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Review Article

Microbiome matters: the hidden influence of gut flora on male reproductive health

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ABSTRACT

Male infertility is a complex disorder that affects about half of all cases worldwide. An increasing amount of research shows that the human microbiome has a significant impact on male reproductive health. Current understanding of how the gut, semen, and testicular bacteria affect reproductive results is studied in this review. Although bacteriospermia and poor semen parameters were linked in early culture-based investigations, microbial cultivation limits forced the use of sophisticated molecular approaches. The previous presumption of sterility has subsequently been challenged by metagenomic sequencing, especially next-generation sequencing (NGS), which has shown complex microbial communities in semen and testes. Changes in microbial composition, particularly in semen samples linked to assisted reproductive technologies (ART) and idiopathic non-obstructive azoospermia (iNOA), indicate dysbiosis may jeopardise sperm quality and the effectiveness of ART. Furthermore, a reciprocal relationship impacting hormonal balance and fertility is highlighted by interactions between the gut microbiota and androgen metabolism via the brain–gut–testis axis. Although the exact mechanisms are yet unknown, prebiotic and probiotic treatments have demonstrated promise in enhancing sperm motility, morphology, and DNA integrity. Current research is hindered by uneven methodology, a dearth of longitudinal data, and a lack of functional evaluations of spermatozoa, despite promising results. To demonstrate causality and therapeutic promise, future microbiome research must include stringent controls, longitudinal sampling, and thorough fertility evaluations. Knowing how the microbiome affects male fertility may help develop new probiotic-based therapies and diagnostic biomarkers, especially for cases of idiopathic infertility.

Keywords: Microbiota, Dysbiosis, Male infertility, Semen microbiome NGS

INTRODUCTION

The human microbiota refers to the composition of bacteria, fungi and viruses that are present within the human body within its organ systems.¹ The microbiome in the human gastrointestinal (GI) tracts constitutes an approximate of about 10^{14} , this includes a wide variety of microorganisms such as yeast, bacteria, bacteriophages.² In addition to the volume and variety of microorganisms, the gut microbiome (GM) is distinguished by its ability to provide resistance to inflammation and tumors, influence the metabolism or autoimmune responses of the host, and influence the function of the brain and endocrine system

(the brain–gut axis).³ Through the release of a variety of chemicals, the microbiome is responsible for regulating a variety of activities, including intestinal permeability, the composition and function of the mucus layer, epithelial cell function, innate and adaptive immunity, and neurotransmission.⁴ Despite the fact that the terms "microbiota" and "microbiome" are sometimes used interchangeably, there are nonetheless some differences between the two. The phrase "microbiota" refers to the living microbes that are present in a particular habitat, whereas the term "microbiome" refers to the collection of genomes from all of the microorganisms that are present in the environment.⁵

According to the conventional definition, infertility is defined as the inability of a couple to conceive during a period of one year after engaging in sexual activity that is both frequent and unprotected. In approximately twenty percent of cases, men are the only factor contributing to infertility. Additionally, males are a contributing component in between thirty and forty percent of the instances likewise. In general, the male component is responsible for a considerable contribution to approximately fifty percent of all cases of infertility.⁶ There is a wide range of factors that can influence male fertility, including disorders that can be reversed and some that cannot be reversed. Age, drugs, surgical history, exposure to environmental pollutants, genetic abnormalities, and systemic disorders are some of the other factors that can have an effect on male partner. When a male is evaluated for infertility, the primary goals are to discover the causes that are contributing to his inability to conceive, to provide treatment for those factors that are reversible, to decide whether or not the patient is a candidate for assisted reproductive methods (ART), and to evaluate and provide counselling for issues that are irreversible and cannot be treated.⁷

EARLY STUDIES ON SEMEN MICROBIOME AND MALE INFERTILITY

In earlier times, the approaches that were considered to be the gold standard for identifying and characterising the diversity of microorganisms were culture-based. Some microorganisms, however, are challenging to isolate and need specific conditions to thrive in a laboratory. On the other side, certain microorganisms are easy to cultivate, which may cause them to overcrowd or hinder the growth of bacteria that are growing at a faster rate. Considering some species may be over- or under-represented, it is challenging to evaluate data based on microbial culture techniques. Despite these drawbacks, a variety of bacterial species, including *Escherichia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., and *Ureaplasma* spp., have been recovered from semen using culture-based techniques.⁸

The majority of culture-based research revealed that men with bacterio-spermia had significantly lower sperm concentrations and a lower proportion of increasingly motile spermatozoa.⁹ However, contaminated semen appears to have the least impact on sperm morphology. Other groups, however, contested such conclusions. The prevalence of bacterio-spermia in normo-zoospermic males were comparable to that of infertile men. Furthermore, research has indicated that sperm quality is unaffected by the presence or absence of bacteriospermia.^{10,61-64} More sensitive identification and detection of certain microbial species has been made possible by the development of polymerase chain reaction (PCR) technology.¹¹ Using standard culturing techniques, the technology has detected bacteria that have never been isolated from semen or detected at extremely low concentration.^{12,57-60} In general, PCR data agreed with the

negative impact of bacterio-spermia on semen quality observed using culture-based methods. A recent investigation that used culturing indicated a comparable incidence of bacterio-spermia. Remarkably, infertile men were more likely to have bacterio-spermia than healthy donors.¹³

In summary, earlier studies had established a crucial foundation for understanding the possible contribution of microbiome to the reproductive health of men. It underlines the need for a more standardized and organized research to validate the connections and to delve deeper into microbiome-based research for the management of male factor infertility.

RECENT METAGENOMICS STUDIES

Numerous metagenomics research has investigated various human body parts since the Human Microbiome Project (HMP) began, utilizing high-throughput sequencing methods like next-generation sequencing (NGS).¹⁴ Studies of the microbiota in the female reproductive system have provided a significant amount of information regarding the involvement of the microbiome in human reproductive health. Dysbiosis of the uterine and vaginal microbiomes, for instance, has been extensively studied and connected to negative results in assisted reproductive technology (ART) and pregnancy rates.¹⁵ However, little is understood about how the microbiota contributes to male infertility due to lack of literature.

TESTICULAR MICROBIOME

Since not so long ago the male testes were believed to be completely uninhabited by microorganism or in other words completely sterile. This remained true until the experiment that was conducted by Alfano et al using NGS technology. The study conducted experiment on men with idiopathic non-obstructive azoospermia (iNOA) who had or did not have sperm retrieval following testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) and were compared to men who had undergone orchiectomy for other reasons in terms of the microbiota of their testicular tissue.¹⁶ Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria were the most common phyla in male's testicular tissue with normal germ lines, much like the seminal microbiota. Because of the significantly lower levels of Bacteroidetes and Proteobacteria ($p=0.00002$), they discovered that iNOA patients exhibited more dysbiosis, with more 16sDNA copy counts indicating a higher bacterial load ($p=0.02$) and lower species richness and diversity. Men with negative retrieval during microdissection testicular sperm extraction (micro-TESE) did not have *Clostridium*, according to additional research on the azoospermia group.¹⁷ As a result, Firmicutes and Actinobacteria, the latter of which are the dominating phyla in patients without sperm retrieval, dominated the testicular ecology of iNOA patients. However, no significant research has been done

on how the testicular microbiota shows its effect in the case of obstructive Azoospermia patients.

SEMEN MICROBIOME AND ART

Whilst sperm parameters have traditionally been the main focus of research on the semen microbiome, newer findings indicate that it may also affect the results of assisted reproduction. Following *in vitro* fertilization (IVF), several bacterial species in semen have been connected to the quality of the embryo. In particular, the classifications Alpha-proteobacteria and Gammaproteobacteria have been associated with lower embryo quality, while the family Enterobacteriaceae has been associated with higher embryo quality.¹⁸ It is crucial to remember that these outcomes could be impacted by additional variables such as the cause of infertility, stimulation techniques, and oocyte counts.¹⁸ The relationship between the female reproductive system and the semen microbiome is also crucial for IVF results. Dysbiosis in semen and vaginal or cervical samples was associated with a lower clinical pregnancy rate (19.5%) in a trial of 951 IVF couples than in couples who only had vaginal infections (36.2%).¹⁹ Additionally, other research groups have discovered significant variations in the microbiomes of vaginal and semen samples between couples who did not achieve clinical pregnancy following IVF and those who did.²⁰ *Proteobacteria*, *Prevotella*, and *Bacteroides* were found to be less prevalent in semen from instances with a subsequent clinical pregnancy, but *Lactobacillus jensenii* and *Faecalibacterium* were more colonized.²⁰ Lastly, IVF failure has been linked to certain genital infections. Couples experiencing IVF failure were more likely to have pathogens such as *Enterococcus faecalis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Escherichia coli*.²¹ Significantly more IVF failure cases (36.3%) had the group comprising *E. faecalis*, *U. urealyticum*, and *M. hominis* than successful cases (16.7%).²¹ This emphasizes how crucial it is to test for and treat microbial infections in order to enhance ART results.

Not all research supports the semen microbiome's importance in ART outcomes, in contradiction to the statistics. According to one research, a couples who had successful and unsuccessful intrauterine insemination (IUI) did not significantly differ in their semen microbiomes. According to recent studies on the vertical transmission of microbes into embryo culture media (ECM) and its correlation with assisted reproductive outcomes, semen was the primary source of contamination in traditional IVF cases.

Microbes can also vertically transmit from follicular fluid and semen to embryo culture media.²² The microbiomes of ECM, semen, and follicular fluid did not significantly correlate with the results of ART, although there were strong relationships between certain microbial taxa in semen and sperm quality.

MECHANISM OF ACTION BETWEEN MICROBIOME AND INFERTILITY

Several studies have recently revealed that gender differences impact the composition of the intestinal flora, resulting in type 1 diabetes and systemic lupus erythematosus having a sex-biased incidence.²³⁻²⁵ *Prevotella* is more prevalent in males than in women due to its strong positive link with testosterone, and it is interesting to note that men have a far lower variety of gut microbiota than women.²⁷ Several studies have also verified additional gender variations in gut microbiota.²⁶ Furthermore, *Clostridium*, *Methanobrevibacter*, and *Desulfovibrio* were less common in males than in females, although *Bacteroides* was more prevalent in females. Although the exact mechanisms are yet unknown, differences in sex hormones seem to play a role in this phenomenon.^{28,29} Over the past ten years, there has also been growing evidence that testosterone may significantly alter the gut microbiome through intricate processes.³⁰ Consequently, GM also has a role in controlling testosterone synthesis and metabolism. One of the primary mechanisms of androgen metabolism has also been identified as GM.³¹ Glutaronidated androgens can be expelled into the small intestine via the bile.³² One study discovered that by efficiently deglucuronidated glucuronidated testosterone (T-G) and dihydrotestosterone (DHT-G), GM, particularly GM in the cecum, which is mostly reliant on glucuronidase activity, produced androgens (including DHT and T) that were reabsorbed in the distal intestine.³⁰ Furthermore, glucocorticoids, pregnenolone, and hydroxypregnenolone can be converted into androgens by microbial species like *Clostridium scindens* and *Ruminococcus gnavus*, which raises intestinal DHT and T levels.³³

The capacity of certain gut bacteria to express steroid-processing enzymes and create steroid hormones has become evident as the role of sex steroids in the gut in influencing gastrointestinal system function has been increasingly understood. Another study found that the cerebral cortex had much lower levels of 3 α -diol and the colon had significantly higher levels of T metabolites, including DHT, 3 α -diol, and 17 β -E, than plasma.³³

The concentrations of DHT and 17 β -E in the colon and the cerebral cortex did not differ significantly.³⁴ By using steroid-metabolizing enzymes, certain bacteria, such as *Clostridium scindens* ATCC 35704 and *Butyricoccus desmolans*, convert and use sex hormones.³⁵ Thus, sex steroids may also regulate the structure and function of GM. It was shown that the composition of the gut flora changed after using finasteride, an enzyme 5 α -R inhibitor, and letrozole, an aromatase inhibitor.^{36,37}

PROBIOTIC AND PREBIOTIC THERAPY EFFECTS ON SPERM QUALITY

Probiotics are live microorganisms that have a number of ways to affect the host's health. Most commercial probiotic

products come from food, especially cultured milk products. Four categories can be used to categorize probiotic benefits: suppression of the proliferation of pathogens, enhancement of the function of the intestinal barrier immune system regulation, and pain perception regulation.³⁸⁻⁴² Therefore, probiotics can be utilized as a medicinal technique to treat a variety of illnesses. Probiotics have been employed in numerous trials to boost male fertility, and a number of plausible explanations have been put out. Three weeks of probiotic treatment (*Bifidobacterium longum* CECT7347 and *Lactobacillus rhamnosus* CECT8361) dramatically increased sperm motility and reduced DNA fragmentation, according to an observational study.⁴³ A reduction in intracellular H₂O₂ levels (approximately 3.5-fold change) was also observed, suggesting that probiotic may work through some mechanism to reduce intracellular (OS and hence prevent DNA fragmentation).⁴³

Six months after using probiotics (*Lactobacillus paracasei* B21060, arabinogalactan, oligo-fructosaccharides, and L-glutamine), the same outcomes were observed. By lowering the quantity of free radicals in semen (a nuclear protein that promotes favorable cohesiveness of sperm genetic material), a randomized controlled trial study showed that probiotics, not protamine, can lower OS and lessen sperm DNA damage.⁴⁴ Four weeks after adding *Lactobacillus rhamnosus* PB01 to a high-fat diet-induced obese mouse model, Dardmeh et al observed a significant improvement in the motility and morphological parameters of mouse spermatozoa when compared to controls. Additionally, *Lactobacillus rhamnosus* strains led to a significant increase in the cross section of the spermatogenic tubules and the number of Leydig cells per testis. During the four weeks of probiotic treatment, the mice fed a high-fat diet kept their body weight constant.⁴⁵ Treatment with probiotics (*Lactobacillus* and *Bacillus*) has also shown that they may help infertile mice's sperm dynamics and morphology by lowering sperm damage.⁴⁶ Among the most well-known prebiotics include oligosaccharides found in breast milk, galacto-oligosaccharides, and oligosaccharides found in oligosaccharides.⁴⁷ Prebiotics influence the host by changing the intestinal flora's makeup and serving as food for the good bacteria. According to earlier research, prebiotics can raise levels of specific probiotics, including *Lactobacillus* and *Bifidobacterium*, as well as short-chain fatty acids, which support immunological, metabolic, and other processes.⁴⁸

In addition to promoting spermatogenic tubule formation and spermatogenesis and growth, a study discovered that mice treated with oligo fructose showed notable changes in steroidogenesis, which may have changed plasma corticosterone and testosterone levels via the HPA axis.⁴⁹ Hence it is clear that using probiotic as well as prebiotic therapy significantly enhances male fertility and could possibly optimize IVF outcomes.

CHALLENGES FOR FUTURE MICROBIOME STUDIES

A more realistic representation of the semen microbiota is offered by molecular-based methods. It is unable to discriminate between living and dead microorganisms, however. Data from NGS investigations may therefore reflect both the short-term history of the semen microbiota in the host environment as well as the bacterial numbers at the moment of sample collection. Moreover, every semen microbiome study that has been published to date has examined semen samples at a single moment in time, offering no proof as to whether the microbiome in semen is permanent or temporary. Exploring that could provide insights into the dynamics of changes in the microbial community over time, regardless of the potential cost in maintenance.⁵⁰

Most of the NGS research indicated a connection between male infertility and the microbiota profile in semen. Nevertheless, those research data are inconclusive to draw firm conclusions. The majority of research employed aberrant semen parameters as a marker for male infertility, which is one explanation for this. The functional ability of spermatozoa to undergo crucial processes necessary for fertilization, such as acrosome reaction (AR) or zona pellucida (ZP) binding and oocyte penetration, cannot be accurately assessed based on routine semen analysis, despite the fact that semen analysis is frequently used in male fertility assessment.⁵¹

FUTURE PROSPECTS FOR MICROBIOME STUDIES

All metagenomics research requires both positive and negative control samples. Positive controls reduce the possibility of data misinterpretation and can identify possible biases in the microbial community.⁵² On the other hand, only a few studies have employed the collection of parallel samples, such as samples of urine or stomach, as a positive control.^{53,54} The use of negative controls, on the other hand, allows for the detection of contaminants, the identification of their sources, and the exclusion of those contaminants from the subsequent microbiome study.^{53,55,56} Hence it is highly recommended to keep control samples in all future research regarding the topic.

CONCLUSION

In conclusion, this narrative review focuses on new advances in both human and animal models to better understand the relationship between GM dysbiosis and male fertility. There have only been a few metagenomics research published in this area thus far, and they point to a possible influence of a particular microbial species on the quality of semen. The research that is now available indicates that gut bacteria play a role in the makeup of male reproductive health, and the relationship between GM and male fertility is complicated. Microbiome studies may be able to explain some idiopathic infertility, especially in

males with generally normal semen characteristics, as routine sperm analysis may not fully capture reproductive status. Additionally, researching the male reproductive system's microbiome will help us better understand how microbiome drifting affects male fertility and find bacteria with advantageous profiles that can be employed as probiotics to cure or enhance male fertility. Although the exact processes are yet unknown, probiotic and prebiotic supplements appear to have positive effects and improve several biochemical results in patients with male reproductive issues. The function of these therapies in the treatment or prevention of male infertility requires more investigation.

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