"CLINICAL CORRELATION OF MALE INFERTILITY WITH BACTERIAL INFECTION"

Vikas Mishra[1], Sheela Sharma[2], Sanjay Nigam[3], Shrawan Kumar[4*], Nashra Afaq[5], R Sujatha[6], Rita Mittal[7], Madhu Yadav[8], Desh Nidhi[9]

Abstract

Introduction: In India, infertility has escalated to a serious emotional and societal issue. Up to 15% of male infertility's causes are infections. One of the main causes of male infertility is male urogenital tract infection (UTI), as the presence of bacteria in semen samples may reduce the quality of the sperm.

Aim and Objective: To study the Semen Analysis and its Bacteriological Profile in Infertile Males at a Tertiary Care centre, Uttar Pradesh**.**

Material and Methods: The present study was a cross-sectional study conducted in the Department of Pathology and the Microbiology Department at RMCH&RC, Mandhana for a period of 1 year i.e June 2022 to June 2023. A total of 206 semen samples were collected, after informed written consent, from married males with the complaint infertility. Semen analysis was carried out according to WHO guidelines. The specimens were processed as per the latest CLSI guidelines 2022 for isolation and identification of the organisms, followed by Antibiotic susceptibility testing.

Results: A total of 206 semen samples was included in our study out of which 62 (20.09%) 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 (53.22%) followed by 31-35 years (22.58%) and least in 20-25 years (6.45%). The GPC accounts for 44 (21.33.4%) isolates and 18 (8.73%) isolates were Gram negative bacilli (GNB). The commonest isolates was the *Coagulase Negative Staphylococcus species* (8.73%) followed by *Enterococcus species* (4.85%), *Staphylococcus aureus* with 4.36% and least for *Streptococcus species* with 3.39%. In case of GNB the maximum isolates was from *E.coli* with 5.82% followed by *Pseudomonas aeruginosa* (2.91%) .

It was found that all the GPC isolates were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (88.88%).

Among the GNB isolated, most were sensitive to Amikacin (88.8%) and Piperacillin- Tazobactum (88.8%), and lesser sensitivity was seen for Nitrofurantoin, Ciprofloxacin 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 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.

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

[1]Professor, Department of Microbiology, G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India
[2]Professor and Head, Department of Obstetrics & Gynaecology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India
[3]Professor, Department of Pathology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India
[4*]Professor and Head, Department of Medicine, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India
[5]Research Associate, Department of Microbiology and Central Research Laboratory, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India
[6]Professor and Head, Department of Microbiology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India
[7]Professor, Department of Obstetrics & Gynaecology, Apollo Spectra Hospital, Kanpur, Uttar Pradesh, India.
[8]Associate Professor, Department of Microbiology, G.S.V.M. Medical College, Kanpur, Uttar Pradesh, India
[9]Professor, Department of Microbiology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India.

***Corresponding author:** Dr. Shrawan Kumar
*Professor and Head, Department of Medicine, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India, Email: drshrawanknp@gmail.com

DOI: - 10.48047/ecb/2023.12.si10.00528

INTRODUCTION

The biological inability of a man or woman to contribute to conception is known as infertility. To put it another way, infertility refers to a woman's inability to carry a pregnancy to term [1]. Normally a woman is fertile around their ovulation period (48 hours before to 48 hours after the ovulation) before returning to a normal state of infertility for the rest of their menstrual cycle [2] According to the study done by American National Institute of Health on infertility, male factors are responsible in 1/3rd of the cases, female factor in another 1/3rd of the cases, and in rest 1/3rd of the cases both male and female factors are responsible or no apparent cause is detected [3]. In other words we can say in around 40% of infertile couples, male is either sole cause or a contributing cause of infertility. In males, sperm deficit is the main factor responsible for infertility whereas in female it is more complex. Some evidence revealed that untreated urogenital infections in male and female can lead to infertility. Perhaps semen analysis is the most important laboratory investigation of male partner of an infertile couple [4] According to a number of studies, 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 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]. According to experimental study, there is a relationship between semen-derived bacteria isolation and degradation of spermatozoal activity and spermatogenesis, which can eventually result in infer case of infertile marriage, female partners are usually blamed because of lots of misconceptions about what a fertile man is. People think that once a man is able to have intercourse, ejaculate semen, then problem must lie in the wife and not in the male partner. However, with improvement in the level of education and awareness these days, trends are gradually changing. Many male partners are now visiting infertility clinics to verify their reproductive status if they are in doubt.

Therefore, the present study was undertaken to study the Semen Analysis and its Bacteriological Profile in Infertile Males at a Tertiary Care centre, Uttar Pradesh**.**

MATERIAL AND METHODS

This was a cross-sectional study conducted in the Department of Pathology and Microbiology Department at RMCH&RC, Mandhana for a study period of 1 year i.e, June 2022 to June 2023. A total of 206 semen samples were collected, after informed written consent, from married males with the complaint of infertility.

Inclusion Criteria:

1. The married males with the complaint of infertility and clinical sign and symptoms of infection.
2. The specimen analysed was semen obtained by masturbation.
3. The semen analysis was undertaken as per the WHO criteria.
4. In studies investigating the association of bacteriospermia with fertility, the male population were diagnosed with infertility.

Exclusion criteria:

1. Evidence of male urogenital infection such as male accessory gland infections (MAGI) or bacterial prostatitis.
2. Evidence of other causes of male infertility, for example drug use, hypogonadism.
3. Specimens other than semen used for assessment of microbiome composition (eg urine, urethral culture).
4. Studies reporting only on bacteriospermia without analysing the impact on semen parameters or the association with male fertility.
5. Patients having other than bacterial infection have been excluded.

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].

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 organism, followed by antibiotic susceptibility testing by Kirby-Bauer disc diffusion method as per the latest CLSI guidelines 2022 [12].

The test results were interpreted according to the clinical correlation of male infertility with bacterial infection. The variables of the study were collected in Microsoft excel sheet. Data was analyzed by descriptive statistical analysis using suitable statistical tools available in Microsoft excel 2022.

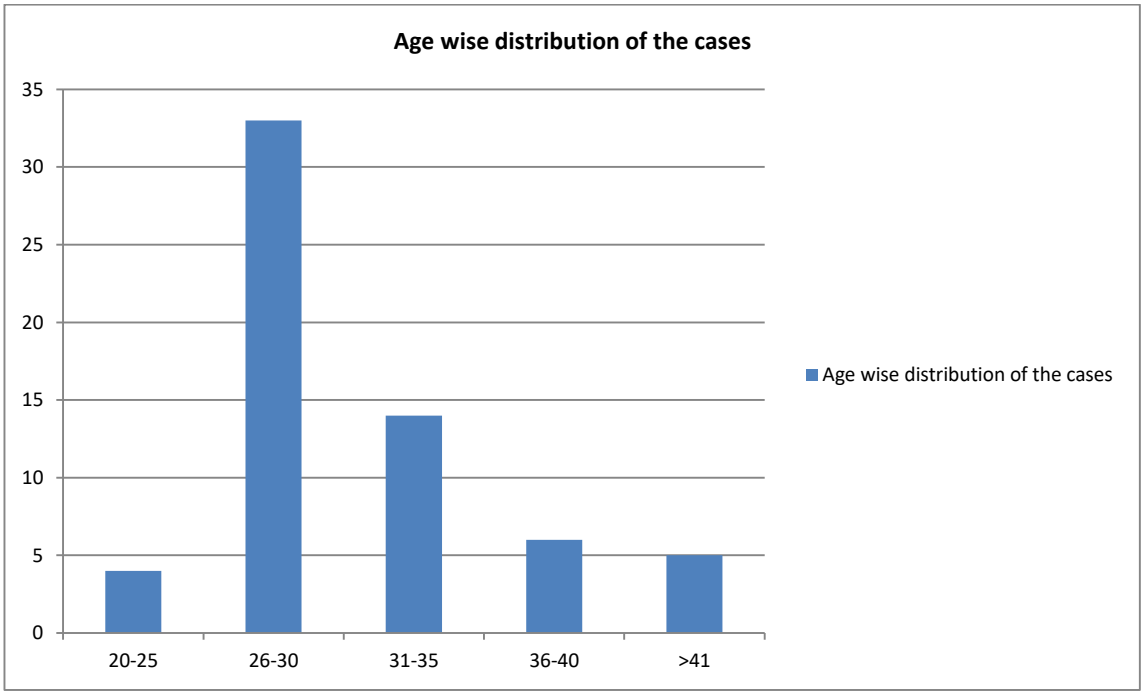
The study was approved by the Ethical Committee of Rama Medical College, Hospital & Research Centre, Kanpur, U.P. India.

RESULTS

A total of 206 semen samples was included in our study after informed written consent, from married males with the complaint of infertility , out of which 62 (30.09%) 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 (53.22%) followed by 31-35 (22.58%) and least in the age group of 20-25 years of age (6.45%), 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=62)	Percentage (%)
20-25	4	6.45%
26-30	33	53.22%
31-35	14	22.58%
36-40	6	9.67%
>41	5	8.06%



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

In our study 44 (21.33%) isolates were from the Gram positive cocci (GPC) and 18 (8.73%) isolates were from the Gram negative bacilli (GNB).

The commonest isolates was the *Coagulase Negative Staphylococcus species* (8.73%) followed by *Enterococcus species* (4.85%), *Staphylococcus aureus* with 4.36% and least for *Streptococcus species* with 3.39%. In case of GNB the maximum isolates was from *E.coli* with 5.82% followed by *Pseudomonas aeruginosa* (2.91%) which is illustrated in the Table no. 2

Table No. 2: Bacteriological Profile of Semen Culture.

Pathogens	**Number (N = 206)**	**Percentages (%)**
Gram Positive Cocci	**44**	**21.33%**
Enterococcus species	10	4.85%
Staphylococcus aureus	9	4.36%
CoNS	18	8.73%
Streptococcus species	7	3.39%
Gram Negative Bacilli	**18**	**8.73%**
Escherichia coli	12	5.82%
Pseudomonas aeruginosa	6	2.91%
Contaminants	**22**	**10.6%**
No Growth	**122**	**59.22%**

It was observed that all the GPC isolated, were sensitive to, Vancomycin, Teicoplanin and Linezolid. Among urinary antiseptic Nitrofurantoin (88.88%) was most effective drug. (Table No. 3).

Among the GNB isolated, most were sensitive to Amikacin (88.8%) and Piperacillin- Tazobactum (88.8%), and lesser sensitivity was seen against 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 isolated from Semen

Organisms	***S. aureus* (N = 9)**	***Enterococcus spp* (N = 10)**	**CoNS (N = 18)**	***Streptococcus species* (N = 7)**
Antibiotics	**S (%)**	**S (%)**	**S (%)**	**S (%)**
Cefoxitin	8 (88.88%)	-	5 (27.7%)	100
Linezolid	9 100%	10 100%	18 100%	7 100%
Vancomycin	9 100%	10 100%	18 100%	7 100%
Teicoplanin	9 100%	10 100%	18 100%	7 100%
Penicillin	8 88.88%	7 70%	2 11.1%	7 100%
Nitrofurantoin	8 88.88%	8 80%	16 88.8%	7 100%
Ciprofloxacin	8 88.88%	4 40%	5 27.7%	-
Co-trimoxazole	6 66.6%	-	7 38.8%	7 100%
Gentamicin	6 66.6%	7 70%	11 61.1%	-

* S = Sensitive, R = Resistant

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

Organisms	**Escherichia coli (N = 12)**		**Pseudomonas aeruginosa ((N =6)**		**Gram Negative Bacilli (N = 18)**	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	10 83.3	2 16.7%	3 50%	3 50%	16 88.8%	2 11.1%
Gentamycin	7 58.3%	5 41.6%	6 100%	 00	15 83.3%	3 16.6%
Imipenem	7 58.3%	5 41.6%	6 100%	00	15 83.3%	3 16.6%
Piperacillin-Tazobactum	9 75%	3 25%	6 100%	00	16 88.8%	2 11.1%
Nitrofurantoin	9 75%	3 25%	3 50%	3 50%	15 83.3%	3 16.6%
Ciprofloxacin	3 25%	9 75%	3 50%	3 50%	14 77.7%	4 22.2%
Co-trimoxazole	10 83.3%	2 16.6%	-	-	13 72.2%	5 27.7%

* S = Sensitive, R = Resistant

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

Organisms	Oligozoospermia	Normozoospermia	Azoospermia
Enterococcus species	6	5	1
Staphylococcus aureus	3	2	1
CoNS	18	12	1
Streptococcus species	1	-	0
Escherichia coli	6	2	2
Pseudomonas aeruginosa	1	1	0
Total	35	22	5

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

DISCUSSION

The Male urogenital tract infections is one of the most important causes of bacterospermia and male infertility worldwide. Genital tract infection and inflammation have been associated to 8-35% of male infertility cases [13-15]. Asymptomatic bacteriospermia may play a major role [16]. Male accessory sex glands infection is a major risk factor in infertility [17].

The significant of pathophysiology of bacteriospermia has been discussed in recent years. Some possible pathomechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology, etc), deterioration of spermatogenesis, auto-immune processes induced by inflammation and dysfunction of accessory sex glands [16,18]. Hence, microbiology investigation of male partners in infertile couple can be useful to detect the male urogenital tract infection, especially asymptomatic infections.

In the present study a total of 206 semen samples was included in our study out of which 62 (30.09%) 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 (53.2%) years followed by 31-35 (22.5%) and least in the age group of 20-25 (6.4%) years of age. 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 44 (21.33%) isolates were from the Gram positive cocci (GPC) and 9 (8.7%) 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* (8.73%) followed by *Enterococcus species* (4.85%), *Staphylococcus aureus* with 4.36% and least for *Streptococcus species* with 3.39%. In case of GNB the maximum isolates was from *E.coli* with 5.82% followed by *Pseudomonas aeruginosa* (2.91%). 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].

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 (88.88%).

Among the GNB isolated, most were sensitive to Amikacin (88.8%) and Piperacillin- Tazobactum (88.8%), 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 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 [26].

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 [27]. In-view of the above, there is the need to institute a Pathological and the Microbiological intervention to detect the probable microbial agents. 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

This study concluded that because of the important role of bacteriospermia in male infertility, more attention should be attached to young sexually active men health. 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.

REFERENCES

1. Makar R.S, Toth T.L. The Evaluation of Infertility. Am. J. Clin. Pathol. 2002; 117: 95-103.
2. Abebe MS, Afework M, Abaynew Y. Primary and secondary infertility in Africa: systematic review with meta-analysis. *Fertil Res Pract* 2020; 6(1): 20.
3. Enwuru C.P, Agbasi U.M, Umeh S.I, Egbuobi R.C. Bacterial isolates & semen analysis of male partners of infertile couples. J. Pharmacy. Biol. Sci. Jul.-Aug. 2013; 7(3):15-20.
4. Franken D.R and Dada O.A. Does Training Assist Medical Laboratory Scientists with Better Evaluation of Sperm Morphology? African J. Reproductive Health. 2007; 11(1): 107-12. .
5. Ivanov I.B, Kuzmin M.D and Gritsenko V.A. Microfl ora of the seminal fl uid of healthy men and men suffering from chronic prostatitis syndrome. International J. Andrology. 2009; 32(5): 462-67.
6. Keck C, Gerher-Schafer C, Clad A, Wilheim C, Beckwoldt M. Seminal tract infections: Impact on male fertility and treatment options. Hum. Reprod. Update. 1998; 4(6): 891-903
7. Shalika S, Dugan K, Smith RD, Padilla SL. The effect of positive semen bacterial
8. and Ureaplasma cultures on in-vitro fertilization success. Hum Reprod. 1996; 11: 2789-92.
9. Ibadin OK, Ibeh IN. Bacteriospermia and sperm quality in infertile male patient at University of Benin Teaching Hospital, Benin City, Nigeria. Mala J Microbiol 2008; 4: 65-7.
10. Keck C, Gerber-Schafer C, Clad A, Wihelm C, Breckwoldf M. Seminal tract infections: impact on male fertility and treatment options. Hum Reproduct .1998; 4: 891-903.
11. World Health Organization (WHO), *WHO Laboratory Manual for the Examination and Processing of Human Semen*, World Health Organization, Geneva, Switzerland, 5th edition, 2010.
12. T. Domes, K. C. Lo, E. D. Grober, J. B. M. Mullen, T. Mazzulli, and K. Jarvi, "The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters," Fertility and Sterility. 2012; 97: 5 :1050–1055.
13. Clinical Laboratory Standards Institute, M100-s24: Performance Standards for Antimicrobial Susceptibility testing: Twenty-First Informational Supplement, Clinical Laboratory Standards Institute, Wayne, Pa, USA, 2022.
14. Elnhar A. Male genital tract infection: the point of view of the bacteriologist. *Gynecol Obstetrique Fertili.* 2005; 33(9):691–697.
15. Ibadin OK, Ibeh IN. Bacteriospermia and sperm quality in infertile male patient at University of Benin Teaching Hospital, Benin City, Nigeria. *Mala J Microbiol.* 2008; 4(2):65–67.
16. Keck C, Gerber–Schafer C, Clad A, Wihelm C, Breckwoldf M. Seminal tract infections: impact on male fertility and treatment options. *Hum Reproduct Updat.* 1998; 4(6):891–903.

17. Bukharin OV, Kuzmin MD, Ivanov IB. The role of the microbial factor in the pathogenesis of male infertility. *Zhurnal Microbiologii Epidemiologii I Immuno biologii*. 2003; (2):106–110.
18. Li H Y, Lui JH. Influence of male genital bacteria infection on sperm function. *Zhonghoa Nan Ke Xue*. 2002; 8(6A):442–444.
19. Diemer T, Ludwig M, Huwe P, Haler DB, Weidner W. Influence of genital urogenital infection on sperm function. *Curr Opin Urol*. 2000; 1(1):39–44.
20. Moretti E, Capitani S, Figura N, Pammolli A, Federico MG, Giannerini V, et al. The presence of bacteria species in semen and sperm quality. J Assist Reprod Genet 2009; 26: 47-56
21. Mogra N, Dhruva A, Kothari LK. Nonspecific seminal infection and male infertility: A bacteriological study. J PostGrad Med 1981; 27: 99-104
22. Riddhi Hathiwala, Archana Bhimrao Wankhade, Poornima Dhandale. JMSCR. 2018; 06: 04 :830-837
23. . Enwuru CA, Iwalokun B, Enwuru VN, Ezechi O, Oluwadun A. The effect of presence of facultative bacteria species on semen and sperm quality of men seeking fertility care. Afr J Urol 2016; 22: 213-22
24. Ibeh Nnana Isaiah,[1] Bikwe Thomas Nche,[2] Ibeh Georgina Nwagu,[3] and Ibeh Isaiah Nnanna[4]. Current studies on bacterospermia the leading cause of male infertility: a protégé and potential threat towards mans extinction. N Am J Med Sci. 2011 ; 3(12): 562–564.
25. Isaiah IN, Nche BT, Nwagu IG, Nnanna II. Current studies on bacterospermia the leading cause of male infertility: a protégé and potential threat towards mans extinction. North Am J Med Sci 2011; 3: 562-564.
26. Madhuvanti Karthikeyan, Kubera N.S. , and Rakesh Singh· Association of Semen Bacteriological Profile with Infertility:– A Cross-Sectional Study in a Tertiary Care Center. J Hum Reprod Sci. 2021 ; 14(3): 260–266.
27. Bukharin OV, Kuzmin MD, Ivanov IB. The role of the microbial factor in the pathogenesis of male infertility. Zh Mikrobiol Epidemiol Immunobiol 2000; 2: 106-10.
28. Syriou V, Papanikolaou D, Kozyraki A, Goulis DG. Cytokines and male infertility. *Eur Cytokine Netw*. 2018; 29:73–82.