus*, which showed spermagglutinating and spermicidal properties in vitro (69).

2) Microorganisms trigger a local inflammatory reaction. The inflammatory response of the genitourinary tract to the invasion of microorganisms and inflammation is considered to be extremely similar to the reaction observed in other sites of the body (70). This physiological response activates leukocytes and inflammatory mediators such as cytokines and reactive-oxygen species (ROS) which are known to play important roles in sperm DNA fragmentation and male infertility (67). The inflammatory process caused by pathogenic bacteria in the genital tract may lead to a deterioration of spermatogenesis and obstruction of the seminal tract (71). The induction all of the inflammatory reactions in the seminal tract through the activation of neutrophils and macrophages may indirectly exert a deleterious effect on male fertility, where most of the leukocytes attracted to the semen during bacterial semen infection are phagocytic cells such as polymorphonuclear granulocytes (PMNs) and macrophages. The tight adhesion of neutrophils, and macrophages to the surface of the sperm results in phagocytic process (1) (Figure 1). The sperm abnormal form associated with elongation and reduced acrosomal inducibility have been found in men with inflammatory chronic prostatitis and these changes were attributed to leukocytes (72).

3) Induction of sperm autoantibodies (73).

4) Some microbial pathogens may affect the sperm, resulting in the expression of some surface virulent factors such as lipopolysaccharides (LPS), cytotoxic necrotising factor, α-haemolysins and β-haemolysins, and from the release of soluble spermatotoxic factors such as sperm immobilisation factor (SIF) (74,75).

A single incubation with *E. faecalis*, *E. coli* and *S. aureus* induced apoptosis in human sperm with two possible, putative mechanisms: a direct cytotoxic activity of bacterial toxins and the contact with pili and flagella. It has also been demonstrated that *E. coli* can start the apoptotic process by activating several caspases, proteases responsible for mitochondrial changes, alterations in membrane symmetry, and DNA fragmentation (48).

Other study revealed that the *E. coli* showed a significant increase in apoptosis in sperm, and the bacterial infection
of male genital tract decrease the motility and increase in non-viable sperm, as well as causing sperm DNA fragmentation (29).

*Escherichia* is the most extensively studied microorganism in relation to infertility as a result of interaction with spermatozoa (76). It is also the primary bacteria associated with prostatitis and epididymitis (77). *E. coli* has a passive effect on sperm motility and acrosomal function (48). Several authors were described spermaggulation and immobilization by *E. coli* (52,78).

In rats, infection with uropathogenic *E. coli* (UPEC) results in severely impaired spermatogenesis, characterized by, for example hypospermatogenesis, germ cell loss and reduced sperm number (79). Kaur and Prabha (69) isolated Sperm agglutination factor from *S. aureus* which showed sperm agglutinating and spermicidal properties in vitro.

In human, *E. coli* and *S. aureus* are the predominant flora in infertile men (80). Other authors reported that these species of bacteria can cause a significant decrease in sperm motility (81). Emokpae et al (82) studied the contribution of seminal tract infection to sperm density, asthenozoospermia and teratozoospermia, where they observed *S. aureus* as the causative organism accounting for 68.2% of seminal infections. *S. aureus* is known to produce various toxins and enzymes that may exert a damaging effect on human sperm.

The increased prevalence of genital tract infections caused by *E. faecalis* is associated with a deterioration of semen quality in terms of sperm concentration and morphology. Also the presence of micrococci and alpha-haemolytic streptococci does not appear to exert any detrimental effect on sperm quality (46).

Although no significant depressor effect of enterococci on sperm motility was observed (48), some researchers described, in an in vitro study, a negative influence on membrane integrity of human sperm head, neck and mid-piece (83), probably mediated by haemolysin, a well-known virulence factor of enterococci.

5) Infection treatment with antibiotics

In spite of the sperm parameters being improved after the treatment of UTIs (84), antibiotics have negative effects on sperm motility and morphology (67).

**The Effects of Bacterial Infection on Sperm Chromatin Condensation and DNA Integrity**

Different bacterial species such as *S. aureus*, *E. coli*, *P. aeruginosa* can cause sperm DNA fragmentation (29). The effect of the male genital tract infection depends on the pathogen type, acute or chronic condition as well as the site of infection, where the inflammation can occur in the epididymis, prostate gland or seminal vesicles (66). In certain situation the genital tract inflammation become difficult to diagnose because the symptoms may not be apparent (Asymptomatic infection) and the patients only suffering from some local discomfort (85).

Human patients infected with *C. trachomatis* and *Mycoplasma* had a significant (P<0.05) increased of sperm DNA damage compared to control individuals (86). This effect has also been seen in other animal species (87). However, Rybar et al concluded that the contaminated semen with *C. trachomatis*, *Ureaplasma* and *Mycoplasma* spp. were not associated with sperm DNA fragmentation (85).

The exact molecular mechanism of how bacteria infections affect chromatin and sperm nuclear protein still unknown. In mammals, postmeiotic spermatogenesis is characterized by a dramatic reorganization and compaction of the chromatin. The nucleosomal histone-based structure is largely replaced by a transition protein-based structure and eventually by a protamine-based structure (88-91).

Dottermusch-Heidel et al (92) reported that the bacterial infections lead to the premature emergence of trimethylated H3K79 and hyperacetylated H4, which simultaneously occur with the transition protein TNP1. In contrast, they were never observed in the spermaticids of infected rats. Furthermore, upon bacterial infection, only histone-based spermatid chromatin showed abnormalities; whereas protamine compacted chromatin seemed to be unaffected.

Hyperacetylation of histone H4 occurs during the histone-to-protamine transition, perhaps causing a more open chromatin structure to facilitate histone replacement (93-96) and serving as a signal for the bromodomain protein BRDT to initiate the histone-to-protamine transition (97). In mammals, reduced levels of histone H4 hyperacetylation correlates with impaired fertility (95,98).

Many researchers found that there are different bacteria species can affect sperm DNA integrity for examples: *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *C. trachomatis*, *U. urealyticum*, *Mycoplasma* spp. and *C. albicans*. They can induce the expression of apoptosis in the male genital tract during inflammatory processes (68).

The increased sperm DNA fragmentation in an infertile patient with the male accessory gland infection is due to influence of reactive oxygen species produced by activated leukocytes at the level of apoptosis in mature human spermatozoa, *E. coli* showed significant increase in apoptosis on spermatozoa as well as cause alteration of human sperms (29).

Gallegos et al (86) demonstrated that human patients infected with *C. trachomatis* and *Mycoplasma* have increased values of sperm DNA damage compared to control individuals.

Burrello et al (99) reported that the male accessory gland infection with *C. albicans* increased sperm DNA fragmentation and sperm chromatin packaging damage.

**Pathogenicity of Some Bacterial Species on Sperm**

Some gram-negative Enterobacteriaceae such as *E. coli*, *Klebsiella* spp., *Proteus*, *Serratia*, *Pseudomonas* spp., etc are considered pathogens for the urogenital system (100). The major difficulty in interpreting microbiological findings is the presence of contaminating, indigenous microbiota, or of inhibitory substances known to be present in...
the prostate secretions, as well as previous courses of antibiotics (77). The diagnosis of semen bacterial infection may be confirmed by semen quantitative bacteriological cultures. The semen cultures were considered positive when the number of bacteria colonies was >10^6 CFU/mL, according to Domes et al (58).

Several of the bacterial species have negative effects on sperm conventional parameters, chromatin condensation and DNA integrity (100). In the experimental infection module, the incubation of human sperm with suspensions of some bacterial species such as *E. coli*, *S. haemolyticus*, and *B. ureolyticus* resulted in a reduction of sperm motility (53).

**Staphylococcus aureus**

*Staphylococcus aureus* is one of the most pathogenic bacteria as it can infect various organs in the body (101). Various studies revealed that *S. aureus* was the most common isolated bacterial species from seminal fluid samples. Prabha et al (102) found that 51.85% of seminal fluid samples were contaminated with *S. aureus*. Additionally, Emokpae et al (82) detected *S. aureus* in 68.2% of the seminal fluid. Contamination of seminal fluid with *S. aureus* significantly increased the risk of recurrent pregnancy abortion (103). *S. aureus* produces a protein molecule (MW = 20 kDa) called SIF. It was isolated and purified by Prabha et al (104), where they reported that SIF can lead to complete immobilization of spermatozoa at a concentration of 150 mg/ml; whereas 200 mg/mL of this factor is required to kill spermatozoa.

**Escherichia coli**

There were several investigations that have described the harmful effects of *E. coli* on sperm fertilization potentials. Fraczek et al (53) concluded that *E. coli* and serotype O75:H1NT have a negative effect on human sperm motility. An inhibitory effect of *E. coli*, serotype 06, on sperm motility has been investigated by some authors (105). Sperm incubated with *E. coli* demonstrated significant alterations in motility (78,106). Some investigators revealed that the immobilization of spermatozoa may occur as a result of direct contact of the sperm with bacterial cells (105,107).

Villegas et al (68) concluded that the direct exposure of spermatozoa to *E. coli* is enough to decrease sperm quality. They noticed that the early apoptosis incident (phosphatidyl serine [PS] externalization) was significantly increased in spermatozoa after incubation with *E. coli* alone. Other in vitro studies demonstrated that the soluble products of *E. coli* decreased sperm motility by causing defects in the sperm's mitochondrial function (108,109). SIF was isolated from *E. coli* by Prabha et al (110), where they reported that the incubation of spermatozoa with SIF causes sperm immobilization and structural modification.

*Escherichia coli* have certain virulence characteristics and have the ability to adhere to sperm cells and to colonize tissues of the male genital tract, thereby causing asymptomatic male infertility (76).

**Neisseria gonorrhoeae**

*Neisseria gonorrhoeae* is diplococcus bacteria, which infect men and women alike, causing gonorrhea (111). The seminal fluid acts as a mediator triggering the motility of *N. gonorrhoeae* and microcolony formation via an increase in the number of pili on the bacterial surface (112). *N. gonorrhoeae* may attach to the spermatozoa by pili as T1 gonococci or by direct contact as T4 gonococci (113). Gonococcal infection caused by *N. gonorrhoeae* triggers the flow of PMNs into the infected tissue (114). As mentioned previously, the presence of leucocytes in the genital tract can increase the level of reactive oxygen species which have harmful effects on spermatozoa (40,42). On the contrary, Liu et al (115), did not find any effects on the sperm by *N. gonorrhoeae* after in vitro incubation.

**Ureaplasma urealyticum**

*Ureaplasma urealyticum* is a common bacteria present in the genitourinary tract and it is more prevalent in infertile men, where it has the ability to affect the sperm morphology (116). Various authors noted that there was a significant correlation between lower sperm concentration and the presence of *U. urealyticum* in the male genital tract (100). Furthermore, one study revealed that infected patients with *U. urealyticum* showed a significant impairment of sperm concentration, motility, and vitality. The authors found that the seminal plasma alpha-glucosidase decreased in the infected patients when compared with in the non-infected patients (117). Kohn el al (118) studied the effect of *U. urealyticum* on human sperm. It was noticed that 69% of infected patients with *U. urealyticum* had a decreased capacity of sperm acrosome reaction.

**Chlamydia trachomatis**

Chlamydial infection is a popular sexually transmitted disease that is caused by *C. trachomatis*. It affects approximately 90 million people yearly worldwide (51). *C. trachomatis* infection has a positive correlation with apoptosis rate in the human spermatozoa (119). Furthermore, Gallegos et al (86) demonstrated the negative effect of chlamydial infection on the sperm DNA.

Kokab et al (51) found a significant relationship between chlamydial infections and an increase the level of IL-8 and seminal leukocytes. While the progressivly motile sperm decreased in infected patients with *C. trachomatis*, another bacterial species had a negative effect on sperm parameters. *E. faecalis*, for instance, had negative effects on sperm motility and morphology (46). The results of another study clearly showed a spermicidal activity of *Streptococcus anginosus*, thereby, affecting sperm concentration and triggering necrosis. Additionally, *Staphylococcus epidermidis* had negative effects on sperm concentration and progressive motility (48).

In an in vitro study, the incubation of spermatozoa with *Mycoplasma hominis* reduced sperm motility and acrosome reaction property (118). Moreover, *Mycoplasma
had a negative effect on sperm DNA integrity (86). The positive correlation between Klebsiella spp. infection and morphologically abnormal spermatozoa were also reported (120).

Conclusions
The bacteria in semen samples have negative effects on sperm parameters and may be an important factor negatively influencing fertility status and worsening reproductive potential (53).

The molecular mechanism of how bacteria affect chromatin and sperm nuclear protein still unknown exactly. The bacterial infection should be treated with care, especially in patients consulting for infertility and advising for assisted reproduction techniques.

Ethical Issues
Not Applicable.

Conflict of Interests
The authors declare no conflicts of interests.

Financial Support
This study received no funding.

Acknowledgments
I am grateful to thank, Dr. Majed Alhudhud, Consultant Obstetrician and Gynaecologist, UK for revising the text language.

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