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Molecular and Cellular Mechanisms of Microbial Infections in Male Reproductive Health and Fertility

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Abstract

Microbial infection poses a severe threat to male reproductive health, leading to a decline in fertility, reproductive dysfunction, and the well-being of individuals. This review aimed to provide a comprehensive understanding of the molecular and cellular mechanisms of microbial infection in altering male fertility and how the crosstalk of the microbial pathogens, host immune responses, and reproductive tissues could manifest in a spectrum of reproductive tract disorders, including urethritis, prostatitis, epididymitis, and orchitis, a leading cause of human infertility. Insight into the molecular mechanisms of pathogenesis in microbial infections reveals the nature of the infection and disease progression, as well as informs the search for suitable therapeutic targets, which is crucial for curbing the infection. Microbes' virulence factors, attachment, and immune evasion strategies are exploited to colonize, invade, and maintain their persistence within the male reproductive tract. The diagnosis of genital tract infections in male reproductive health involves a thorough work-up, including microbiological, molecular, serological, and imaging procedures, as detailed in this review. Antimicrobial therapy remains a crucial component in treating microbial infections affecting male reproductive health. Nevertheless, the emergence of resistance, frequent adverse effects, and an increasing number of therapeutic failures have fueled the search for novel therapeutic options. Targeting new drugs against emerging pathogens also requires a clear understanding of resistance mechanisms. Development of precision medicine approaches, immunomodulatory therapies, identification of novel therapeutic targets, and preventive strategies are expected to top the research priorities in the field of male reproductive health. In summary, a combined scientific effort is necessary to overcome the complex challenges posed by microbial infections in male reproductive health. This study will enhance our understanding of the molecular basis of disease pathogenesis, aid in the development of targeted therapeutic interventions, and ultimately lead to the adoption of preventive and prophylactic strategies to mitigate the effects of microbial infections on male reproductive health and fertility.

Keywords: Male fertility, Microbial infection, Testis, Apoptosis, Sperm

Introduction

Microbial infections are a significant cause of male infertility, causing molecular and cellular anomalies during the reproductive process (1,2). Approximately 15% of couples globally are infertile, with problems in the male accounting for 50% of the cases. Although microbial infections have been associated with female reproductive health, emerging studies have shown molecular evidence of their widespread incidence and impact on male fertility (1,3). Microbial pathogens are a diverse group of microorganisms, including bacteria, viruses, fungi, and parasites, that can colonize and infect various tissues of the male reproductive tract (MRT) (1,2). These pathogens include sexually transmitted pathogens such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and human papillomavirus (HPV), as well as non-sexually transmitted microbes like *Escherichia coli*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* (4,5). When these pathogens invade the male reproductive system, they initiate a series of molecular events that impair spermatogenesis and sperm function, resulting in a significant reduction in fertility potential (5,6).

At the molecular level, microbial infections cause inflammation, chemotaxis, and immune activation within the MRT. Microbial pathogen-associated molecular patterns (PAMPs) in the environment initiate immuno-stimulation signaling by acting on Pattern recognition receptors (PRRs) on the immune nuclei to induce the synthesis of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). Since infection-derived inflammation can be long-standing due to the persistent microbial colonization, it can lead to tissue damage and fibrosis, as well as changes in the testicular and accessory gland gene expression and function that will affect fertility (7-9).

Furthermore, pathogens that are directly toxic to spermatogenesis are capable of infecting germ cells or Sertoli cells, which play crucial roles in supporting spermatogenesis and maintaining Blood-testis barriers (BTBs), respectively. Agents such as *C. trachomatis*

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and *Mycobacterium tuberculosis* have been reported to infect Sertoli cells, impairing their function and the structural integrity of male germ cells. This disruption can undermine the nutritional and immunoprotective function of developing germ cells, which can contribute to spermatogenic arrest, impaired spermatogenesis, and therefore male factor infertility (10,11).

Besides affecting spermatogenesis, infections may also affect sperm functions and quality, where sperm viability, progressive motility, morphological deterioration, and DNA fragmentation are significantly compromised. Possible causative agents of sperm CD include *U. urealyticum*, *M. genitalium*, and other pathogens that cause or correlate with sperm DNA fragmentation, damaging the potential fertilizing ability of sperm. Additionally, some of the non-recalcitrant viruses that have been identified to cause changes in sperm quality are HIV and Zika virus (ZIKV); studies have revealed that men infected with these viruses experience decreases in sperm concentration and motility, as well as viral RNA exposition in semen, which is a risk factor leading to sexual transmission and reproductive implications (12-14).

Therefore, elucidating the effects of microbial infections on the molecular and cellular constituents involved in male fertility will be crucial to providing novel and specific approaches to diagnose and treat the effects of microbes on male fertility. Recent studies in molecular biology, immunology, and microbiology have shed light on key factors and targets for therapeutic intervention in microbial-induced male infertility. More pointedly, understanding the complex relationships between microbial pathogens and the male anatomical structures that govern masculine aesthetic reproduction gives rise to pending attempts to augment reproductive health quality and increase fertility capacity in infected males.

Overview of Microbial Infections in Male Fertility

It is evident that bacterial infections, in particular, play a crucial role in impaired male fertility, yet these factors are overlooked. However, throughout history, male reproductive health has not been given much concern. Although present investigations demonstrated that microbial pathogens have negative effects on male fertility, there is still a long way to go. Bacterial infections of the male reproductive system may be acquired from sexually transmitted infections (STIs), recurrent or uncomplicated urinary tract infection (UTI), and systemic infections that involve the genitourinary (GU) tract (15-17).

The *C. trachomatis*, *N. gonorrhoeae*, and HPV are the pathogens known to cause STIs, and are among the common causes of male infertility (18,19). These infections can render sperm production, maturation, and transport impaired due to inflammation and structural damage to the male accessory organs, such as the testes, epididymis, and seminal vesicles (12). Moreover, STIs may elicit inflammatory reactions that interfere with the physiologic homeostasis in the MRT, which could be a driving factor for infertility as well (20).

Many other scientifically proven infectious agents, apart from those that are sexually transmissible, can also affect fertility in males; these include bacterial, viral, fungal, and parasitic agents (6). Some bacteria capable of causing colonization in the GU system include *E. coli*, *M. genitalium*, and *U. urealyticum*, among others. These bacteria may cause UTIs, prostatitis, and similar conditions. These infections might cause changes in sperm attributes such as viability, swimming ability, and their overall genomic integrity, which in turn affects male fertility (6,21).

VBs, such as HIV, HBV, and ZIKV, have been implicated in the reduction of sperm production and function among men (12). Both HIV and HBV are sexually transmitted diseases or are transmitted through blood contact, affecting the systemic immune system, and might also lead to damage to sperm characteristics (12). Non-sexual transmission of ZIKV through the Aedes mosquito threatens testicular inflammation, impaired spermatogenesis, and the continuation of the virus in semen, which increases the likelihood of sexual transmission and issues related to reproduction (7).

In addition, fertility can also be affected by a few other factors, such as fungal and parasitic infections (5). Smear: Fungal infections, including candidal balanitis and epididymo-orchitis, can affect sperm quality and productivity. Some parasitic diseases, such as schistosomiasis and filariasis, can cause GU pathology, including azoospermia, which occurs when sperm are obstructed at the seminal vesicles, thereby hindering sperm delivery and fertilization (22,23).

In total, microbial infections reflect a spectrum of pathogens with considerable potential to exert significant influences on male fertility through various inflammations, tissue damage, immune dysfunction, and specific toxicities to spermatozoa. It is crucial to ascertain the prevalence of microbial infections among male infertility patients to understand the development of such infections and their medical implications, to ensure accurate diagnosis, to administer appropriate treatment, and ultimately to prevent the dissemination of infections that compromise reproductive health in men (23).

An overview of these pathogens and their impact is provided in Figure 1, and Table 1 summarizes common microbial pathogens affecting male fertility and their effects.

Molecular Mechanisms of Microbial Pathogenesis

Microorganisms have diverse strategies to acquire tissue access, tropism for specific host cells, and immune evasion mechanisms to perpetrate infection and impair male fertility within the male reproductive system (24). Knowledge of these complex cellular mechanisms is essential for identifying the molecular basis of various

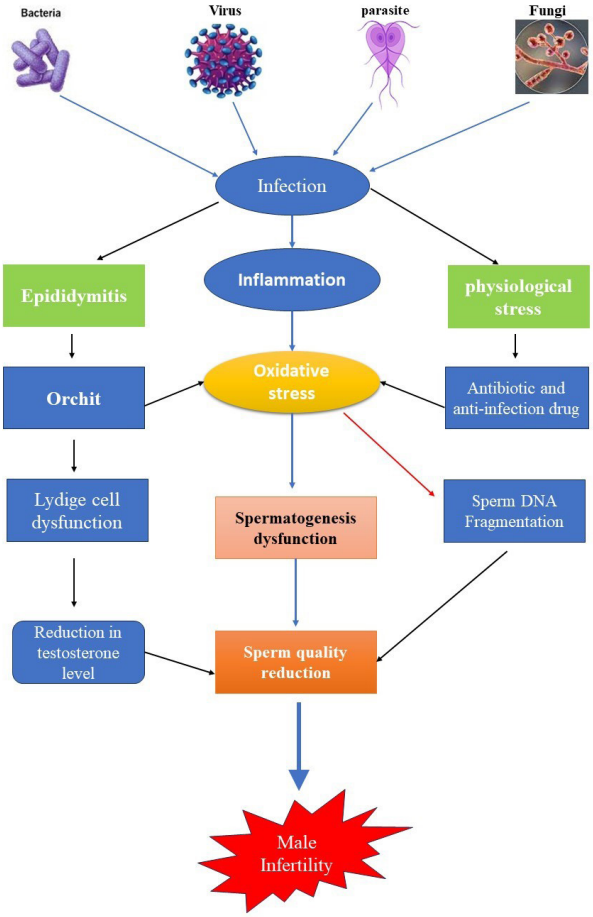

Figure 1. Overview of Microbial Infections in Male Fertility.

infections leading to reproductive complications and for creating prospective treatment strategies (24,25).

Adhesion and invasion: Microbial pathogens interact with their host by attaching themselves to epithelial cells found in the male reproductive system through adhesins and by effectively entering these cells. Attachment to the mucosal layers helps microbes settle, while entering host cells enables them to remain within the host and disseminate throughout the body. For instance, *C. trachomatis* and *N. gonorrhoeae* indirectly adhere to epithelial cells with the help of adhesins and outer membrane proteins to penetrate the mucosal epithelium, thus causing infection (26-28).

Immune evasion: For example, microbial pathogens that infect the male reproductive system have developed multiple mechanisms by which they can circumvent host immune responses and maintain themselves as chronic Residents in the tract (29,30). They include changes in surface proteins as a means of evading the host's immune system, the ability to alter host cytokines and chemokines, as well as the inhibition of host production of antimicrobial peptides (M. genitalium and U) (31). *Candida urealyticum* has been found to cause acute infections due to its ability to change its membrane antigens and inhibit cytokine production, thereby evading immune system attacks and leading to chronic colonization of host tissues and persistent inflammation (31,32).

Tissue damage and inflammation: Testicular inflammation and tissue injury are consequences of microbial pathogens, toxins secreted by pathogens, and inflammatory molecules present in male reproductive tissues (33,34). These factors interfere with the epithelial lining, attract more leukocytes, and induce the synthesis of pro-inflammatory cytokines that cause tissue damage and impaired function. For example, *E. coli* can secrete toxins and endotoxin lipopolysaccharides (LPS), which cause inflammation in the prostate and seminal vesicles, leading to the development of chronic prostatitis and seminal vesiculitis (35,36).

Modulation of spermatogenesis: Bacteria and other microbes, therefore, potentially have a direct effect on spermatogenesis and sperm function, as they are known to interfere with the testes and epididymal microenvironments. Intracellular pathogens, including Mycobacterium tuberculosis and viruses such as the ZIKV, can affect germ cells and Sertoli cells, leading to degeneration of spermatogenesis and impacts on sperm quality. Further, infection, inflammation, and oxidative stress that often occur with microbial infections cause sperm DNA damage and reduce sperm intensity and vitality (7,36,37).

Dysregulation of host signaling pathways: In this context, microbial pathogens can subvert cellular signaling to maintain their viability and proliferation in the male reproductive tissue. A pathogen is not only able to

Table 1. Summary of Common Microbial Pathogens Affecting Male Fertility and Their Effects

Pathogen	Transmission Mode	Primary Target in the Male Reproductive Tract	Main Effects on Fertility
Chlamydia trachomatis	Sexual	Sertoli cells, epididymis	Inflammation, meiotic defects, and reduced motility
Neisseria gonorrhoeae	Sexual	Urethra, prostate, and seminal vesicles	Structural damage, sperm quality reduction
Mycoplasma genitalium	Sexual, urogenital colonization	Germ cells, sperm DNA	DNA fragmentation, impaired motility
Escherichia coli	Ascending UTI, sexual	Prostate, seminal vesicles	Inflammation, toxin-mediated sperm damage
Zika Virus (ZIKV)	Mosquito, sexual	Testis, semen	Decreased sperm count/motility, RNA in semen
HIV	Sexual, blood	Testis, immune system	Reduced concentration/motility, immune suppression

modify extracellular signals but also to interfere with cellular signaling pathways, which regulate events like cell division, death, and immune response. For instance, HPV disrupts the host cell cycle regulation and interferes with apoptosis to enable the virus to remain in the host cells and promote oncogenic alteration to cells lining the male genital tract (38-40).

In conclusion, the three aspects of microbial pathogenesis—the processes of microbial adhesion, invasion, and evasion of the host's immune defenses, damage to host tissue, and alteration of spermatogenesis and signal transduction pathways—are intricate processes that are not yet fully understood. Understanding these mechanisms is imperative for the creation of new anti-microbial therapeutic approaches and the management of microbial influence on male fertility (41,42). The key molecular mechanisms are summarized in Table 2.

Impact of Microbial Infections on Spermatogenesis

Various infections by microbes in the male reproductive organs have been shown to interfere with spermatogenesis significantly – the process by which spermatogonia or male germ cells differentiate through mitosis, meiosis, and spermatogenesis to form mature spermatozoa. Spermatogenesis occurs in the seminiferous tubules of the testes, involving coordinated cell signaling processes at the molecular level. Through diverse mechanisms, bacterial and viral pathogens can disrupt spermatogenesis, sperm morphology, and function, adding to male infertility (16,43,44).

Disruption of spermatogonial stem cells: spermatogonial stem cells can also be affected by microbial infections because they are involved in essential spermatogenesis processes throughout adulthood. These include direct infection of spermatogonial stem cells and induction of apoptosis, which reduces the pool and the capability of spermatogonial stem cells to sustain spermatogenesis, for example, through the pathogen of mumps that causes orchitis. Also, the inflicted microbial infections can cause inflammation and oxidative stress to the body, hence altering the seminiferous tubules' microenvironment, which is detrimental for spermatogonial stem cell (43,45-47).

Impairment of meiosis: Meiosis is another vital process of spermatogenesis. The spermatocytes at this stage of spermatogenesis are made to undergo two divisions of cell division to form haploid spermatids. Since microscopic organisms are capable of disrupting meiotic functions, disturbances in chromosomal structures and spermatogenesis may result in meiotic arrest. For instance, *C. trachomatis* and *M. genitalium* are causative agents for meiotic defects and aberrations in the sperm morphology, which suggest increased interference with the meiotic process (48,49).

Sertoli cell dysfunction: Sertoli cells play a crucial role in spermatogenesis, providing key factors that support the architectural and endocrine development of germ cells (48,49). It was also found that microbial infections may potentially affect Sertoli cells, altering their functions in the process and subsequently impacting germ cells (43). For example, intracellular pathogens such as HIV and Mycobacterium tuberculosis can directly infect Sertoli cells, leading to a decrease in the production of growth factors and cytokines essential for regulating the maturation process of germ cells. Hence, this disruption can cause loss of germ cells, spermatogenic arrest, and, in the process, hinder the formation of sperm (43,50).

Testicular inflammation and fibrosis: Microbial infections are often acute and may ascend to the testicles and cause inflammation (orchitis) and fibrosis, which will impair spermatogenesis even further (5). The production of pro-inflammatory cytokines and chemokines during infection leads to tissue injury, disruption of the blood-testis barrier, and affects the development of germ cells. It is crucial for spermatozoa production, and a distorted interstitial structure may impair the movement of germ cells or alter the micro-environment required for spermatogenesis and result in azoospermia (50, 51).

Oxidative Stress and DNA Damage: The organisms infecting the males cause oxidative stress in the testes, which in turn creates free radicals known as ROS that affect the germ cells' DNA and negatively impact sperms. In this regard, recent data evidence that ROS, depending on their quality and concentration, may cause chromosomal aberrations, DNA fragmentation, and apoptosis of developing germ cells. Furthermore, the exercise can result in oxidative stress that affects the mitochondria and sperm mobility, thereby affecting fertility in males (33,46,47).

In summary, microbial infections have numerous direct and indirect consequences on spermatogenesis, destabilizing the intricate balance of signaling pathways

Table 2. Molecular Mechanisms of Microbial Pathogenesis in the Male Reproductive Tract

Mechanism	Examples	Effect on Fertility
Adhesion & invasion	Adhesins in *C. trachomatis*, OMPs in *N. gonorrhoeae*	Establish infection, disrupt mucosal barrier
Immune evasion	Antigenic variation in *M. genitalium*, *U. urealyticum*	Chronic infection, persistent inflammation
Tissue damage & inflammation	LPS from *E. coli*	Prostatitis, seminal vesiculitis
Modulation of spermatogenesis	*M. tuberculosis*, ZIKV infection	Germ cell loss, sperm defects
Host signaling dysregulation	HPV alters the cell cycle/apoptosis	Oncogenesis, testicular dysfunction

responsible for generating morphologically and biochemically competent spermatozoa. Overall, to effectively manage the bacterial infections that affect spermatogenesis and the resulting negative effects on male fertility, it is crucial to understand the impacts that these organisms cause on spermatogenesis in an attempt to formulate efficient and effective treatment methods to counter them (33). The multifaceted impact of infections on spermatogenesis is illustrated in Figure 2 and detailed in Table 3.

Cellular Responses to Microbial Infections in the Male Reproductive Tract

The prostate is an organ in the MRT. It could be regarded as an organ of immune defense, capable of recognizing and eliminating microbial threats to the host organism. To highlight the primary characters and events of the research, the following statements have been constructed as headings: Roles of the MRT in initiating immune response against microbial invasions; Overview of the immune cells and molecules involved in the defense responses against infections in the MRT. Nevertheless, the invading microbial pathogens have ways of escaping the immune system's detection and remain resident in the Prostate and other accessory glands of the MRTs, where they cause actual inflammation, tissue damage, and impaired male reproduction (52,53).

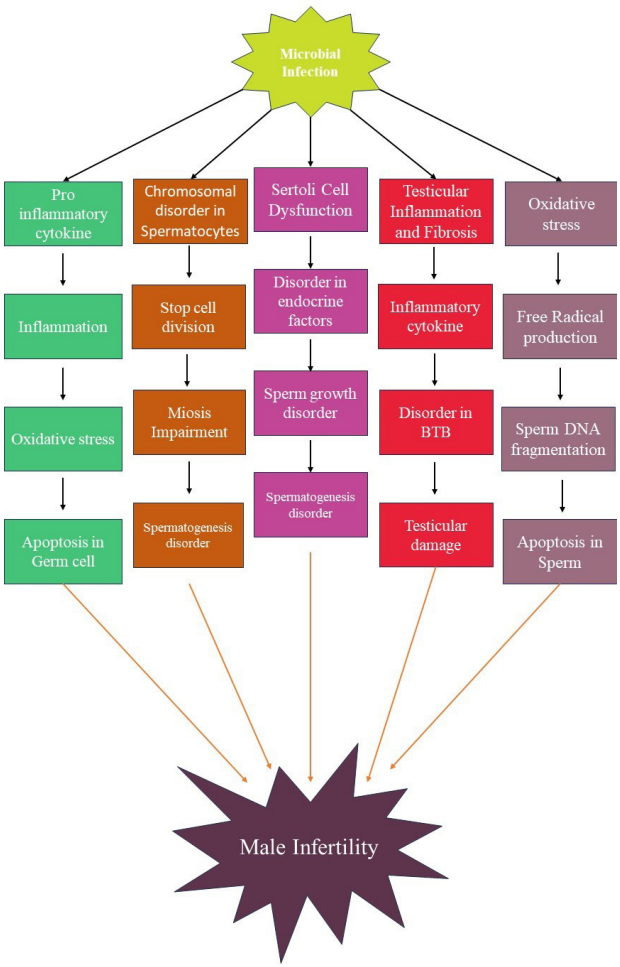

Figure 2. Effect of Microbial Infections on Spermatogenesis and Male Fertility.

Innate immune responses: Males' gonadal tissue exhibits innate immune responses, including macrophages, dendritic cells, neutrophils, and epithelial cells, which defend the body against microbial infections. These cells contain PRRs, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), that detect PAMPs — molecules found on the surfaces of microbes. This is because activation of PRRs results in the activation of intracellular signaling pathways that result in the generation of pro-inflammatory cytokines, chemokines, as well as antimicrobial peptides, thus enhancing inflammation and recruitment of other immune cells to the site of infection (54,55).

Adaptive immune responses: In addition to non-specific defensive mechanisms, the MRT has specific advantages in antimicrobial defense, including T cells, B cells, and other cells that generate active, specific reactions to microbial pathogens. The effector T cells include the T helper cells and cytotoxic cells, which coordinate cell-mediated immunity and directly help in the immune response against the infected cells (56,57).

T cell activation and differentiation are then observed in effector T cell subsets, including Th and cytotoxic T cells, which coordinate cell-mediated immunity and direct the immune response against infected cells. Unlike T cells, B cells secrete and release molecules into the bloodstream called antibodies that identify and coat potential hazardous pathogens, also known as microbial antigens, to make them recognizable by other cells in the immune system and render them harmless (58-60).

Immunoregulatory mechanisms: The immunomodulatory aspects of male reproductive system organs help control inflammation and tissue damage associated with the overactivation of the immune system. Tregs are uniquely positioned to exert suppressive influences on immune functions, thereby abrogating autoreactivity and reactivity to microbial symbionts. Also, there are other immunosuppressive cytokines, including interleukin 10 (IL-10) and transforming growth factor beta (TGF-β), which inhibit immune response and encourage healing and tissue repair when microbes are cleared from the affected

Table 3. Impact of Microbial Infections on Spermatogenesis

Process Affected	Mechanism	Example Pathogens
Spermatogonial stem cell survival	Direct infection, apoptosis	Mumps virus
Meiosis	Chromosomal defects, meiotic arrest	*C. trachomatis*, *M. genitalium*
Sertoli cell function	Direct infection, reduced growth factors	HIV, *M. tuberculosis*
Testicular structure	Orchitis, fibrosis	Various bacteria/ viruses
DNA integrity	ROS-induced fragmentation	*U. urealyticum*, HIV

tissue (60-62).

Immunoevasion strategies of microbial pathogens: These microorganisms have developed various factors for immune evasion and are capable of setting up resident, chronic infections in the male reproductive organs. These alterations involve modifications to the host cell signaling pathway, including the suppression of mechanisms that facilitate the engulfment and degradation of pathogens and antigens, as well as the synthesis of factors that counteract the host immune system. For instance, *C. trachomatis* is an intracellular bacterium that promotes bacterial survival by subverting host cell apoptosis and inhibiting phagosome-lysosome fusion. In contrast, extracellular bacterial pathogens such as *E. coli* can secrete agents that hinder phagocytic ingestion and neutrophil migration (62-64).

Dysregulation of immune responses: The aforementioned chronic microbial infections establish microenvironments in the MRT where immune responses may become dysregulated, contributing to inflammation, tissue injury, and potentially impaired reproductive function. *Mycobacterium* can theoretically interfere with T cells by exposing them to microbial antigens over a prolonged period, eventually exhausting and impairing their function and ability to control the source of the infection. Furthermore, pro-inflammatory cytokines and chemokines lead to further tissue damage, as well as fertility problems (65,66).

A conclusion, based on the findings regarding cellular responses to microbial infections, is that these processes in the MRT are complex and invulnerable, integrating the intricate interactions of the initial and the secondary immune responses. Knowledge of mechanisms of intracellular host defense is critical to dissecting the complexity of RTIs' pathophysiology and for the development of strategies to protect male fertility and RE reproductive health (66, 67). The molecular pathways leading to testicular apoptosis are depicted in Figure 3.

Immunological Responses and Inflammation

Having established the immunological deployment in the protection against microbial pathogens in the male reproductive system, let us delve deeper into immunological responses and inflammation (68). When they come into contact with microbial pathogens, the immune system synchronously initiates mechanisms that may remove the pathogens and rebuild tissue balance. It is also essential to appreciate that inflammation may be associated with pathologic conditions, which can contribute to tissue injury and reproductive disorders. There is an apparent need for understanding how immunological reactions and inflammation interact with each other for better comprehension of the microbial pathogens that cause reproductive tract infections, and to devise an appropriate therapy (69,70).

Innate immune recognition: The innate immune system is a defense mechanism in mammals that responds immediately to microbial infection through the use of PRRs that recognize conserved molecular patterns on the surface of infectious pathogens. Other PRRs include TLRs, NLRs, and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which play a crucial role in detecting microbial molecules that help trigger an immune response. Stimulation of PRRs initiates signaling pathways within the host cells that have constructive consequences, such as the production of inflammatory mediators, cytokines, chemokines, and peptides, as well as antimicrobial peptides to fight the pathogen and immune cell recruitment to the infection site (71,72).

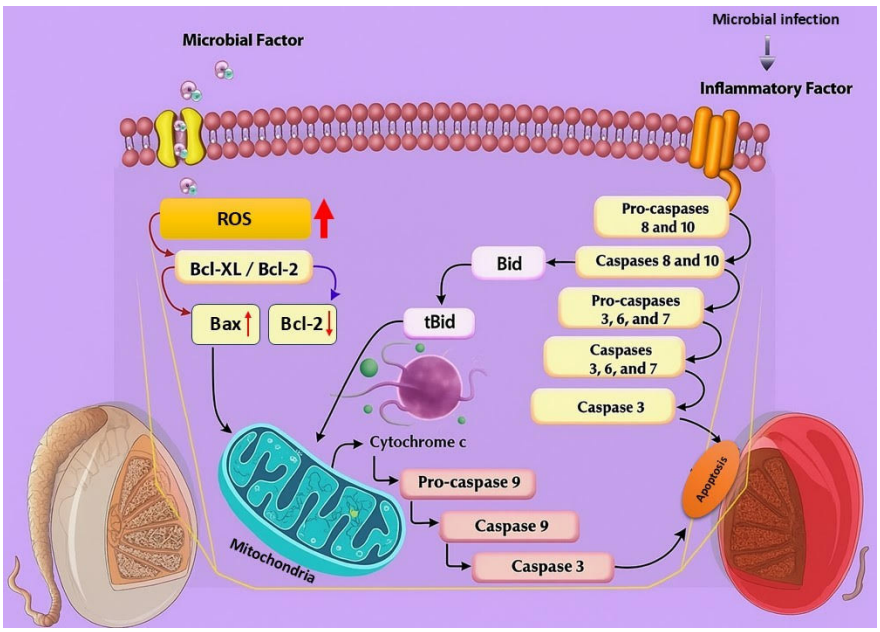

Figure 3. Cellular and Molecular Effects of Microbial Factors on Testicular Apoptosis.

Cellular infiltration and activation: When a microbial pathogen infects tissues, various immune cells, such as neutrophils, macrophages, and dendritic cells, are recruited to the infected sites, where they neutralize the pathogens through phagocytosis and the release of ROS and antimicrobial peptides. Anti-inflammatory drugs inhibit the production of prostaglandins and other lipid mediators involved in inflammation. At the same time, immunosuppressive agents suppress the activation of T cells and other components of the immune system. Indeed, differentiation of CD4 T helper cells and CD8 cytotoxic T cells plays critical roles in the organization of immune reactions by producing cytokines, stimulating other immune cells, and direct lysis of cells affected by the virus (73-76).

Inflammatory mediators: Pro-inflammatory mediators, including cytokines, chemokines, and lipid mediators, play a crucial role in orchestrating inflammation through immune activation, immobilizing cells, and regulating their functions. TNF-α and IL-1 stimulate the immune response and enhance inflammation, while IL-6 has both inflammatory and anti-inflammatory properties. On the other hand, while IL-10 and TGF-β reduce inflammation and facilitate tissue repair or the resolution process (77,78).

Tissue damage and repair: In this case, inflammation, which is crucial in combating microbial pathogens, may become detrimental through the production of free radicals that can cause tissue destruction and compromise reproductive capabilities. These include pathogen-derived toxins and host-derived inflammatory mediators / cellular components such as complement, free radicals, and cytotoxic T cells, which, while participating in the immune response, can damage host tissues and impair their function. On the other hand, anti-inflammatory cytokines and pro-resolving lipid mediators that occur naturally also work to resolve inflammation by mediating tissue healing following the eradication of microbes (79,80).

Dysregulated inflammation and reproductive dysfunction: Chronic inflammation or inflammation that is prolonged within the male genital area can lead to male reproductive disease and some extent of infertility. Inflammation resulting from chronic microbial infections or autoimmune reactions tends to affect spermatogenesis, reducing sperm motility and overall fertilizing capacity. These inflammatory conditions cause pathological changes to the testes through processes like orchitis, epididymitis, and prostatitis, leading to obstructions in the epididymis and impaired functions of the seminal vesicles, conclusively disturbing the production, maturation, and transport of sperms (81).

Combinedly, the immune system and inflammation are highly significant in the protection against microbial invasions in the MRT. Conversely, inflammation can also be pathogenic, causing tissue injury and impairing fertility. Understanding immunological responses and inflammation in microbial-induced reproductive tract infections is crucial, as advances in treating conditions affecting male fertility and resolving inflammation are essential to safeguarding male fertility.

Role of Microbiota in Male Fertility

The MRT harbors a complex community of microorganisms, known as the microbiota, which contributes to the overall health and fertility of the individual. Recent literature suggests that the microbiota plays a role in several processes of male reproductive physiology, including sperm development, maturation, and motility. The relationship between the microbiota and male fertility involves a complex interaction between the human body and the microbiota of the MRT, and an understanding of this interplay is vital for the elucidation of reproductive disorders as well as the identification of new therapeutic targets (82,83).

Microbiota composition: The MRT is colonized by a diverse array of microorganisms that form a dynamic microbial community, comprising bacteria, viruses, fungi, and others. The male urogenital system includes the urethra, prostate gland, seminal vesicles, and epididymis, each of which harbors distinct microbiota. Species of bacteria that are usually isolated from the male urethra consist of lactobacilli, corynebacteria, staphylococci, and ureaplasmas, among others (17,84).

Mechanisms of a healthy microbiota: A balanced, commensal-dominant microbiota supports male fertility through multiple mechanisms. Beneficial bacteria produce metabolites, such as short-chain fatty acids (e.g., butyrate), that possess anti-inflammatory properties, helping to maintain testicular and accessory gland homeostasis and reduce oxidative stress. Furthermore, commensals provide colonization resistance by competing with pathogens for nutrients and adhesion sites, thereby preventing their overgrowth and colonization. They also contribute to maintaining the integrity of the epithelial barrier within the reproductive tract, thereby limiting the translocation of pathogens and their inflammatory products.

Sperm quality and function: Recent literature reveals that MRT microbiota play a significant role in spermatogenic capacity and sperm functionality. Microbiota in the reproductive tract of female also secretes metabolites and signaling molecules that affect the physiological condition of sperm, such as their motility, viability, and DNA damage. Disruption of the RT microbiota has been implicated in poor sperm quality, including low concentration, low motility, poor morphological appearance, and high levels of induced sperm DNA fragmentation, which affects fertility in men (17,82).

Immune modulation: The author identifies that the microbiota present within the MRT modulate or influence immune cells, cytokines, and inflammation. This suggests that commensal bacteria play a crucial role in immune homeostasis and tolerance by influencing the development of regulatory T cells, also known as Tregs,

as well as by releasing anti-inflammatory cytokines. Conversely, a state of dysbiosis, characterized by an overgrowth of pathobionts (e.g., certain *Ureaplasma* or *Mycoplasma* species), can trigger a pro-inflammatory response. This includes the production of cytokines such as IL-6 and TNF-α, which lead to chronic inflammation, autoimmune-like responses, and oxidative stress within the testes and seminal plasma. This inflammatory milieu is directly detrimental to sperm production and function. Killer microbial communities affect the immune system, resulting in chronic inflammatory response, autoimmune responses, and inflammation, causing damage to tissues, certain hormones, and the testes that hinder sperm production and their activity (82,85).

Protection against pathogens: The MRT microbiota is an ecosystem of microorganisms that inhabit the male tracts, which is useful in preventing pathogenic microorganisms from colonizing the body by providing competition and thereby enhancing the health of the male reproductive organs. Alteration of the microbiota can also impede the growth of potential pathogens through competition for nutrients, sharing of adhesion sites, synthesis of peptides that inhibit microbial growth, and immunomodulation. Violation of this protective barrier by, for instance, the use of antibiotics, changes in the individual's environment, or several other factors may disrupt the microbiota composition, resulting in a higher susceptibility to infections, which may further affect fertility in males (86,87).

Reproductive tract disorders: Mendis explains that changes in the MRT microbiota are considered to play a causal role in various reproductive system disorders, including prostatitis, epididymitis, and infertility. The alteration of the microbiota's composition may result in inflammation, oxidative stress, and tissue damage, ultimately leading to these diseases. In a dysbiotic state, the direct interaction of pathobionts with sperm can further impair motility. The associated oxidative stress leads to sperm membrane lipid peroxidation and DNA fragmentation. In addition, infections with pathogenic microorganisms such as *C. trachomatis*, *M. genitalium* group C, and *U. urealyticum* have also been implicated in low sperm densities, essential impairment of sperm qualities, inflammation of the accessory gland, and infertility in males (88,89).

In conclusion, the microbiota plays a crucial role in regulating male fertility by influencing sperm quality, immune function, and the ability to eliminate pathogens. Imbalances in microbial communities within the MRT have been found to interfere with fertility and lead to reproductive disease, confirming the significance of a proper microbiota for preserving fertility. These findings, while contributing towards increasing the understanding of the mechanisms of male fertility and reproductive health, call for further studies on microbiota and its impact on men's reproductive health for effective targeted intervention in cases of male reproductive disorders and infertility.

Diagnostic Methods for Detecting Microbial Infections in Male Reproductive Health

Microbial infections have a significant impact on male fertility or potential fatherhood; therefore, identification of these infections in the male reproductive system for diagnostics, therapy, and prevention of reproductive health disorders is crucial. There are several conventional and innovative methods of identifying microbial pathogens in the male reproductive system that possess strengths and weaknesses (90-92).

This section provides an overview of the diagnostic methods commonly used to detect microbial infections in male reproductive health:

Microbiological culture: Microbiological culture remains the gold standard method for detecting microbes in clinical samples collected from the male genital area. For example, the medium used for specimen inclusion includes semen, urethral swabs, or prostatic fluid, etc. The biochemical reactions on selective and differential media, as well as in specific conditions favoring the growth of the microbiological organism, are then initiated. The identification of microbial colonies through their differentiation by using colonial morphology, biochemical tests, and susceptibility profile helps in reaching an accurate etiological diagnosis of the bacteriological, mycological, and virological infections (17,93,94).

Polymerase chain reaction (PCR): PCR-based methods can recognize microbial nucleic acids present in infected clinical specimens, making them suitable for diagnosing genital infections in males. PCR assays can be designed to amplify conserved DNA sequences present in all members of a particular microbial species or to target pathogen-specific virulence genes, thereby confirming the presence of actinobacterial, bacterial, viral, and fungal pathogens with a very high level of sensitivity and specificity. High-sensitivity real-time PCR techniques enable the identification of microbial DNA targets with efficiency and specificity, useful in diagnosing infections and assessing disease progress or response to treatment (95,96).

Nucleic acid amplification tests (NAATs): NAATs encompass a broad category of molecular methods that utilize PCR, transcription-mediated amplification, and loop-mediated isothermal amplification to amplify and detect microbial nucleic acids in clinical samples. They offer high sensitivity, specificity, and rapidity, along with the features of a multiplex format, for detecting multiple pathogens simultaneously. NAATs are commonly applied for routine detection of STIs, including chlamydia, gonorrhea, and *M. genitalium* in male urethral swabs (97, 98).

Serological assays: Serological assays involve detecting the presence of specific antibodies to microbial antigens

in serum or other body fluids, indicating past infection with the microorganisms. The screening using serological assays can be a standard method for identifying viral diseases, including HIV, Herpes simplex virus (HSV), and HPV, in males. Starch-iodine complex formation has already been established as an analytical signal, and optimization of this reaction is a promising approach for developing new, highly sensitive immunochemical assays (99).

Microscopic examination: Histopathological examination of clinical specimens enhances the observation of microbial pathogens, host cells, and inflammatory characteristics when viewed under the microscope. Gram staining, acid-fast staining, and potassium hydroxide (KOH) wet mounts are the most commonly used impregnation methods employed in microscopy for identifying the presence of different bacteria, mycobacteria, fungi, or parasites in male genital samples. Besides, immunofluorescence microscopy can be used in clinical diagnosis to visualize specific viral antigens/nucleic acids in clinical specimens (100,101).

Point-of-care tests (POCTs): Several advantages are associated with POCT devices, including the rapid and decentralized diagnosis of microbial infections within male reproductive health clinics. A principal characteristic that has been developed in POCTs is the need to allow for easy accessibility without the need for professional expertise or special equipment as well as being convenient to transport from one place to another rapid antigen tests, immunochromatographic assays, and nucleic acid-based POCTs that are currently available for diagnosing various STIs and other genital infections in males are noninvasive, easy to perform, rapid and acceptable in resource restrictive settings (102,103).

As mentioned above, when diagnosing microbial infections in male reproductive health, a combination of microbiological, molecular/serological, microscopic, and point-of-care diagnostic methods can be employed. The selection of diagnostic tests depends on factors such as the likely causative pathogen, clinical signs and symptoms, the type of sample used for laboratory analysis, and the technology available in the lab, aiming for an accurate and timely diagnosis to improve patient management and prognosis. The primary diagnostic methods, along with their advantages and limitations, are summarized in Table 4.

Therapeutic Approaches and Management Strategies

Male reproductive health is a complex field that involves microbial infections that can only be cured or managed using an approach that targets the pathogen while offering relief to the patient, and uses tactics that prevent the development of complications and fertility issues. Treatment may involve antimicrobial agents, supportive care, and lifestyle modifications in the context of long-term care and possible surgical intervention, where the etiology of the infection is known and the severity and clinical manifestation of symptoms are considered (104).

This section outlines the therapeutic approaches and management strategies commonly employed for treating microbial infections in male reproductive health:

Antimicrobial therapy: Antimicrobial agents are the foundation of managing microbial infections in male reproductive health, encompassing bacteria, viruses, fungi, and parasites of various types. The appropriateness of antimicrobial agents varies depending on the potential or confirmed pathogen, along with its resistance profile and evidence-based protocols. Antimicrobial drugs might be systemic, including antibiotics, antivirals, antifungal, and antiparasitic, and might be local, like topical or IV, if the infection is severe (105).

Empiric therapy: This is a treatment administered in cases of uncertain etiology or when the results of related diagnostic tests are pending. Empiric therapy should thus depend on such factors as the prevalence of organisms in the region, levels of resistance, and clinical manifestations. *C. trachomatis*, *N. gonorrhoeae*, and *E. coli* are the most frequently observed pathogens of the male genital tract; Candida species can also be present. In extended-spectrum STIs, the initial broad-spectrum antimicrobial agents can be prescribed (106,107).

Targeted therapy: Thus, after the causative agent has been identified and the results of the antibiotic sensitivity test are reported, it is possible to select an individualized approach to prescribing antimicrobial drugs for patients. For the identified pathogen, narrow-spectrum antibiotic

Table 4. Diagnostic Methods for Microbial Infections in Male Reproductive Health

Method	Description	Advantages	Limitations
Microbiological culture	Growth of pathogens from semen/urine	Identifies live organisms, resistance testing	Time-consuming, some microbes are unculturable
PCR	Amplifies pathogen DNA/RNA	High sensitivity/specificity	Requires lab equipment
NAATs	Multiplex nucleic acid detection	Rapidly detects multiple pathogens	Costly
Serology	Detects antibodies to pathogens	Useful for viral infections	May not detect acute infections
Microscopy	Staining, histopathology	Quick, low-cost	Low sensitivity for some pathogens
POCT	On-site rapid tests	Immediate results	Often less sensitive than lab methods

or antiviral treatment may be deemed appropriate to reduce the risk of development or resistance, toxicity, and harm to the host's other bacteria and viruses (108).

Combination therapy: In instances where polymicrobial anaerobic or mixed microbial infections prevail, combination antimicrobial therapy is often necessary to ensure optimal outcomes by targeting the different organisms. The combined use of drugs with synergistic or additive anti-microbial activity can overcome existing resistance factors, thereby decreasing the chances of treatment failure and the development of resistance to the drugs used. Nevertheless, a general knowledge of the combinations of antimicrobial medicines and an understanding of the possible interconnections and side effects of the drugs in a patient are critical in achieving the best results (109,110).

Supportive care: In conjunction with antimicrobial treatment, symptomatic measures can be employed as an integral part of supportive therapy to alleviate suffering and facilitate the healing process. This can be managed by providing pain-relieving agents, anti-inflammatory drugs, antipyretics, and topical applications in cases of lesions, itching, and discomfort related to genital infections. Other measures of supportive care that would also be essential in getting back on one's feet and avoiding complications are taking enough fluids, getting enough sleep, and observing good hygienic practices, as advised by the doctors (111-113).

Surgical interventions: At times, conditions of genital infections can worsen, necessitating medical surgery to perform incision and evacuation for abscesses, debridement of necrotic tissue, or to repair structural damage, thereby facilitating the growth of the disease. Absolute indications for surgery are abscess drainage, debridement, urethral stricture dilation, or circumcision in cases where the symptoms represent an impediment to the patient and the infection has the potential to spread systemically or compromise tissue integrity. The physician is advised to consider the specific surgical approach based on clinical examination and investigation results, as well as in collaboration with other specialists if necessary (114).

Generally, treating bacterial infections in the MRT requires a mostly personalized approach that considers the causative agents, their antibiotic susceptibility, the severity of the infection, and patient-specific factors. Because UTIs can affect fertility and reproductive health, urologists, infectious disease specialists, microbiologists, and reproductive health experts should be involved in a patient's care to ensure effective treatment and reduce the risk of complications and impacts on male fertility and reproductive function. A summary of these treatment strategies is provided in Table 5.

Future Directions and Emerging Research Areas

Given these perspectives on male microbial-associated health, several research horizons and apparent nascent research directions have emerged in the present study, which can provide clues to the development of pathogenesis, diagnostics, treatment, and prevention strategies.

This section highlights some of the future directions and emerging research areas in the field of male reproductive health:

Microbiome studies: Indeed, the emergence of high-throughput sequencing technologies, coupled with the use of metagenomic approaches to study the microbiome of the MRT, has significantly altered our understanding of the microbiome in health and disease. Subsequent investigation endeavors should be directed toward understanding the components and structures, as well as the functional variability, of the MRT microbiota in diverse groups of men and beyond healthy subjects. Future investigations utilizing longitudinal designs to evaluate the dynamic relationships among the microbiota, host immunity, and their impacts on reproductive health may help to unravel the underlying causes of microbial imbalance, and to identify potential intervention methods to re-establish the optimal balance of the microbiota (115-117).

Host-pathogen interactions: Since the MRT is a specialized site for microbial colonization and propagation, it is crucial to identify the molecular interactions between the host and pathogen, as well as the immune defense systems and pathogen physiology. Prospective studies should focus on the mechanisms by which microbial pathogens that target male reproductive organs gain access to, attach to, and settle on, invade, and survive in host tissues. Of this, targeting the host-pathogen interactions could potentially yield novel therapeutic approaches in averting microbial infections and treating reproductive tract diseases (118).

Precision medicine approaches: The gradual application of precision medicine tools, such as genomics, proteomics,

Table 5. Therapeutic Approaches

Strategy	Examples	Notes
Empiric antimicrobial therapy	Broad-spectrum antibiotics	Used before pathogen identification
Targeted therapy	Narrow-spectrum agents based on sensitivity tests	Reduces resistance risk
Combination therapy	Synergistic antibiotic pairs	For polymicrobial or resistant infections
Supportive care	Analgesics, anti-inflammatories	Symptom relief
Surgical intervention	Abscess drainage, stricture repair	For severe or complicated cases

and metabolomics, in microbial infections affecting male reproductive health can be attributed to their diagnostic and therapeutic modalities. Stromal gene profiles likely reflect the interplay between multicellular and microbial communities and may help elucidate biomarkers correlating to susceptibility to infection, response to treatment, and fertility. Precision medicine solutions can also be used to advance the concept of personalized antimicrobial therapies that address the specificity of the patient and microbial phenotypes and resistances, thereby increasing the effectiveness of treatment and reducing adverse impacts (119).

Immunomodulatory therapies: Therapies designed to modulate the host immune response to microbial pathogens, MRT infections, and infertility represent a rational and innovative approach to addressing this significant clinical problem. Further research should investigate the immune-modulating effects of MRT on immunity and inflammation by examining immune specimens, immune nests, cytokines, and the impact of immune checkpoints involved in microbial pathogen clearance and tissue restoration. Another strategy, which may help improve antimicrobial immunity, decrease inflammation, and foster tissue repair in the MRT, is the targeted regulation of host immune responses through the application of immunomodulatory agents like cytokine inhibitors or agonists to Toll-like receptors, or Treg-based therapies (120).

Novel therapeutic targets: The discovery of new targets to augment the therapeutic pipeline for antimicrobial resistance and to develop additional treatment approaches for microbial infections in male reproductive health has been deemed a priority. Lastly, future studies should investigate alternative approaches to combating MDR pathogens: the use of natural compounds, short antimicrobial peptides, and bacteriophages. Moreover, intervention strategies such as protocol approaches against virulence factors, quorum-sensing molecules, and biofilm development may restore microbial pathogenesis and enhance existing organizations for antimicrobial treatment (121).

Preventive strategies: It is crucial to adopt effective preventative methods to control the spread of disease and reduce disease burden while supporting healthy male reproductive function. Future research should focus on identifying factors, such as vaccination, pre-exposure prophylaxis, behavioral practices, and hygiene trends, that can decrease the incidence of STIs, GU tract infections, and reproductive tract disorders in males. Increasing sexual health education, preventive education for sexually transmitted diseases, expanding access to reproductive health services, and promoting vaccination could significantly help lessen the impact of microbial invasions and improve male fertility.

Subsequently, the following research recommendations should be emphasized: Future studies should focus on expanding knowledge about how microbial infections affect male reproductive health, identify effective diagnostic and treatment options to be used routinely in clinics, and improve prevention strategies to enhance the quality of assisted fertility and the overall health and well-being of subfertile men. Collaboration among multidisciplinary teams, international groups, and community engagement is crucial to addressing complex issues related to microbial-related challenges in male reproductive health and to translating basic research findings into effective antimicrobial practices.

Conclusion

Bacterial GU infections remain a major hurdle to male reproductive health and impact fertility, reproductive function, and quality of life in men. In this paper, we discuss the various mechanisms by which microbial pathogens can affect the male reproductive system, leading to conditions such as urethritis, prostatitis, epididymitis, orchitis, and infertility. Although numerous diagnostic tools for microbial infections have emerged and therapeutic advances have been made, microbial infections continue to pose challenges in terms of diagnosis and therapy, underscoring the need for further research to understand disease causation better, develop improved diagnostic tests, and discover new therapeutic approaches.

This review has sought to juxtapose the molecular and cellular outcomes of microbial invasions during male fertility and the delicate balance between pathogens, host immune systems, and the reproductive tract. In the present work, we have analyzed the mechanisms by which microbes interfere with male fertility, the effects of certain infections on spermatogenesis and sperm characteristics, as well as the cellular responses to microbial invasion in the male genitalia. Furthermore, the paper has elucidated diagnostic procedures for identifying microbial infections affecting male reproductive health and treatment perspectives for managing these infections.

Examining the current state of literature, potential future works and practices, and newly developing fields or concepts would provide a solid basis for furthering the knowledge of microbe-related infections in male fertility. Microbiome analyses, host-pathogen relationships, personalized medicine treatments, immunotherapeutic approaches, new targets for therapy, and strategies to prevent the decline in male fertility are among the crucial areas to be explored in future research programs designed to enhance reproductive health in men. A high level of heterogeneity arising from the nature of such pathologies and their risk factors requires multi-disciplinary team work partnerships, international consortia, and active community participation in research if the discovery of solutions to such infections is to be achieved and translated into practice.

In conclusion, it is therefore vital that more researchers

and stakeholders invest their efforts in combating the growing threat of microbial infections to male fertility and bring about improvements in the identification, management, and control of reproductive system pathologies. By advancing our understanding of disease mechanisms, developing targeted therapeutic interventions, and implementing preventive strategies, we can improve reproductive outcomes, enhance quality of life, and promote male reproductive health and well-being.

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Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

Not applicable.

References

1. Farsimadan M, Motamedifar M. Bacterial infection of the male reproductive system causing infertility. J Reprod Immunol. 2020;142:103183. doi:10.1016/j.jri.2020.103183
2. Pai MO, Venkatesh S, Gupta P. The role of infections in infertility: a review. Int J Acad Med. 2020;6(3):189-196. doi:10.4103/IJAM.IJAM_18_20
3. Babakhanzadeh E, Nazari M, Ghasemifar S, Khodadadian A. Some of the factors involved in male infertility: a prospective review. Int J Gen Med. 2020;13:29-41. doi:10.2147/IJGM.S241099
4. Dasari S, Anandan SK, Rajendra W, Valluru L. Role of microbial flora in female genital tract: a comprehensive review. Asian Pac J Trop Dis. 2016;6(11):909-917. doi:10.1016/S2222-1808(16)61153-0
5. Das S, Roychoudhury S, Roychoudhury S, Agarwal A, Henkel R. Role of infection and leukocytes in male infertility. In: Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility-Volume One. Springer; 2022:115-140. doi:10.1007/978-3-030-89340-8_6
6. Henkel R. Infection in infertility. In: Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants. Springer; 2020:409-429. doi:10.1007/978-3-030-32300-4_32
7. Almeida RdN, Braz-de-Melo HA, Santos IdO, Corrêa R, Kobinger GP, Magalhaes KG. The cellular impact of the ZIKA virus on male reproductive tract immunology and physiology. Cells. 2020;9(4):1006. doi:10.3390/cells9041006
8. Saadedine M, El Sabeh M, Borahay MA, Daoud G. The influence of COVID-19 infection-associated immune response on the female reproductive system. Biol Reprod. 2023;108(2):172-182. doi:10.1093/biolre/ioac203
9. Li L, Liu W, Fan N, et al. Scallop IKK1 responds to bacterial and virus-related pathogen stimulation and interacts with MyD88 adaptor of toll-like receptor pathway signaling. Front Immunol. 2022;13:869845. doi:10.3389/fimmu.2022.869845
10. Willems M. Unravelling the mechanisms behind testicular fibrosis in Klinefelter men [Dissertation]. Universität Würzburg; 2022.
11. Alharthi F. The functional characterisation of potential oncogenic roles of the human germ line genes PRDM9 and TEX19 [Dissertation]. Bangor University; 2020.
12. Akhigbe RE, Dutta S, Hamed MA, Ajayi AF, Sengupta P, Ahmad G. Viral infections and male infertility: a comprehensive review of the role of oxidative stress. Front Reprod Health. 2022;4:782915. doi:10.3389/frph.2022.782915
13. Pagliuca C, Cariati F, Bagnulo F, et al. Microbiological evaluation and sperm DNA fragmentation in semen samples of patients undergoing fertility investigation. Genes (Basel). 2021;12(5):654. doi:10.3390/genes12050654
14. Kumaresan A, Das Gupta M, Datta TK, Morrell JM. Sperm DNA integrity and male fertility in farm animals: a review. Front Vet Sci. 2020;7:321. doi:10.3389/fvets.2020.00321
15. Boeri L, Pederzoli F, Capogrosso P, et al. Semen infections in men with primary infertility in the real-life setting. Fertil Steril. 2020;113(6):1174-1182. doi:10.1016/j.fertnstert.2020.01.034
16. Smolarczyk K, Mlynarczyk-Bonikowska B, Rudnicka E, Szukiewicz D, Meczekalski B, Smolarczyk R, Pieta W. The impact of selected bacterial sexually transmitted diseases on pregnancy and female fertility. Int J Mol Sci. 2021;22(4):2170. doi:10.3390/ijms22042170
17. Zuber A, Peric A, Pluchino N, Baud D, Stojanov M. Human male genital tract microbiota. Int J Mol Sci. 2023;24(8):6939. doi:10.3390/ijms24086939
18. Dorosti H, Eskandari S, Zarei M, Nezafat N, Ghasemi Y. Design of a multi-epitope protein vaccine against Herpes simplex virus, Human papillomavirus and Chlamydia trachomatis as the main causes of sexually transmitted diseases. Infect Genet Evol. 2021;96:105136. doi:10.1016/j.meegid.2021.105136
19. Xie L, Li Q, Dong X, et al. Investigation of the association between ten pathogens causing sexually transmitted diseases and high-risk Human papillomavirus infection in Shanghai. Mol Clin Oncol. 2021;15(1):1-7. doi:10.3892/mco.2021.2300
20. Ye L, Huang W, Liu S, et al. Impacts of immunometabolism on male reproduction. Front Immunol. 2021;12:658432. doi:10.3389/fimmu.2021.658432
21. Wang MK, Copp HL. Bacterial infections of the genitourinary tract. In: McAninch JW, Lue TF, eds. Smith & Tanagho's General Urology. 19th ed. McGraw Hill; 2020.
22. Wang H, Chen T, Chen Y, et al. Evaluation of the inhibitory effects of vaginal microorganisms on sperm motility in vitro. Exp Ther Med. 2020;19(1):535-544. doi:10.3892/etm.2019.8211
23. Babaei A, Kheradmand N, Baazm M, Nejati N, Khalatbari M. Protective effect of vitamin E on sperm parameters in rats infected with *Candida albicans*. Andrologia. 2020;52(7):e13593. doi:10.1111/and.13593
24. Cerdeira CD, Brigagão MRPL. Targeting Macrophage Polarization in Infectious Diseases: M1/M2 Functional Profiles, Immune Signaling and Microbial Virulence Factors. Immunol Invest. 2024;53(7):1030-1091. doi: 10.1080/08820139.2024.2367682.
25. Dutta S, Sengupta P, Izuka E, Menuba I, Jegasothy R, Nwagha U. Staphylococcal infections and infertility: mechanisms and management. Mol Cell Biochem. 2020;474:57-72. doi:10.1007/s11010-020-03833-2
26. Mix A-K, Goob G, Sontowski E, Hauck CR. Microscale communication between bacterial pathogens and the host epithelium. Genes Immun. 2021;22(5):247-254. doi:10.1038/

s41435-021-00146-0
27. Lin Q, Lin S, Fan Z, Liu J, Ye D, Guo P. A review of the mechanisms of bacterial colonization of the mammal gut. Microorganisms. 2024;12(5):1026. doi:10.3390/microorganisms12051026
28. Yang T. Functional insights into the role of a bacterial virulence factor and a host factor in *Neisseria gonorrhoeae* infection [Dissertation]. Universität Würzburg; 2021.
29. Wei M, Liu H, Wang Y, Sun M, Shang P. Mechanisms of male reproductive sterility triggered by dysbiosis of intestinal microorganisms. Life. 2024;14(6):694. doi:10.3390/life14060694
30. Le Tortorec A, Matusali G, Mahé D, et al. From ancient to emerging infections: the odyssey of viruses in the male genital tract. Physiol Rev. 2020;100(3):1349-1414. doi:10.1152/physrev.00021.2019
31. Li H, Zang Y, Wang C, et al. The interaction between microorganisms, metabolites, and immune system in the female genital tract microenvironment. Front Cell Infect Microbiol. 2020;10:609488. doi:10.3389/fcimb.2020.609488
32. Chapartegui-González I, Fernández-Martínez M, Rodríguez-Fernández A, et al. Antimicrobial susceptibility and characterization of resistance mechanisms of *Corynebacterium urealyticum* clinical isolates. Antibiotics (Basel). 2020;9(7):404. doi:10.3390/antibiotics9070404
33. Dutta S, Sengupta P, Slama P, Roychoudhury S. Oxidative stress, testicular inflammatory pathways, and male reproduction. Int J Mol Sci. 2021;22(18):10043. doi:10.3390/ijms221810043
34. Shokoohi M, Khaki A, Roudi Rasht Abadi A, et al. Minocycline can reduce testicular apoptosis related to varicocele in male rats. Andrologia. 2022;54(4):e14375. doi:10.1111/and.14375
35. Nirenjen S, Narayanan J, Tamilanban T, et al. Exploring the contribution of pro-inflammatory cytokines to impaired wound healing in diabetes. Front Immunol. 2023;14:1216321. doi:10.3389/fimmu.2023.1216321
36. Fraczek M, Kurpisz M. Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. Folia Histochem Cytobiol. 2015;53(3):201-217. doi:10.5603/FHC.a2015.0025
37. Liu W, Han R, Wu H, Han D. Viral threat to male fertility. Andrologia. 2018;50(11):e13140. doi:10.1111/and.13140
38. Heilmann RM, Allenspach K. Pattern-recognition receptors: signaling pathways and dysregulation in canine chronic enteropathies—brief review. J Vet Diagn Invest. 2017;29(6):781-787. doi:10.1177/1040638717724747
39. Edwards JL, Apicella MA. The molecular mechanisms used by *Neisseria gonorrhoeae* to initiate infection differ between men and women. Clin Microbiol Rev. 2004;17(4):965-981. doi:10.1128/CMR.17.4.965-981.2004
40. Medda A, Duca D, Chiocca S. Human papillomavirus and cellular pathways: hits and targets. Pathogens. 2021;10(3):262. doi:10.3390/pathogens10030262
41. Zhang W, Tettamanti G, Bassal T, Heryanto C, Eleftherianos I, Mohamed A. Regulators and signalling in insect antimicrobial innate immunity: functional molecules and cellular pathways. Cell Signal. 2021;83:110003. doi:10.1016/j.cellsig.2021.110003
42. Casadevall A, Pirofski LA. The damage-response framework of microbial pathogenesis. Nat Rev Microbiol. 2003;1(1):17-24. doi:10.1038/nrmicro732
43. Oghbaei H, Rastgar Rezaei Y, Nikanfar S, et al. Effects of bacteria on male fertility: spermatogenesis and sperm function. Life Sci. 2020;256:117891. doi:10.1016/j.lfs.2020.117891
44. Ding N, Zhang X, Zhang XD, et al. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. Gut. 2020;69(9):1608-1619. doi:10.1136/gutjnl-2019-319594
45. Abdelaal NE, Tanga BM, Abdelgawad M, et al. Cellular therapy via spermatogonial stem cells for treating impaired spermatogenesis, non-obstructive azoospermia. Cells. 2021;10(7):1779. doi:10.3390/cells10071779
46. Abadi ARR, Boukani LM, Shokoohi M, et al. The flavonoid chrysin protects against testicular apoptosis induced by torsion/detorsion in adult rats. Andrologia. 2023;2023:6500587. doi:10.1155/2023/6500587
47. Dolatkhah MA, Khezri S, Shokoohi M, Alihemmati A. The effect of *Fumaria parviflora* on the expression of sexual hormones along with their receptors in testicles of adult rats induced by varicocele. Andrologia. 2022;54(9):e14512. doi:10.1111/and.14512
48. Suede SH, Malik A, Sapra A. Histology, Spermatogenesis. StatPearls Publishing; 2023.
49. Ameli M, Moghimian M, Saeb F, et al. The effect of clomiphene citrate and human chorionic gonadotropin on the expression of CatSper1, CatSper2, LHCGR, and SF1 genes, as well as the structural changes in testicular tissue of adult rats. Mol Reprod Dev. 2019;86(6):738-748. doi:10.1002/mrd.23147
50. Wanjari UR, Gopalakrishnan AV. A review on immunological aspects in male reproduction: an immune cells and cytokines. J Reprod Immunol. 2023;158:103984. doi:10.1016/j.jri.2023.103984
51. Hasan H, Bhushan S, Fijak M, Meinhardt A. Mechanism of inflammatory associated impairment of sperm function, spermatogenesis and steroidogenesis. Front Endocrinol. 2022;13:897806. doi:10.3389/fendo.2022.897806
52. Akhigbe RE, Dutta S, Hamed MA, Ajayi AF, Sengupta P, Ahmad G. Viral infections and male infertility: a comprehensive review of the role of oxidative stress. Front Reprod Health. 2022;4:782915. doi:10.3389/frph.2022.782915
53. Monin L, Whettlock EM, Male V. Immune responses in the human female reproductive tract. Immunology. 2020;160(2):106-115. doi:10.1111/imm.13136
54. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009;21(4):317-337. doi:10.1093/intimm/dxp017
55. Kumar S, Ingle H, Prasad DVR, Kumar H. Recognition of bacterial infection by innate immune sensors. Crit Rev Microbiol. 2013;39(3):229-246. doi:10.3109/1040841X.2012.706249
56. Areschoug T, Gordon S. Pattern recognition receptors and their role in innate immunity: focus on microbial protein ligands. In: Herwald H, Schmidt A, Egesten A, eds. Trends in Innate Immunity. Vol 15. S. Karger AG; 2008:45-60. doi:10.1159/000135614
57. Li N, Wang T, Han D. Structural, cellular and molecular aspects of immune privilege in the testis. Front Immunol. 2012;3:152. doi:10.3389/fimmu.2012.00152
58. Verdon DJ, Mulazzani M, Jenkins MR. Cellular and molecular mechanisms of CD8+ T cell differentiation, dysfunction and exhaustion. Int J Mol Sci. 2020;21(19):7357. doi:10.3390/ijms21197357
59. Redgrove KA, McLaughlin EA. The role of the immune response in *Chlamydia trachomatis* infection of the male genital tract: a double-edged sword. Front Immunol. 2014;5:534. doi:10.3389/fimmu.2014.00534
60. Gong J, Zeng Q, Yu D, Duan Y-G. T lymphocytes and testicular immunity: a new insight into immune regulation in testes. Int J Mol Sci. 2021;22(1):57. doi:10.3390/ijms22010057
61. Olejnik B, Ferens-Sieczkowska M. Seminal plasma glycoproteins as potential ligands of lectins engaged in immunity regulation. Int J Environ Res Public Health. 2022;19(17):10489. doi:10.3390/ijerph191710489
62. Komai T, Inoue M, Okamura T, et al. Transforming growth factor-β and interleukin-10 synergistically regulate humoral immunity via modulating metabolic signals. Front Immunol. 2018;9:1364. doi:10.3389/fimmu.2018.01364
63. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front Immunol. 2023;14:1161067. doi:10.3389/fimmu.2023.1161067
64. Ren Y, Khan FA, Pandupuspitasari NS, Zhang S. Immune evasion strategies of pathogens in macrophages: the potential for limiting pathogen transmission. Curr Issues Mol Biol. 2017;21(1):21-40. doi:10.21775/cimb.021.021

65. Loveland KL, Klein B, Pueschl D, et al. Cytokines in male fertility and reproductive pathologies: immunoregulation and beyond. Front Endocrinol. 2017;8:307. doi:10.3389/fendo.2017.00307
66. Flannagan RS, Heit B, Heinrichs DE. Antimicrobial mechanisms of macrophages and the immune evasion strategies of *Staphylococcus aureus*. Pathogens. 2015;4(4):826-868. doi:10.3390/pathogens4040826
67. Gouin J-P, Hantsoo L, Kiecolt-Glaser JK. Immune dysregulation and chronic stress among older adults: a review. Neuroimmunomodulation. 2008;15(4-6):251-259. doi:10.1159/000156468
68. Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT. Introduction. Ecological immunology. Philos Trans R Soc Lond B Biol Sci. 2009;364(1513):3-14. doi:10.1098/rstb.2008.0249
69. Liu Y, Hammer LA, Daamen J, Stork M, Egilmez NK, Russell MW. Microencapsulated IL-12 drives genital tract immune responses to intranasal gonococcal outer membrane vesicle vaccine and induces resistance to vaginal infection with *Neisseria gonorrhoeae*. mSphere. 2023;8(1):e00388-22. doi:10.1128/msphere.00388-22
70. Kuttiyarthu Veetil N, Henschen AE, Hawley DM, et al. Varying conjunctival immune response adaptations of house finch populations to a rapidly evolving bacterial pathogen. Front Immunol. 2024;15:1356062. doi:10.3389/fimmu.2024.1356062
71. Al-Nasiry S, Ambrosino E, Schlaepfer M, et al. The interplay between reproductive tract microbiota and immunological system in human reproduction. Front Immunol. 2020;11:378. doi:10.3389/fimmu.2020.00378
72. de Oliveira Mann CC, Hornung V. Molecular mechanisms of nonself nucleic acid recognition by the innate immune system. Eur J Immunol. 2021;51(8):1897-1910. doi:10.1002/eji.202048945
73. Underhill DM, Goodridge HS. Information processing during phagocytosis. Nat Rev Immunol. 2012;12(7):492-502. doi:10.1038/nri3244
74. Pidwill GR, Gibson JF, Cole J, Renshaw SA, Foster SJ. The role of macrophages in *Staphylococcus aureus* infection. Front Immunol. 2020;11:620339. doi:10.3389/fimmu.2020.620339
75. Ryan RYM, Seymour J, Loukas A, Lopez JA, Ikonomopoulou MP, Miles JJ. Immunological responses to envenomation. Front Immunol. 2021;12:661082. doi:10.3389/fimmu.2021.661082
76. Chiurchiù V, Leuti A, Smoum R, Mechoulam R, Maccarrone M. Bioactive lipids ALIAmides differentially modulate inflammatory responses of distinct subsets of primary human T lymphocytes. FASEB J. 2018;32(10):5716-5723. doi:10.1096/fj.201800107R
77. Zhao H, Wu L, Yan G, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther. 2021;6(1):263. doi:10.1038/s41392-021-00646-5
78. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta. 2014;1843(11):2563-2582. doi:10.1016/j.bbamcr.2014.05.014
79. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-7218. doi:10.18632/oncotarget.23208
80. Tu H, Li YL. Inflammation balance in skeletal muscle damage and repair. Front Immunol. 2023;14:1133355. doi:10.3389/fimmu.2023.1133355
81. Azenabor A, Ekun AO, Akinloye O. Impact of inflammation on male reproductive tract. J Reprod Infertil. 2015;16(3):123-129.
82. Wang Y, Xie Z. Exploring the role of gut microbiome in male reproduction. Andrology. 2022;10(3):441-450. doi:10.1111/andr.13126
83. Kiecka A, Macura B, Szczepanik M. The influence of the reproductive system microbiota on female and male fertility. The role of bacteria. Adv Microbiol. 2021;60(2):151-159. doi:10.33073/pjm-2021-021
84. Koedooder R, Mackens S, Budding A, et al. Identification and evaluation of the microbiome in the female and male reproductive tracts. Hum Reprod Update. 2018;25(3):298-325. doi:10.1093/humupd/dmy048
85. Altmäe S, Franasiak JM, Mändar R. The seminal microbiome in health and disease. Nat Rev Urol. 2019;16(12):703-721. doi:10.1038/s41585-019-0250-y
86. Lv S, Huang J, Luo Y, et al. Gut microbiota is involved in male reproductive function: a review. Front Microbiol. 2024;15:1344057. doi:10.3389/fmicb.2024.1344057
87. Maciel-Fiuza MF, Muller GC, Campos DMS, et al. Role of gut microbiota in infectious and inflammatory diseases. Front Microbiol. 2023;14:1098386. doi:10.3389/fmicb.2023.1098386
88. Kaltsas A, Zachariou A, Markou E, Dimitriadis F, Sofikitis N, Pournaras S. Microbial dysbiosis and male infertility: understanding the impact and exploring therapeutic interventions. J Pers Med. 2023;13(10):1481. doi:10.3390/jpm13101481
89. Zuber A, Peric A, Pluchino N, Baud D, Stojanov M. Human male genital tract microbiota. Int J Mol Sci. 2023;24(8):6939. doi:10.3390/ijms24086939
90. Teixeira TA, Oliveira YC, Bernardes FS, et al. Viral infections and implications for male reproductive health. Asian J Androl. 2021;23(4):335-347. doi:10.4103/aja.aja_88_20
91. Keck C, Gerber-Schäfer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. Hum Reprod Update. 1998;4(6):891-903. doi:10.1093/humupd/4.6.891
92. Mändar R. Microbiota of male genital tract: impact on the health of man and his partner. Pharmacol Res. 2013;69(1):32-41. doi:10.1016/j.phrs.2012.10.019
93. Abayasekara LM, Perera J, Chandrasekharan V, et al. Detection of bacterial pathogens from clinical specimens using conventional microbial culture and 16S metagenomics: a comparative study. BMC Infect Dis. 2017;17(1):631. doi:10.1186/s12879-017-2727-8
94. Franco-Duarte R, Černáková L, Kadam S, et al. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019;7(5):130. doi:10.3390/microorganisms7050130
95. Yamamoto Y. PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. Clin Diagn Lab Immunol. 2002;9(3):508-514. doi:10.1128/CDLI.9.3.508-514.2002
96. Artika IM, Dewi YP, Nainggolan IM, Siregar JE, Antonjaya U. Real-time polymerase chain reaction: current techniques, applications, and role in COVID-19 diagnosis. Genes (Basel). 2022;13(12):2387. doi:10.3390/genes13122387
97. Wang M, Liu H, Ren J, et al. Enzyme-assisted nucleic acid amplification in molecular diagnosis: a review. Biosensors (Basel). 2023;13(2):160. doi:10.3390/bios13020160
98. Kang T, Lu J, Yu T, Long Y, Liu G. Advances in nucleic acid amplification techniques (NAATs): COVID-19 point-of-care diagnostics as an example. Biosens Bioelectron. 2022;206:114109. doi:10.1016/j.bios.2022.114109
99. Bahrulolum H, Tarrahimofrad H, Rouzbahani FN, et al. Potential of CRISPR/Cas system as emerging tools in the detection of viral hepatitis infection. Virol J. 2023;20(1):91. doi:10.1186/s12985-023-02058-2
100. Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results. Br J Ophthalmol. 2006;90(10):1271-1276. doi:10.1136/bjo.2006.096263
101. Vijayakumar T, Divya B, Vasanthi V, Narayan M, Kumar AR, Krishnan R. Diagnostic utility of Gram stain for oral smears—a review. J Microsc Ultrastruct. 2023;11(3):130-134. doi:10.4103/JMAU.JMAU_81_21
102. Nayak S. Development and evaluation of point-of-care diagnostic technologies for providers and consumers. Dissertation. Columbia University; 2018.
103. Murgia L. Development of a new medical device for the diagnosis and management of urinary tract infections (UTIs) [Dissertation]. Rome, Italy: Roma Tre University; 2020.
104. Teixeira TA, Oliveira YC, Bernardes FS, et al. Viral infections and implications for male reproductive health. Asian J Androl. 2021;23(4):335-347. doi:10.4103/aja.aja_88_20

105. Di Martino P. Antimicrobial agents and microbial ecology. AIMS Microbiol. 2022;8(1):1-4. doi:10.3934/microbiol.2022001
106. Afrakhteh M, Mahdavi A, Beyhaghi H, Moradi A, Gity S, Zafargandi S, Zonoubi Z. The prevalence of *Chlamydia trachomatis* in patients who remained symptomatic after completion of sexually transmitted infection treatment. Iran J Reprod Med. 2013;11(4):285-292.
107. Van Ommen CE, Malleson S, Grennan T. A practical approach to the diagnosis and management of chlamydia and gonorrhea. CMAJ. 2023;195(24):E844-E849. doi:10.1503/cmaj.221821
108. Gajic I, Kabic J, Kekic D, et al. Antimicrobial susceptibility testing: a comprehensive review of currently used methods. Antibiotics (Basel). 2022;11(4):427. doi:10.3390/antibiotics11040427
109. Rybak MJ, McGrath BJ. Combination antimicrobial therapy for bacterial infections. Guidelines for the clinician. Drugs. 1996;52(3):390-405. doi:10.2165/00003495-199652030-00005
110. Worthington RJ, Melander C. Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol. 2013;31(3):177-184. doi:10.1016/j.tibtech.2012.12.006
111. Adeniji OO, Nontongana N, Okoh JC, Okoh AI. The potential of antibiotics and nanomaterial combinations as therapeutic strategies in the management of multidrug-resistant infections: a review. Int J Mol Sci. 2022;23(23):15038. doi:10.3390/ijms232315038
112. Wang EJ, Karri J, Tontisirin N, Cohen SP. Antimicrobial therapies for chronic pain (part 1): analgesic mechanisms. Korean J Pain. 2023;36(3):281-298. doi:10.3344/kjp.23135
113. Alorfi NM. Pharmacological methods of pain management: narrative review of medication used. Int J Gen Med. 2023;16:3247-3256. doi:10.2147/IJGM.S419239
114. Ronghe V, Modak A, Gomase K, Mahakalkar MG. From prevention to management: understanding postoperative infections in gynaecology. Cureus. 2023;15(10):e46319. doi:10.7759/cureus.46319
115. Filardo S, Di Pietro M, Sessa R. Current progresses and challenges for microbiome research in human health: a perspective. Front Cell Infect Microbiol. 2024;14:1377012. doi:10.3389/fcimb.2024.1377012
116. Yen S, Johnson JS. Metagenomics: a path to understanding the gut microbiome. Mamm Genome. 2021;32(4):282-296. doi:10.1007/s00335-021-09889-6
117. Molina MA, Melchers WJG, Andralojc KM, Leenders WPJ, Huynen MA. Longitudinal analysis on the ecological dynamics of the cervicovaginal microbiome in hrHPV infection. Comput Struct Biotechnol J. 2023;21:4424-4431. doi:10.1016/j.csbj.2023.08.026
118. Jo EK. Interplay between host and pathogen: immune defense and beyond. Exp Mol Med. 2019;51(12):1-3. doi:10.1038/s12276-019-0281-2
119. Gonzalez-Covarrubias V, Martínez-Martínez E, Del Bosque-Plata L. The potential of metabolomics in biomedical applications. Metabolites. 2022;12(2):194. doi:10.3390/metabo12020194
120. Chen J, Chen J, Fang Y, et al. Microbiology and immune mechanisms associated with male infertility. Front Immunol. 2023;14:1139450. doi:10.3389/fimmu.2023.1139450
121. Pillay S, Calderón-Franco D, Urhan A, Abeel T. Metagenomic-based surveillance systems for antibiotic resistance in non-clinical settings. Front Microbiol. 2022;13:1066995. doi:10.3389/fmicb.2022.1066995