*International Journal of Basic and Applied Sciences*, 14 (7) (2025) 123-129

International Journal of Basic and Applied Sciences

*Website: www.sciencepubco.com/index.php/IJBAS*
*https://doi.org/10.14419/qwr5b132*
***Research paper***

# Inflammatory Biomarkers and Sperm Dysfunction in Bacterial Semen Infections

**Satyanarayan Samantaray [1] *, Soumya Jal [1], Gopal Krishna Purohit [2], Soumya Dash [3]**

*[1] School of Paramedics and Allied Health Sciences; Centurion University of Technology and Management; Odisha*
*[2] Heredity Biosciences, Plot No:818, Mayfair Lagoon Road, Jayadev Vihar, Bhubaneswar, Odisha*
*[3] Fertility specialist, Ankura Medical and research Center, Near Sisubhavan Bus Stop, Bapuji Nagar, Bhubaneswar, Odisha 751009*
**Corresponding author E-mail: rimmi.aktu.edu@gmail.com*

*Received: July 21, 2025, Accepted: September 2, 2025, Published: November 4, 2025*

**Abstract**

Male infertility is a global concern because bacterial infections of the reproductive tract play a significant role. These infections trigger inflammatory responses that impair sperm quality. This study explored the relationship between bacterial semen infection, inflammatory biomarkers, and sperm dysfunction using PCR and quantitative real-time PCR (qPCR). Semen samples were collected from 60 male participants attending the infertility clinics. Semen analysis was performed according to the WHO 2021 guidelines to assess concentration, motility, morphology, and viability. Bacterial DNA was isolated from the samples and screened by PCR targeting 16S rRNA genes to detect common seminal pathogens. The expression levels of inflammatory cytokine genes (IL-6, TNF-α, and IL-1β) were quantified using SYBR Green-based qPCR. Gene expression was normalized to that of GAPDH, which was used as a housekeeping gene. Correlations between bacterial presence, cytokine expression, and sperm parameters were analyzed. PCR revealed bacterial DNA in 40% of the semen samples, with Escherichia coli and Staphylococcus aureus being predominant. Samples positive for bacterial DNA showed significantly higher expression of IL-6, TNF-α, and IL-1β ($P < 0.01$), indicating an inflammatory response. These elevated cytokine levels correlated with decreased sperm motility, viability, and morphological integrity ($r > -0.6$, $p < 0.05$). The study showed that bacterial infections in semen are associated with the upregulation of inflammatory cytokines, contributing to sperm dysfunction. PCR and qPCR effectively detect the presence of bacteria and quantify inflammation at the molecular level. These findings underscore the need for molecular diagnostics for male infertility evaluation and highlight inflammatory biomarkers as potential therapeutic targets.

***Keywords*** *: Male Infertility; Semen Microbiota; Inflammatory Biomarkers; 16S rRNA; PCR; Cytokine Gene Expression.*

## 1. Introduction

Male infertility is a significant global health issue, contributing to approximately 50% of all infertility cases in couples (Khan et al, 2024; Raghuwanshi and Kanwal, 2025). Among the various etiological factors, infections of the male reproductive tract are notable contributors, often leading to inflammation and the subsequent impairment of sperm function (Babakhanzadeh et al., 2020). In particular, bacterial infections can induce inflammatory responses that adversely affect spermatogenesis and sperm parameters, including motility, morphology, and DNA integrity (Oghbaei et al. 2020, Wang et al. 2021, Dutta and Sengupta 2025, Samantaray et al. 2024).

The male reproductive tract, once considered sterile, is now known to harbor diverse microbiota. Advances in molecular techniques, such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), have facilitated the detection and quantification of bacterial DNA in semen samples, revealing associations between specific bacterial species and altered semen quality (Kaltsas et al, 2023; Syafrizayanti et al., 2022). Notably, pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, and Ureaplasma urealyticum have been implicated in the pathogenesis of male infertility through mechanisms involving direct sperm damage and induction of inflammatory responses (Shen et al. 2011).

Inflammation within the male reproductive system is characterized by leukocyte infiltration and the release of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β). These cytokines can disrupt the blood-testis barrier, impair Sertoli cell function, and induce oxidative stress, leading to sperm DNA fragmentation and apoptosis (Potiris et al., 2025). Elevated levels of these inflammatory markers in semen have been correlated with decreased sperm motility and increased morphological abnormalities, underscoring their role in male infertility (Akinloye et al., 2015).

Leukocytospermia, defined as the presence of more than 1 × 10^6 leukocytes per milliliter of semen, is a common finding in infertile men and is often associated with bacterial infections. The presence of leukocytes contributes to the generation of reactive oxygen species (ROS), exacerbating oxidative stress and further compromising sperm function (Khodamoradi et al, 2020). Despite its clinical relevance, the

diagnosis and management of leukocytospermia remain challenging, necessitating the development of reliable biomarkers for early detection and targeted therapy.

The use of PCR and qPCR techniques has revolutionized the detection of bacterial pathogens and the quantification of inflammatory gene expression in semen samples. These molecular approaches offer high sensitivity and specificity, enabling the identification of subclinical infections and assessment of inflammatory status. By quantifying the expression levels of cytokine genes, researchers can elucidate the extent of inflammation and its impact on sperm quality (Ricchi et al., 2017).

This study focuses on exploring the complex connections between bacterial infections, inflammation, and sperm dysfunction by examining the link between the presence of bacteria in semen, inflammatory biomarkers, and sperm quality indicators. Using PCR and qPCR techniques, we aimed to identify bacterial DNA and measure the levels of significant pro-inflammatory cytokines in semen samples from infertile men. A better understanding of these relationships will deepen our understanding of the pathophysiological processes involved in infection-related male infertility, and could guide the development of diagnostic and treatment approaches.

# 2. Methods

## 2.1. Study population

Between August 2024 and March 2025, 60 semen samples were collected from male participants undergoing fertility evaluations at the Ankura Fertility Center, Bhubaneswar. The study group consisted of 40 men diagnosed with idiopathic infertility and 20 fertile age-matched controls. All participants were instructed to maintain a period of sexual abstinence for 3–5 days before sample collection in strict adherence to the World Health Organization (WHO, 2021) guidelines for semen analysis.

## 2.2. Semen sample collection and analysis

Semen samples were obtained via masturbation and collected in sterile, wide-mouthed, non-toxic containers. Immediately after collection, each sample was carefully labeled and transported to the andrology laboratory under controlled conditions. All samples were analyzed within one hour to ensure the accuracy of parameters, such as motility, concentration, morphology, and viability. This standardized collection and handling process ensured the reliability of the semen quality assessments and subsequent molecular and microbiological analyses conducted during the study.

## 2.3. Bacterial DNA isolation and PCR amplification

Following semen analysis, aliquots of each sample were subjected to microbial DNA extraction using a Qiagen DNA Mini Kit (Qiagen, Germany), strictly adhering to the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed using a NanoDrop Lite Plus spectrophotometer (Invitrogen, USA), ensuring an A260/A280 ratio of 1.8 and 2.0.

For bacterial identification, the 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Acharya et al. 2025). The PCR reaction mixture (25 µL total volume) consisted of 12.5 µL of PCR master mix, 1 µL of each primer (10 µM), 2 µL of template DNA, and nuclease-free water to adjust the volume.

PCR was performed in a Takara thermal cycler (Takara Bio, Japan) under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 10 min.

The amplified products were later analyzed using agarose gel electrophoresis to confirm the presence of ~1500 bp amplicons, indicative of successful 16S rRNA gene amplification.

## 2.4. Agarose Gel Electrophoresis and Sequencing

The PCR amplicons were analyzed by electrophoresis on a 1.5% agarose gel with SYBR Safe DNA stain in 1X TAE buffer. Electrophoresis was performed at 100 V for 45 min using a 100 bp DNA ladder as a marker. Clear bands at 1500 base pairs confirmed successful 16S rRNA gene amplification. Purified PCR products were subjected to Sanger sequencing. Bioinformatics analysis using NCBI BLASTn identified bacterial species. Sequences were submitted to NCBI GenBank, and the accession number is in Table 4

## 2.5. RNA extraction and cDNA synthesis

Total RNA was extracted from semen cell pellets using a Sperm RNA isolation kit (Transgene, India) according to the manufacturer's instructions. RNA purity was confirmed with an A260/A280 ratio of 1.8 and 2.0. Complementary DNA (cDNA) was synthesized using the PrimeScript™ RT Reagent Kit (Takara, Japan) according to the manufacturer's instructions. The synthesized cDNA was stored at -20°C until use.

## 2.6. Quantitative real-time PCR (qPCR)

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using Tata MD CHECK Express real-time PCR (TATA, India) at Heredity Biosciences, Bhubaneswar. The target genes included IL-6, TNF-α, and IL-1β, with GAPDH serving as the housekeeping gene (Table 1). Gene-specific primers were selected based on validated sequences reported in peer-reviewed journals and their specificity was confirmed using the Primer-BLAST tool. Reactions were performed using SYBR Green Master Mix (Takara, Japan). Before the main qPCR runs, each primer set was optimized for annealing temperature (Tm), concentration, and amplification efficiency to ensure accurate and reproducible quantification.

All primers with more than 95% efficiency were considered for qPCR using the $^{2\text{-}\triangle\triangle}$CT method. During each run, the melt curve stage was considered along with the run method to verify primer specificity. Each qRT-PCR reaction consisted of 1.5 µL cDNA, 0.5 µL forward primer, 0.5 µL reverse primer, 5 µL master mix, and 3 µL nuclease-free water. The reactions were performed in duplicates. The qRT-PCR protocol involved an initial denaturation step (2 min at 95°C), followed by 40 cycles of amplification at 95°C for 15 s, 58°C for 60°C, or

64°C (depending on the specific primer) for 30 s. Melt curve analysis confirmed the amplification specificity. Data analysis for relative gene expression was performed using the $^{2-\triangle\triangle CT}$ method.

**Table 1:** Primer Sequences for Qrt-PCR Analysis of the IL-6, TNF-A, IL-1β, and GAPDH Genes Have Been Confirmed for Specificity Using Literature and Primer-BLAST

| Primer Name | Forword Sequence (5'→3') | Reverse Sequence (5'→3') | Annealing Temp (°C) | Product Size (bp) | References |
|---|---|---|---|---|---|
| IL-6 | ATTCTGCGCAGCTTTAAGGA | AACAACAATCTGAGGTCGCC | 58 | 121 | Hana Attia et al, 2021 |
| TNF-α | GGTGCTTGTTCCTCAGCCTC | AGATGATCTGACCTGCCTGGG | 60 | 142 | Hana Attia et al, 2021 |
| IL-1β | AGCTCGCCAGTGAAATGATG | TGTAGTGGTGGTCGGAGATT | 55 | 156 | Mirghanizadeh Baf-ghi et al |
| GAPDH | AAATCAAGTGGGGCGATGCTG | GCAGAGATGATGACCCTTTTG | 53 | 118 | Mirghanizadeh Baf-ghi et al |

### 2.7. Statistical analysis

All experiments were conducted in triplicate. Data are expressed as mean ± SEM. Statistical comparisons between groups were performed using Student's t-test or ANOVA with Tukey's post-hoc test. Pearson's correlation analysis was used to evaluate the associations between cytokine expression levels and semen parameters. Statistical significance was set at $P < 0.05$. Analyses were performed using GraphPad Prism version 10.

## 3. Results

### 3.1. Semen sample collection and analysis

Semen samples were obtained from 60 male participants, including 40 infertile and 20 fertile men. Semen parameters were assessed according to the WHO 2021 guidelines. As shown in Table 2, infertile men exhibited significantly reduced sperm concentration (12.5 ± 4.3 × $10^6$/mL vs. 48.7 ± 6.2 × $10^6$/mL), total motility (28.4 ± 6.1% vs. 58.9 ± 7.3%), and progressive motility (20.3 ± 5.7% vs. 45.2 ± 6.8%) compared to fertile controls. Additionally, the incidence of normal morphology was notably lower in the infertile group (2.1 ± 0.9% vs. 6.5 ± 1.2%). Leukocyte counts were elevated in infertile samples (1.2 ± 0.4 × $10^6$/mL) relative to fertile men (0.4 ± 0.2 × $10^6$/mL), exceeding the WHO threshold (<1.0 × $10^6$/mL), suggesting possible inflammation or infection.

**Table 2:** Comparison of Semen Parameters between Infertile and Fertile Men

| Parameter | Infertile Men (n=40) | Fertile Men (n=20) | WHO Reference Range (2021) |
|---|---|---|---|
| Volume (mL) | 1.8 ± 0.5 | 3.2 ± 0.6 | ≥1.4 ml |
| Sperm Concentration ($10^6$/mL) | 12.5 ± 4.3 | 48.7 ± 6.2 | ≥16 million |
| Total Motility (%) | 28.4 ± 6.1 | 58.9 ± 7.3 | ≥42 million |
| Progressive Motility (%) | 20.3 ± 5.7 | 45.2 ± 6.8 | ≥30 % |
| Morphology (% normal forms) | 2.1 ± 0.9 | 6.5 ± 1.2 | ≥4 % |
| Leukocyte Count ($10^6$/mL) | 1.2 ± 0.4 | 0.4 ± 0.2 | <1.0 |

Data are presented as the mean ± standard deviation.
The infertile group showed a marked decrease in semen volume, sperm concentration, motility, and morphology, along with higher leukocyte counts, compared to the fertile controls.

### 3.2. Bacterial DNA isolation, PCR amplification, primer selection, and sequence submission

Genomic DNA was successfully extracted from all the semen samples. The PCR technique, targeting the conserved 16S rRNA gene, employed universal primers 27F and 1492R (Table 3) with an annealing temperature of 55°C, producing a product approximately 1500 bp in length. The presence of amplicons in these samples was verified using gel electrophoresis (Figure 1). Bacterial sequencing data were submitted to the NCBI GenBank database for validation and future reference. Table 3 provides the sample ID, bacterial species, and NCBI accession numbers.

**Table 3:** Primer Sequences and PCR Conditions

| Primer Name | Sequence (5'→3') | Target Region | Annealing Temp (°C) | Product Size (bp) |
|---|---|---|---|---|
| 27F | AGAGTTTGATCMTGGCTCAG | 16S rRNA | 58 | ~1500 |
| 1492R | GGTTACCTTGTTACGACTT | | | ~1500 |

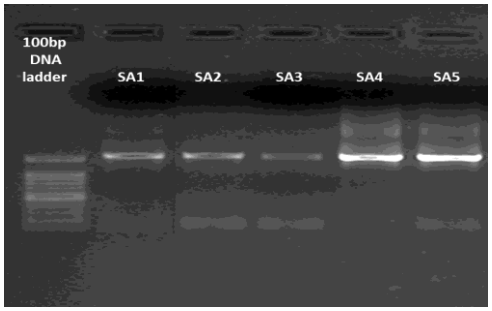


**Fig. 1:** Agarose Gel Electrophoresis Showing PCR Amplification of Bacterial 16S rRNA Genes. Lanes 1–5: Amplified Products (~1500 Bp); Lane M: 100 Bp DNA Ladder. Clear Bands At ~1500 Bp Confirmed the Successful Amplification, Indicating the Presence of Bacterial DNA in the Samples.

**Table 4:** The sequences Were Submitted to the NCBI GenBank Database under the Accession Numbers Provided

| Sample ID | Bacterial Species Identified | NCBI Accession Number |
|---|---|---|
| SA1 | Escherichia coli | PV124038 |
| SA2 | Staphylococcus aureus | PV202446 |
| SA3 | Pseudomonas aeruginosa | PV124045 |
| SA4 | Bacillus cereus | PV202415 |
| SA5 | Staphylococcus pasteuri | PV202445 |

## 4. Quantitative Real-Time PCR (qPCR) Ct Values and Expression Analysis

The expression levels of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β were quantified using SYBR Green-based qPCR to assess the inflammatory response associated with bacterial infection in semen. Primer sequences and amplification parameters are summarized in Table 5 & Figure 2. Gene expression was normalized to GAPDH as an internal control, and relative expression was calculated using the comparative ΔΔCt method. Infertile male subjects exhibited significantly lower Ct values and elevated expression of all three cytokines compared to fertile controls, indicating the upregulation of inflammatory pathways. The calculated average fold increases were as follows: IL-6, 6.9-fold (ΔΔCt = 2.8); TNF-α, 8.5-fold (ΔΔCt = 3.1); and IL-1β, 5.7-fold (ΔΔCt = 2.5). These findings suggest that the presence of bacteria in the semen is associated with a heightened inflammatory gene expression profile, potentially contributing to impaired sperm function and reduced fertility. qPCR analysis revealed significantly elevated expression levels of IL-6, TNF-α, and IL-1β in semen samples of infertile men compared to fertile controls, indicating an inflammatory response associated with bacterial infections.

**Table 5:** qPCR Ct Values and Relative Expression Levels

| Gene | Group | Mean Ct ± SD | ΔCt ± SD | ΔΔCt ± SD | Fold Change (2^-ΔΔCt) |
|---|---|---|---|---|---|
| IL-6 | Infertile | 23.5 ± 0.6 | 5.2 ± 0.4 | 2.8 ± 0.3 | 6.9 |
| | Fertile Control | 26.3 ± 0.5 | 8.0 ± 0.3 | — | — |
| TNF-α | Infertile | 24.1 ± 0.7 | 5.8 ± 0.5 | 3.1 ± 0.4 | 8.5 |
| | Fertile Control | 27.2 ± 0.6 | 8.9 ± 0.4 | — | — |
| IL-1β | Infertile | 22.9 ± 0.5 | 4.6 ± 0.3 | 2.5 ± 0.2 | 5.7 |
| | Fertile Control | 25.4 ± 0.4 | 7.1 ± 0.3 | — | — |

Gene expression was quantified using the comparative Ct method, where ΔCt is the difference between the target gene and GAPDH Ct values (ΔCt = Ct_target gene - Ct_GAPDH). ΔΔCt was calculated by subtracting the fertile control group ΔCt from the infertile group ΔCt (ΔΔCt = ΔCt_infertile - ΔCt_fertile control). Relative expression was determined using 2^–ΔΔCt, measuring regulation between conditions.

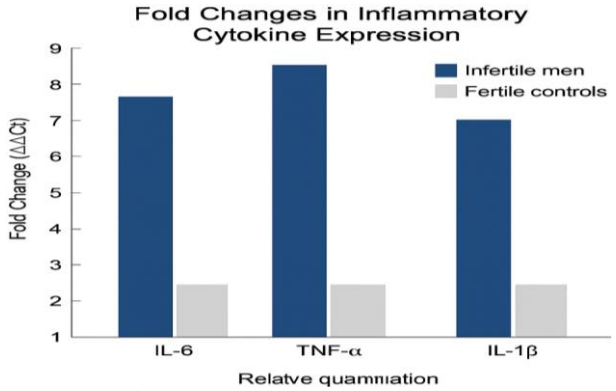


**Fig. 2:** Relative Expression Levels of Inflammatory Cytokines: Bar Graph Depicting Fold-Changes in IL-6, TNF-α, and IL-1β Expression between Infertile and Fertile Men.

Differential Expression of Cytokines in Bacteria-Positive vs. Bacteria-Negative Samples

Quantitative analysis of cytokine gene expression revealed significantly higher levels of IL-6, TNF-α, and IL-1β in bacteria-positive semen samples than in bacteria-negative samples. The presence of bacteria was confirmed by PCR amplification of 16S rRNA. The expression of cytokines was normalized to GAPDH, and relative quantification was determined using the $^{2^{-\Delta\Delta}}$Ct method.

In bacteria-positive samples, IL-6 showed a mean ΔCt of 5.2 ± 0.4, compared to 8.1 ± 0.3 in bacteria-negative samples, corresponding to a 6.9-fold increase in expression ($p < 0.01$). TNF-α expression was similarly elevated, with a mean ΔCt of 5.6 ± 0.5 in positive samples versus 8.5 ± 0.4 in negatives, reflecting a 7.8-fold increase ($p < 0.01$). IL-1β exhibited a 5.6-fold upregulation, with ΔCt values of 4.8 ± 0.3 in positive samples and 7.3 ± 0.3 in negatives ($p < 0.01$) (Table 6 and Figure 3). These findings indicate a robust inflammatory response associated with the presence of bacteria in semen and support the role of cytokines as potential biomarkers of infection-related sperm dysfunction.

**Table 6:** Comparative Cytokine Expression in Bacteria-Positive vs. Bacteria-Negative Semen Samples

| Cytokine | Bacteria-Positive (Mean ΔCt ± SD) | Bacteria-Negative (Mean ΔCt ± SD) | Fold Change (2^-ΔΔCt) | p-value |
|---|---|---|---|---|
| IL-6 | 5.2 ± 0.4 | 8.1 ± 0.3 | 6.9 | < 0.01 |
| TNF-α | 5.6 ± 0.5 | 8.5 ± 0.4 | 7.8 | < 0.01 |
| IL-1β | 4.8 ± 0.3 | 7.3 ± 0.3 | 5.6 | < 0.01 |

Cytokine expression was normalized to GAPDH, and relative quantification was performed using the $^{2^{-\Delta\Delta}}$Ct method.

These results reinforce the role of bacterial infections in the activation of inflammatory signaling pathways in the male reproductive tract. Elevated cytokine levels in bacteria-positive samples correspond to compromised semen quality and may serve as a molecular biomarker for subclinical infection-induced infertility.

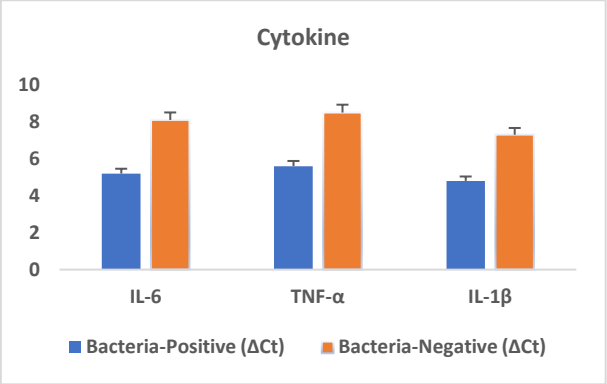


**Fig. 3:** Bar Graph Showing Pro-Inflammatory Cytokine Gene Expression (IL-6, TNF-A, IL-1β) in Bacteria-Positive and Bacteria-Negative Semen Samples By 16S Rrna PCR. Mean Δct Values (± SD) Are Plotted, With Lower Δct Values Indicating Higher Expression. Blue and Red Bars Represent Bacteria-Positive and Negative Samples, Respectively. All Cytokines Showed Significantly Higher Expression in Bacteria-Positive Samples ($P < 0.01$).

## 5. Discussion

This study revealed a clear link between the presence of bacteria in semen, increased expression of proinflammatory cytokines, and reduced sperm quality in infertile men. Through molecular diagnostics, employing PCR for bacterial detection, and SYBR Green-based qPCR for gene expression profiling, we established that subclinical infections are associated with inflammation-driven sperm dysfunction.

Our research found that men with infertility exhibited notably lower levels of sperm concentration, movement, and structure than fertile individuals, consistent with previous studies (Agarwal et al. 2015, Cooper et al. 2010). Elevated leukocyte counts in semen further indicate an immune response, which is often triggered by microbial invasion (Comhaire et al. 1999). Seminal leukocytospermia is recognized as a hallmark of genital tract infections and has been linked to oxidative stress and DNA fragmentation [Aitken and Baker 2004]. The presence of bacterial DNA in 40% of semen samples aligns with earlier findings of asymptomatic bacteriospermia in infertile populations (La Vignera et al. 2011, Ochsendorf 1999). Predominant isolates, such as Escherichia coli and Staphylococcus aureus, are known to impair sperm function through direct interaction and production of reactive oxygen species (ROS) [Fraczek 2007]. These bacteria can adhere to spermatozoa, impair motility, and induce membrane damage (Prabha et al., 2010).

Our analysis of cytokine expression indicated a marked increase in IL-6, TNF-α, and IL-1β levels in the semen samples containing bacterial DNA. These cytokines play a vital role in mediating inflammation and have been associated with male infertility (Huleihel and Lunenfeld 2004, Azenabor et al. 2015). IL-6 and TNF-α can potentially interfere with spermatogenesis and reduce sperm motility by inducing mitochondrial dysfunction (Said and Agarwal, 2006). IL-1β is known to trigger germ cell apoptosis and compromise the blood-testis barrier (Hedger, 2011). Antioxidant therapies and investigation of potential anti-cytokine strategies have been suggested.

Furthermore, the negative correlations between cytokine expression and sperm motility, viability, and morphology were consistent with findings from other studies showing that inflammatory cytokines are inversely associated with sperm quality parameters (Ricci et al., 2002). These relationships highlight the potential mechanistic role of chronic inflammation in mediating sperm damage during infection.

The integration of molecular tools, such as 16S rRNA PCR and qPCR, for inflammatory markers could serve as a robust diagnostic adjunct in infertility clinics, particularly in cases of idiopathic infertility where standard semen analysis falls short (Weng et al. 2014). Exploring inflammatory pathways could present a potential treatment strategy; for example, therapies targeting cytokines or antioxidant treatments have demonstrated potential in early trials (Tremellen et al., 2008). Practicality of incorporating molecular diagnostics in clinical environments with limited resources.

This study demonstrated upregulation of pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β) in semen samples positive for bacterial DNA, indicating a link between microbial presence and inflammation in the male reproductive tract. The elevated cytokine expression aligns with the finding that infections can induce inflammatory cascades that compromise sperm function. IL-6 and TNF-α impair spermatogenesis and reduce sperm motility, whereas IL-1β disrupts the blood-testis barrier(Loveland 2017, Saing et al. 2016). The ΔCt values showed higher cytokine mRNA expression in infected individuals, suggesting that bacterial colonization triggers immune responses that affect semen quality. These findings reinforce that genital tract infections are a significant contributor to male infertility, highlighting the need for molecular tools, such as PCR, in infertility assessments to identify inflammatory conditions undetectable by standard analysis (Azenabor et al. 2015).

This study was constrained by its cross-sectional design, which limits its ability to draw causal conclusions. Additionally, while we verified bacterial presence through PCR, achieving species-level identification and performing community analysis could be enhanced by employing 16S rRNA next-generation sequencing. Future research should include more detailed microbial profiling, oxidative stress markers, and intervention studies to evaluate the therapeutic impact of infection control and anti-inflammatory treatments. Utilizing Illumina MiSeq-based 16S sequencing in future studies could offer a deeper understanding of seminal microbiota diversity.

## 6. Conclusion

This study provides compelling evidence that bacterial infections in semen are closely associated with elevated levels of pro-inflammatory cytokines and a significant decline in sperm quality. Employing molecular techniques such as PCR for pathogen detection and qPCR for cytokine profiling offers a valuable diagnostic approach for identifying inflammatory causes of male infertility. Our findings highlight the potential of inflammatory biomarkers, particularly IL-6, TNF-α, and IL-1β, as diagnostic indicators and possible therapeutic targets. Incorporating molecular diagnostics into standard semen analysis could enhance the clinical evaluation of unexplained infertility and enable the development of more targeted treatments to restore male reproductive health.

## Acknowledgments

The authors express their gratitude to the laboratory staff and the participating fertility centers for their technical assistance and cooperation in sample collection and processing. Special thanks are extended to Heredity Biosciences, Bhubaneswar, for their support in bacterial culture and molecular identification.

## Funding

This study did not receive any specific funding from the public, commercial, or not-for-profit sector.

## Conflict of Interest

The authors declare no conflicts of interest.

## Author Contributions

Satyanarayan Samantaray: Conceptualization, methodology design, data analysis, and manuscript writing. Soumya Jal: Sample processing, laboratory analysis, and data curation. Gopal Krishna Purohit: Overall supervision, critical review, editing, and final approval of the manuscript. All the authors have read and approved the final version of the manuscript.

## Ethics Approval

Ethical approval was not required for this study, as it involved a retrospective analysis of anonymized semen samples obtained during routine clinical care. Informed consent was obtained from all patients, and no additional interventions beyond standard clinical procedures were conducted. All patient data were anonymized and handled confidentially in accordance with institutional guidelines.

## Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## References

[1] Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. Reproductive Biology and Endocrinology, 13, 37. https://doi.org/10.1186/s12958-015-0032-1.
[2] Acharya, P. P., Jal, S., Purohit, G. K., & Pati, P. K. (2025). Molecular identification of uropathogenic bacteria via 16S rRNA gene amplification and sequencing. Journal of Neonatal Surgery, 14(32s), 467–475.
[3] Aitken, R. J., & Baker, M. A. (2004). Oxidative stress and male reproductive biology. Reproduction, Fertility and Development, 16(5), 581–588. https://doi.org/10.1071/RD03089.
[4] Akinloye, O., Azenabor, A., & Ekun, A. O. (2015). Impact of inflammation on male reproductive tract. Journal of Reproduction & Infertility, 16(3), 123–129.
[5] Azenabor, A., Ekun, A. O., & Akinloye, O. (2015). Impact of inflammation on male reproductive tract. Journal of Reproduction & Infertility, 16(3), 123–129. PMID: 26913230; PMCID: PMC4508350.
[6] Babakhanzadeh, E., Ghasemifar, S., Nazari, M., & Khodadadian, A. (2020). Some of the factors involved in male infertility: A prospective review. International Journal of General Medicine, 13(8), 29–41. https://doi.org/10.2147/IJGM.S241099.
[7] Comhaire, F. H., Mahmoud, A., Depuydt, C. E., & Zalata, A. A. (1999). Mechanisms of the spermotoxic effect of leukocytes in semen. Fertility and Sterility, 72(3), 816–820.
[8] Cooper, T. G., Noonan, E., von Eckardstein, S., et al. (2010). World Health Organization reference values for human semen characteristics. Human Reproduction Update, 16(3), 231–245. https://doi.org/10.1093/humupd/dmp048.
[9] Dutta, S., & Sengupta, P. (2025). Bacterial infections and male fertility. In Bentham Science (pp. 122–138). https://doi.org/10.2174/9789815305302125010009.
[10] Fraczek, M., & Kurpisz, M. (2007). Inflammatory mediators exert toxic effects of reactive oxygen species on human spermatozoa. Journal of Andrology, 28(2), 325–333. https://doi.org/10.2164/jandrol.106.001149.
[11] Hedger, M. P. (2011). Immunophysiology and pathology of inflammation in the testis and epididymis. Journal of Andrology, 32(6), 625–640. https://doi.org/10.2164/jandrol.111.012989.
[12] Hirano, T. (2021). IL-6 in inflammation, autoimmunity and cancer. International Immunology, 33(3), 127–148. https://doi.org/10.1093/intimm/dxaa078.
[13] Huleihel, M., & Lunenfeld, E. (2004). Regulation of spermatogenesis by cytokines. International Journal of Andrology, 27(4), 198–199.
[14] Kaltsas, A., Zachariou, A., Markou, E., Dimitriadis, F., Sofikitis, N., & Pournaras, S. (2023). Microbial dysbiosis and male infertility: Understanding the impact and exploring therapeutic interventions. Journal of Personalized Medicine, 13(10), 1491. https://doi.org/10.3390/jpm13101491.
[15] Khan, M. S., Ullah, A., & Mehmood, A. (2024). Male infertility: Its diagnosis and management. BMC Journal of Medical Sciences, 5(1), 1–2. https://doi.org/10.70905/bmcj.05.01.0283.
[16] Khodamoradi, K., Kuchakulla, M., Narasimman, M., Khosravizadeh, Z., Ali, A., Brackett, N., Ibrahim, E., & Ramasamy, R. (2020). Laboratory and clinical management of leukocytospermia and hematospermia: A review. Therapeutic Advances in Reproductive Health, 14(Suppl 4), 263349412092251. https://doi.org/10.1177/2633494120922511.
[17] La Vignera, S., Vicari, E., Condorelli, R., D'Agata, R., & Calogero, A. E. (2011). Male accessory gland infection and sperm parameters. International Journal of Andrology, 34(5 Pt 2), e330–e347. https://doi.org/10.1111/j.1365-2605.2011.01200.x.
[18] Loveland, K. L., Klein, B., Pueschl, D., Indumathy, S., Bergmann, M., Loveland, B. E., Hedger, M. P., & Schuppe, H. C. (2017). Cytokines in male fertility and reproductive pathologies: Immunoregulation and beyond. Frontiers in Endocrinology, 8, 307. https://doi.org/10.3389/fendo.2017.00307.
[19] Oghbaei, H., Rastgar Rezaei, Y., Nikanfar, S., Zarezadeh, R., Sadegi, M., Latifi, Z., Nouri, M., Fattahi, A., Ahmadi, Y., & Bleisinger, N. (2020). Effects of bacteria on male fertility: Spermatogenesis and sperm function. Life Sciences, 256, 117891. https://doi.org/10.1016/j.lfs.2020.117891.

[20] Ochsendorf, F. R. (1999). Infections in the male genital tract and reactive oxygen species. Human Reproduction Update, 5(5), 399–420. https://doi.org/10.1093/humupd/5.5.399.
[21] Potiris, A., Moustakli, E., Trismpioti, E., Drakaki, E., Mavrogianni, D., Matsas, A., Zikopoulos, A., Sfakianakis, A., Tsakiridis, I., Dagklis, T., et al. (2025). From inflammation to infertility: How oxidative stress and infections disrupt male reproductive health. Metabolites, 15(4), 267. https://doi.org/10.3390/metabo15040267.
[22] Prabha, V., Sandhu, R., Kaur, S., et al. (2010). Characterization of E. coli isolated from semen samples of infertile males. Indian Journal of Medical Research, 131, 83–87.
[23] Raghuwanshi, P. T., & Kanwal, M. R. (2025). Causes and associated factors with male infertility among couples visiting infertility/andrologist clinics. Journal of Drug Delivery and Therapeutics, 15(3), 21–29. https://doi.org/10.22270/jddt.v15i3.7008.
[24] Ricchi, M., Bertasio, C., Boniotti, M. B., Vicari, N., Russo, S., Tilola, M., Bellotti, M. A., & Bertasi, B. (2017). Comparison among the quantification of bacterial pathogens by qPCR, dPCR, and cultural methods. Frontiers in Microbiology, 8, 1174. https://doi.org/10.3389/fmicb.2017.01174 .
[25] Ricci, G., Perticarari, S., Fragonas, E., et al. (2002). Cytokines and male fertility: IFN-γ and IL-6 in human semen. European Journal of Obstetrics & Gynecology and Reproductive Biology, 100(2), 165–170.
[26] Saing, T., Valdivia, A., Hussain, P., Ly, J., Gonzalez, L., Guilford, F. T., Pearce, D., & Venketaraman, V. (2016). Data on pro-inflammatory cytokines IL-1β, IL-17, and IL-6 in the peripheral blood of HIV-infected individuals. Data in Brief, 8(11), 1044–1047. https://doi.org/10.1016/j.dib.2016.07.023.
[27] Said, T. M., & Agarwal, A. (2006). Role of sperm oxidative stress in male infertility: A review. Journal of Andrology, 27(6), 737–748.
[28] Samantaray, S., Jal, S., & Purohit, G. K. (2024). Microbial contaminants in semen and their effects on sperm motility, morphology, and fertility outcomes. Journal of Neonatal Surgery, 53(3). https://doi.org/10.48047/4st0qc93.
[29] Shen, C.-C., Peng, H., & Zhu, X. (2011). Male infertility due to Ureaplasma urealyticum of semen analysis. International Medicine and Health Guidance News, 17(2), 151–154.
[30] Syafrizayanti, S., Nurhayati, N., & Safni, S. (2022). Detection and absolute quantification of porcine DNA in sausages using quantitative polymerase chain reaction (qPCR) method. Jurnal Kimia Unand, 11(2), 5–13. https://doi.org/10.25077/jku.11.2.5-13.2022.
[31] Tremellen, K. (2008). Oxidative stress and male infertility—a clinical perspective. Human Reproduction Update, 14(3), 243–258. https://doi.org/10.1093/humupd/dmn004.
[32] Wang, S., Zhang, K., Yao, Y., Li, J., & Deng, S. (2021). Bacterial infections affect male fertility: A focus on the oxidative stress-autophagy axis. Frontiers in Cell and Developmental Biology, 9, 727812. https://doi.org/10.3389/fcell.2021.727812.
[33] Weng, S. L., Chiu, C. M., Lin, F. M., et al. (2014). Bacterial communities in semen from men of infertile couples: Metagenomic sequencing reveals relationships with semen quality. PLoS ONE, 9(10), e110152. https://doi.org/10.1371/journal.pone.0110152.