IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

TO EVALUATE THE PRESENCE OF PATHOGENIC MICROBES IN SEMEN OF PATIENTS FROM BILASPUR AND THEIR OUTCOME IN IVF

Arijeet Mukherjee[1], Research Scholar, Dept. of Life Science (Microbiology), CVRU Bilaspur (C.G.)

Dr. Shweta Sao[2], HOD, Dept of Life Science, CRVU Bilaspur (C.G.)

Abstract:

The objective of this study is to evaluate the seminal fluid for the presence of pathogenic microbes and their effect on the fertility of infertile male patients from bilaspur. For achieving a desired interpretation I have collected 60 semen samples from infertile and regular male patients. After collection following procedures like streaking, morphological study and biochemical tests are done for identifying pathogenic microbes present in semen samples. After studying every sample I came to the conclusion that spp. Of Staphylococcus, E. coli, Enterococcus, Candida spp. etc are the most common pathogens present in semen. The result also correlates the transmission of microbes in some patients from their female partners through vagina (vaginal micro flora). Due to the presence of these microbes the male patients are facing low sperm quantity as well as quality.

Keywords: Semen, Pathogenic Microbes, Transmission, Sperm Quantity, Sperm Quality

Subject Classification: Medical Microbiology

1. INTRODUCTION

Infections of the male genitourinary tract account for up to 15% of cases of male infertility. (Pellati D 2008). Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process, causing qualitative and quantitative sperm alterations (Henkel R, Schill WB 1998). Recent studies have shown that the simple presence of bacteria in semen samples may compromise the sperm quality (. Urata K, Narahara H 2001). The bacteria responsible for

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse (Sanocka-Maciejewska D 2005).

The most frequently isolated microorganism in male patients with genital tract infections or semen contamination is *Escherichia coli* (Purvis K, Christiansen E 1993). The negative influence of this species on sperm quality is partially due to its effect on motility and to the impaired acrosomal function, as demonstrated at the ultra structural level by Diemer (Diemer T. 2003).

During the process of ejaculation in healthy men sperm pass through the ejaculatory ducts and mix with fluids from seminal vesicles, the prostate, and the bulbourethral glands to form semen that is transported through the entire male reproductive tract including the urethra (Willen M 1996, Hillier SL 19990). Semen quality and quantity are both measures of fertility (Keck C 1998, Dohle GR 2005) and can be classified as asthenozoospermia, oligoasthenozoospermia, severe oligoasthenozoospermia, and azoospermia (Pant N 2003).

Semen has been found to serve as a medium for the transmission of bacteria and viruses between men and women (Gallo MF 2011, Swidsinski A 2010) contributing to the development of sexually transmitted diseases (STDs) (Elsner P 1987, Ott MA 2011). Moreover, certain bacteria, fungi, viruses and parasites are known to interfere with reproductive functions in both sexes and infections of the genitourinary tract account for about 15% of male infertility cases. This is generally accepted as one of the potentially correctable causes of male infertility (Pellati D 2008). Infections and consequent inflammation in the male reproductive tract may compromise spermatogenesis and sperm cell function (Henkel R 1998, Urata K 2001). The relationship between the presence of pathogenic microorganisms in the reproductive tract and infertility is widely documented (Sanocka-Maciejewska D 2005, Rehewy MS 1979). Several kinds of microorganisms found in the male urogenital tract are associated with sperm abnormalities, especially aberrant motility, deficient mitochondrial function, and loss of DNA integrity (Eggert-Kruse W 1995 Gdoura R 2005). These microorganisms include *Escherichia coli*, *Enterococcus faecalis*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Candida albicans*, and*Trichomonas vaginalis*. Most of these microorganisms are also associated with sexually transmitted infections (La Vignera S 2011, Merino G1995). Therefore, it is important to understand the bacterial species composition of seminal fluids to

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

better understand the etiology and pathogenesis of urogenital tract infections and associations between urogenital infections and infertility (Lacroix JM 1996, Nelson DE 2010).

2. REVIEW OF LITERATURE

Accorrding to **Joshua S. Jue and Ranjith Ramasamy** in 2017 there are currently no WHO guidelines on the indications for semen culture; however, semen cultures are performed in the evaluation of male infertility and the assisted reproductive technology (ART) process. The relevance and significance of positive semen cultures is widely debated in the literature, with no current consensus on the usefulness of this test in relation to male infertility. We review the pathogenic mechanisms of potentially pathogenic bacteria, general bacteria, urethral flora, and skin flora on sperm parameters. We also present, possible routes of semen contamination, measures to reduce contamination, and the clinical significance of culture contamination. First, it is critical to distinguish round cells in semen as leukocytes from immature germ cells. Second, it is critical to distinguish leukocytospermia from infected semen in order to guide management.

According to **D.Sanocka-Maciejewska, M.Ciupińska, M.Kurpisz** in 2005 We have analyzed two infertile male cohorts with (n = 39) and without genital tract infection (n = 14) comparing their selected seminological parameters with healthy controls (n = 30). Genital tract infection (GTI) has been defined by the presence of leukocytes and pathological bacterial strains identified with Bio-Merieux tests. We have found statistically significant deteriorated semen volume, sperm concentration, motility, morphology and vitality in ejaculated samples of patients with genital tract infection in comparison to healthy controls. Statistically significant negative influence towards sperm reproductive potential has been revealed in case of *Escherichia coli*, *Ureaplasma urealyticum* and *Staphylococcus aureus*.

Acoording to **J. Villegas, M. Schulz, L. Soto & R. Sanchez MD** 2005 an increased number of sperm undergoing apoptosis has been observed during inflammatory processes in the male genital tract, which might be associated with elevated reactive oxygen species (ROS) levels. However, another factor to stimulate apoptosis could be the direct contact with bacteria or its products, even in the absence of ROS. The aim of this study was to investigate whether bacteria can directly initiate apoptosis in human spermatozoa. Human spermatozoa selected by density gradient centrifugation were incubated with polymorphonuclear granulocytes (PMN) isolated from blood and/or *E. faecalis*, *E. coli* or *S. aureus*. As ROS inductor in PMN,

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

phorbol-12-myristate-13-acetate was used. After incubating the cells for 60 min at 37°C, ROS were determined by chemiluminescence and phosphatidyl serine (PS) externalization was analyzed by flow cytometry with Annexin V-FITC and propidium iodide (PI). The increase in the percentage of spermatozoa Annexin V-FITC-positive/ PI-negative (early event of late apoptosis) was significant after the incubation with PMN plus PMA, PMN plus *E. coli* and *E. coli* alone. The percentage of spermatozoa Annexin V-FITC-positive/ PI-positive (apoptosis/necrosis) increased significantly in sperm incubated with *E. coli* and *S. aureus*(20.3% ± 3 and 13.6% ± 3.2 compared to sperm alone, 6% ± 0.5). Sperm incubated with PMN-PMA activated showed only a relative increase in apoptosis/necrosis (8.4% ± 1). Our results show that bacteria directly increase the PS externalisation in ejaculated human sperm. This way of inducing apoptosis does not require external ROS and may result from anyone of the molecular mechanisms that account for changes in motility, vitality and DNA integrity, that are characteristics of spermatozoa in male genital tract infection.

According to **RHMehta, HSridhar,BR VijayKumar,TC Anand Kumar** 2002 Bacterial culture of semen samples from 100 male partners in infertile couples revealed the presence of aerobic bacteria in 49 cases. *Streptococcus faecalis* (*Enterococcus*) was isolated from 53%, micrococci species from 20% and α-haemolytic streptococci from 16% of the infected samples. The incidence of oligozoospermia and teratozoospermia was significantly ($P < 0.05$) higher in men whose semen samples contained *S. faecalis* than those whose semen samples contained micrococci or α-haemolytic streptococci or those that did not contain bacteria. The mean sperm concentration, as well as the mean percentage of morphologically normal spermatozoa, was significantly ($P < 0.03$) lower in semen infected with *S. faecalis* compared with that containing micrococci or α-haemolytic streptococci and the uninfected samples. There is a high incidence of semen infection with *S. faecalis*, and it is associated with compromised semen quality in terms of sperm concentration and morphology. The presence of micrococci or α-haemolytic streptococci does not appear to have any detrimental effect on sperm quality.

3. MATERIAL AND METHOD

MATERIALS

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

For the completion of this research work I am using a no. of materials and these materials are as follows:

- Samples
- Glassware's
- Laboratory Equipments
- Culture Medias
- Biochemical Reagents

We took total 150 samples in which type of is as follow

1. Semen sample

METHOD

We used different types of method as follows

1. **Streaking method**- Streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested (Black, Jacquelyn G. 1999).

2. **Culture media-** culture medium is a solid, liquid or semi-solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation, or small plants like the moss Physcomitrella patens. Different types of media are used for growing different types of cells (Madigan M 2005, Birgit Hadeler 1995).

    a. **MacConkey Agar**- MacConkey agar is an indicator, a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and differentiate them based on lactose fermentation (tmc edu. 2004).

    b. **Blood Agar-** Blood agar plates (BAPs) contain mammalian blood (usually sheep or horse), typically at a concentration of 5–10%. BAPs are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity. β-Hemolytic activity will show lysis and complete digestion of red blood cell contents surrounding a colony(Archived from the original on 2012*)*.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

c. **A7 Agar-** The A7 Agar is a modified Shepard medium, containing serum, peptones, yeast extract and a mixture of vitamins. It is lacking in sugar, but contains urea and arginine as a source of energy. The agar is selective due to the addition of antibiotic and antifungal drugs, thereby inhibiting the development of Gram-positive and Gram-negative bacteria, and fungi. The inclusion of magnesium sulphate results in the Ureaplasma urealyticum colonies having a black coloration in the presence of urea. On agar media, the mycoplasma colonies are small and should be identified with the aid of a microscope (Hi media Article 2023).

3. **Biochemical Test-** The term biochemical refers to something relating to biochemistry, the application of the tools and concepts of chemistry to living systems We are doing a no. of biochemical tests for which we need biochemical reagents, the tests are:

a. **Citrate agar-** Simon's citrate agar media was used for differentiating the intestinal bacteria and other micro organisms on the basis of citrate utilization. Citrate utilization is followed by alkaline reaction e.g., change of color from light green to blue.

b. **Urease agar-** Urea Agar was devised by Christensen for use as a solid medium for the differentiation of enteric bacilli. It differentiates between rapid urease-positive *Proteus* organisms. Some bacteria produce the enzyme Urease, which catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. Organisms that do not produce this enzyme cannot metabolize urea.

c. **Triple sugar iron-** This media was used for initial identification of Gram negative bacilli, particularly members of *enterobacteriaceae*. Three primary characteristics of a bacterium was detected by this media, include ability to ferment carbohydrate (lactose, sucrose, glucose,), ability to produce gas, and the production of hydrogen sulfide gas.

d. **Indole test-** Indole production was tested for some bacteria, which has the ability to degrade tryptophan to indole. Indole production was detected by Kovac's reagent (4-dimethyl amino benzaldehyde, isoamyl alcohol, hydrochloric acid).

e. **Catalase test-** Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas.

f. **Gram's Stain-** There are two types of micro-organism seen: first gram positive cocci shows violet colour and gram negative bacilli shows pink colour under microscope.

g. **Spot Oxidase Disc-** This test was used to identify the organisms, which produce the enzyme Oxidase. A positive reaction indicates by a deep purple blue within 5-10 seconds.

Sample collection:

1. **Semen sample-** Masturbation, directing the sample into a clean cup. This is the most common way to collect a semen sample.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

Inoculation of Samples

All samples were routinely cultured on MacConkey and blood agar plates. These plates were routinely incubated at 37^0C aerobically and after overnight incubation, they were checked for bacterial growth.

Isolation and Identification of Organisms

Suspected Gram negative organisms were identified by colony characteristics, motility, Oxidase reaction, citrate utilization, Indole and gas production and sugar fermentation reactions .Triple sugar iron agar was used for H_2S production, sugar fermentation.

Phenotypic Characteristics

Morphology

1. Microscopically morphology
2. Cultural characteristics

(a) Colonial morphology

(b) Biochemical Identification

4. RESULT

We took total 150 semen samples, in which 122 samples were positive with micro-organism and 28 semen samples were negative with no micro-organisms present in culture media. We gave an example of positive micro-organism in semen sample as below

S.NO	SMPLE ID	TYPE OF SAMPLE	ISOLATION TEST	
			MACCONKEY AGAR	BLOOD AGAR
1.	SF	Seminal Fluid		

SAMPLE ID	TYPE OF SAMPLE	IDENTIFICATION TEST	RESULT	NAME OF ORGANISM
SF	SAMINAL FLUID	GRAM STAIN	Negative	*Escherichia coli*

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

		MOTILITY	Motile	
		INDOLE	Positive	
		UREASE AGAR	Negative	
		CITRATE	Negative	
		TSI AGAR	Acid/Acid, Gas Positive	
		OXIDASE DISC TEST	Negative	
		CATALASE TEST	positive	
		COAGULASE TEST	Negative	

Table No.01 Species wise distribution of semen pathogens

Pathogens	Number	Percentage
Gram negative	39	62.9%
Escherichia coli	26	41.9%
Klebsiella pneumoniae	6	9.6%
Pseudomonas aeruginosa	3	4.8%
Proteus mirabilis	2	3.2%
Proteus vulgaris	2	3.2%
Gram positive	23	37%
Staphylococcus aureus	11	8%
Streptococcus faecalis	7	11.2%
Ureaplasma urealyticum	5	17.7%
Total	62	100

5. CONCLUSION

Genital ureaplasmas and mycoplasmas and other bacteria may colonize male urethra and contaminating the semen during ejaculation. However, these micro-organisms and particularly Ureaplasma urealyticum potentially pathogenic species playing an etiologic role in both genital infections and male infertility.The bacteria may affect the number of sperm and their ability to move in men. In women, it may cause an infection that makes pregnancy more difficult to achieve.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

7. REFERENCES

1. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol 2008;140:3–11. doi:10.1016/j.ejogrb.2008.03.009.

2. Henkel R, Schill WB. Sperm separation in patients with urogenital infections. Andrologia 1998;30:91–7.

3. Urata K, Narahara H, Tanaka Y, Egashira T, Takayama F, Miyakawa I. Effect of endotoxin-induced reactive oxygen species on sperm motility. Fertil Steril 2001;76:163–6. doi:10.1016/S0015-0282(01)01850-7.

4. Sanocka-Maciejewska D, Ciupińska M, Kurpisz M. Bacterial infection and semen quality. J Reprod Immunol 2005;67:51–6. doi:10.1016/j.jri.2005.06.003.

5. Purvis K, Christiansen E. Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Int J Androl 1993;16:1–13. doi:10.1111/j.1365-2605.1993.tb01146.

6. Diemer T, Huwe P, Ludwig M, Schroeder-Printzen I, Michelmann HW, Schiefer HG, et al. Influence of autogenous leucocytes and Escherichia coli on sperm motility parameters in vitro. Andrologia 2003;35:100–5. doi:10.1046/j.1439-0272.2003.00523.

7. Diemer T, Huwe P, Michelmann HW, Mayer F, Schiefer HG, Weidner W. Escherichia coli-induced alterations of human spermatozoa. An electron microscopy analysis. Int J Androl 2000;23:178–86. doi:10.1046/j.1365-2605.2000.00224.

8. Willen M, Holst E, Myhre EB, Olsson AM. The bacterial flora of the genitourinary tract in healthy fertile men. Scand J Urol Nephrol. 1996;30:387–93.

9. Hillier SL, Rabe LK, Muller CH, Zarutskie P, Kuzan FB, Stenchever MA. Relationship of bacteriologic characteristics to semen indices in men attending an infertility clinic. Obstet Gynecol. 1990;75:800–4.

10. Keck C, Gerber Schafer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update. 1998;4:891–903

11. Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. EAU guidelines on male infertility. Eur Urol. 2005;48:703–11.

12. Pant N, Upadhyay G, Pandey S, Mathur N, Saxena DK, Srivastava SP. Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality. Reprod Toxicol. 2003;17:447–50.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

13. Gallo MF, Warner L, King CC, Sobel JD, Klein RS, Cu-Uvin S, et al. Association between Semen Exposure and Incident Bacterial Vaginosis. Infect Dis Obstet Gynecol.2011;2011:842652.

14. Swidsinski A, Dorffel Y, Loening-Baucke V, Mendling W, Verstraelen H, Dieterle S, et al. Desquamated epithelial cells covered with a polymicrobial biofilm typical for bacterial vaginosis are present in randomly selected cryopreserved donor semen. FEMS Immunol Med Microbiol. 2010;59:399–404.

15. Elsner P, Hartmann AA. Gardnerella vaginalis in the male upper genital tract: a possible source of reinfection of the female partner. Sex Transm Dis. 1987;14:122–16.

17. Ott MA, Katschke A, Tu W, Fortenberry JD. Longitudinal associations among relationship factors, partner change, and sexually transmitted infection acquisition in adolescent women.Sex Transm Dis. 2011;38:153–7.

18. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol. 2008;140:3–11.

19. Henkel R, Schill WB. Sperm separation in patients with urogenital infections. Andrologia.1998;30 (Suppl 1):91–7.

20. Urata K, Narahara H, Tanaka Y, Egashira T, Takayama F, Miyakawa I. Effect of endotoxin-induced reactive oxygen species on sperm motility. Fertil Steril. 2001;76:163–6.

21. Sanocka-Maciejewska D, Ciupinska M, Kurpisz M. Bacterial infection and semen quality.J Reprod Immunol. 2005;67:51–6.

22. Rehewy MS, Hafez ES, Thomas A, Brown WJ. Aerobic and anaerobic bacterial flora in semen from fertile and infertile groups of men. Arch Androl. 1979;2:263–8.

23. Eggert-Kruse W, Rohr G, Strock W, Pohl S, Schwalbach B, Runnebaum B. Anaerobes in ejaculates of subfertile men. Hum Reprod Update. 1995;1:462–78.

24. Gdoura R, Kchaou W, Ammar-Keskes L, Chakroun N, Sellemi A, Znazen A, et al. Assessment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*,*Mycoplasma hominis*, and *Mycoplasma genitalium* in semen and first void urine specimens of asymptomatic male partners of infertile couples. J Androl. 2008;29:198–206.

25. La Vignera S, Vicari E, Condorelli RA, D'Agata R, Calogero AE. Male accessory gland infection and sperm parameters (review) Int J Androl. 2011;34:e330–47.

26. Merino G, Carranza-Lira S, Murrieta S, Rodriguez L, Cuevas E, Moran C. Bacterial infection and semen characteristics in infertile men. Arch Androl. 1995;35:43–7.

27. Lacroix JM, Jarvi K, Batra SD, Heritz DM, Mittelman MW. PCR-based technique for the detection of bacteria in semen and urine. J Microbiol Meth. 1996;26:61–71.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

28. Nelson DE, Van Der Pol B, Dong Q, Revanna KV, Fan B, Easwaran S, et al. Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. Plos One. 2010;5:e14116.

29. Joshua S. Jue and Ranjith Ramasamy Transl Androl Urol. 2017 Oct; 6(5): 916–922. doi: 10.21037/tau.2017.06.23PMCID: PMC5673804 PMID: 29184792.

30. D.Sanocka-Maciejewska, M.Ciupińska, M.Kurpisz Bacterial infection and semen quality Journal of Reproductive Immunology Volume 67, Issues 1–2, October 2005, Pages 51-56.

31. J. Villegas, M. Schulz, L. Soto & R. Sanchez MD Bacteria induce expression of apoptosis in human spermatozoa *Apoptosis* volume 10, pages105–110(2005)Cite this article 231 Accesses 72 Citations Metrics.

32. RHMehta, HSridhar,BR VijayKumar,TC Anand Kumar High incidence of oligozoospermia and teratozoospermia in human semen infected with the aerobic bacterium *Streptococcus faecalis* Reproductive BioMedicine Online Volume 5, Issue 1, 2002, Pages 17-21https://doi.org/10.1016/S1472-6483(10)61591-X

33. Black, Jacquelyn G. Microbiology: Principles and Explorations Marymount University, 1999.

34. Madigan M, Martinko J, eds. (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall. ISBN 0-13-144329-1.

35. Birgit Hadeler, Sirkka Scholz, Ralf Reski (1995) Gelrite and agar differently influence cytokinin-sensitivity of a moss. Journal of Plant Physiology 146, 369–371

36. "tmc.edu". Archived from the original on 2008-11-04.

37. "Blood Agar Plates and Hemolysis Protocols". *Archived from* the original *on 2012-02-02*. Retrieved 2014-10-28

38. A7Agar Media base 2023.