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RESEARCH ARTICLE
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TO STUDY THE CLINICAL CORRELATION OF MALE INFERTILITY WITH BACTERIAL INFECTION

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Introduction: In India, infertility has become a severe emotional and social issue. Infertility can cause painful emotional experiences throughout the life mainly known as quality of life impairment. Infections account for up to 15% of male infertility cases. 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: A study on the Semen Analysis and its Bacteriological Profile in Infertile Males at a Tertiary Care centre.
Material and Methods: This was a Cross-sectional study carried out in the Department of Pathology and the Microbiology Department at a tertiary care centre for a period of 1 year i.e, July 2023 to July 2024. A total of 312 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 2023 for isolation and identification of the organisms, followed by Antibiotic susceptibility testing.
Results: In the present study a total of 312 semen samples were screened out of which 86 (27.5%) 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 (48.8%) followed by 31-35 (26.7%) and least in the age group of 20-25 years of age (4.6%). In our study 58 (18.5%) isolates were from the Gram positive cocci (GPC) and 26 (8.3%) 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* (7.6%) followed by *Enterococcus species* (6%), *Staphylococcus aureus* with 3.2% and least for *Streptococcus species* with 1.6 %.
In case of GNB the maximum isolates was from *E.coli* with 5.4% followed by *Pseudomonas aeruginosa* (2.8%).
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 and Piperacillin- Tazobactum The maximum number of cases recorded was from the Oligozoospermia and least from Azospermia.

Conclusion: There should be routine awareness programs for testing the bacteriological profile of infertile males' sperm and studying their antibiotic susceptibility pattern to control infection, as bacteria can affect the quality of sperm because infections have been shown to have a negative impact on semen parameters.

Keywords: Correlation, Semen analysis**,** Infertility, UTI, Antibiotic susceptibility testing, CLSI

INTRODUCTION

Infertility refers to a man or woman's biological incapacity to contribute to conception. To put it another way, infertility is a woman's inability to carry a pregnancy to completion [1]. Typically, a woman is fertile around her ovulation phase (48 hours before to 48 hours after ovulation) before returning to a typical state of infertility for the duration of her menstrual cycle [2]. According to an infertility study conducted by the American National Institute of Health, male factors are involved in one-third of instances, female factors in another one-third of cases, and in the other one-third of cases, both male and female factors are implicated or no apparent cause is discovered [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].

The glands and organs that produce semen are deemed infertile. The normal flow of urine maintains sterility in the internal urethra, but the distal urethra is not considered sterile. As a result, culturing semen samples typically produces the growth of organisms, many of which are regarded to be normal genitourinary tract flora [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. Various bacteria have been isolated and identified from the male reproductive system, including *Escherichia coli*, *Staphylococcus aureus*, *Ureaplasma urealyticum*, *C. trachomatis*, *N. gonorrhoeae*, *Streptococcus agalactia*, and *Staphylococcus saprophyticus* . These bacteria lead to all kinds of diseases, such as chlamydiosis, gonorrhea, and ureaplasmosis, which can cause male reproductive system infections.

After the pathogenic bacteria invade the male reproductive system, oxidative stress and autophagy could be induced by cells in the gonads. Moderate oxidative stress helps clear the pathogen, but excessive oxidative stress can induce testicular damage or even lead to infertility.

According to experimental studies, there is a link between semen-derived bacteria isolation and the degradation of spermatozoal activity and spermatogenesis, which can eventually lead to infertility. 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 Semen Analysis and its Bacteriological Profile in Infertile Males attending a Tertiary care centre

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, July 2023 to June 2024. A total of 312 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 sample appears homogenous gray opalescent. It may be less opaque if the sperm concentration is very low. Volume was measured into a graduated centrifuge tube and the level was recorded in ml. It was measured using Pasteur pipette. Normal semen sample leaves the pipette as small discrete drops whereas in abnormal cases the semen drop forms a thread of >2 cm length.

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].

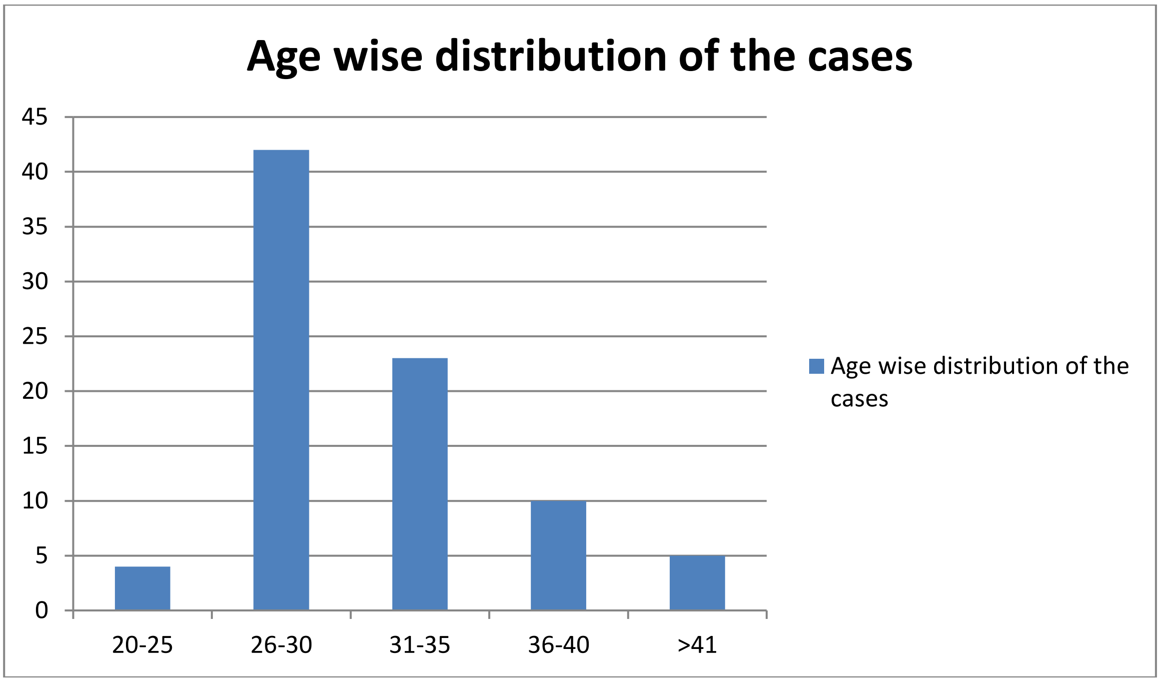
Samples were collected in sterile containers by masturbation after a minimum abstinence period of 3 days. None of the patients had taken prior antibiotics. Gram stain and culture of the samples in blood agar and MacConkey agar were done in microbiology laboratory within 3 hours of specimen collection as per WHO guidelines [10]. Cultures were incubated at 37°C. Those organisms which were isolated in a concentration of $>10^3$ cfu/mL were considered as significant [11]. The specimens were processed for isolation and identification of the the organism, followed by antibiotic susceptibility testing by Kirby-Bauer disc diffusion method as per the latest CLSI guidelines 2023 [12].

RESULTS

In the present study a total of 312 semen samples was included in our study after informed written consent, from married males with the complaint of infertility , out of which 86 (27.5%) 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 (48.8%) followed by 31-35 (26.7%) and least in the age group of 20-25 years of age (4.6%), 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=86)	Percentage (%)
20-25	4	4.6%
26-30	42	48.8%
31-35	23	26.7%
36-40	10	11.6%
>41	5	5.8%



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

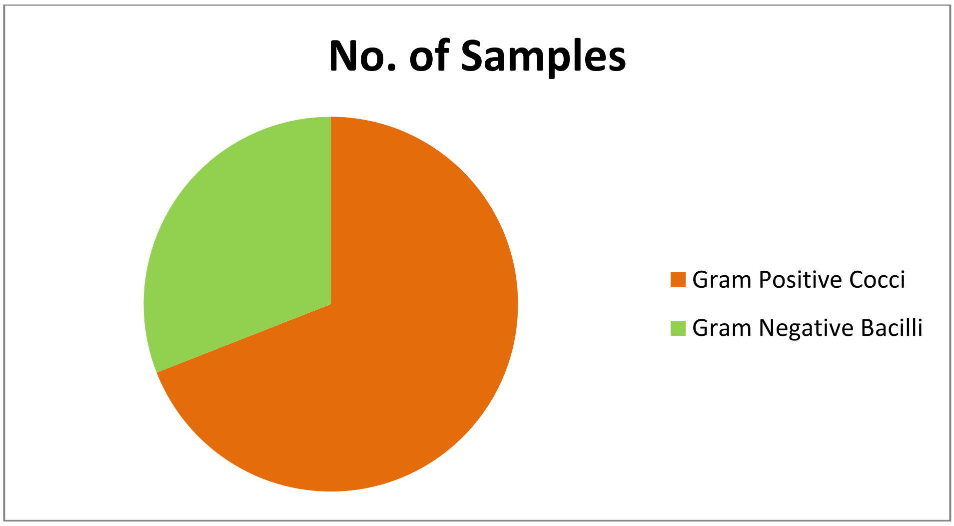
In our study 58 (18.5%) isolates were from the Gram positive cocci (GPC) and 26 (8.3%) isolates were from the Gram negative bacilli (GNB).

The commonest isolates was the *Coagulase Negative Staphylococcus species* (7.6%) followed by *Enterococcus species* (6%), *Staphylococcus aureus* with 3.2% and least for *Streptococcus species* with 1.6%. In case of GNB the maximum isolates was from *E.coli* with 5.4% followed by *Pseudomonas aeruginosa* (2.8%) which is illustrated in the Table no. 2

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

Pathogens	Number (N = 312)	Percentages (%)
Gram Positive Cocci	**58**	**18.5%**
Enterococcus species	19	6%
Staphylococcus aureus	10	3.2%
CoNS	24	7.6%
Streptococcus species	5	1.6%
Gram Negative Bacilli	**26**	**8.3%**
Escherichia coli	17	5.4%
Pseudomonas aeruginosa	9	2.8%
Contaminants	**24**	**7.6%**
No Growth	**204**	**65.3%**

It was observed that all the GPC isolated, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (94.8%). (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 (88.4%) and Piperacillin- Tazobactum (88.4%), 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 = 10)**		**Enterococcus species (N = 19)**		**CoNS (N = 24)**		**Streptococcus species (N = 5)**		**Gram Positive Cocci (N = 58)**	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R(%)**
Cefoxitin	9 90%	1 10%	-	-	6 25%	18 75%	5 100%	00	18 31%	40 68%
Linezolid	10 100%	00	19 100%	00	24 100%	00	5 100%	00	58 100%	00
Vancomycin	10 100%	00	19 100%	00	24 100%	00	5 100%	00	58 100%	00
Teicoplanin	10 100%	00	19 100%	00	24 100%	00	5 100%	00	58 100%	00
Penicillin	9 90 %	1 10%	16 84.2%	3 15.7%	2 8.3%	22 91.6%	5 100%	00	12 20.6%	46 79.3%
Nitrofurantoin	9 90%	1 10%	17 89.4%	2 10.5%	22 91.6%	2 8.3%	5 100%	00	55 94.8%	3 5.1%
Ciprofloxacin	9 90%	1 10%	8 42.1%	11 57.8%	6 25%	18 75%	-	-	15 25.8%	43 74.1%
Co-trimoxazole	7 70%	3 30%	-	-	8 33.3%	16 66.6%	5 100%	00	32 55.1%	26 44.8%
Gentamicin	7 70%	3 30%	16 84.2%	3 15.7%	16 66.6%	8 33.3%	-	-	34 58.6%	24 41.3%

* S = Sensitive, R = Resistant

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

Organisms	**Escherichia coli (N = 17)**		**Pseudomonas aeruginosa ((N =9)**		**Gram Negative Bacilli (N =26)**	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	15 88.2%	2 11.7%	5 55.5%	4 44.4%	23 88.4%	3 11.5%
Gentamycin	10 58.8%	7 41.1%	9 100%	00	22 84.6%	4 15.3%
Imipenem	10	7	9	00	22	4

	58.8%	41.1%	100%		84.6%	15.3%
Piperacillin-Tazobactum	13 76.4%	4 23.5%	9 100%	00	23 88.4%	3 11.5%
Nitrofurantoin	13 76.4%	4 23.5%	5 55.5%	4 44.4%	22 84.6%	4 15.3%
Ciprofloxacin	4 23.5%	13 76.4%	5 55.5%	4 44.4%	21 80.7%	5 19.2%
Co-trimoxazole	15 88.2%	2 11.7%	-	-	20 76.9%	6 23%

* S = Sensitive, R = Resistant

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

Organisms	**Oligozoospermia**	**Normozoospermia**	**Azoospermia**
Enterococcus species	8	4	2
Staphylococcus aureus	5	2	2
CoNS	20	14	2
Streptococcus species	3	-	1
Escherichia coli	9	2	6
Pseudomonas aeruginosa	3	1	0
Total	48	23	13

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

DISCUSSION

Infertility affects approximately one-sixth of couples globally, with the incidence of male infertility steadily increasing [13]. Male urogenital tract infections are a leading cause of bacterospermia and male infertility worldwide. Genital tract infection and inflammation have been linked to 8–35% of male infertility cases [14, 15]. Asymptomatic bacteriospermia could have a significant role [16]. Male accessory sex gland infection is a significant risk factor for infertility[17]. 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 [16,18]. As a result, microbiological testing of male partners in infertile couples can help diagnose male urogenital tract infections, particularly silent illnesses.

In the present study a total of 312 semen samples was included in our study out of which 86 (27.5%) 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 (48.8%) followed by 31-35 (26.7%) and least in the age group of 20-25 years of age (4.6%).

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 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 the current study 58 (18.5%) isolates were from the Gram positive cocci (GPC) and 26 (8.3%) 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* (7.6%) followed by *Enterococcus species* (6%), *Staphylococcus aureus* with 3.2% and least for *Streptococcus species* with 1.6 %.
In case of GNB the maximum isolates was from *E.coli* with 5.4% followed by *Pseudomonas aeruginosa* (2.8%). Simliar result was 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 our 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 (86.2%) and Piperacillin- Tazobactum (76.4%),Co-trimoxazole (88%).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].

Male infertility can be caused by various microorganisms, although the direct effects of bacterial and viral infections on male infertility are still debated. Bacterial infection was considered a significant element of infertility in the semen of asymptomatic infertile men.
Generally the risk of infertility increases by age but most of our investigated patients were young. The idea that bacterial infection may be partly responsible for male infertility arises from the clinical observation of the patients' male reproductive system. Infection processes may lead to deterioration of spermatogenesis, impairment of sperm functions, and obstruction of the seminal tract [28]. For infertility females are always blamed, but advancing knowledge proves males as an equal contributor to this problem. In recent years, male infertility has garnered increasing attention [29,30]. Screening of males by semen analysis provides some insight about the underlying pathological problems occurring in the male genital tract. In-view of the above, there is the need to institute a Pathological and the Microbiological intervention to detect the probable microbial agents [31-33].. It should be noted that presence of urogenital tract infection and inflammation poses a danger to the fertility profile of male patient and should be eradicated by the use of appropriate prescribed antibiotics and anti-inflammatory treatment.

CONCLUSION

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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