ORIGINAL ARTICLE

Role of Microbial Agents in Reproductive Health

Deborah Purushottam M, Nesepogu Padmaja, Rajeshwari Pilli

Abstract

Background: According to a systematic analysis of national health surveys, in 2010, approximately 10.5% of couples around the world experienced secondary infertility, and roughly 2% experienced primary infertility. The prevalence of secondary infertility, in particular, varies widely by region and country, ranging from 6% to 16% of couples. ***Purpose:*** To evaluate the presence of genito-urinary infections in infertile couples who sought counselling to investigate their condition. ***Material and Methods:*** Microbiological analysis was performed on semen and vaginal/cervical samples of both partners of each couple. The samples were cultured to find out the possible bacterial agent which might be the causative agent of reproductive health. ***Results:*** Among the collected samples 27% males and 30% females of the couples showed positive microbial isolates identified by Gram stain, darkground microscopy and by doing culture by conventional method. Among males 53 (27.6) samples are positive. *Escherichia coli* (54.7%) is the most frequent microbial followed by *Streptococcus agalactiae* (2.4%), *Enterococcus fecalis* (9.4%) and *Proteus spp* (7.5%). Among females 58 (30.2%) samples are positive. *Escherichia coli* (56.8%) is the most frequent microbial followed by *Enterococcus fecalis* (20.6%), *Staphylococcus haemolyticus* (17.2%) and *Streptococcus agalactiae* (12.1%). Multibacterial infections were more common in women than men (25.9% vs 20.7 %). The majority of patients were negative and the percentage of positive subjects was higher in women (30.2%) than men (27.6%) though it was not statistically significant (p value=0.65). ***Conclusion:*** It is concluded that the role of urogenital infections should be taken into account while evaluating the cases of infertility and appropriate antibiotic treatment should be given before investigating other possible cause of infertility as encouraging results are achieved in our study.

Key Words

Bacterial agents, Infertility, Antimicrobial susceptibility, Antimicrobial therapy

Introduction

Available data indicate that at least 50 million couples worldwide experience infertility (1). Secondary infertility, refers to women who have had at least one pregnancy and live birth previously, is more common than primary fertility, which refers to women without a biological child (2). According to a systematic analysis of national health surveys, in 2010, approximately 10.5% of women around the world experienced secondary infertility, and roughly 2% experienced primary infertility. The majority of researchers agree that infectious disease, which can lead to fallopian tube blockage, contributes largely to variation

Department of Microbiology, Konaseema Institute of Medical Sciences and Research Foundation (KIMS RF), Chaitanya Nagar, Amalapuram, East Godavari, Andhra Pradesh, India

Correspondence to: Dr Deborah Purushottam M, Assistant Professor, Department of Microbiology, Konaseema Institute of Medical Sciences and Research Foundation (KIMS RF), Chaitanya Nagar, Amalapuram, East Godavari, Andhra Pradesh

Manuscript Received: 27 April 2020; **Revision Accepted**: 05 October 2020;

Published Online First: 10 June 2021

Open Access at: https://www.jkscience.org/

Cite this article as: Purushottam MD, Padmaja N, Pilli R. Role of microbial agents in reproductive health. JK Science 2021;23(2):106-110.

among populations and changes over time (1). Since infertility risk tends to increase with age, differences and changes in the age at childbearing likely play a role.

Human infertility is defined as the inability for a couple to conceive and produce offspring after at least twelve consecutive months of unprotected sexual intercourse (3). Infertility results from multiple factors that are responsible for impairments of reproductive function in men and/or women. These factors include congenital and hormonal disorders, lifestyle, environmental hazards and psychological state (4). Among several causes for this condition, an important role seems to be played by urogenital infections (5). All these factors can lead to impairments in the function of genital organs, the production of reproductive cells, semen quality, sperm cell transport to the oocyte, fertilization and embryo implantation steps (6-9).

In the case of many couples, the cause of infertility remains unknown. Sexually transmitted infections (STI) can target different tissues along the genital tract in both men and women and lead to functional alterations (10). This can result in reduced fertility or even infertility. It is widely accepted that bacterial infections with *Neisseria gonorrheae* and *Treponema pallium*can impair fertility (10,11). In fact, in women, bacterial infections may cause pelvic inflammatory disease (PID) and lead to chronic pelvic pain, ectopic pregnancy and infertility (5,12,13). *Neisseria gonorrhoeae* is also known to be involved in damage to the female reproductive tract, and has been recognised as a cause of PID, together with *Gardnerella vaginalis* and *Trichomonas vaginalis* (5,14,15).

Some studies showed that bacteria, yeasts and protozoa may interact directly with sperm. These interactions result in attachment between bacteria and sperm, agglutination phenomena and morphological alterations to sperm (16). Among these species, there are pathogens which cause genito-urinary infections both in men and women, such as *E. coli*, *E. faecalis,* and *Candida spp* (17). These organisms are present in the human urogenital tract and may be responsible for damage. Most of these phenomena have been observed in *in vitro* experimental studies, but *in vivo* experimental studies confirmed that the presence of sperm agglutinant strains of *E. coli* and *S. agalatiae* in the vagina might be responsible for infertility because their removal from the vagina resulted in the recovery of fertility (16,18).

By keeping in mind the above facts, this study was undertaken to evaluate the presence of infections in couples with fertility disorders in and around Amalapuram an urban area in Andhra Pradesh, India.

Material and Methods

The study population consisted of 192 couples with fertility disorders who contacted the Obstetrics and Gynecological Department of (KIMS & RF) and also the patients who attended Obstetrics and Infertility Clinics in and around Amalapuram to investigate their cause of infertility of age below 40 years for both male and females. Institutional ethical committee approval was obtained before commencement of the study. Consent was taken from the couples whose samples were taken. Microbiological analyses were performed on both members of the couples at Microbiology Department of KIMS & RF.

Semen samples were collected by masturbation after 3 days of sexual abstinence. Patients were asked to urinate and wash hands and external genitalia before ejaculation to avoid possible contamination. The vaginal/ cervical sample collection was done by senior resident from gynaecology department. Vaginal or cervical samples are collected by introducing sampling swabs and rotating them for atleast 10 seconds along the vaginal walls and in the cervix before withdrawal.

Gram Stain is done for the detection of common bacteria and fungi like candida, the samples were observed under darkground microscopy also to detect Treponema pallidum cases. After direct microscopy the samples were inoculated on non-selective and selective media and incubated at 37 °C for 48 hours for presumptive pathogen identification. Antibiotic sensitivity is done for common aerobic bacteria and the sensitive drugs were used by the couple. The couples after antimicrobial study were followed for 6 months to know whether they conceived / turned fertile after receiving appropriate antimicrobial therapy.

Results

In this study we did microbiological analysis on 192 couples by collecting semen in males and vaginal /cervical swabs in females. Among 192 semen samples 53 (27.6%) samples were positive for microorganisms. Among 53 samples 29 (54.7%) samples were *Escherichia coli,* 14 (26.4%) samples were *Streptococcus agalatiae*, 5 (9.4%) samples were *Enterococcus fecalis*, 4 (7.5%) samples were of *Proteus spp*. In addition to bacterial isolates *Candida spp* was isolated from 9 (16.9%) samples, few have mixed bacterial (11 i.e., 20.7% samples) and some mixed bacterial and *candida* infection

(7 i.e., 13.2% samples). 4 (7.5%) samples were positive for *Neisseria gonorrhea* which were identified by Gram's stain and 3 (5.6%) samples were positive for *Treponema pallidum* which were identified by darkground microscopy (*Figure 1*). Among 192, samples 53 (27. 6%) were positive for microorganisms. Among them 11 (20.7%) showed mixed infection, while in others 42 (79.2%) it was due to single agent.

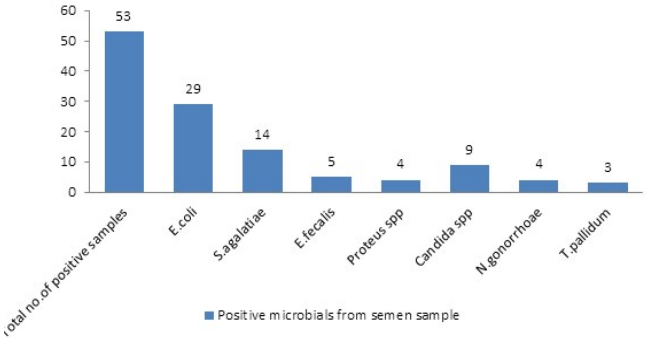

Figure 1: Positive Microbials from Semen Sample

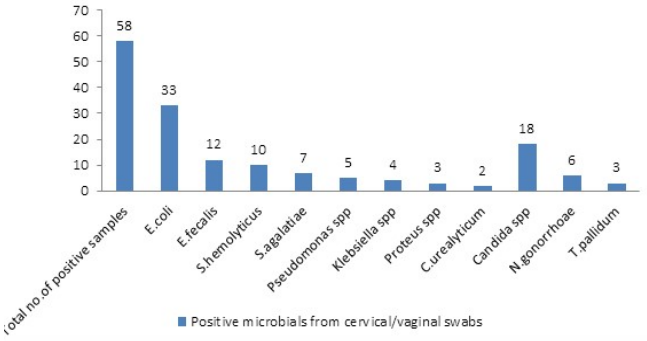

Figure 2: Positive Microbials from Cervical/Vaginal Swabs

Among 192 vaginal/cervical swabs 58 (30.2%) samples were positive and among them 33 (56.8%) samples were *Esherichia coli*, 12 (20.6%) samples were *Enterococcus fecalis*, 10 (17.2%) samples positive for *Staphylococcus haemolyticus*, 7 (12.06%) samples positive for *Streptococcus agalatiae*, 5 (8.6%) samples positive for *Pseudomonas spp*, 4 (6.8%) samples positive for *klebsiella,* 3 (5.1%) samples positive for *Proteus* and 2 (3.4%) samples positive for *Corynebacterium urealyticum*. In addition to bacterial isolates *Candida spp.* was isolated from 18 (31%) samples. 15 (25.9%) mixed bacterial samples were identified and 12 (20.6%) were mixed bacterial and candida infection. 6% samples were positive for *Neisseria gonorrhoea* which were identified by Gram's stain and 3% samples were positive for *Treponema pallidum* which were identified by darkground microscopy (*Figure 2*). In 58 (30.2%) of positive samples, infection was due to single agent is about 43 (74.1%) and in 15 (25.9%) due to mixed infection. Multi bacterial infections were more common in women than men (25.9% vs 20.7%).

The majority of patients were negative and the percentage of positive subjects was higher in women (30.2%) than men (27.6%) though it was not statistically significant (*p* value=0. 65). Among the couples who were infected, the organism isolated from both of them were almost having same isolates with mixed infection and in females *S. haemolyticus* and *Corynebacterium ureaelyticum* were additionally isolated.

All the aerobic bacteria were cultured and antibiotic sensitivity done by Kirby-bauer method and the sensitive drugs were given to the couples. Appropriate treatment for *Neisseria gonorrhea* and *Treponema pallidum* was also given. The patients were followed for next 6 months after giving antimicrobial therapy. Among 58 couples having bacterial isolates, 18 (31%) couples conceived or turned fertile.

Discussion

Infections of the urogenital tract are directly or indirectly a secondary major cause of infertility in couples since they may impair the reproductive organs both in men and women, and because microorganisms may cause the agglutination of motile sperm, an impairment of acrosome reaction and alteration in cell morphology (4,5,19).

The majority of patients were negative and the percentage of positive subjects was higher in women than men and study coinciding with Ruggeri *et al.* (17). Moreover, in women there was a wider variety of bacterial species responsible for the infections than in men. Both these aspects may be explained considering that women are more susceptible to genitourinary infections because the female urethra is shorter than the male and because the area around the vaginal opening is colonised with potential pathogens. Females with minimum health care and unhygienic practices during menstruation and after early abortions might also be the reason for urogenital infections. So, women also have more mixed infections 25.9% compared to men having 20.7% mixed infections.

The most frequently isolated microorganisms in women were *Escherichia coli, Enterococcus faecalis* and *Streptococcus agalactiae* among bacteria and *Candida albicans* among yeasts (17). In men most frequently isolated organisms were *Escherichia coli* followed by *E. faecalis* and these organisms are associated with compromised semen quality in terms of sperm concentration and morphology (20). *Enterococci* do not seem to influence sperm motility, but they seem to have a negative influence on membrane integrity of the human sperm head, neck and mid-piece (21). So, it might be reason of infertility in men in our study correlating with Quang *et al*. (21). Our study showed more number of *E. coli* isolates which has a sperm agglutinating activity *in vivo*. Moreover, studies at ultrastructural level showed its ability to impair the acrosomal function (16,22). In our study 31% positives also had *candida species* in females and 13.2% in males. Species of the genus *Candida* are able to interact with sperms causing reduced motility and profound changes to their ultrastructure (23).

Acute and chronic infections and consequent inflammation in the male reproductive system may compromise sperm cell function and the whole spermatogenetic process, resulting in quantitative and qualitative sperm alterations (24,25). The role of other pathogens, which are rarely encountered as causes of urogenital infections, such as *Klebsiella pneumoniae* or *Proteus spp.* has not yet been clarified (17). Most pathogenic bacteria isolated from patients with urogenital infection do not seem to have any impact on sperm motility. However, some works demonstrated that most strains of a pathogenic species do not affect sperm quality, but some strains can cause *in vivo* sperm agglutination or morphological alteration of sperm (16). The existence of different strains, of which only some may interact with sperm may explain why not all subjects with urogenital infections are infertile.

Infections occurring in the lower female genital tract represent a complex topic from several different viewpoints. First, there is a clinical consequence associated with these infections. Second, the complexity is increased by the biological diversity of infectious organisms involved including bacteria, fungi, virus, and protozoan life forms. Third, the interaction between the host and infectious organisms through the inflammatory responses to infection and through the elaboration of host factors such as secreted antibodies and defense in molecules inhibitory to intravaginal microorganisms. Finally, the vagina is host to an indigenous microbials which is credited with contributing to vaginal health, but the flora also interacts with exogenous microorganisms involved in the pathogenesis of vaginal infections. The vaginal infections derive from a biologically diverse collection of microbes.

We have to continue to examine both the science behind vulvovaginal infectious pathology and to devise clinically relevant therapeutic approaches to the problems that continue to make women uncomfortable, undermine their quality of life, and in some cases threaten their overall health and the health of their foetuses and infants (26).

Conclusion

Women with vaginal or cervical infections which are not treated is the leading cause of pelvic inflammatory disease and fallopian tube blockade leading to cause of infertility. The majority of researchers agree that infectious disease, which can lead to fallopian tube blockage, contributes largely to variation among populations and changes over time. Infertility in men could be due to reduction of sperm quality, sperm agglutinations by urogenital infections. In this study we found that 31% of urogenital microbial infections could be treated after testing and giving appropriate antimicrobial therapy. So, for investigating the cause of infertility the urogenital samples can be taken and test for presences of antimicrobials and treat them to decrease the burden of infertility.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

References

1. Sarah Hodin. The burden of infertility: global prevalence and women's voices from around the world. *Maternal Health Task Force* [Internet] 2017. Available from: https://www.mhtf.org/2017/01/18/the-burden-of-infertility-global-prevalence-and-womens-voices-from-around-the-world/
2. Tabong PT, Adongo PB. Infertility and childlessness: a qualitative study of the experiences of infertile couples in Northern Ghana. *BMC Pregnancy Childbirth* 2013;13:72.
3. Beckmann CRB, Ling FW, Barzansky BM, Herbert WNP, Laube DW, Smith RP. Infertility. In: Beckmann CRB, Ling FW, Barzansky BM, Herbert WNP, Laube DW, Smith RP, editors. Obstetrics and gynecology. 6th ed. Baltimore:

Wolters Kluwer Health/Lippincott Williams & Wilkins; 2010. p. 337-46.

4. Sleha R, Boštíková V, Salavec M, Mosio P, Kusáková E, Kukla R, *et al.* Bakteriální infekce jako pøíèina neplodnosti u lidí [Bacterial infection as a cause of infertility in humans]. *Epidemiol Mikrobiol Imunol* 2013;62(1):26-32.
5. Novy M, Eschenbach D, Witkin SS. Infections as a cause of infertility. *Glob Libr Women's Med* 2008. doi: https://doi.org/10.3843/GLOWM.10328.
6. Abrao MS, Muzii L, Marana R. Anatomical causes of female infertility and their management. *Int J Gynaecol Obstet* 2013;123(Suppl 2):S18-24.
7. Inoue S, Tomasini R, Rufini A, Elia A, Agostini M, Amelio I, *et al.* TAp73 is required for spermatogenesis and the maintenance of male fertility. *Proc Natl Acad Sci USA* 2014; 111(5):1843-48.
8. Pirkalani KK, Rad ZT. Psychological stress causes relative infertility through direct change in the frequency pattern of Gnrh release from the hypothalamus. *Eur Psychiatry* 2013;28(Suppl 1):1
9. Safarinejad MR, Shafiei N, Safarinejad S. The role of endothelial nitric oxide synthase (eNOS) T-786C, G894T, and 4a/b gene polymorphisms in the risk of idiopathic male infertility. *Mol Reprod Dev* 2010;77(8):720-27.
10. Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. *Am J Obstet Gynecol* 2017;216(1):1-9.
11. Fernandes LB, Arruda JT, Approbato MS, García-Zapata MT. Infecção por *Chlamydia trachomatis* e *Neisseria gonorrhoeae*: fatores associados à infertilidade em mulheres atendidas em um serviço público de reprodução humana [*Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection: factors associated with infertility in women treated at a human reproduction public service]. *Rev Bras Ginecol Obstet* 2014;36(8):353-58.
12. Zanetti S, Usai D, Molicotti P, Deriu A, Sechi LA. Presence of *Chlamydia trachomatis* in young women in Northern Sardinia. *New Microbiol* 2007;30(1):63-64.
13. European Centre for Disease Prevention and Control. Chlamydia control in Europe - Qualitative evaluation of the impact of the 2009 ECDC guidance document Chlamydia control in Europe. Stockholm: ECDC; 2015. Available from: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/chlamydia-control-europe-guidance-evaluation-march-2015.pdf
14. Mastromarino P, Hemalatha R, Barbonetti A, Cinque B, Cifone MG, Tammaro F, *et al.* Biological control of vaginosis to improve reproductive health. *Indian J Med Res* 2014;140(Suppl 1):S91-97.
15. Paavonen J. Immunopathogenesis of pelvic inflammatory disease and infertility - what do we know and what shall we do? *J Br Fer Soc* 1996;1(1):42-45.
16. Kaur K, Prabha V. Spermagglutinating *Escherichia coli* and its role in infertility: *in vivo* study. *Microb Pathog* 2014;69-70:33-38.
17. Ruggeri M, Cannas S, Cubeddu M, Molicotti P, Piras GL, Dessole S, *et al.* Bacterial agents as a cause of infertility in humans. *New Microbiol* 2016;39(3):206-09.
18. Cools P. The role of *Escherichia coli* in reproductive health: state of the art. *Res Microbiol* 2017;168(9-10):892-901.
19. 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(1):47-56.
20. Mehta RH, Sridhar H, Kumar BRV, Kumar TCA. High incidence of oligozoospermia and teratozoospermia in human semen infected with the aerobic bacterium *Streptococcus faecalis*. *Reprod Biomed Online* 2002;5(1):17-21.
21. Qiang H, Jiang MS, Lin JY, He WM. Influence of *enterococci* on human sperm membrane *in vitro*. *Asian J Androl* 2007;9(1):77-81.
22. 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(3):178-86.
23. Tian YH, Xiong JW, Hu L, Huang DH, Xiong CL. *Candida albicans* and filtrates interfere with human spermatozoal motility and alter the ultrastructure of spermatozoa: an *in vitro* study. *Int J Androl* 2007;30(5):421-29.
24. 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(1):163-66.
25. Sanocka-Maciejewska D, Ciupińska M, Kurpisz M. Bacterial infection and semen quality. *J Reprod Immunol* 2005;67(1-2):51-56.
26. De Seta F, Guaschino S, Larsen B, Donders G, Andabati G. The infections of lower genital tract. *Infect Dis Obstet Gynecol* 2012;2012:926435.