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The Microbial Dimension of Male Sterility: A Pathological Review of Dysbiosis, Virulence, and the Emergent Diagnostic Imperative

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Abstract

For decades, the male genitourinary tract was considered sterile in healthy individuals. However, advances in culture-independent molecular techniques, particularly next-generation sequencing, have revealed that semen contains a diverse microbial ecosystem known as the seminal microbiome. This paradigm shift has led to growing interest in the relationship between microbial composition and male reproductive health. Current evidence suggests that male infertility is associated not merely with the presence of bacteria in semen, but with alterations in microbial balance, termed seminal dysbiosis. Beneficial genera such as *Lactobacillus* are often associated with normal sperm parameters and genomic stability, whereas increased abundance of opportunistic and pathogenic bacteria—including *Escherichia coli, Enterococcus faecalis, Ureaplasma urealyticum,* and *Prevotella*—is associated with reduced sperm motility, abnormal morphology, and decreased sperm concentration. The pathological mechanisms underlying this relationship involve both host-mediated and pathogen-mediated pathways. Dysbiosis has been suggested to promote chronic inflammation and leukocytospermia, leading to excessive production of reactive oxygen species and consequent oxidative stress. This environment promotes lipid peroxidation and sperm deoxyribonucleic acid (DNA) fragmentation, key contributors to impaired fertilization and poor reproductive outcomes. Despite increasing recognition of the seminal microbiome's role in infertility, current diagnostic approaches remain limited. Culture-based methods frequently fail to detect viable but non-culturable microorganisms, while molecular assays cannot reliably distinguish viable pathogens from residual DNA. Improved diagnostic strategies targeting microbial viability and virulence may therefore play an important role in addressing unexplained male infertility.

Keywords: Bacteriospermia, dysbiosis, male infertility, seminal microbiome

Introduction

I. The Paradigm Shift: From Sterile Assumption to Dynamic Ecosystem

A. Deconstruction of the "Sterile" Hypothesis

For decades, male reproductive biology was framed by the assumption that the male genitourinary tract was sterile in healthy, asymptomatic individuals. This concept was shaped less by definitive evidence than by the limitations of the diagnostic methods available at the time. Conventional microbiological evaluation relied on culture-dependent techniques, which detect only organisms capable of growing on laboratory media. As a result, a large proportion of microorganisms that are difficult to culture, metabolically quiescent, or present in low abundance remained undetected (1). Consequently, bacteria isolated from semen were often dismissed as contaminants or interpreted only as overt pathogens rather than members of a resident microbial ecosystem (2).

This view has now been fundamentally overturned. With the advent of culture-independent molecular methods, particularly next-generation sequencing targeting the 16S ribosomal ribonucleic acid gene, it has become clear that semen from healthy fertile men is not sterile (2,3). Instead, it contains a diverse and relatively complex microbial community. This shift mirrors the broader transformation in microbiome research seen in the gut and other mucosal systems. The male reproductive tract is therefore better understood as a dynamic microbial ecosystem with potentially important effects on fertility, reproductive tract homeostasis, and assisted reproduction outcomes (3).

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The clinical importance of this shift lies in the fact that male infertility can no longer be explained solely by endocrine, genetic, anatomical, or classic infectious causes. A microbial dimension must now be considered, one that lies between overt infection and health. This new framework provides a biologically plausible explanation for many cases that have historically been labeled idiopathic.

B. Characterizing the Core Seminal Microbiome and Defining Dysbiosis

The semen bacteriome, defined as the total bacterial genetic content present in semen, has become a major area of research interest (4). Studies consistently demonstrate that semen is microbiologically rich and diverse in both fertile and infertile men (5). This observation is critical because it indicates that bacteriospermia alone is not necessarily pathological. The mere presence of microorganisms is insufficient to define disease; rather, the structure, relative abundance, and functional behavior of the microbial community determine whether it is beneficial, neutral, or harmful.

This may contribute to the concept of seminal dysbiosis, which describes a disturbance in the normal microbial composition and function of the male reproductive tract (6). As in other organ systems, dysbiosis refers not simply to microbial presence, but to an imbalance that alters local metabolism, immune signaling, and tissue homeostasis. In the seminal environment, this disturbance may compromise sperm viability, motility, membrane integrity, and genomic stability.

Comparative studies between fertile and infertile men have begun to identify microbial signatures associated with eubiosis and dysbiosis. A eubiotic seminal microbiome is often characterized by predominance of genera such as *Lactobacillus*, including *Lactobacillus iners* and *Lactobacillus gasseri* (3). These organisms are generally associated with better sperm quality and deoxyribonucleic acid (DNA) integrity and may exert protective effects by maintaining ecological balance and limiting overgrowth of opportunistic pathogens (3).

By contrast, dysbiotic states are often characterized by relative overrepresentation of genera such as *Prevotella*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Enterococcus faecalis* (*E. faecalis*), *Anaerococcus*, *Corynebacterium*, *Pseudomonas*, and uropathogenic strains of *Escherichia coli* (*E. coli*) (3,5). A core group of detrimental microbes are summarized in Table 1. These organisms have been repeatedly associated with poorer semen parameters and impaired reproductive outcomes. A growing body of evidence, including systematic reviews and meta-analyses, supports a significant association between microbial dysbiosis and male infertility (6).

The important conceptual point is that the microbiological problem in male infertility is often not an acute infection but an ecological imbalance. This reframes the discussion from the presence or absence of pathogens to whether the seminal microbial community has shifted from a protective to a pathogenic configuration.

C. Broader Implications of the New Paradigm

The implications of the seminal microbiome extend beyond the male patient. Semen is not an isolated fluid; it is exchanged during intercourse and may influence the reproductive ecology of the female partner. Sexual transmission of microorganisms can alter the vaginal microbiome, supporting the concept of a "couple genital microbiota" (7,8). In this model, dysbiosis in the male partner may contribute to dysbiosis in the female reproductive tract, thereby affecting conception, implantation, and pregnancy maintenance (7,9).

This broader perspective is important because it reframes male-factor infertility as a couple-level biological interaction rather than an isolated male issue. Studies suggest that couple-level dysbiosis may impair fertility, disturb embryo implantation, and increase miscarriage risk (9). It is therefore increasingly plausible that seminal microbiome abnormalities may exert effects even when conventional semen parameters are only modestly altered.

The seminal microbiome may also influence outcomes of assisted reproductive technologies (ART). Microbial composition in semen used for in vitro fertilization or intracytoplasmic sperm injection (ICSI) may affect fertilization, embryo quality, blastocyst development, and implantation success (3). This is particularly relevant in ICSI, where sperm that appear motile may still carry profound molecular damage related to microbial-induced oxidative stress.

Another emerging implication concerns paternal programming of offspring health. Semen transmits more than paternal DNA; it also contains cytokines, metabolites, extracellular vesicles, and microbial signals (2). The seminal microbiome has therefore been proposed as a contributor to the Developmental Origins of Health and Disease framework, potentially influencing embryo development and later offspring phenotype (2).

Perhaps the most immediate clinical implication is its relevance to idiopathic infertility. A substantial proportion of male infertility, often estimated at 30-50%, remains unexplained after standard evaluation (10). The discovery of a structured and functionally relevant seminal microbiome suggests that many of these "idiopathic" cases may in fact reflect undiagnosed dysbiosis or microbial persistence that escapes routine diagnostic methods (11). This possibility fundamentally challenges current diagnostic algorithms in andrology.

Table 1. Microbial signatures of seminal dysbiosis: a comparison of genera associated with fertile and infertile states

Bacterial genus/species	Associated state	Reported impact on sperm parameters & reproductive health	Key supporting sources
Lactobacillus (e.g., *L. iners, L. gasseri*)	Beneficial	Associated with higher sperm quality and DNA integrity; appears to protect sperm quality	(3)
Prevotella	Detrimental	Opposing effect to *Lactobacillus*; associated with poor sperm quality, lower sperm counts, and motility issues	(3)
Enterococcus faecalis	Detrimental	Negatively impacts semen parameters; linked to sperm apoptosis and hyperviscosity. Commonly identified in chronic bacterial prostatitis	(3,5,15)
Ureaplasma urealyticum	Detrimental	Negatively impacts semen parameters; associated with reduced sperm motility and quality. Linked to chronic prostatitis	(3,5,6)
Mycoplasma hominis	Detrimental	Negatively impacts sperm motility and quality	(3,5)
Escherichia coli	Detrimental	Linked to acrosome damage and sperm DNA fragmentation; associated with chronic prostatitis	(3,6,15)
Pseudomonas	Detrimental	Imbalance of *Pseudomonas* relative to *Lactobacillus* is associated with excessive semen viscosity and OAT	(3,9)
Corynebacterium	Detrimental	Increased prevalence in infertile men; may reduce sperm motility and morphology	(3,15)
Anaerococcus	Detrimental	Found to be negatively correlated with sperm quality	(3)
Bacteroides	Detrimental	Systematically affects sperm morphology and DNA; can lead to mitochondrial disruption	(3)

OAT: Oligoasthenoteratozoospermia, DNA: Deoxyribonucleic acid, *L. iners: Lactobacillus iners, L. gasseri: Lactobacillus gasseri*

II. Pathologies of Seminal Dysbiosis: The Dual-mechanism Assault on Sperm

Seminal dysbiosis typically does not present as an acute symptomatic infection. Rather, it often manifests as chronic, low-grade, asymptomatic inflammation with persistent effects on spermatozoa. This pathology is especially insidious because there may be no pain, fever, or obvious urinary symptoms to trigger urologic investigation. However, sperm function may be continuously impaired through two main pathways: an indirect, host-mediated inflammatory pathway and a direct, pathogen-mediated virulence pathway.

A. Indirect Pathology: The Inflammatory-oxidative Axis

One of the clearest clinical correlates of this state is National Institute of Health category IV prostatitis, an asymptomatic inflammatory condition diagnosed by inflammatory findings in semen, prostatic secretions, or biopsy tissue (12,13). Research demonstrates that men with this condition often harbor abundant polymicrobial communities and higher total bacterial concentrations than controls (14). This provides a strong link between seminal dysbiosis and chronic subclinical reproductive tract inflammation, as further supported by the microbial patterns outlined in Table 1, where inflammation-associated species such as *E. coli* and *E. faecalis* are recurrently identified in conjunction with impaired semen parameters

A major manifestation of this inflammatory state is leukocytospermia, defined by elevated peroxidase-positive leukocytes in semen (15). These leukocytes, particularly polymorphonuclear cells, act as a major source of damage. Once activated in response to microbial signals, they produce large amounts of reactive oxygen species (ROS) through an oxidative burst intended to destroy microorganisms (16).

However, spermatozoa are highly vulnerable bystanders in this environment. Excessive ROS generation overwhelms the limited antioxidant defense capacity of seminal plasma, resulting in oxidative stress (16). This oxidative milieu damages sperm membranes, disrupts motility, alters membrane fluidity, and contributes to DNA fragmentation. ROS, therefore, represent one of the central mechanistic links between dysbiosis and infertility.

A notable feature of this system is that oxidative stress arises from two sources. Host leukocytes generate ROS as part of the inflammatory response, but pathogenic bacteria themselves also contribute to oxidative stress (17,18). For example, *E. faecalis* produces extracellular superoxide and hydrogen peroxide (19), *E. coli* generates hydrogen peroxide as a by-product of aerobic metabolism, and *Ureaplasma urealyticum* also contributes hydrogen peroxide capable of directly injuring sperm membranes (20). Thus, seminal dysbiosis produces a compounded oxidative environment in which both host and pathogen contribute to sperm damage.

Chronicity is further sustained by microbial immune modulation. *Staphylococcus aureus* (*S. aureus*), for example, produces superantigens that provoke broad, non-specific activation of

T-cells and favor a Th1/Th17-dominant inflammatory response (21-23). Instead of promoting efficient pathogen clearance, this process amplifies cytokine production and perpetuates tissue-damaging inflammation. Likewise, Gram-negative organisms such as *E. coli* activate toll-like receptor pathways through lipopolysaccharide (LPS), driving additional production of tumour necrosis factor-α, interleukin-6 (IL-6), and IL-8 (24). In this way, dysbiosis sustains a low-grade inflammatory microenvironment that is highly damaging to sperm but often clinically silent.

B. Direct Pathology: Virulence and Toxicity

In addition to indirect inflammatory injury, many bacteria within a dysbiotic seminal microbiome have evolved direct mechanisms for impairing sperm function. These effects are not merely collateral consequences of infection but, rather, represent sperm-specific or sperm-relevant virulence actions. Table 2 provides a detailed summary of these direct "sperm-centric" virulence mechanisms, demonstrating how different pathogens exert targeted molecular effects on spermatozoa. For example, *S. aureus* produces a sperm immobilization factor that inhibits Mg++ adenosine triphosphatase (ATPase) activity; *E. coli* employs fimbriae-mediated adhesion and toxin-induced membrane damage; and *Ureaplasma urealyticum* disrupts membrane phospholipids via enzymatic degradation and oxidative injury. Collectively, these findings highlight that bacterial effects on fertility are not merely indirect consequences of inflammation but also involve direct, pathogen-specific interference with sperm structure and function.

S. aureus provides one of the most striking examples. *In vitro* studies show that exposure of human spermatozoa to live *S. aureus* is linked to rapid, dose-dependent loss of motility and marked agglutination, whereas heat-killed organisms do not produce the same effect (25). This indicates an actively secreted factor rather than a passive structural interaction. That factor, termed sperm immobilization factor, is a protein of approximately 20 kilodalton that binds directly to sperm and inhibits Mg++ ATPase activity (26). Because this enzyme is essential for energy transduction involved in flagellar motion, its inhibition may contribute to sperm immobilization. The factor also interferes with acrosome reaction, further compromising fertilizing capacity (26).

Other pathogens in the dysbiotic community employ their own "weapons" to neutralize sperm, as detailed in Table 2.

E. coli affects sperm through several complementary pathways. It can attach directly to sperm via pili and flagella, particularly through interactions with mannose-containing receptors (20). This adhesion may itself reduce motility. In addition, *E. coli* secretes LPS, porins, and other factors that can damage the acrosome, impair membrane function, and is suggested to promote apoptosis (3,27). Outer membrane vesicles shed by uropathogenic *E. coli* have been shown to impair sperm function in experimental settings and to be associated with reduced semen quality. Moreover, they promote oxidative stress and DNA fragmentation (28).

E. faecalis exerts pathogenic effects through virulence factors such as hemolysin, which can disrupt membrane integrity of the sperm head, neck, and midpiece (20). Even when effects on motility are less dramatic than those caused by *S. aureus*, membrane disruption and oxidative injury may still significantly impair reproductive potential.

Ureaplasma urealyticum secretes phospholipases A and C, which directly degrade membrane phospholipids, weakening sperm

Table 2. Direct pathogenic mechanisms of sperm impairment

Pathogen	Virulence factor(s)	Mechanism of action	Consequence for sperm	Supporting sources
Staphylococcus aureus	Sperm immobilization factor (SIF)	A secreted ~20 kDa protein. Binds to a receptor on the sperm surface. Inhibits the sperm's Mg++ ATPase activity	Rapid, 100% sperm immobilization; sperm agglutination (clumping); inhibition of acrosome reaction	(25,26)
Escherichia coli	LPS, porins, fimbriae	Fimbriae/pili bind directly to mannose receptors on the sperm surface. Secreted toxins (LPS, porins) damage membranes and bind to cell receptors	Sperm immobilization/agglutination; severe acrosome damage; induction of apoptosis (programmed cell death)	(3,20,27)
Enterococcus faecalis	Hemolysin	A well-known cytotoxic virulence factor that functions as a toxin to lyse cell membranes	Direct damage to the membrane integrity of the sperm head, neck, and mid-piece	(20)
Ureaplasma urealyticum	Phospholipase A & C; H_2O_2	Secreted enzymes enzymatically degrade and "injure" the phospholipid components of the sperm membrane. H_2O_2 adds peroxidative damage	Loss of sperm membrane integrity; reduced sperm motility and morphology	(20)
Pseudomonas aeruginosa	Exotoxin A; porin	Exotoxin A specifically targets proteins in the sperm tail. Porin binds to sperm membrane receptors	Direct reduction in sperm motility; induction of apoptosis	(27)

kDA: Kilodalton, LPS: Lipopolysaccharide, ATPase: Adenosine triphosphatase

membranes and making them more susceptible to oxidative damage (20). Its production of hydrogen peroxide contributes to further chemical injury. *Pseudomonas aeruginosa* also demonstrates sperm-directed pathogenicity through exotoxins and porins that impair motility and promote apoptosis (27).

A common endpoint of these direct bacterial effects is apoptosis (20). Sperm may enter programmed cell death either as a consequence of cumulative oxidative and membrane injury or due to direct triggering by bacterial toxins (27). This contributes to a reduced number of viable sperm, reduced functional quality, and, ultimately, infertility.

Taken together, these indirect inflammatory processes and direct pathogen-mediated virulence mechanisms do not operate in isolation but converge on a shared downstream pathway of cellular injury. Persistent oxidative stress, membrane disruption, and toxin-mediated damage collectively create a molecular environment that compromises sperm integrity at its most fundamental level.

Among these downstream effects, oxidative stress-induced sperm DNA fragmentation (SDF) emerges as a central and unifying endpoint. This molecular lesion integrates the cumulative impact of both host- and pathogen-driven injury and provides a measurable link between microbial dysbiosis and impaired reproductive outcomes.

Accordingly, the following section focuses on the molecular consequences of seminal dysbiosis, with particular emphasis on oxidative stress and SDF as key mediators of infertility.

III. The Molecular Nexus of Damage: Oxidative Stress and SDF

Although seminal dysbiosis affects sperm through multiple pathways, many of these converge at one critical molecular endpoint: SDF. This lesion provides a measurable and clinically meaningful summary of the oxidative and inflammatory burden imposed by the dysbiotic reproductive tract. SDF is increasingly considered an extended functional test in the evaluation of male infertility, complementing conventional semen analysis (10).

Spermatozoa are biologically predisposed to oxidative vulnerability. Their plasma membranes contain high concentrations of polyunsaturated fatty acids, which are necessary for membrane fluidity and fertilization-related membrane dynamics (16). However, these lipids are highly susceptible to ROS-mediated lipid peroxidation. Once peroxidation begins, membrane architecture deteriorates, reducing flexibility, impairing motility, and compromising the ability of sperm to fuse with the oocyte (16,24).

Sperm have very limited intrinsic repair capacity. During maturation, spermatozoa lose most of their cytoplasm, which means they retain only restricted antioxidant defenses and limited capacity for DNA repair (16). They, unlike somatic cells, are unable to effectively repair oxidative lesions once these occur. They therefore depend heavily on extracellular antioxidant protection from seminal plasma. When ROS production from leukocytes and bacteria exceeds this protective capacity, oxidative injury becomes effectively irreversible (16,18,29).

ROS attack not only membranes but also DNA. They can oxidize DNA bases, generate lesions such as 8-hydroxy-2′-deoxyguanosine, and is suggested to promote both single- and double-strand breaks (16). In this context, SDF emerges as the downstream product of microbially induced pathology. Leukocytospermia is associated with increased oxidative stress and higher rates of DNA fragmentation (16). Likewise, bacteriospermia can induce intracellular ROS generation and trigger mitochondrial and apoptotic pathways leading to fragmented sperm DNA (30). *E. coli* outer membrane vesicles are also sufficient to produce oxidative stress and DNA damage (28).

SDF is therefore not merely an associated laboratory abnormality; it represents the integrated molecular signature of seminal microbial pathology. It captures the cumulative effects of chronic inflammation, bacterial ROS production, membrane damage, and apoptotic signaling. In men with otherwise unexplained infertility, high SDF may therefore serve as an indirect marker of hidden dysbiosis.

Clinical Impact: SDF and ART Failure

The reproductive consequences of high SDF are profound. Damaged sperm may still appear motile and be selected for use in ART, particularly during ICSI. Yet injection of a motile sperm with fragmented DNA introduces a compromised paternal genome into the oocyte (31). Although oocytes possess DNA repair mechanisms, severe SDF may exceed maternal repair capacity (32).

The clinical and statistical findings summarized in Table 3 consistently demonstrate that elevated SDF is associated with adverse reproductive outcomes across multiple ART modalities. High SDF levels are linked to reduced fertilization rates, impaired embryo quality, decreased blastocyst formation, lower implantation rates, and increased miscarriage risk. This convergence of evidence reinforces SDF's role as a clinically meaningful downstream marker of microbially induced reproductive damage and a critical determinant of ART success.

The downstream effects include impaired fertilization, poorer embryo quality, reduced blastocyst formation, embryo arrest, lower implantation rates, reduced clinical pregnancy rates, and increased miscarriage risk (32,33). Even in ICSI cycles, where mechanical sperm selection overcomes some conventional

semen deficits, high SDF remains associated with poorer reproductive outcomes (33).

Thus, the clinical relevance of microbial-induced sperm injury extends far beyond abnormal semen analysis. It affects embryo competence, implantation, and pregnancy maintenance, making dysbiosis a plausible hidden contributor to ART failure.

IV. The Diagnostic Gap: Why "Idiopathic" Infertility is Often a Failure of Detection

Current diagnostic tools in andrology remain limited in their ability to fully capture functional and molecular abnormalities, contributing to the persistence of idiopathic infertility (10). Despite the growing evidence linking seminal dysbiosis to infertility, this pathology is often missed by standard clinical diagnostics. This creates a major diagnostic gap and may explain why idiopathic male infertility remains common (11). The core problem is not necessarily the absence of pathology, but rather the failure to detect it. A central factor in this failure is the viable but non-culturable (VBNC) state.

A. The Hidden Reservoir: VBNC Pathogens

The VBNC state is a survival strategy used by many bacteria under environmental stress, including nutrient deprivation, temperature change, or antibiotic exposure (34). In this condition, bacteria remain alive, maintain cellular integrity, and often retain virulence potential, but they no longer grow on standard culture media (34,35). Importantly, these dormant organisms can later resuscitate and regain active pathogenicity when conditions improve (36).

This phenomenon is highly relevant to chronic and recurrent urogenital infection. Studies on uropathogenic *E. coli* show that after antibiotic treatment, bacteria may disappear from culture yet remain detectable by molecular assays and viability testing, indicating persistence in a VBNC state (37). These cells can act as a reservoir for relapse.

The same logic applies to male infertility. Pathogens associated with chronic prostatitis and impaired sperm quality, such as *E. faecalis*, are known to enter the VBNC state (38-40). This creates a vicious cycle: a patient receives antibiotics for a reproductive tract infection; the culture becomes negative; the patient is considered cured; yet viable, dormant organisms persist in the prostate or seminal tract, where they continue to drive low-grade inflammation, oxidative stress, and sperm damage. The result is a patient with culture-negative semen, high SDF, and infertility, subsequently labeled as idiopathic.

B. Failure of Current Diagnostic Standards

Table 4 systematically outlines the limitations of current diagnostic approaches and highlights a fundamental mismatch between microbial biology and conventional detection methods. Culture-based techniques fail to detect VBNC organisms, leading to false-negative results, whereas standard PCR detects microbial DNA without distinguishing between viable, dormant, and dead cells, resulting in clinical ambiguity and potential overtreatment. This diagnostic gap provides a compelling explanation for the persistence of "idiopathic" infertility despite apparently negative microbiological findings.

Culture-based methods fail because they depend on bacterial growth. If viable bacteria are dormant and non-culturable, the test becomes falsely negative (41). A patient may, therefore, harbor an ongoing pathogenic reservoir despite all standard cultures remaining negative.

Standard polymerase chain reaction (PCR) based methods overcome some sensitivity issues, but they create another problem: they cannot reliably distinguish DNA from live bacteria, dormant bacteria, and dead organisms (42,43). A positive PCR result may reflect an active infection, a dormant VBNC population, or only residual DNA from non-viable cells. This ambiguity limits clinical interpretation and may lead to inappropriate antibiotic use. Moreover, unnecessary antibiotics may reinforce bacterial dormancy or select for persistence.

Table 3. Clinical impact of high sperm DNA fragmentation (SDF) on assisted reproductive technology (ART) outcomes

ART modality	Measured outcome	Quantitative impact of high SDF (from systematic reviews and meta-analyses)	Key supporting sources
IVF	Fertilization rate	Reduced. High SDF (e.g., >27-30%) is associated with a significant reduction in the oocyte fertilization rate	(33)
IVF/ICSI	Embryo quality	Reduced. High SDF is significantly linked to a lower rate of high-quality embryos and altered developmental kinetics	(33)
IVF/ICSI	Blastocyst rate	Reduced. A strong negative correlation exists between SDF levels and blastulation rates. High DNA damage "promotes embryo arrest."	(32)
IVF/IUI	Clinical pregnancy rate	Reduced. High SDF is associated with a reduction in pregnancy rates in IUI and frozen embryo transfer cycles	(33)
IVF	Abortion/miscarriage rate	Increased. Patients with high sperm DNA fragmentation show an increased rate of abortion	(33)
IVF/ICSI	Implantation rate	Reduced. A reduction in implantation rate is observed in patients with high SDF	(33)

IVF: In vitro fertilization, ICSI: Intracytoplasmic sperm injection, IUI: Intrauterine insemination

Table 4. Comparative analysis of diagnostic modalities for seminal pathogen detection					
Diagnostic method	**Principle of detection**	**Ability to detect VBNC cells**	**Common clinical failure**	**Clinical consequence**	**Supporting sources**
Culture-based methods (the "Gold Standard")	Requires bacterial metabolic activity and reproduction (growth) on nutrient-rich media to form a visible colony	No. By definition, VBNC cells are non-culturable	False-negative	Fails to detect the "hidden reservoir" of viable pathogens. Leads to a "culture-negative" status and misdiagnosis of "idiopathic infertility."	(1,39,41,44)
Standard PCR (molecular)	Amplification of specific nucleic acid (DNA/RNA) sequences	Yes (detects their DNA)	False-positive/ ambiguous	Cannot differentiate between live (virulent), dormant (VBNC), and dead (non-pathogenic) cells	(42)
				Leads to clinical ambiguity and the harmful overtreatment of non-viable bacterial DNA with antibiotics	(43)
DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, VBNC: Viable but non-culturable					

Together, these limitations explain why many men with inflammation, leukocytospermia, or high SDF remain microbiologically "negative" by routine testing. The consequence is the underdiagnosis of a potentially treatable microbial component of infertility.

V. The Future Imperative: Next-generation Diagnostics for Viability and Virulence

To address this diagnostic gap, infertility diagnostics must evolve beyond simple presence-or-absence testing. A clinically useful platform must distinguish three microbial states: dead, dormant, and metabolically active. Ideally, it should also determine whether the detected organism is actually expressing virulence traits relevant to reproductive injury (44).

A. Detecting Viability

One promising approach is viability-PCR (v-qPCR) using dyes such as propidium monoazide or ethidium monoazide (44). These agents penetrate cells with damaged membranes, bind their DNA after photoactivation, and prevent amplification during PCR. DNA from viable cells with intact membranes remains amplifiable. This allows selective detection of viable organisms while excluding signal from dead bacteria (44,45).

This approach directly addresses the false-positive limitation of standard PCR and helps determine whether detected bacteria are still biologically relevant.

B. Detecting Metabolic Activity

Viability alone does not distinguish dormant VBNC cells from metabolically active pathogens. To make this distinction, diagnostics must assess metabolic activity.

ATP bioluminescence is one of the most established approaches. ATP is a universal marker of cellular metabolism and is rapidly degraded after cell death (46). In ATP-based assays, light emission generated through luciferase activity is proportional to ATP concentration, enabling quantification of metabolic activity (46,47). In principle, dead cells yield no signal, dormant cells yield low signal, and active cells yield high signal.

Other metabolic approaches include microfluidic systems measuring oxygen consumption, fluorogenic substrates that report esterase activity, and electrochemical sensors detecting metabolic by-products or changes in medium conductance (48-50). These technologies move diagnostics from static identification toward functional assessment.

C. Detecting Virulence

The ultimate clinical question is not simply whether a bacterium is present or alive, but whether it is dangerous. Many organisms in the seminal microbiome may be commensal, context-dependent, or only weakly pathogenic (51). Thus, future diagnostics should target virulence factor expression.

This may include detection of genes or proteins encoding adhesins, toxins, hemolysins, sperm-immobilizing factors, or quorum-sensing molecules (52-54). A pathogen such as *E. coli* may be present in both commensal and virulent forms, and a useful clinical test must discriminate between them (51,52). Virulence-based sensing would offer a much more actionable assessment of fertility risk than simple taxonomic detection alone. Emerging diagnostic technologies addressing these limitations are summarized in Table 5, which illustrates a shift toward functional microbiological assessment. Platforms such as v-qPCR, ATP bioluminescence, microfluidic metabolic assays, and virulence-factor biosensors enable simultaneous evaluation of microbial viability, metabolic activity, and pathogenic potential. These innovations represent a critical transition from static detection to dynamic characterization of microbial behavior, with direct implications for precision diagnostics for male infertility.

Table 5. Advanced biosensor technologies for differentiating pathogen viability and activity

Technology	Principle of detection	Target	Clinical question answered	Supporting sources
Viability-PCR (v-qPCR)	DNA-intercalating dyes (PMA, EMA) penetrate membrane-compromised cells and covalently bind to DNA, inhibiting PCR amplification	Cellular viability (membrane integrity)	"Filters out 'noise' from dead cells. What is the total viable pathogen load?"	(44)
ATP bioluminescence	Firefly luciferin/luciferase enzymatic reaction. Light output is directly proportional to ATP concentration	Metabolic activity (intracellular ATP level)	"Is the viable population active (high ATP) or dormant/VBNC (low ATP)?"	(44,47)
Microfluidic metabolic assays	Fluorogenic substrates (e.g., Calcein AM) or oxygen-sensitive fluorophores integrated into microfluidic chips	Metabolic Activity (e.g., esterase activity, respiration)	"Are these viable cells metabolically functional and healthy?"	(48,49)
Electrochemical biosensors	Measure changes in electrical properties (impedance, conductance) as bacteria metabolize substrates	Metabolic activity (metabolic byproducts)	"Is there active metabolism occurring in this sample?"	(50)
Virulence factor (VFG) biosensors	Detect the active expression of specific genes (e.g., pap, fim), the toxins themselves (e.g., SIF), or their signaling molecules (e.g., AHLs)	Pathogenic virulence (active gene/protein expression)	"Is this viable, active population actually harmful? Is it a pathogen or a harmless commensal?"	(52-56)

ATP: Adenosine triphosphate, DNA: Deoxyribonucleic acid, AHL: N-acyl homoserine lactone, SIF: Simulated intestinal fluid, PCR: Polymerase chain reaction, PMA: Propidium monoazide, EMA: Ethidium monoazide

D. Point-of-Care Integration

The ideal endpoint of these innovations is a point-of-care platform capable of rapidly assessing the viability, activity, and virulence of microbes in semen in an infertility clinic. Advances in microfluidics, electrochemical biosensing, and smartphone-assisted diagnostics suggest that this is feasible (55-63).

A future andrology clinic could, therefore, perform a standard semen analysis alongside an integrated microbial screen, thereby yielding a practical "diagnostic triumvirate":

1. Viability: Is there a viable bacterial load?

2. Activity: Is that population dormant or metabolically active?

3. Virulence: Is it expressing fertility-damaging traits?

Such a platform would greatly improve stratification of patients currently labeled as idiopathic.

From a clinical perspective, these mechanistic insights have important implications for the diagnostic approach to male infertility. In patients with unexplained infertility or discordance between standard semen parameters and reproductive outcomes, assessment of SDF may provide additional functional information beyond conventional semen analysis (64). Furthermore, the recognition of seminal dysbiosis as a potential contributing factor suggests that negative culture results should not exclude a microbial component, particularly in the presence of leukocytospermia or elevated oxidative stress markers. In such cases, adjunctive testing strategies, including molecular assays or emerging functional diagnostics targeting microbial viability and activity, may help refine patient stratification. Although these approaches are not yet fully standardized, they highlight a shift toward a more integrated diagnostic model combining conventional semen analysis with molecular and functional assessments to better guide individualized clinical decision-making.

In selected patients, particularly those with non-obstructive azoospermia, surgical sperm retrieval techniques such as microsurgical testicular sperm extraction may influence clinical outcomes, with factors such as follicle-stimulating hormone levels and surgical approach affecting success rates (65).

Despite the growing body of evidence linking seminal microbiome alterations to male infertility, several important limitations should be acknowledged. First, there is substantial heterogeneity in study methodologies, including differences in sample collection, processing, sequencing platforms, and bioinformatic pipelines, which complicates direct comparisons across studies. Second, the lack of standardized protocols and reference frameworks limits reproducibility and hinders the translation of research findings into clinical practice. Finally, a fundamental challenge lies in distinguishing pathogenic organisms from commensal or context-dependent microbiota. Many bacterial species identified in semen may exert variable effects depending on microbial community structure, host factors, and local immune responses. While current data support an association between microbial dysbiosis and impaired reproductive outcomes, caution is warranted when interpreting these findings, and further well-designed, standardized, and longitudinal studies are needed to clarify causal relationships and clinical applicability.

Conclusion

From Pathological Review to Clinical Strategy

The evidence reviewed here supports a major transformation in the understanding of male reproductive health. Semen is not sterile but is part of a dynamic microbial ecosystem. Within this ecosystem, seminal dysbiosis may represent a clinically important driver of male infertility.

The pathological sequence is increasingly coherent. A shift from a protective, eubiotic microbiome toward a dysbiotic community dominated by opportunistic or pathogenic organisms initiates chronic, low-grade inflammation and direct, sperm-targeted toxicity. Through host leukocyte activation, bacterial ROS production, membrane injury, toxin activity, and apoptotic signaling, these microbes create a damaging reproductive microenvironment.

The principal molecular endpoint of this pathology is oxidative stress-induced SDF. This lesion is of major clinical relevance because it links hidden microbial dysfunction with poor embryo development, implantation failure, miscarriage, and ART failure. In this framework, SDF becomes not only a marker of sperm quality but also a potential indicator of otherwise undetected seminal microbial pathology.

Current diagnostics fail to adequately capture this biology. Culture-based methods miss VBNC reservoirs, while conventional PCR cannot determine whether detected organisms are alive, dormant, dead, or virulent. This mismatch between biology and diagnostics likely contributes to the persistence of the "idiopathic male infertility" category.

The field, therefore, requires a new diagnostic strategy. Future approaches should integrate conventional semen analysis with microbial assays capable of determining viability, metabolic activity, and virulence. Technologies such as v-qPCR, ATP bioluminescence, metabolic biosensors, and virulence-factor detection offer a plausible path toward that goal.

In clinical terms, this would enable a more rational triage of infertile men. Those with active virulent infections could be treated directly; those with dormant VBNC reservoirs could be managed with strategies designed to address persistence rather than simple empiric antibiotic therapy; and those without evidence of relevant microbial pathology could proceed to evaluation for endocrine, genetic, anatomical, or vascular causes. These insights support a transition from a purely descriptive diagnostic paradigm toward a more mechanism-informed and individualized approach in andrology practice.

The microbial dimension of male infertility is no longer a peripheral hypothesis. It is an increasingly evidence-based framework that links dysbiosis, inflammation, oxidative stress, SDF, and reproductive failure. While the available evidence strongly supports an association between seminal dysbiosis and male infertility, a definitive causal relationship has not yet been established; further longitudinal and mechanistic studies are required. Future progress in male infertility diagnostics will depend on acknowledging this chain and developing tools that detect not just microbial presence, but microbial behavior. Only then will a substantial fraction of so-called idiopathic infertility become biologically explainable and potentially treatable.

From a clinical perspective, two key implications emerge. First, "idiopathic" male infertility may frequently reflect undetected microbial dysbiosis and oxidative sperm DNA damage. Second, precision andrology will require diagnostic platforms that assess not only microbial presence but also biological activity and pathogenic potential.

Footnotes

Authorship Contributions

Concept: M.B.D., A.K., Design: M.B.D., K.K., A.A., A.K., Analysis or Interpretation: S.Ç., Y.Ö., Literature Search: M.B.D., A.K., Writing: M.B.D., K.K., A.A., S.Ç., Y.Ö., A.K.

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