"A study on Semen Analysis and its Bacteriological Profile in Infertile Males: A Cross-Sectional Study at a Tertiary Care Center, Uttar Pradesh"

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Abstract:

Introduction: Infertility has become a grave emotional and social problem in India. Infections constitute up to 15% of the causes of male infertility. Male urogenital tract infection (UTI) is one of the reasons for most of male infertility as presence of bacteria in semen samples may compromise the sperm quality. Aim and Objectives: To study the Semen Analysis and its Bacteriological Profile in Infertile Males, A Cross-Sectional Study in a Tertiary Care Center, Uttar Pradesh.

Material and Methods: The present study was a cross-sectional study conducted in the Department of Microbiology at RMCH&RC, Mandhana for a period of 1 year i.e, August 2021 to August 2022. A total of 102 semen samples were collected, after informed written consent, from married males with the complaint of infertility. Semen analysis was carried out according to WHO guidelines. The specimens were processed using as per the latest CLSI guidelines for isolation and identification of the organism, followed by antibiotic susceptibility testing.

Results: A total of 102 semen samples was included in our study out of which 37 (36.2%) 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. The GPC accounts for 28 (27.4%) isolates and 9 (8.8%) isolates were Gram negative bacilli (GNB). The commonest isolates were the Coagulase Negative Staphylococcus species (10.7%) followed by Enterococcus species (7.8%), and least for Streptococcus species with 1.9%. In case of GNB the maximum isolates was from E.coli. All the GPC isolated, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (87.5%). Among the GNB, most were sensitive to Amikacin (87.5%) and Piperacillin- Tazobactum (71.4%), and lesser sensitivity was seen for Nitrofurantoin and Co-trimoxazole, the maximum number of cases recorded was from the Oligozoospermia and least from Azospermia.

Conclusion: There should be routinely awareness programs for the testing for the bacteriological profile of semen of infertile males as bacteria may affect the quality of semen because infections have been shown to adversely affect semen parameters such as sperm concentration, motility, and DNA fragmentation.

Keywords: Infertility, UTI, Semen,Antibiotic susceptibility testing

Introduction

Infertility is the inability to achieve conception in a period of 1 year in a couple, despite regular and adequate unprotected sexual intercourse [1]. It has significant psychological, sociocultural, economical, demographic, and physical problems [2, 3]. Seminal infection has been shown to affect semen parameters through various mechanisms such as breach of the blood–testis barrier and upregulation of inflammatory cytokines [4, 5].

It is widely accepted that male factor alone accounts for infertility in about 40% couples facing infertility female factor alone in 40% of the couples and in other 20%, there is a combined male and female factor. In India, the prevalence of primary infertility is estimated to be about 10-20% [6].

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The glands and organs that contribute to the semen are considered sterile. The sterility of the internal urethra is maintained by the normal flow of urine however, the distal urethra is not considered a sterile area. Therefore, the culturing of semen samples usually yields growth of organisms, many of which are considered to be normal flora of the genitourinary tract [7]. Semen contamination arises from the urinary tract of patients or can be sexually transmitted from the partner. Male urogenital tract infection is one of the most important causes of male infertility worldwide. Genital tract infection and inflammation have been associated to 8-35% of male infertility cases [8, 9]. The present study was undertaken to study the Semen Analysis and its Bacteriological Profile in Infertile Males in a Tertiary Care Center, Uttar Pradesh.

Material and Methods

This was a cross-sectional study conducted in the Department of Microbiology at RMCH&RC, Mandhana for a study period of 1 year i.e, August 2021 to August 2022. A total of 102 semen samples were collected, after informed written consent, from married males with

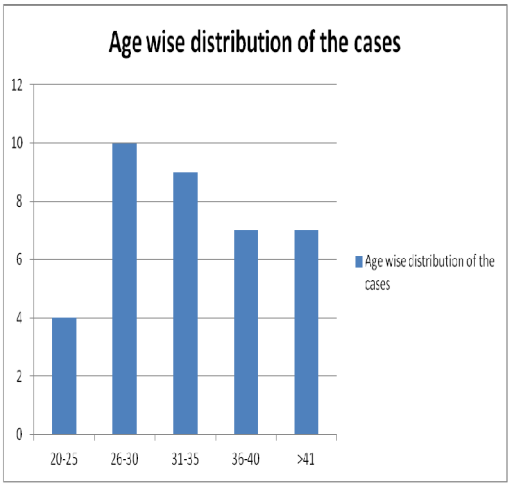
the complaint of infertility. Semen parameters such as appearance, volume, pH, viscosity, liquefaction, count, motility, and morphology, presence of other cells like epithelial cell or round cell, and sperm agglutination were recorded according to the WHO guidelines [10].
Samples were collected in sterile 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 >103 cfu/mL were considered as significant [11].
The specimens were processed as per the latest CLSI guidelines for isolation and identification of the the organism, followed by antibiotic susceptibility testing by Kirby-Bauer disc diffusion method [12].

Results

A total of 102 semen samples was included in our study after informed written consent, from married males with the complaint of infertility , out of which 37 (36.2%) 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 followed by 31-35 is shown in the Table no. 1

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

Age group (Years)	Culture positive (N=37)
20-25	4
26-30	10
31-35	9
36-40	7
>41	7



In our study 28 (27.4%) isolates were from the Gram positive cocci (GPC) and 9 (8.8%) isolates were from the Gram negative bacilli (GNB).
The commonest isolates was the Coagulase Negative Staphylococcus species (10.7%) followed by Enterococcus species (7.8%), Staphylococcus aureus with 6.8% and least for Streptococcus species with 1.9%. In case of GNB the maximum isolates was from E.coli with 6.8% followed by Pseudomonas aeruginosa (1.9%) which is illustrated in the Table no. 2

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

Pathogens	Number (N = 102)	Percentages (%)
Gram Positive Cocci	**28**	**27.4%**
Enterococcus species	8	7.8%
Staphylococcus aureus	7	6.8%
CoNS	11	10.7%
Streptococcus species	2	1.9%
Gram Negative Bacilli	**9**	**8.8%**
Escherichia coli	7	6.8%
Pseudomonas aeruginosa	2	1.9%
Contaminants	**18**	**17.6%**
No Growth	**47**	**46%**

All the GPC isolated, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (87.5%). (Table 3)
Among the GNB isolated, most were sensitive to Amikacin (87.5%) and Piperacillin- Tazobactum (71.4%), and lesser sensitivity was seen for Nitrofurantoin 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 = 7)		Enterococcus species (N = 8)		CoNS (N = 11)		Streptococcus species (N = 2)		Gram Positive Cocci (N = 28)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Cefoxitin	42.8%	71.4%	-	-	27.2%	72.7%	100	00	35.7%	64.2%
Linezolid	100	00	100	00	100	00	100	00	100	00
Vancomycin	100	00	100	00	100	00	100	00	100	00
Teicoplanin	100	00	100	00	100	00	100	00	100	00
Penicillin	42.8%	71.4%	62.5%	37.5%	9%	90.9%	100	00	46.4%	53.5%
Nitrofurantoin	85.7%	14.2%	87.5%	12.5%	90.9%	9%	100	00	92.8%	7.1%
Ciprofloxacin	42.7%	71.4%	37.%	62.5%	27.2%	72.7%	-	-	28.5%	71.4%
Co-trimoxazole	50	50	-	-	36.3%	63.6%	100	00	57.1%	42.8%
Gentamicin	50	50	71.4%	42.8%	63.6%	36.3%	-	-	64.2%	35.7%

* S = Sensitive, R = Resistant

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

Organisms	Escherichia coli (N =7)		Pseudomonas aeruginosa ((N =2)		Gram Negative Bacilli (N = 9)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	85.7%	14.2%	50	50	88.8%	11.2%
Gentamycin	57.1%	42.9%	100	00	77.7%	22.3%
Imipenem	57.1%	42.9%	100	00	77.7%	22.3%
Piperacillin-Tazobactum	71.4%	28.6%	100	00	88.8%	11.2%
Nitrofurantoin	71.4%	28.6%	50	50	66.6%	33.4%
Ciprofloxacin	28.5%	71.5%	50	50	55.5%	44.5%
Co-trimoxazole	42.8%	71.5%	-	-	44.4%	55.6%

* S = Sensitive, R = Resistant

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

Organisms	Oligozoospermia	Normozoospermia	Azoospermia
Enterococcus species	5	3	1
Staphylococcus aureus	4	2	1
CoNS	6	4	1
Streptococcus species	1	-	0
Escherichia coli	4	2	1
Pseudomonas aeruginosa	1	1	0
Total	21	12	4

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

Discussion

Infections are an important cause of male infertility. Seminal infection has been shown to affect semen parameters through various mechanisms such as breach of the blood–testis barrier and upregulation of inflammatory cytokines [4, 5]. Male genital tract infections are often linked to poor sperm motility and function, proper bacteriological testing of the semen including antibiotic resistance should also be included in andrological diagnostic workup for infertility testing. So, a significant number of patients can be treated, as these infections are potentially treatable with an appropriate antibiotic therapy. This in turn will help in preventing the transmission of the infection to the female partner as well as the possible illnesses of the offspring due to infection [13].

In our study a total of 102 semen samples was included in our study out of which 37 (36.2%) 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 followed by 31-35 and least in the age group of 20-25 years of age. This study was in support with the study conducted by the

other authors where the rate of bacterial growth was similar [14-16] but in contrast with the study by Enwuru CA et al, where the rate was 70%.

In our study 28 (27.4%) isolates were from the Gram positive cocci (GPC) and 9 (8.8%) isolates were from the Gram negative bacilli (GNB). Our study was parallel with the study conducted by Riddhi Hathiwala et al.,[16] where the maximum isolates was from GPC, another study by Moretti, et al. [14] [17] isolated 64% and 36%, respectively, which was similar to our study results. Different results was also found in other studies, where isolated about 48% of GPC and 52% of GNB [18].

The commonest isolates were the Coagulase Negative Staphylococcus species (10.7%) followed by Enterococcus species (7.8%), Staphylococcus aureus with 6.8% and least for Streptococcus species with 1.9%. In case of GNB the maximum isolates was from E.coli with 6.8% followed by Pseudomonas aeruginosa (1.9%). Simliar result was found by Riddhi Hathiwala et al.,[16] and Moretti, et al. Enwuru, et al. [17] reported 10.5% of E. coli and 29.6% of Staphylococcus species; while in study done by Bhatt, et al. the commonest isolates were E. coli (41.9%) and S. aureus (17.7%).

In our study all the GPC isolated, were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (87.5%). Among the GNB isolated, most were sensitive to Amikacin (87.5%) and Piperacillin- Tazobactum (71.4%), and lesser sensitivity was seen for Nitrofurantoin and Co-trimoxazole. Our study was in support with the study by Riddhi Hathiwala et al.,[16] 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.

There are studies which suggest that presence of bacteria in semen samples may compromise the sperm quality, by affecting sperm motility, morphology, spermatogenesis, obstruction of the seminal tract, and autoimmune processes [19].

Conclusion

In our study we found that most of the semen culture positive specimens collected were from 25-30 years age so, 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.

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