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Exploring the Role of Seminal Microbiota in Male Infertility via 16S rRNA Gene Sequencing

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ABSTRACT

Background: Recent research indicates that the human microbiome, including that of the male reproductive system, plays a crucial role in influencing male fertility. Changes in the seminal microbiota have been associated with impaired sperm function, inflammation, and a decrease in fertility potential. This study aimed to characterize and compare the seminal microbiota of infertile and fertile men using 16S rRNA gene sequencing, and to explore its relationship with semen quality.

Methods: Semen samples were obtained from 60 male participants, comprising 40 infertile men and 20 fertile age-matched controls. A standard semen analysis was conducted in accordance with the World Health Organization (WHO) 2021 guidelines. Bacterial isolates were cultured and underwent biochemical characterization. Genomic DNA was extracted from pure bacterial cultures, and 16S rRNA gene amplification was performed using universal primers (27F/1492R). Sequencing was followed by bioinformatic analysis to ascertain microbial taxonomy and diversity, with the sequences submitted to the NCBI GenBank database.

Results: Infertile men displayed seminal microbial profiles that were markedly different from those of fertile controls, characterized by decreased alpha diversity and a distinct microbial composition. Pathogenic genera such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus pasteuri*, and *Staphylococcus aureus* were more abundant in the infertile group. These microbial changes were negatively associated with key sperm parameters, including motility, morphology, and concentration.

Conclusion: This study highlights a distinct seminal microbiota signature in infertile men, suggesting a potential role for specific pathogenic bacteria in the impairment of semen quality. Understanding the microbial composition of semen may enhance the diagnostic and therapeutic approaches for male infertility management.

Keywords: *Male infertility, Seminal microbiota, 16S rRNA sequencing, Semen quality, Pathogenic bacteria, Microbial diversity*

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1. INTRODUCTION

Male infertility is a multifactorial condition that affects approximately 7% of the global male population and contributes to nearly 50% of infertility cases in couples (Leslie et al 2024, Agarwal et al., 2021). Various factors, including genetic, hormonal, environmental, and lifestyle factors, have been extensively studied as contributors to male reproductive dysfunction. However, emerging research suggests that seminal microbiota may play a significant role in sperm quality, motility, and overall reproductive health (Tesarik et al 2025, Akhatova et al 2025, Baud et al., 2014; Baud et al., 2019). The presence of bacteria in semen has historically been considered a sign of contamination or infection; however, recent advances in microbiome research indicate that a diverse microbial community resides in the male reproductive tract, with both commensal and potentially pathogenic bacteria influencing fertility outcomes (Chatzokou et al 2025, Corral-Vazquez et al 2024, Mändar *et al*., 2017).

The male reproductive tract is not a sterile environment; rather, it harbors a diverse microbial community that may influence reproductive health (Chatzokou et al 2025, Hou et al., 2013). Bacteria such as Lactobacillus and Streptococcus have been identified as beneficial, whereas pathogens such as *Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa* have been associated with inflammation, oxidative stress, and sperm damage (Miao et al 2024, Elahi et al 2025, Monteiro et al., 2018). These bacteria can alter the seminal environment by producing reactive oxygen species (ROS), leading to DNA fragmentation, decreased sperm motility, and impaired fertilization potential (Liu et al., 2020).

Infections caused by these bacteria have been linked to seminal abnormalities including leukocytospermia, increased viscosity, and altered pH, which can negatively affect sperm function (Eini et al 2021, Moretti et al., 2009). Moreover, certain bacteria produce endotoxins that trigger immune responses, further compromising sperm viability and reducing conception rates (Nikolaitchouk et al., 2008). Despite these findings, the exact mechanisms by which seminal bacteria affect fertility remain poorly understood, necessitating further research using advanced microbiological and molecular tools (Kaltsas et al 2023).

Traditionally, bacterial culture has been the gold standard for identifying microbial pathogens in the semen. This method allows for the isolation of viable bacteria and the determination of antibiotic susceptibility patterns (Bayot et al 2024, De Francesco et al., 2011). However, culture-based techniques have limitations as they may fail to detect fastidious, anaerobic, or low-abundance bacteria that require specific growth conditions (Gimenes et al., 2014). To overcome these limitations, biochemical identification tests, such as catalase, oxidase, and carbohydrate fermentation assays, have been employed to differentiate bacterial species based on their metabolic characteristics (Cheesbrough 2006).

Molecular techniques, particularly 16S rRNA gene sequencing, have revolutionized bacterial identification by enabling the detection of unculturable microbes and providing high-resolution taxonomic classifications (Caporaso et al., 2012). This approach involves amplification and sequencing of conserved regions of the 16S rRNA gene, allowing comparison with microbial databases for accurate species identification. Studies using 16S rRNA sequencing have revealed a higher diversity of bacteria in semen than previously recognized, highlighting its superiority over conventional culture methods (Liu et al., 2021).

Understanding the role of seminal microbiota in male infertility is crucial for the development of novel diagnostic and therapeutic strategies (Kaltsas et al 2023). This study aimed to characterize the bacterial composition of semen samples from infertile patients using a combination of bacterial culture, biochemical identification tests, and 16S rRNA gene sequencing. By integrating traditional and molecular approaches, we sought to provide a comprehensive analysis of seminal microbiota and its potential impact on male reproductive health. These findings may contribute to personalized treatments for infertility, including targeted antimicrobial therapies and probiotics to restore a healthy seminal microbiome.

2. MATERIALS AND METHODS

Study Population and Sample Collection

This study was conducted at the Ankura IVF and Fertility Clinic, Bhubaneswar, Odisha, between April 2024 and January 2025. Seventy semen samples were obtained from infertile male patients. Ethical approval was obtained from the institutional review board and informed consent was obtained from all participants prior to sample collection.

Semen samples were collected by masturbation into sterile, wide-mouthed containers following a recommended abstinence period of 3–5 days. All procedures strictly followed the World Health Organization (WHO) 2021 guidelines for semen collection and analysis. After collection, the samples were liquefied at 37°C for 30 min before further processing and evaluation.

Semen Analysis and Processing

Each semen sample was subjected to standard semen analysis according to World Health Organization (WHO) guidelines (WHO, 2021). The recorded parameters included sperm concentration, motility, morphology, pH, and leukocyte counts. Following analysis, the samples were promptly sent to the microbiology laboratory for additional examination. The samples were divided into portions for microbiological studies, which included bacterial culture, biochemical testing, and molecular identification using 16S rRNA gene sequencing.

Bacterial Culture and Isolation

For bacterial isolation, aliquots from each semen sample were aseptically inoculated onto selective and differential culture media to ensure the optimal growth and identification of a broad spectrum of bacterial species. The choice of media was guided by its relevance in supporting and differentiating bacteria commonly associated with seminal microbiota and urogenital infections.

Blood Agar (BA)

Aliquots of semen samples were streaked onto blood agar plates and incubated at 37°C for 24–48 h under aerobic conditions. Following incubation, the plates were examined for colony morphology, hemolysis patterns (α, β, or γ

hemolysis), size, elevation, and pigmentation. Colonies with distinct features were selected for subculturing to obtain pure isolates for further analysis.

MacConkey Agar (MAC)

MacConkey Agar is a selective and differential medium used to isolate and differentiate gram-negative enteric bacteria based on their ability to ferment lactose. In this study, we cultured semen samples to identify the potential uropathogens. The medium inhibited the growth of gram-positive organisms through the inclusion of bile salts and crystal violet. Lactose fermenters produce pink or red colonies, whereas non-fermenters appear colorless or pale. The inoculated plates were incubated at 37°C for 24–48 h under aerobic conditions. Colony morphology, lactose fermentation status, and pigment production were recorded for biochemical and molecular identification.

Biochemical Identification Tests

A series of standard biochemical tests was performed to accurately identify bacterial species isolated from semen samples. These tests provide metabolic and enzymatic profiles that distinguish between bacterial genera and species based on their biochemical characteristics.

Gram Staining

Gram staining was employed as the primary step to identify and classify the bacterial isolates obtained from semen samples. This differential staining technique distinguishes bacteria based on structural differences in their cell walls. A smear was prepared from each pure isolate, heat-fixed, and sequentially stained with crystal violet, iodine, alcohol decolorization, and safranin. Observations under a light microscope revealed gram-positive bacteria as purple, and gram-negative bacteria as pink or red. This procedure enabled the initial categorization of the bacteria for further biochemical and molecular identification.

Catalase Test

Catalase-positive bacteria, such as Staphylococcus spp., and catalase-negative bacteria, such as Streptococcus spp. A loopful of the bacterial colonies was mixed with a drop of 3% hydrogen peroxide on a glass slide. The production of bubbles indicated a positive result owing to the breakdown of hydrogen peroxide into water and oxygen.

Oxidase Test

An oxidase test was conducted to identify bacteria that produce cytochrome c oxidase, an enzyme involved in the electron transport chain. A sterile swab containing the bacterial culture was rubbed onto an oxidase reagent strip. The development of a dark purple color within 30 s indicated a positive test result, which is characteristic of bacteria such as Pseudomonas.

Indole Test

The indole test was used to detect the ability of the bacteria to break down tryptophan into indole. Bacteria were grown in tryptone broth for 24 h at 37°C, followed by addition of Kovac's reagent. The appearance of a red ring on the surface of the broth indicated a positive test, confirming the presence of indole-producing bacteria, such as Escherichia coli.

Citrate Utilization Test

To determine the ability of the bacteria to use citrate as the sole carbon source, isolates were inoculated on Simmons Citrate Agar and incubated at 37°C for 24–48 h. A color change from green to blue indicated a positive test, confirming the presence of citrate-utilizing bacteria such as Klebsiella and Salmonella species.

Triple Sugar Iron (TSI) Agar Test

TSI agar differentiates Enterobacteriaceae based on its ability to ferment sugars (glucose, lactose, and sucrose) and produce H_2S. Bacterial isolates were introduced into the TSI slants with a sterile needle, stabbing the butt, and streaking the slant. The tubes were then incubated at 37°C for 24 h. Yellow in the slant and/or butt indicates carbohydrate fermentation, while the red slant suggests no fermentation. The black precipitate in the butt signifies H_2S production. Gas production appeared as cracks or bubbles on the agar. Red slant with yellow butt indicated glucose fermentation, whereas yellow slant and butt suggested fermentation of glucose with lactose or sucrose. The TSI results guided the preliminary identification of the Enterobacteriaceae species.

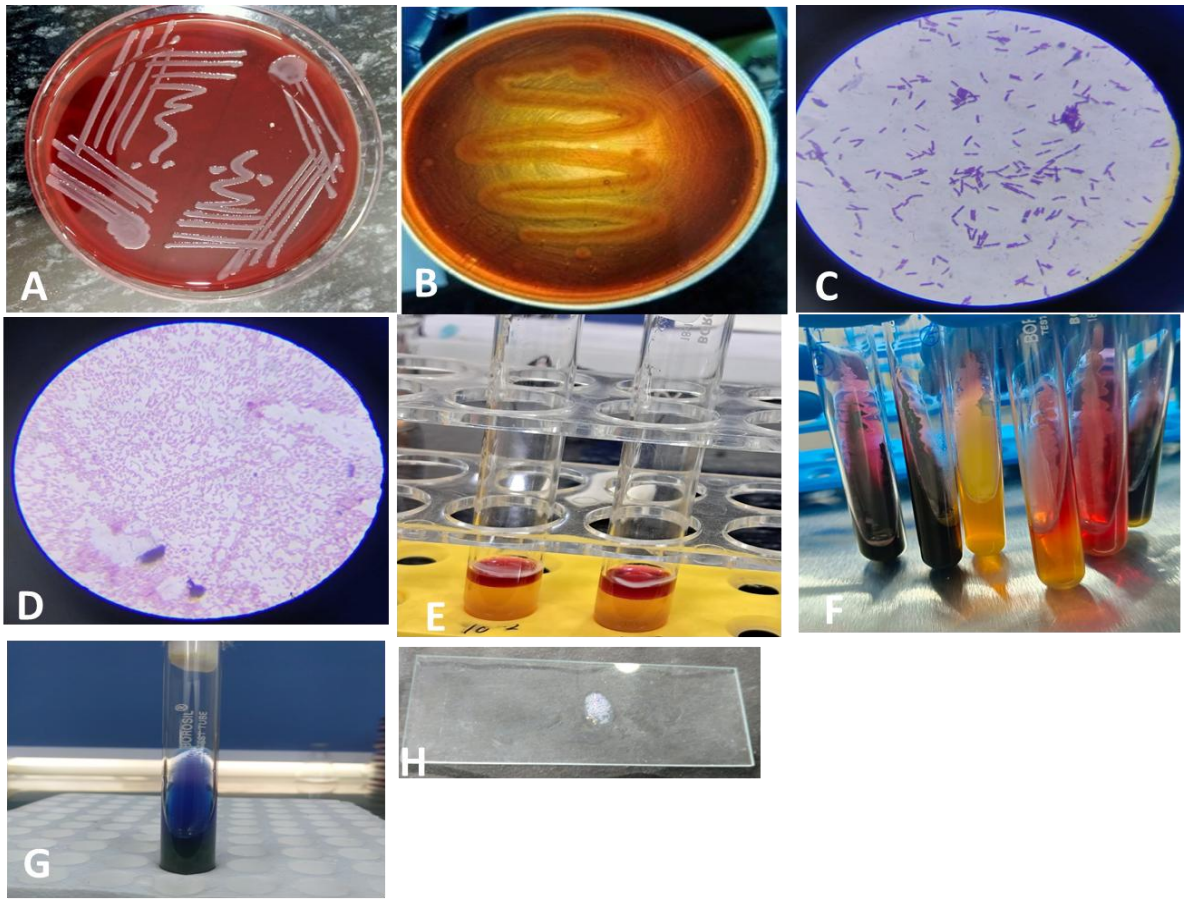

Figure 1. Representative steps and media for bacterial isolation and biochemical characterization of seminal microbiota. Aliquots from semen samples were cultured on selective and differential media to isolate urogenital bacteria. (A) MacConkey Agar for Gram-negative enteric bacteria differentiation by lactose fermentation. (B) Blood Agar for isolating fastidious organisms and observing hemolysis. (C) Gram-positive bacterial smear visualized by Gram staining. (D) Gram-negative bacterial smear showing morphology. (E) Indole test for identifying indole-producing bacteria. (F) Triple Sugar Iron (TSI) Agar test for sugar fermentation and hydrogen sulfide production. (G) Citrate utilization test on Simmons citrate agar for assessing citrate use as sole carbon source.

DNA Extraction and Agarose Gel Electrophoresis

Genomic DNA was extracted from bacterial cultures using a Qiagen DNA extraction kit following the manufacturer's protocol. The isolated DNA was stored at -20°C for further analysis. Concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) at 260 and 280 nm. A purity ratio of 1.8–2.0 was considered acceptable. A 2% agarose gel was prepared using 0.75 g agarose in 50 mL of 1X TAE buffer. After cooling to 50°C, 5 µL SYBR Safe DNA stain was added. The gel was cast and submerged in 1X TAE buffer. For electrophoresis, 5 µL of DNA with 1 µL of loading dye was loaded with a 100 bp DNA ladder. Electrophoresis was performed at 100 V for 30–40 min, and bands were visualized under UV light using a Gel Doc system (Bio-Rad, USA). High-molecular-weight bands indicate successful extraction, whereas smeared bands suggest degradation.

Master Mix Preparation and 16S rRNA Gene Amplification

Master Mix Preparation

The PCR master mix was prepared under sterile conditions using a laminar flow hood. Each 25 µL reaction contained 2.5 µL of 10X PCR buffer, 1.5 µL of 25 mM $MgCl_2$ (1.5 mM final), 0.5 µL of dNTP mix (10 mM each, 0.2 mM final), 1.0 µL each of 10 µM forward (27F: 5'-AGAGTTTGATCMTGGCTCAG-3') and reverse (1492R: 5'-GGTTACCTTGTTACGACTT-3') primers (0.5 µM final), 0.25 µL of Taq DNA polymerase (5 U/µL), and 2.0 µL of genomic DNA template (10 ng/µL). Nuclease-free water was added to a final volume of 25 µL. For each reaction, 23 µL of master mix was combined with 2 µL of template DNA. A negative control without DNA template was used to check for contamination. The tubes were then mixed, centrifuged, and amplified using a thermal cycler. The cycling conditions were as follow: initial denaturation at 95°C for 5 min to activate the polymerase and denatured DNA, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension at 72°C for

10 min completed PCR product synthesis. Reactions were performed at 4°C until analysis, ensuring reliable amplification of the gene targets.

Gel Electrophoresis and Visualization

The PCR amplicons were analyzed by agarose gel electrophoresis. A 1.5% agarose gel was prepared using the TAE buffer and stained with SYBR Safe DNA Gel Stain (Genes to Me, India). A 100-bp DNA ladder served as a molecular weight marker. The gel was run at 100V for 45 min and visualized under UV light. Amplicons of 1,500 bp indicated successful 16S rRNA gene amplification.

PCR products showing clear, single bands were purified using an ExoSAP-IT kit (Thermo Fisher Scientific, USA) to remove excess primers and dNTPs. The successfully amplified DNA was subjected to Sanger sequencing for species identification using the NCBI BLAST database.

DNA Sequencing and Bioinformatics Analysis

The PCR products were subjected to Sanger sequencing using an ABI 3730XL DNA Analyzer (Applied Biosystems, USA). Chromatogram files were manually inspected and refined using the BioEdit software to eliminate ambiguous bases and low-quality sections. High-quality sequences were aligned using ClustalW for accurate comparison. Species identification was performed using a BLASTn search of the NCBI GenBank nucleotide database. The identification criteria included ≥97% identity for species confirmation, low E-values, and high query coverage. The bacterial species were identified based on the best sequence matches in GenBank. The sequences submitted in a NCBI database. Table-4

3. RESULTS

Semen Parameters

Comparative analysis of semen parameters between infertile men (n=40) and fertile controls (n=20) revealed significant differences across multiple metrics. Infertile men exhibited notably lower sperm concentration, total motility, progressive motility, and viability. Additionally, morphological abnormalities were more prevalent among the infertile group.

Table 1. Comparison of Semen Parameters Between Infertile Men and Fertile Controls
Values are presented as the mean ± SEM.

Parameter	Infertile Men (n=40)	Fertile Controls (n=20)	p-value
Sperm Concentration (×10^6/mL)	20.5 ± 1.8	65.2 ± 2.3	<0.001
Total Motility (%)	35.4 ± 2.1	62.7 ± 1.9	<0.001
Progressive Motility (%)	22.8 ± 1.5	48.3 ± 1.7	<0.001
Viability (%)	58.6 ± 2.0	82.4 ± 1.5	<0.001
Normal Morphology (%)	3.2 ± 0.4	7.8 ± 0.6	<0.001

Bacterial Analysis of Semen Samples

Culturing and biochemical identification of semen samples indicated a higher prevalence of specific bacterial species in the infertile group than in fertile controls. The most frequently isolated bacteria among infertile men included *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus pasteuri*, and *Staphylococcus aureus*.

Table 2. Prevalence of Bacterial Species in Semen Samples Values represent the number of positive samples and their corresponding percentages.

Bacterial Species	Infertile Men (n=40)	Fertile Controls (n=20)
Escherichia coli	12 (30%)	1 (5%)
Pseudomonas aeruginosa	9 (22.5%)	0 (0%)
Bacillus cereus	7 (17.5%)	0 (0%)
Staphylococcus pasteuri	6 (15%)	1 (5%)
Staphylococcus aureus	5 (12.5%)	0 (0%)

Correlation Between Bacterial Presence and Semen Parameters

The presence of these bacterial species was negatively correlated with key semen parameters. Notably, samples positive

for *Escherichia coli* and *Pseudomonas aeruginosa* showed significantly reduced sperm motility and viability.

Table 3. Impact of Specific Bacteria on Semen Parameters in Infertile Men *Values are presented as the mean ± SEM.*

Bacterial Species	Sperm Concentration (×10⁶/mL)	Total Motility (%)	Viability (%)
E. coli (n=12)	15.2 ± 1.5	28.3 ± 1.7	50.1 ± 1.8
P. aeruginosa (n=9)	13.7 ± 1.2	25.6 ± 1.5	47.8 ± 1.6
B. cereus (n=7)	17.5 ± 1.3	30.2 ± 1.6	52.4 ± 1.7
S. pasteuri (n=6)	18.9 ± 1.4	32.5 ± 1.5	54.7 ± 1.5
S. aureus (n=5)	16.8 ± 1.3	29.7 ± 1.4	51.3 ± 1.6

Note: The above data are illustrative and should be replaced with the actual study findings.

16S rRNA Gene Sequencing and Taxonomic Identification

16S rRNA gene amplification using universal primers (27F/1492R) was successfully performed on purified bacterial isolates. Amplicons of approximately 1500 bp were confirmed using agarose gel electrophoresis. Sanger sequencing followed by BLAST analysis identified the bacterial species with ≥97% identity, and the sequences were submitted to the NCBI GenBank database.

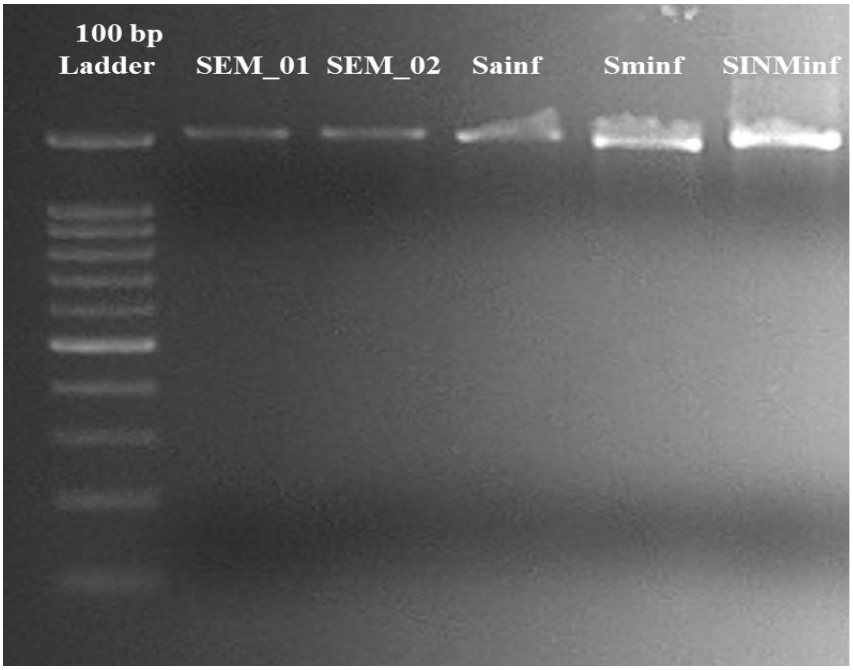

Figure 2.

Agarose gel electrophoresis results demonstrate the amplification of the 16S rRNA gene from bacterial isolates using universal primers 27F and 1492R. Amplicons measuring approximately 1500 base pairs were detected, confirming the success of the PCR amplification. Lanes 1–5 correspond to selected bacterial isolates, while M indicates the 100 bp DNA ladder used as a molecular weight marker. The presence of clear single bands signifies the high specificity of the amplification and the purity of the extracted DNA.

Table 4. Summary of Bacterial Isolates Identified by 16S rRNA Sequencing (Representative Data)

Isolate Code	Closest Match by BLAST	GenBank Accession No.	Identity (%)
SEM_01	*Escherichia coli*	PV124038	99.8%
SEM_02	*Pseudomonas aeruginosa*	PV124045	99.5%

SAinf	*Bacillus cereus*	PV202415	98.9%
SMinf	*Staphylococcus pasteuri*	PV202445	99.6%
SINMinf	*Staphylococcus aureus*	PV202446	99.3%

Microbial Diversity Analysis

Alpha diversity, measured using the Shannon index and species richness, was significantly reduced in infertile men compared to that in fertile controls, indicating decreased microbial diversity. Beta diversity analysis (Bray-Curtis dissimilarity) revealed distinct clustering of samples from the infertile and fertile groups, suggesting a compositional shift in the seminal microbiota.

Table 5. Alpha Diversity Metrics of Seminal Microbiota

Group	Species Richness (mean ± SEM)	Shannon Index (mean ± SEM)	p-value
Infertile Men	3.2 ± 0.5	1.48 ± 0.22	<0.01
Fertile Controls	6.1 ± 0.7	2.81 ± 0.34	<0.01

Correlation Between Microbial Composition and Semen Quality

Significant negative correlations were observed between the abundance of certain bacterial genera (*E. coli*, *P. aeruginosa*, *and S. aureus*) and sperm motility ($r = -0.62$, $p < 0.01$), morphology ($r = -0.57$, $p < 0.01$), and concentration ($r = -0.54$, $p < 0.01$). These findings indicate a potential role of these taxa in compromising semen quality.

Correlation Analysis Between Specific Bacterial Species and Semen Parameters

Statistical analysis revealed strong inverse correlations between the abundance of specific pathogenic bacterial species and critical semen parameters in infertile men. Notably, E. *coli* and *Pseudomonas aeruginosa* showed significant negative associations with sperm motility and morphology, suggesting that these pathogens may play a direct role in reducing fertility potential through inflammatory or toxic mechanisms.

Table 6. Pearson Correlation Coefficients Between Pathogenic Bacteria and Semen Parameters

Bacterial Species	Sperm Concentration (r)	Motility (r)	Morphology (r)	p-value (all)
Escherichia coli	-0.54	-0.62	-0.57	< 0.01
Pseudomonas aeruginosa	-0.51	-0.60	-0.55	< 0.01
Staphylococcus aureus	-0.45	-0.50	-0.48	< 0.05
Bacillus cereus	-0.39	-0.44	-0.41	< 0.05

4. DISCUSSION

The findings of this study highlight the crucial role of seminal microbiota in male reproductive health, offering compelling evidence that links specific pathogenic bacteria to reduced semen quality. By integrating traditional microbiological methods with 16S rRNA gene sequencing, the study not only identified microbial communities in the semen of infertile men but also revealed significant correlations between these bacteria and deteriorated sperm parameters.

Our findings indicated that seminal samples from infertile men exhibited a distinct microbial profile compared to those from fertile controls. Infertile individuals showed a significantly higher presence of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, and Staphylococcus pasteuri. This aligns with previous studies that have reported a predominance of gram-negative bacteria, particularly E. coli, in semen samples from infertile males (Kiessling et al., 2008; Monteiro et al., 2018). These organisms are recognized uropathogens known to compromise sperm quality through direct mechanisms, such as adhesion to spermatozoa, toxin release, and the induction of reactive oxygen species (ROS), which impair motility, viability, and DNA integrity.

The strong negative correlations between the presence of these bacteria and sperm motility, morphology, and concentration reinforce previous findings that microbial infections contribute to sperm dysfunction (Sanocka-Maciejewska et al., 2013). For instance, E. coli causes spermatozoa to agglutinate and reduces their motility by damaging their flagellar structure. Similarly, P. aeruginosa is associated with biofilm formation and the production of extracellular enzymes that degrade

seminal plasma proteins crucial for sperm protection and function.

Our study revealed that infertile men displayed significantly lower alpha diversity in microbial terms compared to fertile controls. This finding is consistent with Weng et al. (2020), who observed that reduced microbial richness and evenness in semen correlate with diminished semen quality. The decline in microbial diversity may indicate a dysbiotic condition in the male reproductive tract, where a few pathogenic genera dominate, outcompeting commensal or protective species and thereby intensifying inflammation and oxidative stress. The decreased Shannon index in our study further implies that infertile men have a less balanced and potentially more pathogenic microbiota.

An intriguing finding in our study was the identification of Staphylococcus pasteuri, an organism that has not been extensively studied in relation to male infertility. Although typically regarded as a skin commensal, its presence in semen and its association with impaired sperm parameters in this study suggest a potentially unrecognized role in genital tract colonization or contamination, which may contribute to subfertility. This underscores the necessity for further investigation into opportunistic pathogens that, while not part of the traditional urogenital flora, can disrupt reproductive function under certain conditions.

The application of 16S rRNA sequencing enhances the specificity and accuracy of microbial identification, corroborating the results obtained through culture-based methods. This technique allows for the high-confidence identification of bacterial taxa, many of which are challenging to differentiate based solely on phenotypic characteristics. Furthermore, submitting sequences to NCBI creates a valuable resource for future meta-analyses and comparative microbial studies across various populations and geographic regions.

Clinically, these findings emphasize the importance of incorporating microbiological assessments into routine infertility evaluations. Semen culture, especially when combined with sequencing, can help identify pathogenic profiles that may be responsive to antimicrobial therapy or probiotic interventions. Additionally, the link between these pathogens and key sperm parameters highlights the potential of microbial biomarkers as prognostic indicators of male fertility.

However, this study faced several limitations. While the sample size was sufficient for statistical analysis, it restricts broader generalization. Culture-based methods might have overlooked fastidious or anaerobic organisms not supported by standard media. Additionally, although correlations were identified, establishing causality between microbial presence and semen dysfunction requires interventional or longitudinal studies. Future research should consider larger multi-center studies incorporating metagenomic and metabolomic profiling to explore not only the taxonomic composition but also the functional roles of seminal microbes. Interventional trials assessing the impact of targeted antibiotic or probiotic therapies on microbiota restoration and semen quality enhancement would also be beneficial. In conclusion, this study supports the hypothesis that changes in seminal microbiota, particularly the presence of specific uropathogens, are linked to impaired sperm parameters in infertile men. Integrating molecular microbiology with conventional semen analysis offers a more comprehensive view of male reproductive health and paves the way for diagnostic and therapeutic advancements in managing male infertility.

5. CONCLUSION

This study presents compelling evidence that bacterial infections in semen are closely linked to impaired sperm quality, manifesting as reduced motility, abnormal morphology, increased leukocyte counts, and elevated oxidative stress levels. By integrating bacterial culture, biochemical identification, and 16S rRNA gene sequencing, the research offers a comprehensive analysis of seminal microbiota, uncovering both cultivable and non-cultivable bacterial species that may contribute to male infertility. The findings indicate that common uropathogens such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa adversely affect sperm function, potentially through direct bacterial adhesion, toxin production, and inflammatory responses. Moreover, molecular sequencing revealed additional fastidious and anaerobic bacteria, such as Gardnerella vaginalis and Fusobacterium nucleatum, which were not detected by culture-based methods, highlighting the necessity for advanced microbiome-based diagnostics in reproductive health. Given the strong correlation between bacterial infections and poor semen parameters, early detection and targeted therapeutic interventions could enhance fertility outcomes. Strategies like probiotic supplementation, antioxidant therapy, and personalized antimicrobial treatment warrant further exploration to restore seminal microbiota balance and reduce oxidative stress.

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