Micromanagement: how the male reproductive microbiome shapes male fertility

Sohei Kuribayashi, M.D., Ph.D.,[a] Noopur Naik, B.S.,[b] Aaron W. Miller, Ph.D.,[c] and Scott D. Lundy, M.D., Ph.D.[a]

[a] Department of Urology, Glickman Urological & Kidney Institute, Cleveland Clinic, Cleveland, Ohio; [b] Cleveland Clinic Lerner College of Medicine of Case Western Reserve University School of Medicine, Cleveland, Ohio; [c] Department of Cardiovascular and Metabolic Sciences, Cleveland Clinic, Cleveland, Ohio

Importance: One in six couples worldwide experience infertility, and male factors contribute to at least half of these cases. The pathophysiology of male infertility is complex and often multifactorial, and the male reproductive microbiome has now been implicated as a potentially critical component in male reproductive health and disease.
Objective: To summarize the emerging evidence describing the male reproductive microbiome with a focus on clinically relevant aspects within spermatogenesis and reproductive outcomes.
Evidence review: We performed a librarian-led search of MEDLINE, Embase, and Web of Science and a targeted PubMed search for antibiotic and probiotic trials, focusing on human studies that reported male reproductive microbiome data together with semen parameters, oxidative, deoxyribonucleic acid (DNA)–damage, or inflammatory indices, or assisted reproduction outcomes. Given heterogeneity in design and laboratory methods, we conducted a narrative synthesis and integrated relevant mechanistic animal work.
Findings: Across heterogeneous cohorts, anaerobe-leaning seminal profiles (often with *Prevotella* or *Atopobium*) are more often linked to higher oxidative stress, greater sperm DNA fragmentation, and lower motility or total motile count. In contrast, *Lactobacillus*-rich profiles more often align with better redox status and DNA integrity. Couple-level data show rapid and robust partner microbiome exchange that can shift semen over short periods. Work on the gut–testis axis has identified a link between gut dysbiosis and systemic inflammation with semen quality via the gut-testis axis. Proposed mechanisms underlying the effect of the microbiome on fertility include local inflammation, oxidative injury, altered seminal rheology (e.g., viscosity/viscoelastic properties of seminal plasma), adherence/biofilms, and immune–metabolic crosstalk. Small trials to date using antibiotics and/or probiotics show variable benefits; durability and reproductive endpoints remain limited.
Conclusion and relevance: Emerging evidence supports a role for the male reproductive microbiome in sperm function and fertility and highlights it as a potential target for clinical intervention. However, predominantly small, cross-sectional studies and methodological heterogeneity limit causal inference and immediate translation, underscoring the need for longitudinal, couple-integrated cohorts and adequately powered randomized trials of targeted microbiome modulation. (Fertil Steril® 2026;125:583–95. ©2026 by American Society for Reproductive Medicine.)

Key Words: male infertility, male reproductive microbiome, seminal microbiome, probiotics, gut-testis axis

Infertility is defined as a failure to conceive after ≥12 months of unprotected intercourse and conveys extensive social, relational, psychological, and medical consequences on the one in six couples worldwide who struggle with it (1). In half of these cases, male factor infertility is either a primary or contributing cause. Although some analyses have suggested an increase in the global burden of male infertility in recent decades, findings remain heterogeneous across regions and methodologies (1–4). Nevertheless, there is growing concern that environmental exposures, lifestyle factors, and the overall decline in global cardiometabolic health may contribute to worsening male reproductive outcomes (5–8). Beyond its reproductive implications, male infertility is increasingly recognized as a sentinel marker of overall health (9), with epidemiologic evidence suggesting that patients with infertility have increased all-cause mortality compared with their counterparts (10,11). Infertility is also associated with a higher prevalence of

Received November 28, 2025; revised January 19, 2026; accepted January 21, 2026; published online January 27, 2026.
Correspondence: Sohei Kuribayashi, M.D., Ph.D. (E-mail: kuribas@ccf.org).

Fertil Steril® Vol. 125, No. 4, April 2026 0015-0282

https://doi.org/10.1016/j.fertnstert.2026.01.019

chronic conditions (12). Several of these conditions, such as obesity, metabolic syndrome, and genetic disorders, are part of a constellation of well-documented factors contributing to male factor infertility and worsened overall health (13–17).

Other established etiologies of male infertility include anatomic factors, such as varicocele and congenital absence of the vas deferens (18), infections, including orchitis and sexually transmitted infections (19), gonadotoxic treatments, such as chemotherapy (20), and environmental/lifestyle triggers (21). A substantial proportion of cases, however, remain idiopathic, underscoring the need for additional insight into the underlying mechanisms.

The human microbiome has emerged as a critical component of general health (22–25). Its composition is shaped by diet, environment, and the presence of various diseases, all of which have also been implicated in male infertility (26, 27). Thus, the male reproductive microbiome has become an emerging focus in efforts to understand how microbial ecology may contribute to male reproductive health.

The human microbiome refers to the community of symbiotic bacteria, viruses, fungi, and microbes residing within and on the body, whereas the term microbiome more broadly refers to their collective genomes, metabolic products, and functional interaction with the host (28). Although the gut microbiome is a principal component, microbial communities exist throughout body systems, including male reproductive organs (29). Although these microbes have important roles in homeostasis, dysbiosis, defined as a shift in their composition or function toward a pathogenic state, has been implicated in diverse disease processes (30). Historically, only overt acute infections, such as urethritis, epididymitis, or acute bacterial prostatitis, were considered relevant to male reproductive health, and microorganisms in the male tract were viewed primarily as pathogens causing sexually transmitted or inflammatory disease (31, 32). However, culture-based reports in the 1970s and 1980s, followed by modern molecular surveys, suggested that this assumption was incomplete (33). Contemporary next-generation sequencing (NGS)–based studies now demonstrate distinct, low-biomass yet diverse microbial communities within semen and related organs, with compositional differences reported between fertile and infertile men, which raises the possibility of microbiome involvement in reproductive function (34, 35). However, because most studies rely on 16S rRNA gene sequencing of extracted deoxyribonucleic acid (DNA), such data cannot distinguish between viable and nonviable organisms, and rigorous contamination control is essential when conducting studies and interpreting results (36). Early investigations focused mainly on identifying pathogens causing urethritis, epididymitis, and prostatitis, whereas current work explores how commensal and opportunistic taxa may relate to inflammatory and oxidative pathways affecting sperm quality (37). This review summarizes current knowledge about microbial communities within the principal male reproductive organs and their clinical contexts. We provide this information as a foundational basis to understand future studies and provide a framework for the clinician interested in understanding how this may impact patient care in the near future.

METHODS

Information sources and librarian-led comprehensive search

A health sciences librarian executed database searches in Ovid MEDLINE ALL (1946–2025-08-26), Embase (1974–2025-08-26), and Web of Science–Science Citation Index (1965–present). Strategies combined controlled vocabulary (MeSH/Emtree) and free-text terms for male reproductive anatomy/infertility and microbiome/microbiota, with animal-only records and nonarticle document types excluded. Language was limited to English, and publication years to 2015–current. Result counts at the final librarian run were as follows: MEDLINE n = 933, Embase n = 921, Web of Science n = 1,027 (details of line-by-line strategies are provided in Supplementary Table S1).

Author-performed PubMed targeted search (interventions)

Because male-focused trials were sparse, we ran a PubMed targeted search restricted to human trials, using two Boolean queries (antibiotics, probiotics) and processing the pooled records as one set (deduplicated together; queries in Supplementary Table S2). PRISMA (combined set): identified 714, duplicates 329, screened 385, full texts 31, included 17 (Supplementary Figure S1).

Eligibility criteria

Inclusion (humans): male reproductive microbiome studies reporting at least one of: (i) semen parameters (concentration, motility, morphology, composite indices), (ii) DNA fragmentation/oxidative stress, (iii) inflammation/symptoms (e.g., leukocytospermia, NIH-CPSI), or (iv) Assisted Reproductive Technology (ART)–relevant outcomes (fertilization, embryo, clinical pregnancy, live birth). Interventions included antibiotics, probiotics/pre-/synbiotics, and related adjuncts.

Exclusion: animal-only, pediatric-only, case reports, conference abstracts only, reviews, or studies lacking primary microbiome data relevant to male reproductive sites/semen.

Deduplication and screening

Across MEDLINE/Embase/Web of Science, 2,881 records were retrieved. After deduplication (1,488 removed), 1,393 titles/abstracts were screened by two reviewers independently; 164 full texts were assessed; 143 studies met inclusion criteria for the main domains (semen, couple-level, gut-testis, other male sites) (Supplementary Figure S2). Of the 143 included reports, 60 were review articles without primary data and were excluded from synthesis. Among the remaining 100, five were subsequently excluded upon detailed appraisal because of scope mismatch, leaving 95 original studies for final synthesis, including 17 intervention trials identified through targeted PubMed search.

Data extraction, quality flags, and risk-of-bias notes

Two reviewers extracted prespecified items (design, participants, specimen/fraction, microbiome method/region, outcomes, key taxa/findings). In situations of disagreement, a third reviewer provided clarification. We flagged low-biomass rigor (negative/kit/extraction blanks, decontamination, abstinence interval, recent antibiotics/antiseptics, leukocytospermia handling). Given heterogeneity, we used narrative synthesis with vote counting (positive/negative/mixed/neutral/unclear), and qualitative sensitivity analyses restricted to higher-rigor subsets. In addition to human studies, relevant animal data and recent human reports identified outside the initial search were reviewed to provide mechanistic and up-to-date context.

Ethics/IRB

Not applicable. This study is a systematic review of published literature and did not involve new human participant research or identifiable private information.

OVERVIEW OF MALE REPRODUCTIVE TRACT MICROBIOME RESEARCH

Early investigations of the male reproductive tract focused on identifying pathogenic bacteria responsible for infections such as urethritis, epididymitis, and prostatitis (38, 39). With the advent of molecular methods, particularly 16S rRNA sequencing that amplifies and profiles bacterial ribosomal gene regions to infer community composition, evidence has accumulated that even asymptomatic men harbor detectable bacterial DNA in the genital tract (35, 40, 41). This section summarizes current knowledge about microbial communities within the principal male reproductive organs and their clinical contexts.

Urethra and periurethral/penile surfaces

The urethra and glans penis are among the most microbially diverse regions of the male reproductive tract. Commonly identified taxa include *Corynebacterium*, *Staphylococcus*, and *Streptococcus* species, reflecting both skin and mucosal sources (42, 43). Their composition varies with hygiene, circumcision, and sexual activity (41, 44–46). Although dysbiosis has been linked to urethritis and other inflammatory conditions (47, 48), the significance of urethral community structure for semen quality in asymptomatic men remains unclear.

Prostate and seminal vesicles

The prostate and seminal vesicles (male accessory glands) have long been considered low-biomass environments, and sequencing studies now detect microbial DNA, particularly in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS; NIH category III), a condition historically considered nonbacterial (49–51). Reports often describe low-level signals from *Lactobacillus*, *Corynebacterium*, or anaerobes such as *Prevotella* (52), alongside elevated cytokines or oxidative markers in semen (53), consistent with a model in which accessory-gland inflammation could alter semen rheology, referring to the physical properties of seminal plasma, and sperm function (54). Seminal plasma also shapes the female genital tract immune response after intercourse, promoting tolerance to paternal antigens and supporting early reproductive events (55, 56). Therefore, accessory-gland inflammation and dysbiosis may plausibly influence not only sperm function but also female tract inflammation and reproductive outcomes. Causality, however, remains unproven, and detection of bacterial DNA by PCR or sequencing does not itself establish infection or pathogenicity, as these methods cannot distinguish live from dead organisms or exclude background contamination (57, 58).

Beyond CP/CPPS, several studies have compared intraprostatic or urinary microbiota between prostate cancer cases and controls (59, 60). Although some cohorts note differences (e.g., anaerobe enrichment or distinct urinary profiles), specific taxa are inconsistent, and mechanisms may also involve gut–prostate inflammatory crosstalk (61).

Epididymis and testis

Historically, epididymis and testis have been viewed as immune-privileged and essentially sterile, and in practice, they represent ultralow-biomass environments (62, 63). Detection of bacterial sequences from these sites has been inconsistent and frequently attributed to contamination (64). A limited number of reports suggest microbial DNA or RNA in association with epididymitis (65), obstructive azoospermia (66), and idiopathic nonobstructive azoospermia (67), yet no evidence supports a stable "testicular microbiome." In oncology, exploratory work has examined associations with testicular germ-cell tumors, often via systemic inflammatory/metabolic pathways (68); however, evidence for a stable local "testicular microbiome" remains unsubstantiated. To substantiate such a microbiome, future studies would need to demonstrate reproducible, site-specific communities across independent cohorts with rigorous low-biomass contamination controls, spatial localization within testicular tissue, and ideally some indication of microbial viability or local host responses.

Seminal microbiome

Semen represents a composite specimen integrating contributions from the prostate, seminal vesicles, and distal urethra. Sequencing studies consistently identify *Lactobacillus*, *Corynebacterium*, and *Gardnerella* among dominant genera (35), with variable detection of anaerobes such as *Prevotella* or *Finegoldia* (69). Because of its accessibility and direct relevance to reproductive outcomes, semen has become the primary specimen for characterizing the male reproductive microbiome and linking community shifts to sperm motility, morphology, DNA integrity, and inflammation (70, 71).

In summary, evidence across anatomical sites indicates that the male reproductive tract harbors low-abundance but biologically active microbial communities. Although many signals are disease-associated or transient, growing

data imply that shifts in these communities, particularly within semen, may contribute to inflammation, oxidative stress, and impaired spermatogenesis.

CURRENT EVIDENCE FOR THE MALE REPRODUCTIVE MICROBIOME

Seminal microbiome

Semen harbors a low-biomass yet polymicrobial community, whose composition varies with fraction (cell pellet vs seminal plasma), abstinence interval, and laboratory pipeline. Signals were most coherent in studies reporting negative controls and leukocyte handling. Across 42 human cohorts, almost all studies have used 16S rRNA gene amplicon sequencing or pan-bacterial qPCR to profile the seminal microbiome, with rare application of shotgun metagenomics (37). In these studies, recurrent genera were *Lactobacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*/*Enterococcus*, *Pseudomonas*, *Escherichia/Shigella, Acinetobacter, Bacteroides, Prevotella, Gardnerella, Atopobium*, and *Finegoldia*, although single-taxon dominance was uncommon (37, 70, 72–75).

Associations. Despite heterogeneity, a reproducible pattern emerges. Anaerobe-leaning profiles, often enriched for *Prevotella*, *Corynebacterium*, or *Sneathia*, more often accompany lower sperm progressive motility or total motile count and, in some cohorts, lower concentration (71, 76–78). In contrast, *Lactobacillus*-enriched states are generally associated with neutral-to-favorable trends in sperm motility and morphology, although results are not entirely consistent across cohorts (71, 79, 80). Across individual studies, some groups have linked higher seminal *Lactobacillus* abundance to lower sperm DNA fragmentation (71), higher motility (71), or normal morphology (79) and concentration (80), whereas others have reported associations between *Lactobacillus* detection and abnormal motility (81) or oligoasthenoteratozoospermia (82). The genus-level signal for *Lactobacillus* likely masks species/strain heterogeneity and amplicon-resolution limits. For example, short-read 16S (V3–V4/V4) can under-resolve *L. crispatus* vs. *L. iners* (83). Nevertheless, several 16S-based studies have reported contrasting associations tentatively attributed to these taxa. *L. iners* has been linked to adverse markers (higher sperm DNA fragmentation, impaired motility) (70, 81), whereas *L. crispatus* tends to align with healthier states or favorable partner-level ART profiles (84, 85). These apparent differences should be interpreted cautiously, as current evidence relies entirely on low-resolution 16S assignments rather than species-specific validation. In the only human semen study to date that applied shotgun metagenomic sequencing, community shifts in a pilot male-infertility cohort were accompanied by alterations in S-adenosyl-L-methionine and related metabolic pathways, suggesting links between microbiome composition, redox imbalance, and DNA methylation biology (37).

Outside the male reproductive tract, strain-resolved shotgun metagenomics has repeatedly shown that clinically relevant heterogeneity is often invisible at the genus or species level. In pregnancy cohorts, strain-level analyses of the vaginal microbiome have demonstrated that within-species nucleotide diversity and gene content in key taxa, such as *Gardnerella* and *Lactobacillus,* discriminate women who subsequently deliver preterm from those delivering at term, even when species-level community state types appear similar (86). In the gut, longitudinal strain-resolved metagenomics in Crohn's disease has shown that only a subset of *Escherichia coli* strains, closely related to adherent-invasive pathotypes, expand in synchrony with inflammatory flares, whereas co-colonizing *E. coli* strains of the same species are preferentially observed during remission (87). These examples underscore that strain-level resolution can fundamentally change disease-risk prediction, reinforcing the need for strain-resolved approaches (e.g., deep shotgun metagenomics) in future studies of the semen microbiome.

Inflammation and leukocytospermia. Cross-sectional cohorts suggest that leukocytospermia co-occurs with distinct seminal community patterns (e.g., higher alpha diversity, a relative increase in Bacteroidetes, and a shift away from *Lactobacillus*-enriched toward *Streptococcus*-enriched profiles) and with less favorable semen parameters (82, 88, 89). These studies support a link between inflammatory tone (baseline inflammatory state, e.g., cytokine/chemokine profiles and leukocyte-associated signals) and the seminal microbiota, but formal mediation through inflammatory intermediates has not been demonstrated, and reporting of leukocyte quantification, recent antimicrobials, and contamination controls remains variable. Leukocytospermia can arise from both infectious and noninfectious etiologies. Noninfectious contributors include oxidative stress (90), prolonged abstinence (91), and local immune activation, so microbial associations in cross-sectional studies should be interpreted with caution when inferring causality (92).

Oxidative stress and DNA-damage endpoints. Where oxidative and DNA endpoints were measured (e.g., ORP [oxidation–reduction potential], MDA [malondialdehyde], 8-OHdG [8-Hydroxy-2'-deoxyguanosine], DFI [DNA Fragmentation Index]), *Lactobacillus*-enriched or anaerobe-reduced profiles more often aligned with lower oxidative load and DFI, whereas anaerobe-rich states aligned with higher values (70, 71). In several studies, taxon–semen associations were attenuated after accounting for inflammatory markers, consistent with partial mediation by inflammation and oxidative stress (70, 71, 93). However, all available data are cross-sectional, so these relationships should be interpreted as associative rather than causal. Although most conceptual models assume that microbial shifts drive inflammation, oxidative stress, and ultimately sperm injury, the reverse direction is also plausible and has been raised in prior work: primary spermatogenic or epididymal dysfunction could alter seminal plasma composition (e.g., protein, lipid, and redox milieu), thereby selecting for particular bacterial communities without those bacteria necessarily being pathogenic. At present, no study has formally disentangled these directions of effect, underscoring the need for longitudinal and interventional designs.

Overall directionality. Determinate findings modestly favor better oxidative/DFI outcomes in Lactobacillus-enriched states, whereas effects on concentration and morphology are mixed. To visualize the overall pattern of associations, we summarized all study-level reports linking major genera with semen parameters (Figure 1). In this bubble chart, each circle represents the cumulative sample size of studies reporting a given association, with color indicating whether the genus was generally beneficial (linked to improved semen quality or reduced oxidative/inflammatory load) or detrimental (associated with impaired parameters, higher oxidative stress, or inflammation). At present, genus-level signatures are best interpreted as contextual markers of inflammatory and oxidative status rather than diagnostic targets; species-resolved, couple-integrated, contamination-aware longitudinal studies are needed to define actionable thresholds.

ART relevance. Evidence directly linking the seminal microbiome to ART outcomes is emerging but limited. In a couple's cohort, a semen *Acinetobacter*-dominated community was associated with higher ART success, and vaginal *L. crispatus* dominance also tracked with better outcomes; couples in which both partners had beneficial community types achieved a higher success rate (53% vs 25%) (84). Assay and cut-off heterogeneity and unmeasured partner factors, however, limit causal interpretation.

Complementary male/female genitourinary couple microbiome

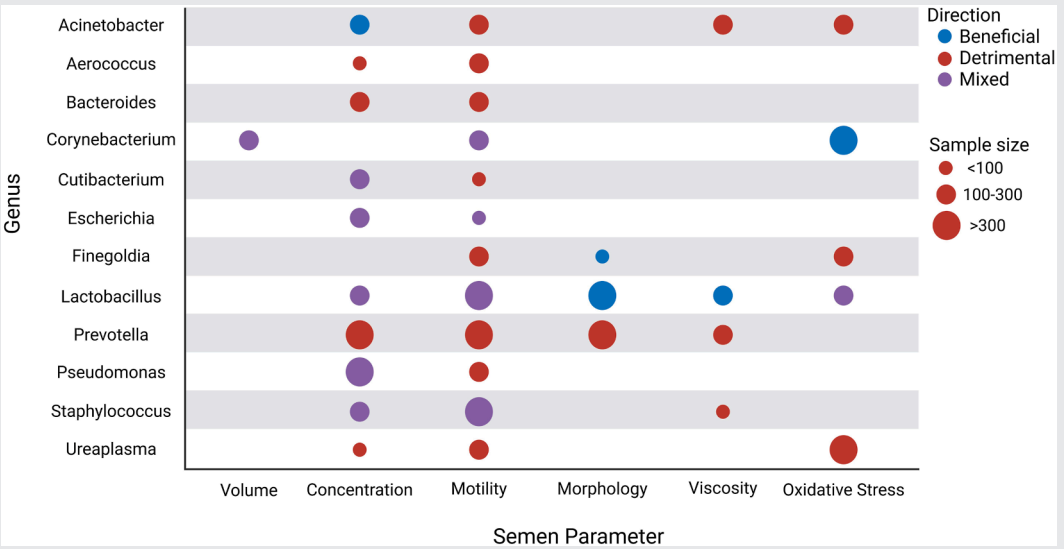
Although unprotected sexual intercourse involves the exchange of a plethora of microbes through bodily fluids, which inevitably interact, these microbiomes have typically been separately studied. A limited number of couple-level studies (n = 9) have characterized both male and female genital tract microbiomes in parallel, revealing substantial within-couple similarity that exceeds between-couple similarity even after accounting for abstinence and hygiene. Overlapping taxa commonly include *Lactobacillus*, *Gardnerella*, and *Prevotella*, supporting substantive microbial exchange at the couple level (94–97).

Directionality and strain resolution. When longitudinal or strain-resolved sampling was performed, the balance of evidence favored transfer from female to male for shared taxa, with less persistent male-to-female transmission (98, 99). Strain- or ASV-level analyses demonstrated that identical *Lactobacillus* or *Gardnerella* lineages can be detected within couples over short intervals but diverge substantially between couples, suggesting that shared strains are transiently acquired from the female partner and gradually replaced by the male's resident microbiota over time (95, 98, 99).

Temporal dynamics. Pre/postintercourse and short-interval designs show that penile and urethral communities can shift within hours of exposure, followed by partial reversion. The magnitude of change depends on vaginal community state types (CSTs), condom use, hygiene, and time since intercourse (94, 98). These rapid dynamics likely inflate cross-sectional heterogeneity when behavioral metadata are incomplete.

Fertility-relevant readouts. In couple-level analyses integrating semen and vaginal profiles, anaerobe-enriched states (e.g., *Gardnerella*, *Prevotella*) were linked to less favorable semen parameters and poorer embryo morphology, whereas

FIGURE 1



Overall directionality of genus-level associations within the seminal microbiome. Bubble area corresponds to the cumulative sample size (infertile and control combined). Colors indicate beneficial (blue), detrimental (red), or mixed (purple) associations. Alternating gray shading distinguishes genera for clarity.

Kuribayashi. Male reproductive microbiome. Fertil Steril 2026.

Lactobacillus-dominant vaginal CSTs corresponded to more favorable semen and embryo outcomes (95). Nonetheless, assay variability and unmeasured couple behaviors limit causal interpretation. These anaerobe-enriched community types broadly overlap with bacterial vaginosis or vaginal dysbiosis. Outside of couple-level studies, bacterial vaginitis (BV), and related vaginal dysbiosis are more prevalent among infertile women and have been associated with tubal factor infertility and an increased risk of early pregnancy loss after in vitro fertilization (IVF) in several cohorts and meta-analyses (100, 101), although effects on implantation and live-birth rates are inconsistent (102, 103). Although assay variability and unmeasured couple behaviors still limit causal interpretation, future couple-based studies may better disentangle these bidirectional microbiome–reproductive interactions.

Clinical implications and future directions. Collectively, these findings support a bidirectional model of couple-level interaction. On short timescales (hours to days), exposure to a female partner appears to be the predominant determinant of male genital tract communities (95, 98), with female-to-male microbial transfer more frequently observed than the reverse. Randomized trials demonstrate that male circumcision reduces BV and Trichomonas infection in female partners (104), and a recent partner-treatment trial showed lower BV recurrence when male partners were treated concurrently with women (105). Although these studies were outside our PRISMA-defined couple-profiling corpus, they reinforce that semen exposure, penile ecology, and sexual behavior jointly shape the microbial landscape of both partners. Future couple-integrated, strain-resolved designs with standardized behavioral metadata will be essential to quantify the relative contributions and persistence of these reciprocal influences in relation to semen quality and ART outcomes.

Gut–testis axis

Like the well-established gut–brain axis, the emerging "gut–testis axis" links intestinal microbes to testicular function through systemic immune–metabolic pathways. The gut–brain axis is firmly supported by mechanistic studies showing that microbial metabolites, such as short-chain fatty acids (106) and tryptophan derivatives (107), modulate neuroendocrine and inflammatory pathways that affect mood, stress, and cognition (108–110). In comparison, evidence for gut–testis communication remains preliminary but increasingly suggestive. Intestinal dysbiosis has been linked to male reproductive phenotypes through systemic immune–metabolic pathways. Across 16 human studies, stool microbiome profiles were paired with semen parameters, inflammatory/oxidative markers, and hormones. Despite heterogeneous pipelines, convergent trends suggest that gut community structure and function track with sperm quality and redox status (37, 80, 111, 112).

Community signatures. Men with abnormal semen parameters more often show reduced gut microbiota alpha diversity and enrichment of taxa associated with endotoxemia (e.g., *Bacteroides*, *Bilophila*) (113, 114), whereas SCFA-producing profiles (e.g., Bifidobacterium, Blautia) tend to associate with higher concentration/motility and lower oxidative or DNA-fragmentation indices (80, 115).

Inflammation and metabolic comorbidity. Gut dysbiosis can facilitate translocation of microbial products and low-grade systemic inflammation, which in turn may impair Leydig and Sertoli cell function. Obesity, insulin resistance, and other metabolic traits frequently cosegregate with dysbiotic signatures and poorer semen quality, supporting a model in which gut-derived metabolic inflammation contributes to oxidative stress and suboptimal sperm function (116, 117).

Metabolite and hormonal pathways. Direct human evidence that specific circulating metabolites or hormones mediate gut–semen links remains limited. However, cross-site profiling in couples has identified genus-level shifts together with inferred differences in microbial molecular functions/pathways between fertile and infertile participants, which is consistent with altered microbial metabolic output that could influence redox or endocrine tone (118). In parallel, two-sample Mendelian randomization (MR) combining MiBioGen (gut-microbiota Genome-Wide Association Study [GWAS]) and FinnGen (male infertility) identified genes with putative causal effects on male infertility risk; whereas MR does not specify mediators, it strengthens the rationale to test metabolite- and hormone-centered mechanisms prospectively (115, 119).

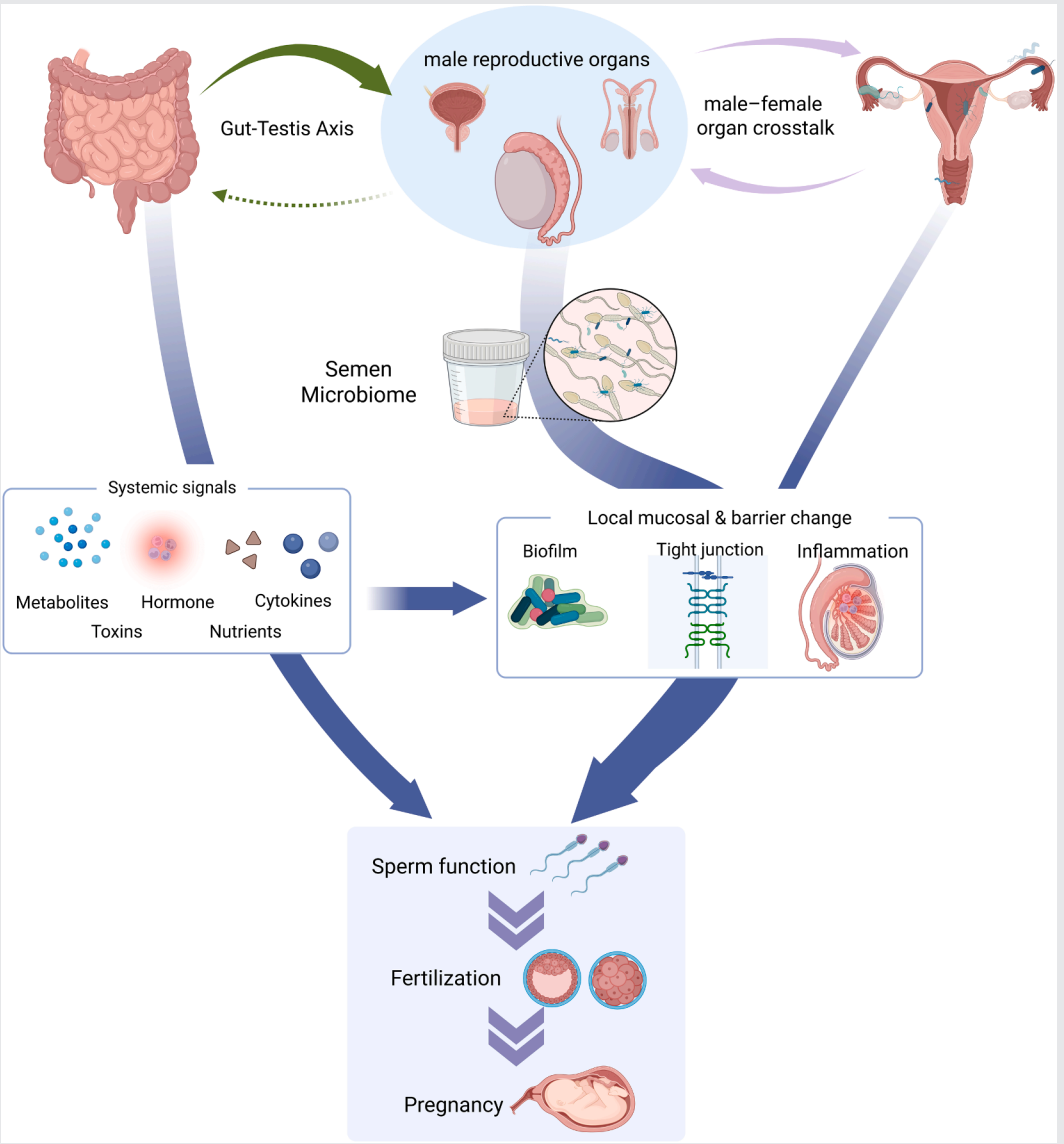
Integrative multiomics. A cross-site multiomic study integrating gut, urine, and semen microbiomes with shotgun metagenomics, reported coordinated taxonomic and functional shifts across all three sites, including alterations in the S-adenosyl-L-methionine cycle that link microbial metabolism to redox, methylation, and polyamine pathways in infertile men (37). A complementary gut-only shotgun metagenomic study in men with nonobstructive azoospermia similarly identified species-level shifts and altered carbohydrate, amino-acid, and methane-metabolism pathways, which correlated with hormonal and clinical parameters (80). Together, these shotgun data support a role for gut microbial metabolic capacity in male reproductive dysfunction. By contrast, most work to date relies on 16S-based functional inference rather than measured metabolomics; future contamination-aware, standardized pipelines that integrate targeted metabolomics/lipidomics and immune panels with longitudinal semen outcomes are needed to establish mechanisms and clinical utility.

Experimental model. Animal studies provide causal support for a gut–testis axis (120–125). In high-fat-diet–induced metabolic-syndrome models, gut dysbiosis alters bile acid composition and impairs intestinal vitamin A absorption, leading to disrupted testicular retinoic-acid signaling and defective spermatogenesis (122). In antibiotic-induced microbiota depletion models, exosome-mediated dysregulation of testicular retinoic acid likewise causes meiotic failure and impaired sperm production, which is mitigated by restoring the gut microbiota or providing short-chain fatty acid supplementation (123). These findings demonstrate causality

between gut microbial perturbation and testicular dysfunction through multiple intersecting metabolic and inflammatory pathways.

Interpretation. Taken together, human data support a biologically coherent link between intestinal dysbiosis and impaired testicular/seminal environments via systemic immune–metabolic crosstalk. Effect sizes are modest, and most studies are cross-sectional, so causality remains unresolved; routine stool microbiome testing for male infertility is not currently justified. Priority should be given to contamination-aware, couple-integrated longitudinal trials that combine strain-level metagenomics with targeted metabolomics/lipidomics and immune profiling, use standardized semen endpoints, including concentration, motility, morphology, and DNA fragmentation index/oxidative reduction potential (DFI/ORP). When possible, incorporation of pregnancy or live-birth outcomes would clarify whether targeted modulation of the gut microbiome offers sustained reproductive benefits.

FIGURE 2



Pathways linking seminal, gut, and male/female microbiomes to male fertility Proposed framework illustrating how distinct microbiome sites may influence sperm function. Evidence is strongest for associations within the seminal microbiome, with potential contributions from the gut–testis axis (via systemic inflammatory or metabolic mediators) and complementary male/female genitourinary couple microbiome (within-couple transfer).

Kuribayashi. Male reproductive microbiome. Fertil Steril 2026.

Mechanistic insights

Emerging evidence suggests several biologically plausible pathways through which the microbiome may influence male reproductive function (Figure 2). These mechanisms are not mutually exclusive and may operate in parallel.

Local inflammation and immune activation. Although leukocytospermia is often used as a marker of inflammation, direct measurements of seminal cytokines and oxidative mediators more accurately reflect the local immune milieu. Elevated IL-6, IL-8, and TNF-α levels have been associated with reduced motility, increased DNA fragmentation, and granulocyte-derived oxidative stress, supporting an inflammatory pathway linking dysbiosis to sperm dysfunction (126, 127).

Adaptive immune response and antisperm antibodies. - Chronic genital-tract inflammation and disruption of the blood–testis or blood–epididymis barrier can expose sperm antigens to the immune system, leading to loss of gamete immune tolerance, which in turn drives inflammation and gamete damage (128) and may, but does not necessarily, result in antisperm antibody (ASA) production (129, 130). In a 16S-based cohort, taxa such as *L. crispatus* and *G. vaginalis* were associated with normal ASA (IgA) levels and favorable semen parameters, whereas anaerobe-dominated profiles coincided with multiple abnormalities, including ASA positivity (126). However, no study has yet established that specific semen microbiome states directly drive ASA induction.

Beyond effects on sperm, seminal plasma also conditions the female genital tract immune milieu after intercourse (131). Thus, inflammation- or dysbiosis-associated changes in seminal plasma may modulate female tract inflammation and tolerance to paternal antigens, with potential downstream effects on implantation and reproductive outcomes (55, 56).

Seminal environment and rheology. Microbial activity may perturb the physicochemical milieu that supports motility and capacitation, most notably the timing/efficiency of liquefaction and semen viscosity. Human cohorts report pathogen-leaning communities in hyperviscosity phenotypes and identify microbiome shifts alongside semen-feature differences, consistent with altered proteolysis and redox pathways; direct taxa–pH associations remain limited in current datasets (132, 133).

Biofilm formation and microbial adherence. Bacteria have been observed to adhere to spermatozoa and, in some reports, to form aggregates or biofilm-like structures within semen or distal ducts (134–136). Such an organization may create localized hypoxic and inflammatory microenvironments that impede motility and perpetuate chronic inflammation, although direct human evidence remains limited.

Barrier integrity and paracrine support. Inflammatory cytokines and reactive oxygen species (ROS) can modulate Sertoli-cell tight junctions (e.g., ZO-1/claudins) (137), compromising the blood–testis barrier and perturbing the paracrine support and redox homeostasis required for spermatogenesis, which are concepts consistent with broader microbiome–gonadal crosstalk models.

Systemic and metabolic crosstalk. Beyond local effects in the male genital tract, systemic immune–metabolic disturbances can shape the testicular/seminal niche and thereby influence observed associations between the seminal microbiome and semen quality. Such disturbances may reflect distal microbiome inputs (particularly the gut) and/or non-microbiome host factors, including hyperglycemia, micronutrient deficiency, systemic inflammation/autoimmunity/infection, and environmental toxin exposures (138, 139). In experimental models, microbially derived metabolites such as short-chain fatty acids, bile acids, and tryptophan derivatives, together with endocrine and cytokine signaling, can modulate oxidative balance, steroidogenesis, and blood–testis–barrier integrity (121, 122). However, direct human data linking specific systemic microbial or metabolite signatures to semen quality remain limited, so these pathways should currently be viewed as mechanistic hypotheses rather than established causal routes.

Interventions

Therapeutic modulation of the male reproductive microbiome has been explored through two main approaches: antimicrobial suppression and probiotic-based restoration. Twenty-two human studies (13 antibiotic, 9 probiotic or pre/synbiotic) form the current evidence base, although most are small, unblinded, and short-term. Despite heterogeneity, a consistent theme emerges: interventions that temper inflammation and oxidative stress tend to yield modest improvements in semen quality.

Antibiotics. Most antibiotic trials enrolled infertile men with leukocytospermia, male accessory gland infection, or asymptomatic urogenital colonization (e.g., *Chlamydia*, *Ureaplasma*, *Mycoplasma*), and all reported semen endpoints. Across randomized and before–after designs, antibiotics frequently reduced seminal inflammation (e.g., leukocyte counts and/or ROS generation) and were often accompanied by modest gains in total or progressive motility; effects on concentration and morphology were inconsistent (140–145). Notably, one randomized control trial found that levofloxacin did not resolve asymptomatic leukocytospermia (146). Contemporary evidence syntheses and American Urologic Association/AUA/American Society of Reproductive Medicine (ASRM) and European Association of Urology guidelines no longer support empiric antibiotic therapy for idiopathic leukocytospermia or abnormal semen parameters in the absence of microbiologic confirmation (e.g., positive culture or nucleic acid amplification test for a specific pathogen) (147, 148). Treatment is now reserved for documented infections such as *C. trachomatis*, *U. urealyticum*, or *M. genitalium*, following organism-specific protocols. Indiscriminate antimicrobial use carries risks of resistance and partner-mediated recolonization. Overall, antibiotics may transiently lower seminal inflammatory tone in selected infection-associated phenotypes, but current data are insufficient to justify routine use solely to improve semen quality.

Probiotics/pre-/synbiotics. Interventions have attracted growing interest as lower-risk adjuncts. Most formulations combined *Lactobacillus* and *Bifidobacterium* species, administered orally for 3–6 months. Three randomized, double-blind, placebo-controlled trials in infertile men reported improvements in at least some semen endpoints, most consistently motility, with concurrent reductions in oxidative-stress or inflammatory indices; effects on concentration and morphology were variable (149–151). These patterns align with a redox-mediated mechanism rather than a simple change in total bacterial load.

Beyond semen readouts, an open-label couple-based intervention using a defined *Ligilactobacillus* salivarius strain documented shifts in local/systemic cytokines, changes in reproductive-tract microbiota, and reported pregnancies during follow-up (152). Although not placebo-controlled, the study supports a gut/urogenital–immune axis in idiopathic infertility and underscores the potential importance of partner ecology.

Mechanistic support also comes from controlled in vitro exposure of purified human spermatozoa to a vaginal probiotic Lactobacillus, which altered immune-related readouts, including sperm-surface human leukocyte antigens class I/II expression and peripheral blood mononuclear cell (PBMC)-derived IgG and antipaternal cytotoxic antibody following sperm-PBMC coculture (153). Related in vitro work further showed that probiotic treatment can modify toll-like receptor (TLR) 2 and TLR4 expression on spermatozoa, although downstream signaling was not directly measured (154). Although these experiments do not establish clinical benefit, they provide a biologically plausible route by which probiotic-dominated environments could dampen inflammatory signaling at the gamete interface.

Taken together, available trials suggest that probiotic supplementation can improve oxidative/DNA-integrity surrogates and modestly enhance motility in some men. Key gaps remain: small sample sizes, heterogeneous study design and technical platforms, limited use of sequencing to confirm strain persistence, and sparse reproductive endpoints.

LIMITATIONS AND FUTURE DIRECTIONS

Limitations

Most studies are small and cross-sectional, which limits causal inference. Low-biomass safeguards and negative controls are inconsistently applied, so contamination cannot be excluded despite field guidelines (36, 155, 156). Sampling and processing differ across studies (fraction profiled, abstinence interval, time to liquefaction/processing, leukocyte handling). Short-read 16S region/primer choices constrain species/strain resolution, and most datasets report relative, not absolute or viable, loads (156, 157). Partner-integrated and longitudinal designs, and pregnancy or live-birth endpoints, remain uncommon; clinic-based sampling may also limit generalizability.

In addition to methodological sources of heterogeneity, a substantial component of the observed inconsistency may reflect the inherently stochastic nature of microbial colonization in the male reproductive tract. Neutral modeling of reproductive tract microbiomes suggests that sexually associated microbial exposure is largely stochastic, with transient seeding events that vary widely across individuals (94). Similar stochastic, exposure-driven assembly has been described in other low-biomass ecosystems, for example, in the lung, where communities largely reflect immigration from the upper airway and occasional enrichment with gut-associated taxa in critical illness (158). Although speculative, these dynamics may amplify between-study variability and contribute to inconsistent genus-level signatures.

Future directions

Progress in this field will depend on overcoming current design and methodological limitations. Longitudinal, couple-resolved cohorts with strain-level resolution and detailed behavioral metadata are needed to define temporal and partner-mediated effects. Standardized protocols for specimen handling, negative controls, and metadata reporting tailored to low-biomass niches will be essential to ensure reproducibility. Mechanistic multiomics studies integrating microbiome, metabolome, and immune profiling could clarify how specific taxa or metabolites mediate oxidative stress, DNA fragmentation, and cytokine signaling. Beyond observational human work, further development and application of germ-free and microbiota-manipulated animal models, ex vivo testicular and epididymal cultures, and in vitro sperm functional assays will be essential to move from association to causality and to dissect tissue-specific pathways by which microbial signals influence spermatogenesis and sperm function. Clinical translation will require blinded, adequately powered randomized trials of species-defined probiotics or targeted antibiotics, ideally incorporating pregnancy and live-birth endpoints with durability assessments beyond 3–6 months. Finally, defining clinically validated quantitative thresholds, such as anaerobe load or species-specific cut-offs, will be crucial for integrating microbiome testing into diagnostic and therapeutic decision-making.

Acknowledgments

The authors thank Mary Schleicher, MLIS, Medical Librarian at the Cleveland Clinic Floyd D. Loop Alumni Library, for expert assistance in developing and executing the database search strategies.

CRediT Authorship Contribution Statement

Sohei Kuribayashi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Noopur Naik:** Writing – original draft, Investigation, Formal analysis, Data curation. **Aaron W. Miller:** Writing – review & editing. **Scott D. Lundy:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of Interests

S.K. has nothing to disclose. N.N. has nothing to disclose. A.W.M. has nothing to disclose. S.D.L. reports consultant fees from Give Legacy. S.D.L. serves as an advisory board member for PS Fertility and as a consultant for Give Legacy.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.fertnstert.2026.01.019.

REFERENCES

1. Huang B, Wang Z, Kong Y, Jin M, Ma L. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of Global Burden of Disease Study. BMC Public Health 2023;23:2195.
2. Cox CM, Thoma ME, Tchangalova N, Mburu G, Bornstein MJ, Johnson CL, et al. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. Hum Reprod Open 2022; 2022:hoac051.
3. Gong J, Wu Y, Qiu C, Yin G, Yuan P. Global, regional, and national burden of diseases associated with male reproduction from 1990 to 2021: a systematic analysis with forecasts to 2050. BMC Public Health 2025;25:2404.
4. Auger J, Eustache F, Chevrier C, Jégou B. Spatiotemporal trends in human semen quality. Nat Rev Urol 2022;19:597–626.
5. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson A-M, Eisenberg ML, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev 2016;96:55–97.
6. Mínguez-Alarcón L, Gaskins AJ, Meeker JD, Braun JM, Chavarro JE. Endocrine-disrupting chemicals and male reproductive health. Fertil Steril 2023; 120:1138–49.
7. Sharpe RM. Endocrine disruption and male reproductive disorders: unanswered questions. Hum Reprod Oxf Engl 2024;39:1879–88.
8. Service CA, Puri D, Al Azzawi S, Hsieh T-C, Patel DP. The impact of obesity and metabolic health on male fertility: a systematic review. Fertil Steril 2023;120:1098–111.
9. Choy JT, Eisenberg ML. Male infertility as a window to health. Fertil Steril 2018;110:810–4.
10. Eisenberg ML, Li S, Behr B, Cullen MR, Galusha D, Lamb DJ, et al. Semen quality, infertility and mortality in the USA. Hum Reprod Oxf Engl 2014;29: 1567–74.
11. Latif T, Kold Jensen T, Mehlsen J, Holmboe SA, Brinth L, Pors K, et al. Semen quality as a predictor of subsequent morbidity: a Danish cohort study of 4,712 men with long-term follow-up. Am J Epidemiol 2017;186:910–7.
12. Ventimiglia E, Pozzi E, Capogrosso P, Boeri L, Alfano M, Cazzaniga W, et al. Extensive assessment of underlying etiological factors in primary infertile men reduces the proportion of men with idiopathic infertility. Front Endocrinol 2021;12:801125.
13. Hammoud AO, Gibson M, Peterson CM, Meikle AW, Carrell DT. Impact of male obesity on infertility: a critical review of the current literature. Fertil Steril 2008;90:897–904.
14. Shiraishi K, Matsuyama H. Effects of medical comorbidity on male infertility and comorbidity treatment on spermatogenesis. Fertil Steril 2018; 110:1006–11.e2.
15. Cohen DJ, Giaccagli MM, Herzfeld JD, González LN, Cuasnicú PS, Da Ros VG. Metabolic syndrome and male fertility disorders: Is there a causal link? Rev Endocr Metab Disord 2021;22:1057–71.
16. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril 2010;93:1–12.
17. Ferlin A, Arredi B, Foresta C. Genetic causes of male infertility. Reprod Toxicol Elmsford N 2006;22:133–41.
18. Persad E, O'Loughlin CA, Kaur S, Wagner G, Matyas N, Hassler-Di Fratta MR, et al. Surgical or radiological treatment for varicoceles in subfertile men. Cochrane Database Syst Rev 2021;4:CD000479.
19. Henkel R. Long-term consequences of sexually transmitted infections on men's sexual function: a systematic review. Arab J Urol 2021;19:411–8.
20. Su HI, Lacchetti C, Letourneau J, Partridge AH, Qamar R, Quinn GP, et al. Fertility preservation in people with cancer: ASCO guideline update. J Clin Oncol 2025;43:1488–515.
21. Tully CA, Alesi S, McPherson NO, Sharkey DJ, Teong XT, Tay CT, et al. Assessing the influence of preconception diet on male fertility: a systematic scoping review. Hum Reprod Update 2024;30:243–61.
22. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG. The gut microbiome in neurological disorders. Lancet Neurol 2020;19:179–94.
23. Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575–84.
24. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.
25. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol 2021;19:55–71.
26. Ashonibare VJ, Akorede BA, Ashonibare PJ, Akhigbe TM, Akhigbe RE. Gut microbiota-gonadal axis: the impact of gut microbiota on reproductive functions. Front Immunol 2024;15:1346035.
27. Wang H, Xu A, Gong L, Chen Z, Zhang B, Li X. The microbiome, an important factor that is easily overlooked in male infertility. Front Microbiol 2022;13:831272.
28. Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome 2020;8:103.
29. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev 2012;70(1):S38–44, Suppl 1.
30. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. N Engl J Med 2016;375:2369–79.
31. Gimenes F, Souza RP, Bento JC, Teixeira JJV, Maria-Engler SS, Bonini MG, et al. Male infertility: a public health issue caused by sexually transmitted pathogens. Nat Rev Urol 2014;11:672–87.
32. Hua L, Gao X, Zhan J, Wu X, Liu H. Prostatitis and male infertility. Aging Male Off J Int Soc Study Aging Male 2025;28:2494550.
33. Lewis RW, Harrison RM, Domingue GJ. Culture of seminal fluid in a fertility clinic. Fertil Steril 1981;35:194–8.
34. Farahani L, Tharakan T, Yap T, Ramsay JW, Jayasena CN, Minhas S. The semen microbiome and its impact on sperm function and male fertility: A systematic review and meta-analysis. Andrology 2021;9:115–44.
35. Hou D, Zhou X, Zhong X, Settles ML, Herring J, Wang L, et al. Microbiota of the seminal fluid from healthy and infertile men. Fertil Steril 2013;100:1261–9.
36. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in low microbial biomass microbiome studies: issues and recommendations. Trends Microbiol 2019;27:105–17.
37. Lundy SD, Sangwan N, Parekh NV, Selvam MKP, Gupta S, McCaffrey P, et al. Functional and taxonomic dysbiosis of the gut, urine, and semen microbiomes in male infertility. Eur Urol 2021;79:826–36.
38. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted infections treatment guidelines, 2021. MMWR Recomm Rep 2021;70:1–187.
39. Krieger JN, Nyberg L, Nickel JC. NIH consensus definition and classification of prostatitis. JAMA 1999;282:236–7.
40. Magill RG, MacDonald SM. Male infertility and the human microbiome. Front Reprod Health 2023;5:1166201.
41. Liu CM, Hungate BA, Tobian AAR, Serwadda D, Ravel J, Lester R, et al. Male circumcision significantly reduces prevalence and load of genital anaerobic bacteria. mBio 2013;4:e00076.
42. Dong Q, Nelson DE, Toh E, Diao L, Gao X, Fortenberry JD, et al. The microbial communities in male first catch urine are highly similar to those in paired urethral swab specimens. PloS One 2011;6:e19709.
43. Zuber A, Peric A, Pluchino N, Baud D, Stojanov M. Human male genital tract microbiota. Int J Mol Sci 2023;24:6939.
44. Mehta SD, Nandi D, Agingu W, Green SJ, Otieno FO, Bhaumik DK, et al. Longitudinal changes in the composition of the penile microbiome are associated with circumcision status, HIV and HSV-2 status, sexual

practices, and female partner microbiome composition. Front Cell Infect Microbiol 2022;12:916437.
45. Price LB, Liu CM, Johnson KE, Aziz M, Lau MK, Bowers J, et al. The effects of circumcision on the penis microbiome. PloS One 2010;5:e8422.
46. Toh E, Xing Y, Gao X, Jordan SJ, Batteiger TA, Batteiger BE, et al. Sexual behavior shapes male genitourinary microbiome composition. Cell Rep Med 2023;4:100981.
47. Plummer EL, Ratten LK, Vodstrcil LA, Murray GL, Danielewski JA, Fairley CK, et al. The urethral microbiota of men with and without idiopathic urethritis. mBio 2022;13:e0221322.
48. Frølund M, Wikström A, Lidbrink P, Abu Al-Soud W, Larsen N, Harder CB, et al. The bacterial microbiota in first-void urine from men with and without idiopathic urethritis. PloS One 2018;13:e0201380.
49. Nickel JC, Stephens A, Landis JR, Chen J, Mullins C, van Bokhoven A, et al. Search for microorganisms in men with urologic chronic pelvic pain syndrome: a culture-independent analysis in the MAPP research network. J Urol 2015;194:127–35.
50. Shoskes DA, Altemus J, Polackwich AS, Tucky B, Wang H, Eng C. The Urinary microbiome differs significantly between patients with chronic prostatitis/chronic pelvic pain syndrome and controls as well as between patients with different clinical phenotypes. Urology 2016;92:26–32.
51. Kermes K, Punab M, Lõivukene K, Mändar R. Anaerobic seminal fluid micro-flora in chronic prostatitis/chronic pelvic pain syndrome patients. Anaerobe 2003;9:117–23.
52. Mändar R, Punab M, Korrovits P, Türk S, Ausmees K, Lapp E, et al. Seminal microbiome in men with and without prostatitis. Int J Urol 2017;24:211–6.
53. Khadra A, Fletcher P, Luzzi G, Shattock R, Hay P. Interleukin-8 levels in seminal plasma in chronic prostatitis/chronic pelvic pain syndrome and nonspecific urethritis. BJU Int 2006;97:1043–6.
54. Rosellen J, Dittmar F, Hauptmann A, Diemer T, Schuppe H-C, Schagdarsurengin U, et al. Impaired semen quality in patients with chronic prostatitis. J Clin Med 2024;13:2884.
55. Robertson SA, Sharkey DJ. Seminal fluid and fertility in women. Fertil Steril 2016;106:511–9.
56. Lyons HE, Arman BM, Robertson SA, Sharkey DJ. Immune regulatory cytokines in seminal plasma of healthy men: a scoping review and analysis of variance. Andrology 2023;11:1245–66.
57. Emerson JB, Adams RI, Román CMB, Brooks B, Coil DA, Dahlhausen K, et al. Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems. Microbiome 2017;5:86.
58. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 2014;12:87.
59. Davidsson S, Mölling P, Rider JR, Unemo M, Karlsson MG, Carlsson J, et al. Frequency and typing of Propionibacterium acnes in prostate tissue obtained from men with and without prostate cancer. Infect Agent Cancer 2016;11:26.
60. Shrestha E, White JR, Yu S-H, Kulac I, Ertunc O, De Marzo AM, et al. Profiling the urinary microbiome in men with positive versus negative biopsies for prostate cancer. J Urol 2018;199:161–71.
61. Matsushita M, Fujita K, Hayashi T, Kayama H, Motooka D, Hase H, et al. Gut microbiota-derived short-chain fatty acids promote prostate cancer growth via IGF1 signaling. Cancer Res 2021;81:4014–26.
62. Zhao H, Yu C, He C, Mei C, Liao A, Huang D. The immune characteristics of the epididymis and the immune pathway of the epididymitis caused by different pathogens. Front Immunol 2020;11:2115.
63. Fijak M, Meinhardt A. The testis in immune privilege. Immunol Rev 2006;213:66–81.
64. Molina NM, Plaza-Díaz J, Vilchez-Vargas R, Sola-Leyva A, Vargas E, Mendoza-Tesarik R, et al. Assessing the testicular sperm microbiome: a low-biomass site with abundant contamination. Reprod Biomed Online 2021;43:523–31.
65. Paira DA, Olmedo JJ, Olivera C, Tissera AD, Molina RI, Rivero VE, et al. Chronic epididymitis due to Chlamydia trachomatis LGV-L2 in an HIV-negative heterosexual patient: a case report. Front Public Health 2023;11:1129166.
66. Sripada S, Amezaga MR, Hamilton M, McKenzie H, Templeton A, Bhattacharya S. Absence of chlamydial deoxyribonucleic acid from testicular and epididymal samples from men with obstructive azoospermia. Fertil Steril 2010;93:833–6.
67. Alfano M, Ferrarese R, Locatelli I, Ventimiglia E, Ippolito S, Gallina P, et al. Testicular microbiome in azoospermic men-first evidence of the impact of an altered microenvironment. Hum Reprod Oxf Engl 2018;33:1212–7.
68. Ciernikova S, Sevcikova A, Mego M. Exploring the microbiome-gut-testis axis in testicular germ cell tumors. Front Cell Infect Microbiol 2024;14:1529871.
69. Weng S-L, Chiu C-M, Lin F-M, Huang W-C, Liang C, Yang T, et al. Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. PloS One 2014;9:e110152.
70. He J, Ma M, Xu Z, Guo J, Chen H, Yang X, et al. Association between semen microbiome disorder and sperm DNA damage. Microbiol Spectr 2024;12:e0075924.
71. Garcia-Segura S, Del Rey J, Closa L, Garcia-Martínez I, Hobeich C, Castel AB, et al. Seminal microbiota of idiopathic infertile patients and its relationship with sperm DNA integrity. Front Cell Dev Biol 2022;10:937157.
72. Alqawasmeh OAM, Jiang X-T, Cong L, Wu W, Leung MBW, Chung JPW, et al. Vertical transmission of microbiomes into embryo culture media and its association with assisted reproductive outcomes. Reprod Biomed Online 2024;49:103977.
73. Zheng J, Liao J, Sun C-G, Yuan Z, Qin Y, Han T-L, et al. Integrated metabolomic and microbiota analysis of semen: seasonal and morphological associations. Asian J Androl 2025:10–4103, In press.
74. Gachet C, Prat M, Burucoa C, Grivard P, Pichon M. Spermatic microbiome characteristics in infertile patients: impact on sperm count, mobility, and morphology. J Clin Med 2022;11:1505.
75. Garcia-Segura S, Del Rey J, Closa L, Garcia-Martínez I, Hobeich C, Castel AB, et al. Characterization of seminal microbiome of infertile idiopathic patients using third-generation sequencing platform. Int J Mol Sci 2023;24:7867.
76. Campbell K, Suarez Arbelaez MC, Ghomeshi A, Ibrahim E, Roy S, Singh P, et al. Next-generation sequencing analysis of semen microbiome taxonomy in men with nonobstructive azoospermia vs. fertile controls: a pilot study. FS Sci 2023;4:257–64.
77. Baud D, Zuber A, Peric A, Pluchino N, Vulliemoz N, Stojanov M. Impact of semen microbiota on the composition of seminal plasma. Microbiol Spectr 2024;12:e0291123.
78. Fu L, Lou Y, Guo Y, Zhou F, Ma J, Wang S, et al. Seminal plasma microbiomes, sperm parameters, and cryopreservation in a healthy fertile population. Front Microbiol 2024;15:1401326.
79. Baud D, Pattaroni C, Vulliemoz N, Castella V, Marsland BJ, Stojanov M. Sperm microbiota and its impact on semen parameters. Front Microbiol 2019;10:234.
80. Cao T, Wang S, Pan Y, Guo F, Wu B, Zhang Y, et al. Characterization of the semen, gut, and urine microbiota in patients with different semen abnormalities. Front Microbiol 2023;14:1182320.
81. Osadchiy V, Belarmino A, Kianian R, Sigalos JT, Ancira JS, Kanie T, et al. Semen microbiota are dramatically altered in men with abnormal sperm parameters. Sci Rep 2024;14:1068.
82. Pochernikov D, Postovoytenko N, Getman V, Galkina I. Diagnostic significance of Lactobacillus spp. identification in ejaculate. Bull Russ State Med Univ 2020;3:38–44.
83. O'Callaghan JL, Willner D, Buttini M, Huygens F, Pelzer ES. Limitations of 16S rRNA gene sequencing to characterize Lactobacillus species in the upper genital tract. Front Cell Dev Biol 2021;9:641921.
84. Koort K, Sõsa K, Türk S, Lapp E, Talving E, Karits P, et al. Lactobacillus crispatus-dominated vaginal microbiome and Acinetobacter-dominated seminal microbiome support beneficial ART outcome. Acta Obstet Gynecol Scand 2023;102:921–34.
85. Amato V, Papaleo E, Pasciuta R, Viganò P, Ferrarese R, Clementi N, et al. Differential composition of vaginal microbiome, but not of seminal microbiome, is associated with successful intrauterine insemination in couples

with idiopathic infertility: a prospective observational study. Open Forum Infect Dis 2020;7:ofz525.
86. Liao J, Shenhav L, Urban JA, Serrano M, Zhu B, Buck GA, et al. Microdiversity of the vaginal microbiome is associated with preterm birth. Nat Commun 2023;14:4997.
87. Fang X, Monk JM, Nurk S, Akseshina M, Zhu Q, Gemmell C, et al. Metagenomics-based, strain-level analysis of *Escherichia coli* from a time-series of microbiome samples from a Crohn's disease patient. Front Microbiol 2018;9:2559.
88. Yao Y, Qiu X-J, Wang D-S, Luo J-K, Tang T, Li Y-H, et al. Semen microbiota in normal and leukocytospermic males. Asian J Androl 2022;24:398–405.
89. Tvrdá E, Ďuračka M, Benko F, Kováčik A, Lovíšek D, Gálová E, et al. Ejaculatory abstinence affects the sperm quality in normozoospermic men-how does the seminal bacteriome respond? Int J Mol Sci 2023;24:3503.
90. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril 2002;78:491–9.
91. Comar VA, Petersen CG, Mauri AL, Mattila M, Vagnini LD, Renzi A, et al. Influence of the abstinence period on human sperm quality: analysis of 2,458 semen samples. JBRA Assist Reprod 2017;21:306–12.
92. Dutta S, Bocu K, Agarwal A. Role of leukocytospermia in the management of male infertility: decoding a mystery for the busy clinicians. World J Mens Health 2025;43:465–76.
93. Mowla S, Farahani L, Tharakan T, Davies R, Correia GDS, Lee YS, et al. Characterisation and comparison of semen microbiota and bacterial load in men with infertility, recurrent miscarriage, or proven fertility. eLife 2025;13:RP96090.
94. Ma ZS. Microbiome Transmission During Sexual Intercourse Appears Stochastic and Supports the Red Queen Hypothesis. Front Microbiol 2021;12:789983.
95. Baud D, Peric A, Vidal A, Weiss JM, Engel P, Das S, et al. Genital microbiota in infertile couples. Reprod Biomed Online 2025;51:105056.
96. Mändar R, Punab M, Borovkova N, Lapp E, Kiiker R, Korrovits P, et al. Complementary seminovaginal microbiome in couples. Res Microbiol 2015;166:440–7.
97. Mehta SD, Zhao D, Green SJ, Agingu W, Otieno F, Bhaumik R, et al. The microbiome composition of a man's penis predicts incident bacterial vaginosis in his female sex partner with high accuracy. Front Cell Infect Microbiol 2020;10:433.
98. Dixon R, Egan S, Hughes S, Chapman B. The Sexome - A proof of concept study into microbial transfer between heterosexual couples after sexual intercourse. Forensic Sci Int 2023;348:111711.
99. Ghemrawi M, Ramírez Torres A, Netherland M, Wang Y, Hasan NA, El-Fahmawi B, et al. Forensic insights from shotgun metagenomics: tracing microbial exchange during sexual intercourse. Forensic Sci Int Genet 2025;78:103266.
100. van Oostrum N, De Sutter P, Meys J, Verstraelen H. Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis. Hum Reprod Oxf Engl 2013;28:1809–15.
101. Salah RM, Allam AM, Magdy AM, Mohamed AS. Bacterial vaginosis and infertility: cause or association? Eur J Obstet Gynecol Reprod Biol 2013;167:59–63.
102. Maksimovic Celicanin M, Haahr T, Humaidan P, Skafte-Holm A. Vaginal dysbiosis - the association with reproductive outcomes in IVF patients: a systematic review and meta-analysis. Curr Opin Obstet Gynecol 2024;36:155–64.
103. Skafte-Holm A, Humaidan P, Bernabeu A, Lledo B, Jensen JS, Haahr T. The association between vaginal dysbiosis and reproductive outcomes in subfertile women undergoing IVF-treatment: a systematic PRISMA review and meta-analysis. Pathog Basel Switz 2021;10:295.
104. Gray RH, Kigozi G, Serwadda D, Makumbi F, Nalugoda F, Watya S, et al. The effects of male circumcision on female partners' genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. Am J Obstet Gynecol 2009;200:e1–7.
105. Vodstrcil LA, Plummer EL, Fairley CK, Hocking JS, Law MG, Petoumenos K, et al. Male-partner treatment to prevent recurrence of bacterial vaginosis. N Engl J Med 2025;392:947–57.
106. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol 2019;16:461–78.
107. Zhou M, Fan Y, Xu L, Yu Z, Wang S, Xu H, et al. Microbiome and tryptophan metabolomics analysis in adolescent depression: roles of the gut microbiota in the regulation of tryptophan-derived neurotransmitters and behaviors in human and mice. Microbiome 2023;11:145.
108. Margolis KG, Cryan JF, Mayer EA. The microbiota-gut-brain axis: from motility to mood. Gastroenterology 2021;160:1486–501.
109. Ohara TE, Hsiao EY. Microbiota-neuroepithelial signalling across the gut-brain axis. Nat Rev Microbiol 2025;23:371–84.
110. Aburto MR, Cryan JF. Gastrointestinal and brain barriers: unlocking gates of communication across the microbiota-gut-brain axis. Nat Rev Gastroenterol Hepatol 2024;21:222–47.
111. Grande G, Graziani A, Scafa R, De Toni L, Garolla A, Ferlin A. Gut dysbiosis in infertile patients with persistent male accessory gland infection. Life Basel Switz 2025;15:894.
112. Pan Y, Wang S, Liu L, Liu X. Characteristics of gut microbiota in patients with asthenozoospermia: a Chinese pilot study. BMC Microbiol 2024;24:22.
113. Cao Y, Wang H, Jin Z, Hang J, Jiang H, Wu H, et al. Characterization of non-obstructive azoospermia in men using gut microbial profiling. J Clin Med 2023;12:701.
114. Wang Z-H, Kang Y-F. Gut microbiota and male fertility: a two-sample Mendelian randomization study. Medicine (Baltimore) 2025;104:e41542.
115. Fu Z, Wang Y, Yan H-L. Male infertility risk and gut microbiota: a Mendelian randomization study. Front Microbiol 2023;14:1228693.
116. Zou H, Xu N, Xu H, Xing X, Chen Y, Wu S. Inflammatory cytokines may mediate the causal relationship between gut microbiota and male infertility: a bidirectional, mediating, multivariate Mendelian randomization study. Front Endocrinol 2024;15:1368334.
117. Jiao J, Xu P, Wang X, Xing Z, Dong S, Li G, et al. Enterotypes in asthenospermia patients with obesity. Sci Rep 2022;12:16993.
118. Manzoor A, Amir S, Gul F, Sidique MA, Kayani MUR, Zaidi SSA, et al. Characterization of the gastrointestinal and reproductive tract microbiota in fertile and infertile Pakistani couples. Biology 2021;11:40.
119. Deng R, Huang Y, Tian Z, Zeng Q. Association between gut microbiota and male infertility: a two-sample Mendelian randomization study. Int Microbiol 2024;27:1655–63.
120. Shan L, Guo X, Hu Y, Zhou H, Meng X, Liu K, et al. L-citrulline protects testicular Sertoli cell function by mitigating DNA damage via the gut-testis axis of sheep fed a high-concentrate diet. NPJ Biofilms Microbiomes 2025;11:202.
121. Yan X, Feng Y, Hao Y, Zhong R, Jiang Y, Tang X, et al. Gut-testis axis: microbiota prime metabolome to increase sperm quality in young type 2 diabetes. Microbiol Spectr 2022;10:e0142322.
122. Zhang T, Sun P, Geng Q, Fan H, Gong Y, Hu Y, et al. Disrupted spermatogenesis in a metabolic syndrome model: the role of vitamin a metabolism in the gut-testis axis. Gut 2022;71:78–87.
123. Chen T, Zhang B, He G, Wang N, Cao M, Shen C, et al. Gut-derived exosomes mediate the microbiota dysbiosis-induced spermatogenesis impairment by targeting *Meioc* in mice. Adv Sci Weinh Baden-Wurtt Ger 2024;11:e2310110.
124. Lin Y, Wang K, Che L, Fang Z, Xu S, Feng B, et al. The Improvement of Semen Quality by Dietary Fiber Intake Is Positively Related With Gut Microbiota and SCFA in a Boar Model. Front Microbiol 2022;13:863315.
125. Hao Y, Feng Y, Yan X, Chen L, Ma X, Tang X, et al. Gut microbiota-testis axis: FMT mitigates high-fat diet-diminished male fertility via improving systemic and testicular metabolome. Microbiol Spectr 2022;10:e0002822.
126. Liu CM, Osborne BJW, Hungate BA, Shahabi K, Huibner S, Lester R, et al. The semen microbiome and its relationship with local immunology and viral load in HIV infection. PLoS Pathog 2014;10:e1004262.
127. Chen J, Chen J, Fang Y, Shen Q, Zhao K, Liu C, et al. Microbiology and immune mechanisms associated with male infertility. Front Immunol 2023;14:1139450.

128. Tung KSK, Harakal J, Qiao H, Rival C, Li JCH, Paul AGA, et al. Egress of sperm autoantigen from seminiferous tubules maintains systemic tolerance. J Clin Invest 2017;127:1046–60.
129. Ingerslev HJ, Walter S, Andersen JT, Brandenhoff P, Eldrup J, Geerdsen JP, et al. A prospective study of antisperm antibody development in acute epididymitis. J Urol 1986;136:162–4.
130. Witkin SS, Kligman I, Bongiovanni AM. Relationship between an asymptomatic male genital tract exposure to Chlamydia trachomatis and an autoimmune response to spermatozoa. Hum Reprod Oxf Engl 1995;10:2952–5.
131. Ahannach S, Gehrmann T, Spacova I, Wittouck S, Hiers J, Bron PA, et al. Microbial and seminal traces of sexual intercourse and forensic implications. Microbiome 2025;13:237.
132. Monteiro C, Marques PI, Cavadas B, Damião I, Almeida V, Barros N, et al. Characterization of microbiota in male infertility cases uncovers differences in seminal hyperviscosity and oligoasthenoteratozoospermia possibly correlated with increased prevalence of infectious bacteria. Am J Reprod Immunol N Y N 1989 2018;79:e12838.
133. Veneruso I, Cariati F, Alviggi C, Pastore L, Tomaiuolo R, D'Argenio V. Metagenomics reveals specific microbial features in males with semen alterations. Genes 2023;14:1228.
134. Busolo F, Zanchetta R, Bertoloni G. Mycoplasmic localization patterns on spermatozoa from infertile men. Fertil Steril 1984;42:412–7.
135. Prabha V, Sandhu R, Kaur S, Kaur K, Sarwal A, Mavuduru RS, et al. Mechanism of sperm immobilization by Escherichia coli. Adv Urol 2010;2010:240268.
136. Wolff H, Panhans A, Stolz W, Meurer M. Adherence of Escherichia coli to sperm: a mannose mediated phenomenon leading to agglutination of sperm and E. coli. Fertil Steril 1993;60:154–8.
137. Mruk DD, Cheng CY. The mammalian blood-testis barrier: its biology and regulation. Endocr Rev 2015;36:564–91.
138. Krzastek SC, Farhi J, Gray M, Smith RP. Impact of environmental toxin exposure on male fertility potential. Transl Androl Urol 2020;9:2797–813.
139. Fijak M, Pilatz A, Hedger MP, Nicolas N, Bhushan S, Michel V, et al. Infectious, inflammatory and "autoimmune" male factor infertility: how do rodent models inform clinical practice? Hum Reprod Update 2018;24:416–41.
140. Yanushpolsky EH, Politch JA, Hill JA, Anderson DJ. Antibiotic therapy and leukocytospermia: a prospective, randomized, controlled study. Fertil Steril 1995;63:142–7.
141. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. Hum Reprod Oxf Engl 2000;15:2536–44.
142. Merino G, Carranza-Lira S. Infection and male infertility: effect of different antibiotic regimens on semen quality. Arch Androl 1995;35:209–12.
143. Pajovic B, Radojevic N, Vukovic M, Stjepcevic A. Semen analysis before and after antibiotic treatment of asymptomatic Chlamydia- and Ureaplasma-related pyospermia. Andrologia 2013;45:266–71.
144. Ahmadi MH, Mirsalehian A, Sadighi Gilani MA, Bahador A, Talebi M. Asymptomatic infection with *Mycoplasma hominis* negatively affects semen parameters and leads to male infertility as confirmed by improved semen parameters after antibiotic treatment. Urology 2017;100:97–102.
145. Branigan EF, Muller CH. Efficacy of treatment and recurrence rate of leukocytospermia in infertile men with prostatitis. Fertil Steril 1994;62:580–4.
146. Krisp A, Hörster S, Skrzypek J, Krause W. Treatment with levofloxacin does not resolve asymptomatic leucocytospermia–a randomized controlled study. Andrologia 2003;35:244–7.
147. Brannigan RE, Hermanson L, Kaczmarek J, Kim SK, Kirkby E, Tanrikut C. Updates to male infertility: AUA/ASRM guideline (2024). J Urol 2024;212:789–99.
148. Minhas S, Boeri L, Capogrosso P, Cocci A, Corona G, Dinkelman-Smit M, et al. European Association of Urology guidelines on male sexual and reproductive health: 2025 update on male Infertility. Eur Urol 2025;87:601–16.
149. Helli B, Kavianpour M, Ghaedi E, Dadfar M, Haghighian HK. Probiotic effects on sperm parameters, oxidative stress index, inflammatory factors and sex hormones in infertile men. Hum Fertil Camb Engl 2022;25:499–507.
150. Asadi M, Gholipour F, Rahavian A, Javanbakht M. Effects of probiotic supplementation on semen parameters after varicocelectomy: a randomized controlled trial. J Res Med Sci 2023;28:74.
151. Abbasi B, Abbasi H, Niroumand H. Synbiotic (FamiLact) administration in idiopathic male infertility enhances sperm quality, DNA integrity, and chromatin status: a triple-blinded randomized clinical trial. Int J Reprod Biomed 2021;19:235–44.
152. Iniesta S, Esteban S, Armijo Ó, Lobo S, Manzano S, Espinosa I, et al. Ligilactobacillus salivarius PS11610 exerts an effect on the microbial and immunological profile of couples suffering unknown infertility. Am J Reprod Immunol 2022;88:e13552.
153. Rafiee M, Sereshki N, Alipour R, Ahmadipanah V, Pashoutan Sarvar D, Wilkinson D. The effect of probiotics on immunogenicity of spermatozoa in couples suffering from recurrent spontaneous abortion. BMC Immunol 2022;23:32.
154. Alipour R, Sereshki N, Rafiee M, Ahmadipanah V, Pashoutan Sarvar D, Rahimian K, et al. The effect of probiotic bacteria on toll-like receptor-2 and -4 expression by spermatozoa in couples with unexplained recurrent spontaneous abortion. Biochem Biophys Rep 2023;33:101390.
155. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 2018;6:226.
156. Mirzayi C, Renson A, , Genomic Standards Consortium, Massive Analysis and Quality Control Society, Zohra F, Elsafoury S, et al. Reporting guidelines for human microbiome research: the STORMS checklist. Nat Med 2021;27:1885–92.
157. Johnson JS, Spakowicz DJ, Hong B-Y, Petersen LM, Demkowicz P, Chen L, et al. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun 2019;10:5029.
158. Lipinksi JH, Ranjan P, Dickson RP, O'Dwyer DN. The lung microbiome. J Immunol 2024;212:1269–75.