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Evaluating The Clinical Correlation Of Male Infertility With Bacterial Infection And Sperm Quality

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ABSTRACT

Introduction: Numerous factors trigger male infertility, including lifestyle, the environment, health, medical resources and pathogenic microorganism infections. Bacterial infections of the male reproductive system can cause various reproductive diseases. Male urogenital tract infection (UTI) is one of the leading causes of male infertility, as bacteria in semen samples can lower sperm quality.

Aim and Objective: Evaluating the clinical correlation of male infertility with bacterial infection and sperm quality

Material and Methods: This was a Cross-sectional study carried out in the Department of Pathology with collaboration to Department of Microbiology at a tertiary care centre for a period of 1 year i.e, November 2023 to November 2024. A total of 410 semen samples were collected, after informed written consent, from married males with the complaint infertility. Semen analysis was carried out according to the WHO guidelines. The specimens were processed as per the latest CLSI guidelines for isolation and identification of the organisms, followed by Antibiotic susceptibility testing.

Results: In the present study a total of 410 semen samples were screened out of which 105 (25.6%) showed significant bacterial growth i.e. ≥ 103 bacteria/ml of semen ejaculate.

The maximum number of cases was found in the age group of 26-30 years (48.9%) followed by 31-35 (25%) and least in the age group of 20-25 years of age and above 41 years of age with (6.5%).

In our study 72 (17.5%) isolates were from the Gram positive cocci (GPC) and 33 (8%) isolates were from the Gram negative bacilli (GNB). In the present study it was observed that the commonest isolate was the *Coagulase Negative Staphylococcus species* (7.5%) followed by *Enterococcus species* (5.3%), *Staphylococcus aureus* with 3.6% and least for *Streptococcus species* with 0.9 %. In case of GNB the maximum isolates was from *E.coli* with 5.1% followed by *Pseudomonas aeruginosa* (2.9%).

It was also noted that all the GPC isolates, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin.

Among the GNB isolated, most were sensitive to Amikacin and Piperacillin- Tazobactum The maximum number of cases recorded was from the Oligozoospermia and least from Azospermia.

Conclusion: Bacterial infection in the male reproductive system is one of the key factors affecting male fertility. The main factors leading to male sterility are inflammation of the reproductive system, injury of the male reproductive organ, and spermatogenesis disorder induced by pathogenic bacterial infection. Since bacteria can affect the quality of sperm because infections have been shown to have a negative impact on semen parameters. We strongly suggest increasing awareness among people and considering screening programs for patients seeking fertility both to avoid transmission and to improve fertility outcomes among them.

Keywords: *Clinical Correlation, Semen analysis, Infertility, AST, CLSI*

1. INTRODUCTION

Infertility defines as the inability to achieve pregnancy after a year of regular intercourse. Approximately 15 % of couples of reproductive ages fail to achieve a wanted pregnancy within a 12-month period, despite regular unprotected sexual intercourse [1]. Reduced male fertility can be a result of congenital and acquired urogenital abnormalities, infections of the male accessory glands, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors. Of all of these factors, inflammatory processes and infections are among the significant and major causes of infertility [2]. In 40%–50% of infertile couples, abnormal semen parameters are considered to be the major male-infertility-associated factor. It plays an essential role in fertility outcomes, either alone or in combination with female factors [3].

Numerous factors trigger male infertility, including lifestyle, the environment, health, medical resources and pathogenic microorganism infections. Bacterial infections of the male reproductive system can cause various reproductive diseases. Several male reproductive organs, such as the testicles, have unique immune functions that protect the germ cells from damage [4].

In other words, in approximately 40% of infertile couples, the male is the sole or contributory cause of infertility. In males, sperm deficiency is the primary cause of infertility, however in females it is more complex. Some data suggests that untreated urogenital infections in both men and women can contribute to infertility. Semen analysis may be the most crucial laboratory investigation of the male member of an infertile relationship [5].

The sperm characteristics like poor concentration, slow motility, and morphological defects of contributing to male infertility are sperm [5]. These elements occasionally linked to the existence of nonspecific squamous-tissue infections [6].

Semen culture is considered an important diagnostic tool in the assessment of a genitourinary tract infection. The presence of bacteria in concentrations greater than 10^3bacteria/mL ejaculate is clinically regarded as a sign of an active infection and is called bacteriospermia. Semen contamination originates from the urinary tract of patients and can be sexually transmitted from the one person to another by pathogens. It is regarded as a major health care concern among people with a significant negative impact on male fertility. Approximately 15% of men with infertility have a significant number of bacterial pathogens in their semen. Depending on the nature of the infection, sperm production, sperm function, and sperm transport can be threatened [7].

Semen contamination occurs in the urinary tract of patients or can be transmitted sexually by the partner. Male urogenital tract infection is a leading cause of male infertility worldwide. Genital tract infection and inflammation have been linked to 8–35% of male infertility cases [8,9]. The effect of bacterial infection on reproductive system function is important.

Different types of bacteria have been isolated from the semen and genital tracts of fertile and infertile men. *Chlamydia trachomatis*, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Mycoplasma hominis, *Escherichia coli*, Enterococcus faecalis, Staphylococcus aureus, Helicobacter pylori, Streptococcus agalactiae, Gardnerella vaginalis, Anaerococcus, Neisseria gonorrhoeae, and *Pseudomonas aeruginosa* are among the most common isolated bacteria, affecting semen quality and interfering with male fertility.

In the case of infertile marriage, female partners are typically blamed due to widespread misconceptions about what a fertile man is. People believe that once a man is able to have intercourse and ejaculate semen, the problem must be with the wife, not the male partner [7]. However, as knowledge and awareness levels grow, trends are increasingly shifting. Many male partners are increasingly attending infertility clinics to confirm their reproductive status if they are unsure. Therefore, the present study was undertaken to study the clinical correlation of male infertility with bacterial infection and sperm quality

2. MATERIAL AND METHODS

This was a Cross-sectional study conducted in the Department of Pathology and the Microbiology Department at a tertiary care centre for a study period of 1 year i.e, November 2023 to November 2024. A total of 410 semen samples were collected, after informed written consent, from married males with the complaint of infertility .

Inclusion criteria: The married males with the complaint of infertility and those ready to give consent were included in our study

Exclusion criteria: The patients who were not ready to give consent were excluded from the study.

Semen parameters such as appearance, volume, pH, viscosity, liquefaction, count, motility, morphology, presence of other cells like epithelial cell or round cell, and sperm agglutination were recorded according to the WHO guidelines [10].

Appearance: Normal semen samples are homogenous, gray, and opalescent; they may be less opaque if the sperm concentration is very low. A Pasteur pipette was used to measure the volume into a graduated centrifuge tube, and the level was recorded in milliliters. Normal semen samples exit the pipette as tiny, distinct drops, but in abnormal cases, the semen drop forms a thread that is more than two centimeters long.

Motility: It was done by applying a drop of semen sample onto a slide covered with cover slip and then examined under

high power (×40) objective lens. Motility was graded active motile, sluggish motile and non motile as per WHO criteria [10].

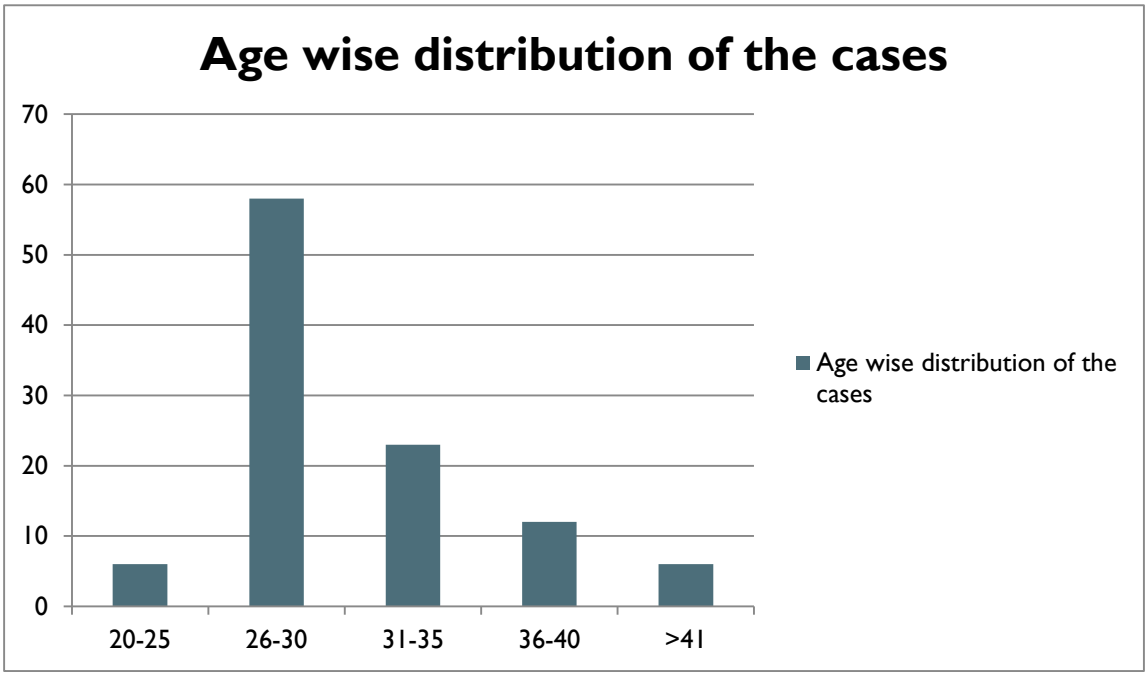
Following a minimum abstinence time of three days, samples were obtained via masturbating in sterile containers. Not a single patient had ever taken antibiotics before. Within three hours of specimen collection, the microbiology lab performed the Gram stain and culture of the samples in blood agar and MacConkey agar in accordance with WHO guidelines [10]. At 37°C, cultures were incubated. Significant organisms were those that were isolated at concentrations more than 103 cfu/mL [11]. In accordance with the most recent CLSI guidelines 2023, the specimens were processed for organism isolation and identification before being subjected to the Kirby-Bauer disc diffusion method for testing for antibiotic susceptibility [12].

3. RESULTS

In the present study a total of 410 semen samples was included in our study after informed written consent, from married males with the complaint of infertility , out of which 105 (25.6%) showed significant bacterial growth i.e. ≥ 103 bacteria/ml of semen ejaculate. The maximum number of cases was found in the age group of 26-30 years (55.2%) followed by 31-35 (21.9%) and least in the age group of 20-25 years of age and above 41 years of age with (5.7%), which is shown in the Table no. 1

Table No. 1: The Age-wise distribution of the study participants

Age group (Years)	Culture positive (N=105)	Percentage (%)
20-25	6	5.7%
26-30	58	55.2%
31-35	23	21.9%
36-40	12	11.4%
>41	6	5.7%



Graph No. 1: Graphical representation of the Agewise distribution of the cases

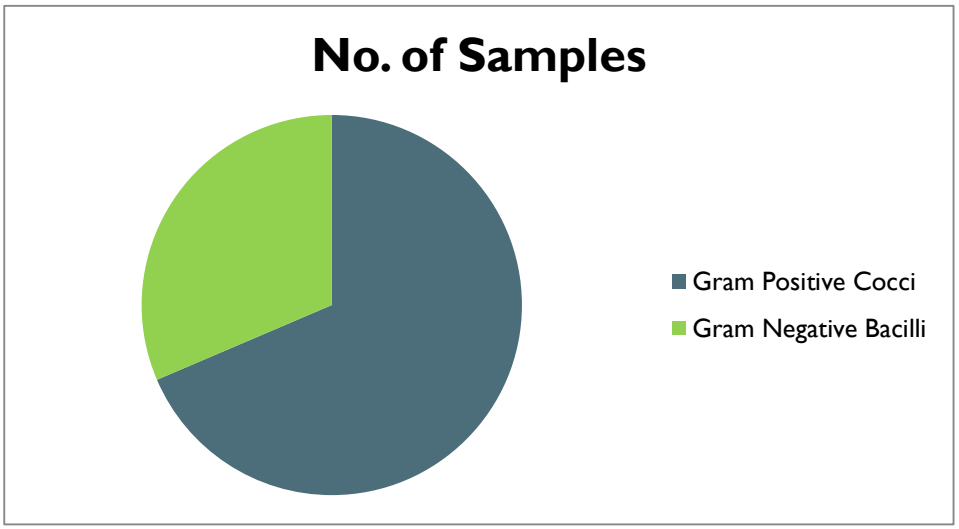
In our study 72 (17.5%) isolates were from the Gram positive cocci (GPC) and 33 (8%) isolates were from the Gram negative bacilli (GNB).

The commonest isolates was the *Coagulase Negative Staphylococcus species* (7.5%) followed by *Enterococcus species* (5.3%), *Staphylococcus aureus* with 3.6% and least for *Streptococcus species* with 0.9%. In case of GNB the maximum isolates was from *E.coli* with 5.1% followed by *Pseudomonas aeruginosa* (2.9%) which is illustrated in the Table no. 2

Table No. 2: Distribution of organisms from the Semen Analysis

Pathogens	Number (N = 410)	Percentages (%)
Gram Positive Cocci	**72**	**17.5%**
Enterococcus species	22	5.3%
Staphylococcus aureus	15	3.6%
CoNS	31	7.5%
Streptococcus species	4	0.9%
Gram Negative Bacilli	**33**	**8%**
Escherichia coli	21	5.1%
Pseudomonas aeruginosa	12	2.9%
Contaminants	**41**	**10%**
No Growth	**264**	**64.3%**

It was observed that all the GPC isolated, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (94.4%). (Table No. 3).



Graph No. 2: Graphical representation of the Distribution of organisms from the Semen Analysis

Among the GNB isolated, most were sensitive to Amikacin (90.9%) and Piperacillin- Tazobactum (84.8%), and lesser sensitivity was seen for Nitrofurantoin, Ciprofloxacin and Co-trimoxazole. Table no. 3 and Table no. 4 below illustrate the sensitivity pattern of the antibiotics.

Table No.3: Antibiotic Sensitivity pattern of Gram Positive organisms in Semen

Organisms	Staphylococcus aureus (N = 15)		Enterococcus species (N = 22)		CoNS (N = 31)		Streptococcus species (N = 4)		Gram Positive Cocci (N = 72)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R(%)**
Cefoxitin	14 93.3%	1 6.6 %	-	-	8 25.8%	23 74%	4 100%	00	22 30.5%	50 69.4%
Linezolid	15 100%	00	22 100%	00	31 100%	00	4 100%	00	72 100%	00
Vancomycin	15 100%	00	22 100%	00	31 100%	00	4 100%	00	72 100%	00
Teicoplanin	15 100%	00	22 100%	00	31 100%	00	4 100%	00	72 100%	00
Penicillin	14 93.3 %	1 6.6 %	19 86.3%	3 13.6%	3 9.6%	28 90.3%	4 100%	00	16 22.2%	56 77.7%
Nitrofurantoin	14 93.3%	1 6.6 %	20 90%	2 10%	29 93.5%	2 6.4%	4 100%	00	68 94.4%	4 5.5%
Ciprofloxacin	9 93.3%	1 6.6 %	10 45.4%	12 54.5%	8 25.8%	23 74%	-	-	17 23.6%	55 76.3%
Co-trimoxazole	12 80%	3 20%	-	-	10 32.2%	21 67.7%	4 100%	00	40 55.5%	32 44.4%
Gentamicin	12 80%	3 20%	19 86.3%	3 13.6%	21 67.7%	10 32.2%	-	-	43 59.7%	29 40.2%

* S = Sensitive, R = Resistant

Table No. 4: Antibiotic Sensitivity pattern of Gram Negative organisms in Semen

Organisms	Escherichia coli (N = 21)		Pseudomonas aeruginosa ((N =12)		Gram Negative Bacilli (N =33)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	18 85.7%	3 14.2%	7 58.3%	5 41.6%	30 90.9%	3 9%
Gentamycin	13 61.9%	8 38%	12 100%	00	29 87.8%	5 15.1%
Imipenem	13 61.9%	8 38%	12 100%	00	29 87.8%	5 15.1%

Piperacillin-Tazobactum	16 76.1%	5 23.8%	12 100%	00	28 84.8%	5 15.1%
Nitrofurantoin	16 76.1%	5 23.8%	7 58.3%	5 41.6%	29 87.8%	5 15.1%
Ciprofloxacin	5 23.8%	16 76.1%	7 58.3%	5 41.6%	27 81.8%	6 18.1%
Co-trimoxazole	19 90.4%	2 9.5%	-	-	25 75.7%	8 24.2%

* S = Sensitive, R = Resistant

Table No. 5: Distribution of the Semen isolates according to sperm count

Organisms	Oligozoospermia	Normozoospermia	Azoospermia
Enterococcus species	10	4	3
Staphylococcus aureus	7	3	2
CoNS	23	8	2
Streptococcus species	3	-	4
Escherichia coli	9	9	6
Pseudomonas aeruginosa	3	9	0
Total	55	33	17

It was also noted that the maximum number of cases recorded was from the Oligozoospermia and least from Azospermia.

4. DISCUSSION

Approximately one-sixth of couples worldwide experience infertility, and the prevalence of male infertility is continuously rising [13,14]. One of the main causes of bacterospermia and male infertility in the world is male urogenital tract infections. Eight to thirty-five percent of male infertility cases have been associated with genital tract infections and inflammation. Bacteriospermia without symptoms might play a big part [15, 16]. An important risk factor for infertility is an infection of the male accessory sex glands.

Microorganisms can affect the male reproductive function directly, causing the agglutination of motile sperm, reducing the ability of acrosome reaction and causing alterations in cell morphology—and indirectly, through the production of reactive oxygen species generated by the inflammatory response to the infection [17].

The aim of this study was to investigate the semen quality in the presence of different bacterial species. Semen samples were processed for bacteriological analysis and examined to evaluate sperm concentration and motility. In recent years, there has been much discussion about the pathophysiology of bacteriospermia. Some possible pathomechanisms for the development of infertility associated with infection are considered: direct effect on sperm function (motility, morphology, etc.), worsening of spermatogenesis, auto-immune processes generated by inflammation, and dysfunction of accessory sex glands . As a result, microbiological testing of male partners in infertile couples can help diagnose male urogenital tract infections, particularly silent illnesses [18].

In the present study a total of 410 semen samples was included after informed written consent, from married males with the complaint of infertility , out of which 105 (25.6%) showed significant bacterial growth i.e. ≥ 103 bacteria/ml of semen ejaculate. The maximum number of cases was found in the age group of 26-30 years (55.2%) followed by 31-35 (21.9%) and least in the age group of 20-25 years of age and above 41 years of age with (5.7%).

This study was in support with the study conducted by the other authors Moretti E et al., Mogra N et al., and Hathiwala R et al, where the rate of bacterial growth was similar [19-21] but in contrast with the study by Enwuru CA et al, [22] where the rate was recorded with 70%. Another study by Isaiah IN et al., was also in contrast to our study where 92 (65.7%) out of a total number of 140 semen samples from infertile males collected yielded bacterial growth [23].

In our study 72 (17.5%) isolates were from the Gram positive cocci (GPC) and 33 (8%) isolates were from the Gram negative bacilli (GNB).

Our study was parallel with the study conducted by Hathiwala R et al.,[21] where the maximum isolates were from GPC, another study by Moretti, et al. [19] [22] isolated 64% and 36%, respectively, which was similar to our study results. Different results was also found in other studies performed by the other investigators where 48% of GPC and 52% of GNB [24] were isolated.

In the present study it was observed that the commonest isolate was the *Coagulase Negative Staphylococcus species* (7.5%) followed by *Enterococcus species* (5.3%), *Staphylococcus aureus* with 3.6% and least for *Streptococcus species* with 0.9 %. In case of GNB the maximum isolates was from *E.coli* with 5.1% followed by *Pseudomonas aeruginosa* (2.9%).

Similar result were found by Hathiwala R et al.,[21] and Moretti, et al. [19]Enwuru, CA et al. [22] reported 10.5% of E. coli and 29.6% of Staphylococcus species. There was another study which was not in accordance to the current study where the most common organism grown was *E. fecalis*, followed by *S. hemolyticus* [25].

The study by Mehrdad Gholami et al in 2022 observed the meta-analysis where in 56 studies, the rate of bacterial infections in the semen of infertile men was 12%.Also, in 26 case-control studies, the association of infertility in men with bacterial infections was evaluated. The results show that the odds ratio of infertility in men exposed to bacterial infections is 3.31 times higher than that in non-infected men [26].

In the present study it was found that all the GPC isolates, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin.

Among the GNB isolated, most were sensitive to Amikacin (90.9%) and Piperacillin- Tazobactum (84.8%), and lesser sensitivity was seen for Nitrofurantoin, Ciprofloxacin and Co-trimoxazole. Our study was in support with the study by Hathiwala R et al.,[21] where most of the GPC were found to be sensitive to Linezolid, Vancomycin, Teicoplanin and Nitrofurantoin and most of the GNB were found to be sensitive to Amikacin.

It was also noted that the maximum number of cases recorded was from the Oligozoospermia and least from Azospermia. This was similar to other studies done for semen analysis by Enwuru, et al. which reported 52.5%, 33.3% and 14.2%, respectively [22]. There was another study by Naina Kuma et al. which was in accordance to the current study where Normozoospermia was observed in 1104 (35.80%), Oligozoospermia in 1053 (34.14%), Asthenoteratozoospermia in 597 (19.35%) and Azoospermia in 330 (10.70%) [27].

Although the direct effects of bacterial and viral infections on male infertility are still up for debate, a variety of microorganisms can induce male infertility. Infertility in the semen of asymptomatic infertile men was thought to be significantly influenced by bacterial infection.

Although the majority of the patients we looked at were young, the risk of infertility generally rises with age. Clinical observations of the patients' male reproductive systems give rise to the theory that bacterial infections may be partially to blame for male infertility. Deterioration of spermatogenesis, impairment of sperm functions, and blockage of the seminal tract are all possible outcomes of infection processes [28].

treatment. Women are always held responsible for infertility, but new research shows that men are just as much to blame. Male infertility has attracted more attention in recent years [29, 30]. Semen analysis screening for males offers some information about the underlying pathological issues affecting the male genital system. Given the foregoing, a pathological and microbiological intervention is required in order to identify the likely microbial agents [31–33]. It should be mentioned that urogenital tract infections and inflammation might harm a male patient's fertility profile and should be treated with the right antibiotics and anti-inflammatory drugs.

5. CONCLUSION

Bacterial infections in the male reproductive tract, including the presence of bacteria in semen (bacteriospermia), can negatively impact sperm quality and function, potentially leading to male infertility. Our findings show that the simple presence of bacteria might alter the sperm quality. The presence of bacterial infections is a risk factor and could impair male fertility potential by decreasing sperm quality. Therefore, there should be routinely awareness programs for the testing for the bacteriological profile of semen of infertile males and to study their antibiotic susceptibility pattern to control the infection as bacteria may affect the quality of semen because infections have been shown to adversely affect semen parameters.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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