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TO UNDERSTAND SEMINAL BACTERIAL DIVERSITY ASSOCIATED WITH NORMOSPERMIA AND ASTHENOSPERMIA THROUGH NEXT-GENERATION SEQUENCING

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ABSTRACT

Background: Male factor is attributable in up to 50% of cases of infertility. Most studies on normal and abnormal sperm microbiota are based on 16S rRNA gene-specific next-generation sequencing (NGS). Microbiome analysis based on subunit 16S rRNA sequencing is a fast tool that can enable the identification of all the pathogenic microorganisms associated with semen in clinical pathology.**Material & Methods:** This study comprised of 280 Male infertility patients attending Pacific IVF Center between July 2019 and July 2020. Seminal microbiome was analyzed using 16S rRNA sequencing is a fast tool that can enable the identification of all the pathogenic microorganisms associated with semen in clinical pathology. **Results:** The major bacterial diversity in asthenozoospermic and normospermic samples belongs to the genera Lactobacillus, Prevotella, Staphylococcus, and Anaerococcus. **Conclusion:** Studies on the impact of semen micro-biomes in asthenozoospermic and normospermic samples could improve the results of assisted reproductive technologies.

Keywords: Male Infertility, Semen Microbes, NGS.

INTRODUCTION:

Infertility is becoming more frequent, and male factors (alone with female variables) thought to play a substantial role in roughly of infertile couples is 40%–50%. Despite the fact that modern therapies improve the chances of conceiving for couples who are experiencing male infertility, they frequently neglect the lack of a specific etiological or pathophysiological diagnosis. Unfortunately, in a vast percentage of cases, male infertility is still classified as "idiopathic (1-3). As a result, there is a significant need for study into the causes (and potential preventative treatments) of male infertility.

Genitourinary (GU) tract acute and persistent infections can promote male factor infertility. There are several infectious etiologies that account for Male factor infertility affects 15% of couples (4). Ochsendorf (5) and Keck et al. (6) indicated that there are so many pathogenic bacteria and viral as well as fungal infection, protozoan species that can

invade in the normal genital-urinary system via sexual transmission, which is infect the intracanicular dissemination of urine, or hematogenous seeding of genital organs. These Infections in the testis, epididymis, and prostate can cause spermatogenesis and reproductive problems (7, 8).

MATERIALS AND METHODS

This study comprised of 280 Male infertility patients attending Pacific IVF Center between July 2019 and July 2020. Depending on the spermiogram results, they were divided into two groups. Group 1 (n=127) was asthenozoospermic, and Group 2 (n=153) normospermic semen samples, Patients were aged between (20-45 years). The ethical approval was obtained from the institute.

Informed Consent was obtained from the participants.

Inclusion Criteria: All of the males were in generally good health, had no sexually transmitted infections or continuing uro-genital problems, and none were receiving antibiotic treatment at the time of the sampling. **Exclusion Criteria**: Importantly, this cohort has already been ruled out for male accessory gland infections due to negative seminal culture results as well as for several sexually transmitted diseases like syphilis and other. The ethical approval was obtained from the institute.

DNA Extraction and Quantification and PCR amplification of ribosomal marker and Sequencing

Genomic DNA was extracted from semen samples using the commercially available DNeasypower soil kit (Qiagen, Germany). For the sequencing, 16S Amplicon-Seq hypervariable regions V1–V9 were amplified using primers, including the forward 16S primer 5'-ATCGCCTACCGTGAC-barcode-AGAGTTTGATCMTGGCTCAG-3' and the reverse 16S primer 5'-ATCGCCTACCGTGAC-barcode-CGGTTACCTTGTTACGACTT-3', with gene-specific sequences, using MinION, Oxford Nanopore.

STATISTICAL ANALYSIS

To compare two data sets, t-test was employed. Mann- Whitney U-test was used for data not-normally distributed. The SPSS (Version 25.0; IBM,) 2016 was used for statistical analyses of the data at 5% significance level. The Average values were presented for continuous variables with a normal distribution, such as patient demographic information.

RESULTS

Different bacterial communities were detected when asthenozoospermic and normospermic semen samples were analyzed. Patients with asthenozoospermic parameter, a higher alpha diversity index tendency was found. In normospermic semen samples p value of bacterial diversity was non-significant (p=0.14 for the Shannon index) and significant differences in asthenozoospermic semen samples (p=0.01 for the Shannon index).In the beta diversity analysis, no significant differences were observed between the both groups, Relative abundance analysis identified the bacterial communities, Lactobacillus, Streptococcus and Ureaplasma in normospermic semen samples, and Anaerococcus and Gardnerella in asthenozoospermic samples, Comparison of asthenozoospermic and normospermic samples with alpha diversity (p=0.06 for the Shannon index and p=0.08 for the Simpson

index) and beta diversity ($p<0.001$) showed significant differences. Relative abundance in normospermic semen samples identified the bacterial communities, Lactobacillus ($p=3.70E-4$), Prevotella ($p=8.11E-4$), and in asthenozoospermic semen samples Anaerococcus ($p=0.004$) and Gardnerella ($p=0.004$). (Table 1).

Table: 1. Comparing various parameters between subjects with normospermia and asthenospermia.

Subjects Total N=280	**Normospermia**	**Asthenospermia**	**P - Value**
Number of subjects	153	127	0.29
Average age (y)	26-32	29-35	0.34
Semen volumes (ml)	1.3-1.5	0.7-1.2	0.28
Semen pH	7.5-7.8	7.1-7.6	0.46
Sperm counts(millions)	50-80	50-60	0.42
Sperm motility (%)	40-43	10-30	0.41
Bacterial diversity	*Lactobacillus*	*Prevotella*	8.11
	Gardnerella	*Anaerococcus*	0.004

DISCUSSION

Infertility affects one out of every seven couples throughout the world, and diagnosing and treating it takes time and money, which can be upsetting for couples (9). There have been several reports of pre-testicular, testicular, and post-testicular causes of male infertility (9). Genetic, immunological, viral, and anatomical variables all have the potential to produce inflammation in the male genital tract (9, 10, 11). As a result, poor sperm quality and, ultimately, male infertility have been associated to inflammatory state (12, 13, 14). Microorganisms, in particular, have been shown to impair spermatozoa functions via a variety of mechanisms. Surprisingly, inflammatory cytokines and an increase in the formation of oxygen reactive species aren't the only factors that contribute to this unfavourable effect (15, 16, and 17).Microbes, on the other hand, appear to be capable of directly interacting with spermatozoa by attaching to them or releasing soluble chemicals that can influence sperm motility or cause death (12,13,14).The molecular processes through which urogenital infections or specific bacteria might impair host physiology, resulting in alterations in semen quality and, as a result, typical infertile traits, are unknown (12).For many years, routine plate culture testing in infertile males has yielded positive results for aerobic bacteria growth in 15–100% of the cases investigated, however similar results have also been recorded in healthy and fertile men (15,16,17,18).As a result, anaerobic bacteria have been studied as well. In this respect, Rehewy et al. cultivated the sperm of infertile and fertile men and found that infertile individuals had more viable bacteria (19).

In the case of male infertility, it would be necessary to see if specific microbiological signatures are linked to the individual's fertility status. In a preliminary investigation, (20). discovered six microbial clusters,

none of which were linked to infertility. Anaerococcus is found in semen, and on the other hand, was linked to its poor quality. The bacterial content of semen was divided into three groups in a second study, two of which, Pseudomonas- and Prevotella-predominant, were linked to aberrant semen parameters (21). The Lactobacillus-predominant group had a higher prevalence of normospermic patients. According to new research, microbiota can be found in almost every part of the human body, including the endocrine niche, such as tumours, blood, and synovial fluid (22,23,24,25). According to new research, microbiota can be found in seminal plasma and play an important role in maintaining host homeostasis (26). The presence of bacteria in sperm has been linked to male infertility (27). Some bacteria in the urogenital tract can disrupt spermatogenesis and lower sperm quality in a variety of ways, including sperm motility, DNA integrity, and mitochondrial function degradation (28). Pathogens associated with male infertility include Escherichia coli, Mycoplasma genitalium, Ureaplasma urealyticum, Mycoplasma hominis, Staphylococcus aureus, and Chlamydia trachomatis (29,30, and 31). Several high-throughput sequencing studies have revealed a bacterial population in seminal plasma that includes Lactobacillus, Pseudomonas, Prevotella, and Gardnerella, among others. Worldwide around 15% of couples which are unable to conceive due to infertility, with men accounting for half of all infertility cases (32,33). Male infertility which can be affected by a variety of circumstances, including genetic, environmental factors (34, 35). (abnormal sperm) is a cause of infertility that affects around half of all male infertility cases (36, 37).

CONCLUSION

The impact of bacterial diseases including Chlamydia, ureaplasmas, and mycoplasmas on male fertility is still up for debate. Dysbiosis of the sperm microbiota can lead to illness. Because they may impair fertility through subtle effects on critical reproductive functions, their potential negative effect may not be apparent through traditional male infertility investigations, such as semen testing.

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