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SEMEN ANALYSIS PROFILE AND ASSOCIATED BACTERIAL INFECTIONS IN INFERTILE MALES FROM PESHAWAR

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Abstract

Background and Purpose: *Infertility is a significant public health issue in Pakistan, with male factors contributing to a substantial proportion of cases. Semen analysis is a fundamental, cost-effective, and non-invasive diagnostic tool for assessing male infertility. This study aimed to evaluate the prevalence and seminal parameters of primary and secondary male infertility in Peshawar, Pakistan, and to raise awareness regarding its diagnosis and management.*

Methodology: *A cross-sectional study was conducted, involving 366 semen samples from infertile males. Samples were collected via masturbation, processed within one hour, and analyzed according to WHO guidelines. Sperm concentration, motility, morphology, and the presence of leukocytes were assessed. Bacterial culture was performed for samples with leukocytospermia.*

Results: *Primary infertility was observed in 264 (72.13%) patients, while 102 (27.86%) had secondary infertility. Among primary infertility cases, normospermia was found in 52 (14.20%), oligozoospermia in 106 (28.96%), oligoasthenozoospermia in 98 (26.77%), and azoospermia in 100 (27.32%). Among secondary infertility cases with leukocytospermia, normospermia was observed in 28 (27.45%), oligozoospermia in 48 (47.05%), oligoasthenozoospermia in 38 (37.25%), and azoospermia in 26 (25.49%). Bacterial growth was identified in 20 (19.60%) of leukocytospermic samples, predominantly Staphylococcus aureus (40%), Escherichia coli (30%), Streptococcus species (20%), and Proteus species (10%).*

Conclusion: *Semen analysis remains a valuable, accessible, and inexpensive test for diagnosing male infertility. In Peshawar, primary infertility is more prevalent than secondary infertility, with oligozoospermia and oligoasthenozoospermia being the most common abnormalities. Leukocytospermia is associated with significant semen parameter alterations and bacterial infections, underscoring the need for targeted diagnostic and therapeutic approaches.*

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INTRODUCTION

Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects approximately 15% of couples globally, with male factors contributing to half of all cases (1, 2). In Pakistan, infertility rates are notably high, with an estimated prevalence of 21.9% (3). The etiology of male infertility is multifactorial, encompassing genetic, endocrine, anatomical, and environmental factors (4, 5). Despite its significance, male infertility remains underdiagnosed and inadequately managed in resource-limited settings due to socioeconomic constraints, cultural barriers, and limited access to specialized diagnostics (6).

The evaluation of the male partner is therefore a critical component of a couple's fertility assessment. Semen analysis serves as the cornerstone of this evaluation. It is an inexpensive, easily performed, and non-invasive laboratory test that provides essential information on seminal parameters. It assesses the quantity and quality of a man's semen, determining sperm count, motility, morphology, and other characteristics of the seminal plasma, which are vital for predicting fertility potential and guiding therapy. The procedure, conducted according to standardized guidelines like the WHO manual, involves collecting a semen sample via masturbation and analyzing its volume, pH, sperm concentration, motility, vitality, and morphology (7). It is a cost-effective test that provides critical information on sperm concentration, motility, morphology, and seminal fluid composition (8). Abnormalities in these parameters, such as oligozoospermia (low sperm count), asthenozoospermia (reduced motility), and teratozoospermia (abnormal morphology), are key indicators of infertility (9). Among the various causes of abnormal semen parameters, infection and inflammation of the male genital tract represent significant and potentially treatable etiologies. The presence of an excessive number of white blood cells (leukocytes) in semen, known as leukocytospermia, is a recognized marker of such inflammation. Infections causing leukocytospermia can be caused by a range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* species, and *Proteus* species, which may directly or indirectly compromise fertility (10, 11).

In the context of Khyber Pakhtunkhwa, particularly Peshawar, data on the patterns of male infertility are scarce. This study aimed to evaluate the seminal profiles of men with primary and secondary infertility, determine the prevalence of leukocytospermia, and identify associated bacterial pathogens. By highlighting local trends and reinforcing the diagnostic utility of semen analysis, this research seeks to inform clinical practice and public health strategies to address male infertility in the region.

Materials and Methods

This cross-sectional study was conducted at the Bashir Clinical Laboratory, Peshawar. A total of 366 semen samples were collected from infertile males aged 18–40 years, who reported inability to conceive after at least one year of unprotected intercourse. Primary infertility was defined as no prior conception, while secondary infertility was defined as the inability to conceive following at least one previous pregnancy (12). Patients with known genetic disorders (e.g., Klinefelter syndrome), prior vasectomy, or systemic illnesses (e.g., diabetes, renal failure) known to affect fertility were excluded. Ethical approval was obtained from the Institutional Review Board of Khyber Medical University, and written informed consent was secured from all participants.

Semen samples were collected by masturbation after a recommended abstinence period of 3–5 days, following the standardized protocols of the WHO laboratory manual (5th edition) (8). Samples were collected in a private room within the laboratory using sterile, wide-mouthed, non-toxic plastic containers. They were allowed to liquefy at 37°C in an incubator and analyzed within one hour of collection.

Analysis included measurement of semen volume (mL), pH, and macroscopic assessment. Sperm concentration (million/mL) was determined using an improved Neubauer hemocytometer. Sperm motility was categorized as progressive motility (PR), non-progressive motility (NP), or immotility (IM), with total motility (PR+NP) reported as a percentage. For morphology assessment, thin smears were prepared, air-dried, and stained with Diff-Quik stain. A

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minimum of 200 spermatozoa were evaluated under oil immersion (1000x magnification) using strict Tygerberg criteria (8). Semen samples exhibiting leukocytospermia were subjected to microbiological culture. Using a sterile loop, samples were inoculated onto blood agar, chocolate agar, and MacConkey agar plates. The plates were incubated at 37°C under appropriate atmospheric conditions (5% CO2 for chocolate agar) for 24–48 hours. Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests (13).

Statistical Analysis

Data were analyzed using SPSS version 20.0. Descriptive statistics were computed for all variables, with results expressed as frequencies and percentages. Chi-square test was used to compare categorical variables between primary and secondary infertility groups, with a p-value of <0.05 considered statistically significant.

Results

Distribution of Infertility Types

Of the 366 participants, 264 (72.13%) were diagnosed with primary infertility, and 102 (27.86%) with secondary infertility (Table 1). The mean age of participants was 31.4 ± 5.2 years, with no significant age difference between the two groups (p=0.42).

Table 1: Semen Parameters in Primary and Secondary Infertility

Semen Parameter	Primary Infertility (n=264)	Secondary Infertility (n=102)	p-value
Normospermia	52 (14.20%)	28 (27.45%)	0.002
Oligozoospermia	106 (28.96%)	48 (47.05%)	<0.001
Oligoasthenozoospermia	98 (26.77%)	38 (37.25%)	0.032
Azoospermia	100 (27.32%)	26 (25.49%)	0.71

Secondary Infertility and Leukocytospermia

All 102 patients with secondary infertility presented with leukocytospermia. Their semen profiles revealed a high burden of abnormalities, with oligozoospermia being the most prevalent finding (47.05%).

Microbiological Findings

Bacterial growth was observed in 20 (19.60%) of the 102 leukocytospermic samples. The spectrum of isolated bacteria is detailed in Table 2

Table 2: Bacterial Isolates from Semen Cultures (n=20)

Bacterium	Number of Isolates (%)
Staphylococcus aureus	8 (40%)
Escherichia coli	6 (30%)
Streptococcus species	4 (20%)
Proteus species	2 (10%)

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Discussion

This study provides a detailed analysis of seminal parameters in a cohort of infertile men from Peshawar, Pakistan. The high prevalence of primary infertility (72.13%) aligns with some regional studies (14) but contrasts with others reporting a higher proportion of secondary infertility (15). This discrepancy may be attributed to cultural factors, such as earlier marriage and childbearing expectations, or to methodological differences in patient recruitment.

Oligozoospermia and oligoasthenozoospermia were the predominant abnormalities in both primary and secondary infertility groups, consistent with global patterns where sperm concentration and motility are the most affected parameters (16, 17). The significant prevalence of azoospermia (27.32% in primary cases) warrants attention, as it suggests severe testicular failure or obstructive pathology requiring specialized interventions like testicular sperm extraction or surgical correction (18).

A key finding of this study is the universal presence of leukocytospermia in patients with secondary infertility. Leukocytospermia is a well-established marker of male genital tract inflammation and is associated with oxidative stress, which can damage sperm membranes and DNA, impair motility, and reduce fertilization potential (10, 19). Our observation that leukocytospermia was strongly correlated with oligozoospermia and asthenozoospermia supports this pathogenic link (20).

The bacterial isolation rate of 19.60% from leukocytospermic samples is comparable to rates reported in other studies, which range from 15% to 30% (21, 22). The spectrum of pathogens *S. aureus*, *E. coli*, *Streptococcus* spp., and *Proteus* spp. is typical of ascending genitourinary tract infections (23). Notably, the majority of leukocytospermic samples (over 80%) yielded no bacterial growth. This suggests that leukocytospermia may often be due to non-infectious inflammation, viral infections, or subclinical conditions not detected by routine culture (24). This underscores the recommendation for additional diagnostic tests, such as polymerase chain reaction (PCR) for sexually transmitted pathogens, in the evaluation of unexplained leukocytospermia (25).

The finding of normospermia in 27.45% of leukocytospermic patients is clinically significant. It indicates that standard semen parameters may appear normal despite underlying inflammatory or infectious processes that could compromise sperm function at a molecular or biochemical level (26). This group of patients may benefit from advanced sperm function tests, such as the reactive oxygen species (ROS) assay or sperm DNA fragmentation index (DFI) testing, to guide appropriate management (27).

Limitations and Strengths

This study has several limitations. Its cross-sectional design precludes causal inference. The lack of data on hormonal profiles (e.g., FSH, LH, testosterone), genetic testing (e.g., Y-chromosome microdeletions), scrotal ultrasonography, and lifestyle factors (e.g., smoking, occupational exposures) limits a comprehensive etiological assessment. Furthermore, the sample was drawn from a clinical setting, which may introduce selection bias.

Nevertheless, the study's strengths include a substantial sample size, strict adherence to WHO protocols for semen analysis, and the integration of microbiological culture. It provides valuable baseline data on the pattern of male infertility in a region with limited prior research.

Clinical Implications

The results reinforce semen analysis as an indispensable first-line investigation for male infertility, even in resource-limited settings. The high prevalence of leukocytospermia in secondary infertility highlights the importance of screening for and treating genital tract infections. Empirical antibiotic therapy should be guided by culture and sensitivity results to avoid antimicrobial resistance. For patients with persistent leukocytospermia and negative cultures, anti-inflammatory or antioxidant therapies could be considered, as some studies have shown improvement in semen parameters with such treatments (28, 29).

Conclusion

Semen analysis remains a crucial, accessible, and cost-effective tool for the initial evaluation of male

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infertility. In Peshawar, primary infertility is more common than secondary infertility, with deficits in sperm concentration and motility representing the most frequent abnormalities. Leukocytospermia is a significant finding in secondary infertility and is often, but not exclusively, associated with bacterial infections. These findings underscore the need for a standardized diagnostic approach that includes semen analysis and targeted cultures, coupled with public health initiatives to improve awareness, early diagnosis, and comprehensive management of male infertility in Pakistan.

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