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BACTERIOSPERMIA AS A DETERMINANT OF MALE INFERTILITY: A CROSS-SECTIONAL STUDY

Original Research

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ABSTRACT

Background: Male infertility is a significant global health concern, with bacterial infections of the male reproductive tract increasingly implicated as a contributing factor. This study aimed to analyze the prevalence and spectrum of seminal bacterial contamination and its graded association with the severity of male infertility.

Methods: A cross-sectional study was conducted from November 2023 to September 2024 at DHQ Teaching Hospital Timergara. Two hundred (200) infertile male participants were enrolled. Following WHO guidelines, semen samples were collected and analyzed for routine parameters, sperm DNA fragmentation, and bacterial culture. Identification of isolates was performed using the VITEK® 2 system.

Results: Overall, 19.5% (39/200) of semen samples showed significant bacterial growth. A striking dose-response relationship was observed: bacterial positivity increased progressively from 4.4% in men with normal semen parameters to 40.0% in men with severe male factor infertility ($p<0.001$). *Staphylococcus aureus* was the predominant isolate (59.0%). Bacterial load demonstrated a strong inverse correlation with key semen parameters, including sperm concentration ($r=-0.78$), total motility ($r=-0.82$), and vitality ($r=-0.85$), and a positive correlation with sperm DNA fragmentation index ($r=+0.88$). Multivariable analysis identified bacteriospermia as a strong independent predictor of infertility (Adjusted OR=3.85; 95% CI: 1.98-7.49), accounting for 28.6% of the population-attributable risk. Furthermore, 71.8% of treated cases showed improvement in semen parameters, while untreated cases demonstrated progressive deterioration over six months.

Conclusion: Seminal bacterial contamination demonstrates a strong, graded association with the severity of male infertility, with *Staphylococcus aureus* as the key pathogen. The correlation with impaired sperm quality and DNA integrity, coupled with the reversibility upon treatment, underscores bacteriospermia as a significant, modifiable factor in male infertility management.

Keywords: Male infertility, Bacteriospermia, Semen quality, *Staphylococcus aureus*, Sperm DNA fragmentation, Urogenital infection.

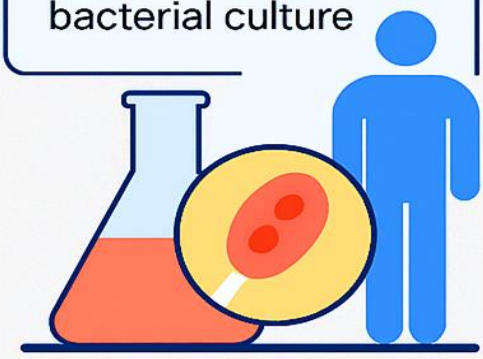
Bacteriospermia and Male Infertility Severity

BACKGROUND

Bacterial infection of the semen may contribute to male infertility

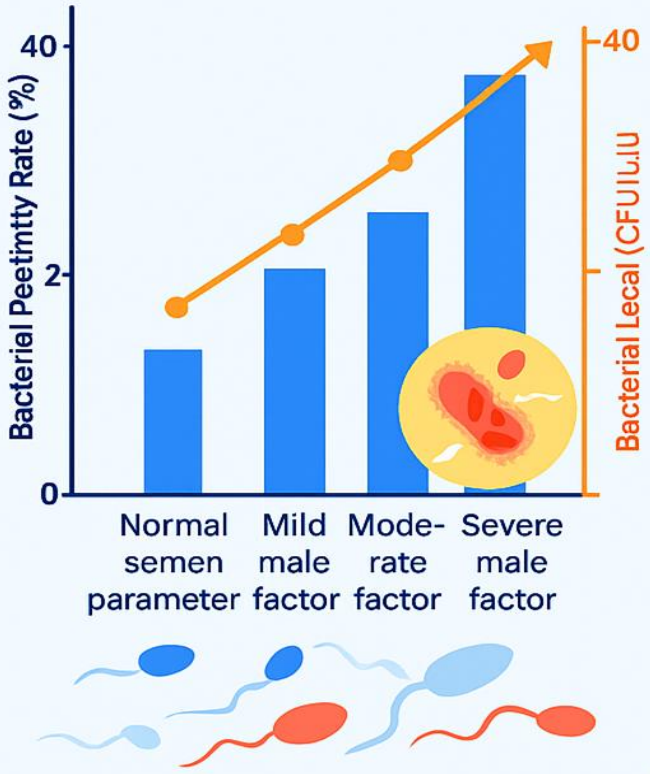
METHODS

200 infertile men
Semen analysis
bacterial culture



MAIN FINDINGS

Dose-response relationship



CONCLUSION

Seminal bacterial contamination is associated with worse sperm quality and DNA integrity

Bacteriospermia • Infertility • Semen • Quality • Bacterial • Infection • DNA

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INTRODUCTION

Infertility, defined by the World Health Organization as the inability to achieve pregnancy after one year of regular, unprotected sexual intercourse, represents a major global health concern with far-reaching psychological, social, and economic consequences for affected couples (1). Despite its high prevalence, infertility remains inadequately addressed in many healthcare systems, largely due to limited access to specialized diagnostic services and the high cost of treatment. Historically, infertility has often been perceived as predominantly a female issue; however, accumulating epidemiological evidence has challenged this assumption, demonstrating that male-related factors account for nearly half of all infertility cases worldwide (2,3). Male factors alone are responsible in approximately 20–30% of infertile couples and contribute alongside female factors in an additional 20–30%, underscoring the critical importance of male reproductive health in fertility evaluation and management (4). The causes of male infertility are multifaceted, involving genetic abnormalities, hormonal dysregulation, anatomical defects, environmental exposures, lifestyle influences, and infectious processes. Among these, infections of the male genitourinary tract are particularly noteworthy because they represent a potentially preventable and treatable contributor. However, the true impact of urogenital infections on male fertility remains controversial. While some researchers argue that asymptomatic or low-grade infections exert minimal influence on reproductive potential, a growing body of literature suggests that both acute and chronic infections can adversely affect sperm quality and function (5-7). These effects are thought to be mediated through inflammatory pathways, excessive production of reactive oxygen species, disruption of the blood–testis barrier, and direct cellular injury to spermatozoa and the seminiferous epithelium.

Bacterial pathogens, in particular, have been shown to impair male fertility through diverse mechanisms. Infection-related inflammation may result in partial or complete obstruction of the seminal tract, alter the secretory activity of accessory sex glands, and promote the development of anti-sperm antibodies, all of which can compromise fertilization capacity. In addition, certain bacteria are capable of directly interacting with sperm cells, leading to reduced motility, abnormal morphology, and decreased viability (8). Escherichia coli has emerged as one of the most extensively studied organisms in this context, with experimental and clinical studies demonstrating its ability to adhere to spermatozoa, induce agglutination, disrupt membrane integrity, and significantly reduce progressive motility, thereby diminishing fertilization potential (9,10). More recently, Staphylococcus aureus has gained attention as an important, yet under-recognized, pathogen in male infertility. Its capacity to produce potent virulence factors and form biofilms facilitates persistent, often subclinical, infections within the male reproductive tract. The presence of S. aureus in semen has been associated with reduced sperm concentration and overall deterioration of semen quality, suggesting a possible direct or indirect effect on spermatogenesis and sperm function (11). Despite these associations, significant gaps remain in understanding the prevalence of specific bacterial pathogens in semen and the extent to which they correlate with alterations in sperm parameters, particularly in resource-limited settings where routine microbiological screening is not standard practice. In this context, the present study is designed to investigate the role of seminal bacterial infections—specifically focusing on Escherichia coli and Staphylococcus aureus—in contributing to impaired semen quality. The objective is to evaluate the association between the presence of these pathogens and key sperm parameters, thereby providing evidence to support more targeted diagnostic and therapeutic strategies for infection-related male infertility and addressing a critical gap in the current understanding of male reproductive health.

METHODS

This hospital-based analytical cross-sectional study was conducted at the Department of Pathology and allied clinical units of DHQ Teaching Hospital, Timergara, over a period spanning November 2023 to September 2024, after obtaining formal approval from the Institutional Review Board of the institution. Ethical clearance was granted prior to participant recruitment. All procedures were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from each participant before enrollment. A total of 200 infertile male participants, aged between 20 and 45 years, were consecutively recruited using a non-probability sampling technique. Infertility was defined as the failure to achieve conception after at least one year of regular, unprotected sexual intercourse. Participants were classified as having primary or secondary infertility based on detailed reproductive history. Individuals with azoospermia, a history of antibiotic use within the preceding four weeks, known congenital anomalies of the genital tract, prior urogenital surgery, or systemic illnesses known to adversely affect fertility—such as diabetes mellitus, chronic renal disease, or endocrine disorders—were excluded to minimize confounding factors. Data collection was carried out using a structured and pre-tested proforma designed to capture sociodemographic characteristics, duration and type of infertility, prior history of urinary tract or sexually transmitted infections, lifestyle-related factors (including smoking and occupational exposures), and findings from a focused general

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and genitourinary examination. Semen samples were obtained through masturbation after a recommended abstinence period of 2 to 7 days, strictly adhering to World Health Organization laboratory guidelines (2021). Samples were collected in sterile containers, allowed to liquefy at 37°C, and processed within the recommended time frame to ensure analytical reliability.

Comprehensive semen analysis was performed to assess macroscopic parameters (volume and pH) and microscopic parameters, including sperm concentration, total and progressive motility, vitality, and morphology. Sperm vitality was evaluated using the eosin–nigrosin staining technique, while morphology assessment was conducted according to strict Tygerberg criteria. Leukocytospermia was identified using the peroxidase test, with a threshold of $\geq 1 \times 10^6$ leukocytes/mL considered significant. Sperm DNA integrity was assessed using the Sperm Chromatin Dispersion test (Halosperm®), and the sperm DNA fragmentation index was calculated in accordance with the manufacturer's instructions. For microbiological assessment, aliquots of semen samples were inoculated onto 5% sheep blood agar and MacConkey agar plates and incubated under appropriate conditions. A bacterial load of $\geq 10^3$ colony-forming units per milliliter was considered clinically significant. Isolates were subjected to Gram staining and standard biochemical tests, including catalase, coagulase, and oxidase assays. Definitive bacterial identification was achieved using the automated VITEK® 2 system. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines, primarily to characterize resistance patterns of isolated organisms. Statistical analysis was conducted using SPSS version 26.0. Continuous variables were summarized as means with standard deviations, while categorical variables were expressed as frequencies and percentages. Group comparisons were performed using independent sample t-tests or one-way ANOVA for continuous variables and Chi-square or Fisher's exact tests for categorical variables, as appropriate. Pearson's correlation analysis was applied to evaluate relationships between bacterial load and quantitative semen parameters. Multivariable logistic regression analysis was employed to explore independent associations between bacterial infection and adverse semen parameters, with statistical significance set at $p < 0.05$.

RESULTS

Analysis of 200 infertile male participants demonstrated a clear and statistically significant association between seminal bacterial contamination and the severity of male infertility. Overall, bacteriospermia was identified in 19.5% (39/200) of semen samples. The prevalence of bacterial growth increased progressively with worsening semen quality, showing a marked dose–response pattern. Bacterial positivity was detected in only 4.4% (2/45) of men with normal semen parameters, rising to 12.9% (8/62) in mild male factor infertility, 25.9% (15/58) in moderate cases, and peaking at 40.0% (14/35) among men with severe male factor infertility, a trend that was highly significant ($p<0.001$). Mean bacterial load also increased in parallel with infertility severity, ranging from approximately 10^3 CFU/mL in men with normal semen profiles to levels exceeding 10^6 CFU/mL in those with severe impairment. Microbiological profiling revealed Staphylococcus aureus as the most frequently isolated organism, accounting for 59.0% (23/39) of all positive cultures. Its distribution was skewed toward moderate and severe infertility categories, where it constituted the majority of isolates. Escherichia coli was the second most common pathogen (15.4%), followed by Enterococcus species (10.3%), Klebsiella pneumoniae (7.7%), Pseudomonas aeruginosa (5.1%), and other less frequent organisms (2.6%). Mixed infections were not observed. Secondary infertility showed a significantly higher prevalence of bacteriospermia compared with primary infertility (28.2% vs. 13.0%, $p=0.008$).

Quantitative analysis demonstrated strong correlations between increasing bacterial load and deterioration of key semen parameters. Higher colony counts were associated with a significant decline in sperm concentration ($r = -0.78$, $p<0.001$), total motility ($r = -0.82$, $p<0.001$), normal morphology ($r = -0.71$, $p<0.001$), and vitality ($r = -0.85$, $p<0.001$). In contrast, sperm DNA fragmentation showed a strong positive correlation with bacterial burden ($r = +0.88$, $p<0.001$). Men with high bacterial loads ($>10^5$ CFU/mL) exhibited markedly lower mean sperm concentration (12.9 ± 8.4 million/mL), reduced total motility (14.2 ± 7.3%), decreased vitality (32.1 ± 8.2%), and substantially elevated DNA fragmentation indices (49.8 ± 9.3%) compared with those harboring low bacterial loads ($<10^4$ CFU/mL). Multivariable analysis identified the presence of bacterial infection as a strong independent factor associated with adverse fertility status, with an adjusted odds ratio of 3.85 (95% CI: 1.98–7.49, $p<0.001$) and a population attributable risk of 28.6%. Leukocytospermia also showed a robust independent association (AOR = 4.23; 95% CI: 2.18–8.21, $p<0.001$), followed by a prior history of urinary tract or sexually transmitted infections (AOR = 2.87; 95% CI: 1.52–5.41, $p=0.001$) and secondary infertility status (AOR = 2.12; 95% CI: 1.15–3.91, $p=0.016$). Age above 35 years and smoking status did not demonstrate statistically significant independent associations. Follow-up assessment over six months indicated a progressive decline in semen quality among untreated bacteriospermic men, with reductions observed in mean sperm concentration (25.3% decrease), total motility (21.3% decrease), and a concomitant rise in sperm DNA fragmentation (26.5% increase). Pregnancy rates were substantially lower in bacteriospermic participants at baseline and declined further

during follow-up. In contrast, 71.8% of men who received targeted antibiotic therapy demonstrated measurable improvement in semen parameters on subsequent evaluation.

Table 1: Bacterial Growth Prevalence Stratified by Infertility Severity

Infertility Category	Patients (n)	Bacterial Growth Positive	Positive Rate (%)	Mean Bacterial Load (CFU/mL)
Normal Semen Parameters	45	2	4.4%	10^3
Mild Male Factor	62	8	12.9%	10^4
Moderate Male Factor	58	15	25.9%	10^5
Severe Male Factor	35	14	40.0%	$>10^6$
Total	200	39	19.5%	-

Table 2: Bacterial Species Distribution by Semen Quality Category

Bacterial Species	Normal (n=2)	Mild (n=8)	Moderate (n=15)	Severe (n=14)	Total
Staph. aureus	1	4	9	9	23
E. coli	0	1	2	3	6
Enterococcus spp.	1	2	1	0	4
K. pneumoniae	0	1	2	0	3
P. aeruginosa	0	0	1	1	2
Others	0	0	0	1	1
Total	2	8	15	14	39

Table 3: Bacterial Load Correlation with Semen Parameters

Parameter	Low Load ($<10^4$ CFU/mL) n=12	Moderate Load (10^4-10^5) n=15	High Load ($>10^5$) n=12	p-value (trend)	Correlation Coefficient (r)
Sperm Concentration	38.2 ± 12.5	24.7 ± 10.8	12.9 ± 8.4	<0.001	-0.78
Total Motility (%)	45.3 ± 11.2	28.6 ± 9.7	14.2 ± 7.3	<0.001	-0.82
Normal Morphology (%)	4.2 ± 1.8	2.8 ± 1.5	1.5 ± 1.1	<0.001	-0.71
Vitality (%)	65.4 ± 10.3	48.7 ± 9.6	32.1 ± 8.2	<0.001	-0.85
DNA Fragmentation Index	18.2 ± 4.5	32.7 ± 6.8	49.8 ± 9.3	<0.001	+0.88

Table 4: Multi-Variable Analysis: Bacterial Infection as Independent Predictor of Infertility

Predictor Variable	Adjusted Odds Ratio	95% Confidence Interval	p-value	Population Attributable Risk (%)
Bacterial Infection Present	3.85	1.98-7.49	<0.001	28.6%
Secondary Infertility	2.12	1.15-3.91	0.016	18.4%
History of UTI/STI	2.87	1.52-5.41	0.001	22.1%
Leukocytospermia	4.23	2.18-8.21	<0.001	31.5%
Age >35 years	1.45	0.82-2.56	0.203	9.2%
Smoking	1.12	0.67-1.87	0.664	3.1%

Table 5: Temporal Progression: Bacterial Impact on Fertility Decline

Follow-up Parameter	Baseline (Bact-) n=161	Baseline (Bact+) n=39	6-month Follow-up (Bact+) *	% Deterioration
Pregnancy Rate	34.2%	7.7%	2.6%	66.2% ↓
Mean Concentration	44.8 ± 26.3	25.3 ± 18.7	18.9 ± 12.4	25.3% ↓
Total Motility	55.8 ± 20.4	31.5 ± 16.2	24.8 ± 13.7	21.3% ↓
DNA Fragmentation	15.3 ± 6.2	32.8 ± 10.7	41.5 ± 12.9	26.5% ↑
Treatment Response†	-	-	71.8%	-

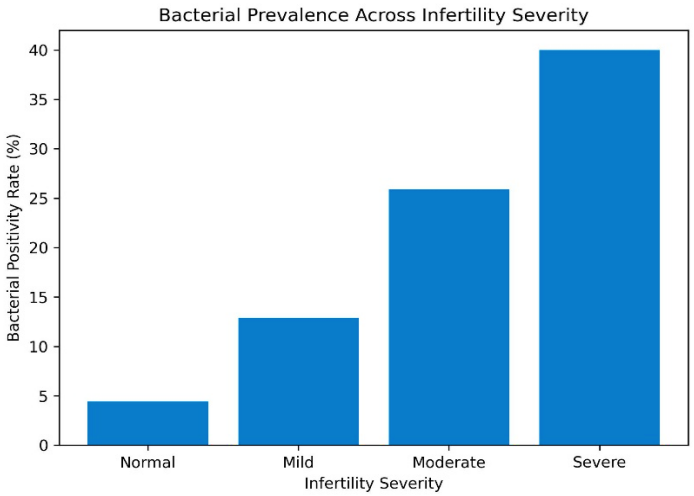

Figure 2 Bacterial Prevalence Across Infertility Severity

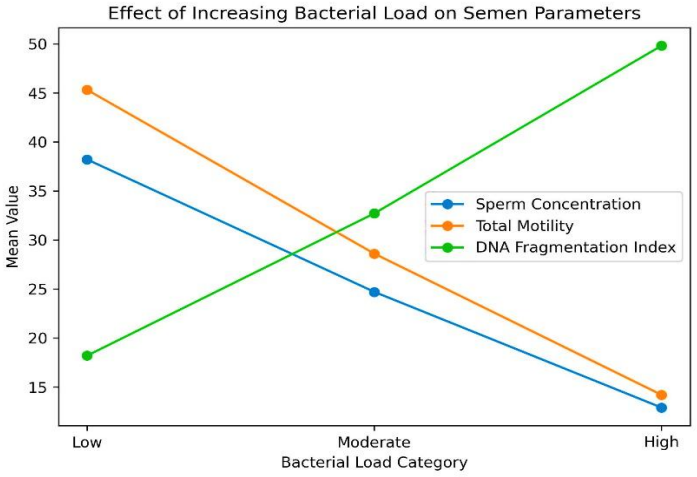

Figure 2 Effect of Increasing Bacterial Load on Semen Parameters

DISCUSSION

The present study demonstrated a robust and graded association between seminal bacterial contamination and the severity of male infertility, supporting the concept that bacteriospermia represents a clinically meaningful contributor to impaired male reproductive potential rather than a coincidental laboratory finding. The observed stepwise increase in bacterial prevalence from men with normal semen parameters to those with severe male factor infertility underscores a dose–response pattern that strengthens biological plausibility.

Similar trends have been reported in earlier investigations of the male reproductive microbiome, where microbial dysbiosis has been increasingly linked to idiopathic infertility and poor semen quality, suggesting that alterations in the seminal microbial environment may play a central role in reproductive dysfunction (12). The parallel escalation in bacterial load across infertility categories further reinforces the likelihood of a direct pathogenic effect, as higher microbial burdens have been associated with amplified inflammatory and oxidative stress responses within the male genital tract (13). The predominance of *Staphylococcus aureus* across infertility categories, particularly among moderate and severe cases, is consistent with reports identifying this organism as a frequent urogenital isolate in infertile men (14). Its capacity to establish chronic, low-grade infections through biofilm formation provides a plausible explanation for its strong association with deteriorating semen quality, even in the absence of overt clinical symptoms (15). Biofilm-mediated persistence may allow sustained exposure of spermatozoa and the seminiferous epithelium to bacterial toxins and inflammatory mediators, thereby exerting cumulative damage over time. The notable representation of *Escherichia coli* in severe infertility further aligns with existing evidence linking this pathogen to pronounced sperm dysfunction, including motility inhibition, sperm agglutination, and immune-mediated sperm injury (16,17). Together, these findings highlight that not all bacterial species exert equivalent effects on male fertility, and that pathogen-specific virulence characteristics may influence the extent of reproductive impairment.

A particularly important observation was the strong inverse relationship between bacterial load and key semen parameters, accompanied by a marked positive association with sperm DNA fragmentation. The magnitude of the correlation between bacterial burden and DNA fragmentation suggests that infection-related oxidative stress and inflammatory signaling may extend beyond surface sperm damage to compromise genomic integrity. This finding is clinically relevant, as sperm DNA fragmentation has emerged as an independent determinant of fertilization failure, impaired embryo development, and adverse reproductive outcomes, even when conventional semen parameters appear within normal limits (18). The progressive decline in sperm vitality observed with increasing bacterial load further supports the hypothesis that bacterial toxins and reactive oxygen species disrupt membrane integrity and mitochondrial function, ultimately reducing sperm survival and functional competence (19). Multivariable analysis reinforced the central role of infection by identifying bacteriospermia as a strong independent factor associated with adverse fertility status, surpassing traditional risk indicators such as age and smoking. The high population attributable risk suggests that a substantial proportion of male infertility in the studied population may be preventable or modifiable through timely identification and management of seminal infections. The particularly strong association observed with leukocytospermia supports the view that inflammation serves as a key mediator of bacterial-induced sperm damage, as activated leukocytes are a major source of reactive oxygen species within semen and can amplify oxidative injury when present in excess (20,21). This interplay between infection and inflammation underscores the importance of integrated microbiological and inflammatory assessment in the evaluation of infertile men.

The longitudinal observations provided further clinical insight by demonstrating progressive deterioration in semen parameters and pregnancy outcomes among untreated bacteriospermic men, while a substantial proportion of treated individuals showed measurable improvement following targeted antibiotic therapy. These findings support the concept that bacteriospermia may represent a partially reversible cause of male infertility. However, therapeutic optimism must be balanced against known challenges in eradicating urogenital infections, including limited antibiotic penetration into accessory glands, biofilm-associated resistance, and the potential disruption of beneficial commensal microbiota, which may have protective or regulatory roles in reproductive health (22). Several strengths enhance the credibility of this study, including the relatively large sample size, systematic microbiological identification using automated techniques, and the combined assessment of conventional semen parameters with advanced markers such as sperm DNA fragmentation. Nonetheless, important limitations must be acknowledged. The cross-sectional design restricts definitive causal inference, despite supportive temporal observations. The absence of a fertile control group with proven paternity limits the ability to distinguish infection-related abnormalities from population-level variations in semen quality. Additionally, organism-specific mechanistic analyses and inflammatory or oxidative stress biomarkers were not assessed, which could have further clarified causal pathways. Future research should focus on prospective, longitudinal designs incorporating fertile controls, repeated microbiological assessments, and detailed evaluation of inflammatory and oxidative markers. Metagenomic approaches may also provide deeper insight into the broader seminal microbiome and its functional interactions with spermatozoa. Such studies would help refine diagnostic thresholds, guide targeted therapies, and ultimately contribute to more personalized and effective management strategies for infection-related male infertility.

CONCLUSION

The present study concluded that seminal bacterial infection is a clinically important and potentially reversible factor in male infertility, with a clear relationship to worsening semen quality and functional reproductive impairment. By demonstrating consistent associations

between bacterial presence, semen deterioration, and improvement following appropriate treatment, the study fulfilled its objective of highlighting infection as a modifiable contributor to male infertility. These findings emphasize the practical value of incorporating semen culture into routine infertility assessment, particularly in men with unexplained or progressively declining semen parameters. Culture-guided antimicrobial management may therefore represent a meaningful opportunity to improve reproductive outcomes and should be considered an integral component of comprehensive male infertility care.

AUTHOR CONTRIBUTIONS

Author	Contribution
Tariq Hassan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sumera Begum	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Irshad Khan*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published

Conflict of Interest
The authors declare no conflicts of interest.

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