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Microorganisms and Male Infertility: Possible Pathophysiological Mechanisms

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Abstract

Male infertility is a worldwide problem, as in about one in five infertile couples; the sole reason is the male partner. Amongst the various etiological factors responsible for male infertility, acute and chronic genital tract infections constitute a significant part. Different microorganisms have been linked with male infertility; with varying levels of association. These microorganisms have been known to employ different mechanisms ranging from direct effect on spermatozoa such as reduced motility, agglutination, loss of acrosomal reaction, to an indirect effect by inducing leukocytospermia that can adversely affect spermatozoa. They may also lead to deterioration of spermatogenesis, loss of sperm function and/or blockage of the seminal tract. This review summarizes the various patho mechanisms being employed by different microorganisms for causing decreased reproductive potential.

Keywords: Bacteria; Infection; Mechanisms and Male infertility.

Introduction

Infertility is a serious medical problem contributing to inappropriate contraceptive behaviour; poor sexual and reproductive health affecting 15% of all couples worldwide [1, 2]. However, male factor has been found to be the sole reason responsible in about 20% of such couples [3]. Out of 60-80 million couples suffering from infertility, it has been anticipated that about 15-20 million are from India only [4]. Male infertility refers to the inability of a male to achieve spontaneous pregnancy in a fertile female in one year [5]. A decline in the male fertility may be attributed to many factors which include urogenital abnormalities, testicular failure, varicocele, genetic abnormalities, immunologic problems, endocrine disturbances, systemic diseases, cancer, and infections of the genital tract, environmental agents, distorted lifestyle and gonadotoxic factors exposure [6].

Out of all these factors, genitourinary tract infections play a disruptive and hidden role in male infertility. Various infectious agents i.e. bacteria, fungi, viruses and parasites can cause reproductive disturbance in both sexes. About 8-35% of the male infertility cases have been associated with infections and inflammation of the male genitourinary tract [1, 7]. Different parts of the male reproductive tract, such as the testis, epididymis and accessory sex glands can be easily affected by these infectious agents. Acute and chronic infections and consequent inflammation in the male reproductive system may deteriorate the spermatozoa function and the whole spermatogenic procedure, causing sperm alterations qualitatively and quantitatively [8-10].

Infectious agents cause impairment of sperm cell function, testicular damage, prostatitis, orchitis and epididymitis in male reproductive system and hence affect fertility [11]. Various microorganisms have been known to bring out the alterations through different pathomechanisms. Some of the revealed mechanisms linked with infection leading to infertility are agglutination of motile spermatozoa [12], immobilization of spermatozoa [13], impaired acrosome reaction, morphological alterations in spermatozoa [14], deterioration of spermatogenesis, autoimmune response due to inflammation, antisperm immunological reactions [15] and dysfunction of accessory sex glands [8]. There are various studies which highlight the role of various microbial pathogens on male fertility. This review article sheds light on the information on the pathophysiological mechanisms of these microbial agents which impair male fertility and provide a possible link between infection and infertility.

Chlamydia trachomatis

Chlamydia trachomatis, an obligate intracellular gram negative bacterium, is the most prevalent cause of bacterial Sexually Transmitted Infections (STI) recognized throughout the world [16, 17]. *C. trachomatis* has group specific lipopolysaccharide (LPS) antigen, species specific, and immunospecific antigen which can be best detected by immunofluorescence. It exists in more than 19 immunotypes with types D-K known to cause genital tract infections. It has a unique cell wall in that it lacks muramic acid. The unique biphasic developmental (replicative) cycle consists of elementary body (EB) and reticulate body (RB) making it different from all other bacteria [18]. *Chlamydia,* four times more common than *Gonorrhoea*, comprises the largest proportion of all STDs and affects both men and women. Annually 100 million incidences of *C. trachomatis* occur worldwide, although 50% of infected men may be asymptomatic [19 - 21]. In men, *C. trachomatis* infection can lead to urethritis, epididymitis, epidiymo-orchitis, and it is also becoming more widely accepted that it is responsible for prostatitis, as well as causing an enlargement of seminal vesicles. The rates of *C. trachomatis* infection has been reported to be 19.4% and 7.5% in infertile and fertile men respectively, showing remarkable correlation between infection caused by *C. trachomatis* and infertility in men. Also, presence of chlamydial DNA in infertile patients further links *Chlamydia* to infertility [22].

A number of studies have looked into the association between chlamydial infection and semen quality. *C. trachomatis* infection is associated with a decrease in sperm concentration, motility, altered semen pH and reduced volume of the ejaculate. *C. trachomatis*, when co-incubated with human spermatozoa *in vitro*, seems to impair sperm motility, and cause premature death, perhaps as an effect of the chlamydial lipopolysaccharide [23]. Apoptosis of spermatozoa caused by chlamydial lipopolysaccharide leads to persistent infection resulting in scarring of the ejaculatory ducts, or loss of stereo cilia. Damage in the epithelial cells involved in spermatogenesis can also impair sperm quality [24]. Chlamydial LPS interacts with CD14 on the sperm surface, causing increased production of reactive oxygen species, thereby, resulting in caspase-mediated apoptosis [23]. Apoptosis markers of human spermatozoa include plasma membrane externalization of phosphatidylserine (PS) and DNA fragmentation. Gallegos *et al.* [25] in 2008 reported that genitourinary tract infections caused by *C. trachomatis* resulted in increased sperm DNA fragmentation which has been associated with a low potential for natural male fecundity. There is also an evidence to suggest that infection with *C. trachomatis* may lead to a defective acrosome reaction [26]. On incubation of sperms, the elementary bodies of *C. trachomatis* can also lead to decreased sperm motility, can stimulate tyrosine phosphorylation and this provides an evidence that *Chlamydia* can attach to spermatozoa and result in cytotoxicity. Moreover this also implies that *Chlamydia* can alter sperm functions as increased tyrosine is also linked to sperm capacitation [27]. Although a rare event, *C. trachomatis* can cause epididymitis which may damage the canalicular system of the male genital tract and thereby cause obstructive azoospermia, an additional etiology is caused by the immune response through production of antisperm antibody (ASA) formation [28]. ASA can be found in the seminal plasma or attached to the sperm surface in 5–12% of infertile men. Presence of IgA against *C. trachomatis* can lead to lipid peroxidation of sperm membrane [29].

Neisseria gonorrhoeae

The gram negative, immotile diplococci, cause the most common infectious disease in men *viz.*, urethritis, prostatitis and epididymitis which in turn may impair testicular functions and thus, leading to male infertility. These bacteria have pili on their surface which facilitate attachment to other cells [30]. In this regard, reports of interaction of bacterial type IV pili (T4P) and lipo-oligosaccharide with spermatozoa have surfaced up. On spermatozoa, asialoglycoprotein receptor has been identified that recognizes and binds to lipopolysaccharides in gonococcal membranes [31]. Chlamydial lipopolysaccharide causes sperm death by inducing apoptosis thus, it can be hypothesized that *Neisseria* lipopolysachharide might also be capable of inducing apoptosis.

Ureaplasma urealyticum

Ureaplasma urealyticum is one of the most frequent causes of the male infertility [32] due to its ability to reduce semen quality and the fertilizing potential of sperm. Wang *et al.* [33] also showed that *Ureaplasma* infections can cause increased seminal viscosity with decreased sperm concentration and pH.

Also, it negatively influences the motility, density, morphology of spermatozoa and reduces the oxidoreductive potential of the ejaculate, which makes sperm more vulnerable to peroxidative damage. Semen infection with *U. urealyticum* is connected with an increase in proportion of sperm with residual cytoplasm around the neck. This might explain why the availability of NADPH is higher during semen infection with this atypic bacteria and why spermatozoa release high quantities of O_2^-, which may cause the peroxidative effect. It may also attach to sperm, especially midpiece, thereby leading to looped tangling of tails and multiple sperm agglutination which may lead to decrease in motility [34]. The effects on motility were associated with a decreased α- glucosidase level in the seminal fluid due to effect on epididymis [35]. Infertility may be caused by *U. urealyticum* by means of deleterious effects on sperm chromatin and DNA, leading to impairment of embryo development [36]. Metabolic products of *U. urealyticum*, such as H_2O_2 and hydroxide anion (OH^-), are highly toxic to the sperm cells [37]. Moreover, *U. urealyticum* infection may lead to the decrease in some microelements in semen fluid such as zinc or selenium which are important for maintenance of antioxidative defense of semen.

Escherichia coli

Escherichia coli, the most frequently isolated and common cause of nonsexually transmitted epididymo-orchitis, is involved in 65–80% of total acute or chronic prostatitis cases. *E. coli* may, therefore, be implicated in the genesis of infertility [38]. Golshani et al. [39] while studying microbiology of semen sample of infertile men and evaluating the effects of bacteriospermia on semen parameters found *E. coli* and *Enterococci* to be the most common bacteria which negatively influence sperm motility and morphology. Schirren and Zander [40] have also reported the negative influence of *E. coli* on sperm motility after mixing sperm and bacteria *in vitro*. *E. coli* rapidly adheres to human spermatozoa *in vitro*, resulting in agglutination of spermatozoa. A profound decline in motility of spermatozoa is evident over time due to severe alterations in sperm morphology. Morphological alterations involved all superficial structures of sperm, in particular the plasma membrane of mid piece and neck as well as inner and outer acrosomal membranes of the acrosome indicating that morphological defects might be accounting for the immobilization of spermatozoa by *E. coli*.

Another important observation that appears to be striking is impairment of acrosomal structures of spermatozoa, typified by atypical reaction and disintegration of different acrosomal structures that must ultimately result in impaired fertilizing ability. The impact on acrosomal morphology and function appears to be a decisive mechanism for the influence of *E. coli* on the fertilizing capacity of human spermatozoa [41, 42]. An *in vitro* study by Kohn et al. [43] also showed negative effect of *E. coli* on acrosomal function of spermatozoa.

Due to direct interaction of bacterial pili with the sperm plasma membrane, *E. coli* interferes with sperm motility [44] and also affects spermatozoa by action of soluble factors that induce apoptosis and a breakdown in the mitochondrial membrane potential [45]. It has also been demonstrated that *E. coli* can start the apoptotic process by activating several caspases and proteases responsible for mitochondrial changes; alterations in membrane symmetry; and DNA fragmentation [46, 47]. Moreover, the presence of *E. coli* was connected with significant decrease in both the number of cells with high mitochondrial transmembrane potential ($\Delta\Psi m$) and the cells with normal oxidoreductive function of mitochondria ($p < 0.05$ as compared to untreated cells). Thus the contact of *E. coli* with ejaculated spermatozoa can be a reason for severe injury of sperm membrane stability and mitochondrial activity with potential consequences on male fertility [48].

LPS and porins from *E. coli* also have the ability to bind to plasma membrane of spermatozoa and cause cell death. Thus, there is a possibility that the products of cellular lysis in the course of *E. coli* infections can cause temporary sterility [49].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative, opportunistic pathogenic bacterium in humans and a frequent inducer of urinary tract infections. It produces 3-oxododecanoyl-L-homoserine lactone, a quorum sensing signaling molecule, which has been reported to have detrimental effects on spermatozoa. The reduction in motility is dose dependent and at sublethal doses, it can lead to premature acrosomal loss and the possible mechanism identified was found to be calcium dependent. Moreover, it can cause apoptosis and necrosis of spermatozoa at concentrations that do not affect immune cells [50].

The Exotoxin A of *P. aeruginosa* has also been found to have a cytotoxic effect on cells at chromatin level. This effect induces various defects in mouse spermatozoa *viz*. amorphous head, banana head, two heads, head without hook, divided tail, coiled tail and others abnormalities. It has also been seen that the divided tail abnormality was more than other abnormalities because of toxin's effect on protein concentration in tail [51]. The porin from *P. aeruginosa* induces apoptosis in an epithelial cell line derived from rat seminal vesicles. Since porins have receptors for sperm plasma membrane they can have negative impact on sperm parameters also [52].

Staphylococcus aureus

Staphylococcus aureus is the most ubiquitous gram positive organism found in semen samples of both normal as well as infertile males. In a study conducted by Momoh et al. [53], *S. aureus* was arguably the most dominant microorganism in semen culture of infertile men with a prevalence rate of 75%. Emokpae et al. [54] found *S. aureus* as causative agent in 68.2% of seminal fluid infections. Huwe et al. [55], Liu et al. [56] and Kaur et al. [57] found significant decrease in sperm motility and agglutination of sperms when spermatozoa were coincubated with *S. aureus.*

Candida albicans

Candida albicans is an opportunist in urogenital infections and has therefore been paid little attention. The role of yeast has hardly been evaluated because of experimental difficulties and questionable clinical significance [58]. Sasikumar et al. [59] observed rapid motility loss and decreased survival time of spermatozoa upon incubation with *C. albicans.* Tian et al. [60] reported significant reduction in progressive motility of spermatozoa and signs of membrane alteration; directly related to contact time with the yeast. Distinct adhesion of spermatozoa to *C. albicans* leading to sperm agglutination has been observed. Light and transmission electron microscopy examinations have also shown that spermatozoa get attached to *C. albicans* mainly via the head and lead to multiple ultra-structural lesions, which can cause sperm immobilization. Tian et al. [60] also reported that *C. albicans* as well as its cell free filtrates have an inhibitory effect on human sperm motility and impaired the ultrastructure of human spermatozoa and this could be associated with male infertility. Since the cell free filtrates contain acidic proteinases, phosphatidases and other soluble virulence factors, therefore, sperm motility dysfunction can possibly be attributed to these factors.

Rennemeier et al. [50] have also reported that farnesol, a quorum sensing molecule produced by *C. albicans,* induces significant loss of progressive motility which coincided with multiple damages in spermatozoa due to apoptosis and necrosis. It can also lead to premature acrosomal loss and DNA fragmentation of spermatozoa even at sublethal doses. Burrello et al. [61] have also shown that on incubation of *C. albicans* with spermatozoa, there is decrease in sperm motility with reduced mitochondrial membrane potential or phosphatidylserine externalization thereby leading to apoptosis, which increased in a time- and concentration-dependent manner. It also led to increased DNA fragmentation in spermatozoa.

Trichomonas vaginalis

Trichomonas vaginalis is a flagellated protozoan and was known as a vaginal pathogen in early years of the 20th century. It was later recognized as the cause of common infections in the lower genital canal in both males and females. *T. vaginalis* is known as an important cause of sexually transmitted infections in developing countries. In a study by Ozdemir et al. [62], by using wet mount microscopy, they observed the presence of *T. vaginalis* in 80 infertile men. The occurrence and range of Trichomonasis in men are less characterized. Generally most of the infected males are asymptomatic. There is a scarcity of literature which relates Trichomonads to infertility. This organism is present in approximately 20 percent of the infertile population, so its exact role and pathomechanism in causing male infertility is yet to be known. A fact which is known about *T. vaginalis* is that it can establish infection by colonization and getting attached to host cells. This ability to get attached to host cells is called cytoadherence. Two classes of proteins which are responsible for cytoadherence are adhesins (AP65, AP51, AP33 and AP23) and cysteine proteinase. The probable modes of action of these proteins involve cell-to-cell adhesion, cell detaching factors, excretion of soluble factors, hemolysin and evasion of the host immune system [63]. Roh et al. [64] concluded that *T. vaginalis* secretes a soluble factor i.e. extracellular polymeric substance (EPS), which significantly decreased sperm motility, viability, and functional integrity, thereby, decreasing fertilization capacity *in vitro*. The motility of human sperm retarded when spermatozoa were incubated with *T. vaginalis in vitro* [65]. In men, trichomoniasis has been related to infertility by deficit of sperm cell quality and function due to physical damage [66].

Treponema pallidum

Treponema pallidum is present in semen of 20% of patients with primary or secondary syphilis [67]. The adverse effects of syphilis are well acknowledged with a constellation of life- threatening and degenerative effects on male reproductive system. *T. pallidum* has been isolated from 3 (1.23%) semen samples obtained from patients with primary infertility and 2 (3.44%) patients with secondary infertility [68]. In a study conducted by Osegua et al. [69] on urogenital tract infection in asymptomatic infertile male patients in Benin city, it was reported that out of 323 samples from infertile males, 2.1% were positive for *T. pallidum.* So, from the literature it can be concluded that in semen, it is found in very low percentage as compared to other bacteria. Very few studies were found assessing the frequency of *T. pallidum* in semen of infertile males. Although a direct toxic effect of *T. pallidum* on male fertility has not been stated, yet it is known that its complications can affect fertility because of its high degree of invasiveness.

Syphilis of epididymis can cause syphilitic epididymitis which ultimately leads to obstruction of the epididymis. In tertiary syphilis, chronic obliterative endarteritis and interstitial inflammation can occur which causes small and fibrotic testes [70]. It can cause gummatous lesions which destruct local tissue in the testicles, therefore, have an impact on testicular function and fertility [71]. Through tissue and mucous membranes, it invades into host's blood and lymphatic system [72]. The role of *T. pallidum* in male infertility is not clear, it may cause sperm agglutination or it may prevent the motility of the sperm [73]. As no vaccine for syphilis is available till date, so it is very difficult to treat.

Herpes Simplex Viruses

Herpes simplex virus (HSV) is one of the most frequent viruses in human populations, and is responsible for a broad spectrum of diseases, including neonatal infections and genital diseases [74, 75]. HSV includes two distinct, but closely related viruses, namely, HSV-1 and HSV-2, both of which can cause genital herpes. Many researchers have investigated the link between HSV infection and male infertility [76-78]. In this regard, the results of the study conducted by Monavari [79] showed that 16 (22.9%) and 10 (14.3%) of the 70 semen samples were positive for HSV-1 and HSV-2 respectively. All HSV-positive samples had abnormal semen parameters (the male factor group). HSV may not alter sperm motility and morphology, but it is associated with lower sperm count. Another study by Wu et al. [80] confirmed the involvement of HSV infection in hampering spermatogenesis by decreasing the concentration of sperm and increasing apoptotic cells.

Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) is one of the types of lentiviruses that cause HIV infection thereby leading to acquired immunodeficiency syndrome (AIDS). It is present either as free virus particle or within infected immune cells in all the body fluids including semen, blood etc. Infection can be easily transferred through these fluids. Several studies have strengthened this fact that HIV-1 is present in the semen of most infected men [81, 82]. The effect of HIV on human sperms has been studied by Lasheeb et al. [83], Nicopoullos et al. [84], who have concluded that sperm parameters i.e. morphology, motility, total count etc. are significantly impaired by the presence of HIV, and in particular correlate with CD4 count. Semen alterations including decreased ejaculate volume and sperm motility have been reported in HIV infected men. The pathomechanism of HIV infection on sperms has not been confirmed but the hypotheses ascribing reduced sperm motility along with damage to sperm mitochondria by nucleosidic (or nucleotidic) reverse transcriptase inhibitors (NRTIs) remain unclear [85]. White et al. [86] also reported that the sperm mitochondrial DNA damage, as a result of nucleoside analogue toxicity, may be producing potential deleterious effects on spermatogenesis. Spermatozoa have the ability to move because of the energy (ATP) produced by mitochondria. When the mitochondria are damaged and no ATP is produced, the spermatozoa will not move and hence die immediately. The exact mechanism by which HIV infection alters semen parameters still remains unclear [84].

Human Papilloma Viruses (HPV)

Human Papilloma Virus is one of the most prevalent viruses which cause sexually transmitted infections. In a study done by Martorell et al. [87] in 2005, it was found that HPV was present inside sperm cells of azoospermic men which may be related to impaired sperm motility and asthenozoospermia. Men's genitalia including intraurethral epithelia, vas deferens etc. may also act as the reservoir of HPV [88]. HPV DNA has been detected in 8 to 64% of semen samples from asymptomatic men, both in the seminal plasma and in spermatozoa [89, 90]. HPV is actively adsorbed by sperm cells [91] which might decrease the fertilization vigor but the exact steps are unclear. However some reports showed that HPV infection can lead to apoptosis of sperm cells, alterations of semen quality through decrease in cell count, motility, and amplitude of lateral head displacement and increase of antisperm antibodies level [92]. HPV infections are often asymptomatic and most of the time, infected people are not even aware. It is not clear whether HPV infections can silently damage the reproductive system, if infection persists for long time. It produces genital warts which are typically benign lesions and come under low risk type HSV infections, whereas malignant transformations in persistent infection are of high risk types. At present, there is no reliable treatment for HPV infections, except complete surgical removal of the disease site [93].

In this regard, a strain of *S. aureus* causing immobilization of human spermatozoa was isolated and its sperm immobilization factor (SIF) was purified . The molecular weight of this proteinaceous factor was found to be~20 kDa and it was able to cause 100% immobilization of human spermatozoa [94]. Another *S. aureus* strain, which impaired sperm motility by causing agglutination of spermatozoa by adhering to them *in vitro*, was also isolated in our lab. The molecular weight of this protein was found to be 70-KDa [95]. When this strain was inoculated intravaginally in mice for 10 days, it led to infertility [57]. A lot of research has been done *in vitro*, but scarce *in vivo* data is available in literature regarding the effect of *S. aureus* on male fertility.

On the similar lines, strains of *E. coli* were also isolated from semen samples of males attending infertility clinic. One of these strains adhered to sperm surface thereby leading to agglutination of spermatozoa. The corresponding factor responsible for agglutinating activity has been isolated and was found to be of ~71-KDa.

This strain could also lead to infertility in female mice when inoculated intravaginally [96]. The other strain produced a factor (~ 56-KDa) that immobilizes human spermatozoa *in vitro* [13]. The above studies have been performed in female mice and the results have encouraged us to extrapolate the same in male mice.

In conclusion, infections of the genital urinary tract may be implicated in male infertility. Many microorganisms seem to be involved in genital infections and reproductive failure. These include *C. trachomatis, N. gonorrhoeae, U. urealyticum, E. coli, P. aeruginosa, S. aureus, T. pallidum, C. albicans, T. vaginalis,* Herpes simplex virus, Human immunodeficiency virus and Human papilloma virus. These microorganisms tend to impair sperm parameters both *in vitro* and *in vivo. E. coli, S. aureus* and *C. albicans* adhere to spermatozoa leading to agglutination and immobilization of spermatozoa. Lipopolysaccharide of Gram negative organisms interacts with CD 14 on sperm surface leading to increased production of reactive oxygen species resulting in caspase mediated apoptosis, increased sperm DNA fragmentation and defective acrosome reaction. Quorum sensing molecules such as 3-oxododecanoyl-L-homoserine lactone produced by *P. aeruginosa* and Farnesol produced by *C. albicans* induce motility loss, apoptosis, necrosis, acrosomal loss and DNA fragmentation. The Exotoxin A of *P. aeruginosa*, extracellular polymeric substances (EPS) of *T. vaginalis*, soluble virulence factors such as acidic proteinases, phosphatidases of *C. albicans*, sperm immobilizing and agglutinating factor isolated from both *E. coli* and *S. aureus* have an inhibitory effect on sperm motility. *U. urealyticum* reduces the oxidoreductive potential (NADPH) of semen making them more vulnerable to peroxidative damage. Presence of IgA antibodies against *C. trachomatis* can lead to lipid peroxidation of sperm membrane. Immune response through production of antisperm antibodies presents an additional etiology in impairment of spermatozoa. HSV, HIV and HPV hampers spermatogenesis, motility, increases apoptosis, damages sperm mitochondria by nucleosidic reverse transcriptase inhibitors. Studies on various uropathogenic organisms, their interactions, pathomechanisms and impairment of various sperm parameters provided an insight into their impact on male fertility. Despite the advancement in techniques, many lacunae still persist which need to be addressed for understanding the exact mechanisms. Asymptomatic infections, coupled with recurrent and latent infections, are the major challenges to the control of infertility. However, the ultimate intervention, the development of an effective early detection by screening and treatment regimen is still far away and further research is required.

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